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FORUM: Metastasis

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Metastasis

OVERVIEW

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This Forum marks the formation of the Australasian Chapter of the International Metastasis Research Society - OzMRS. OzMRS grew out of the strong local interest in metastasis research, which became obvious when the 14th International Biennial Congress of the Metastasis Research Society (MRS) was held in Brisbane in 2012. OzMRS was formally established in 2013 and became an affiliated organisation of the Clinical Oncology Society of Australia (COSA). Articles in this Forum have been contributed by OzMRS members and illustrate the comprehensive approaches being taken in Australia to understand the molecular and cellular basis of metastasis and thereby provide better outcomes for those afflicted with metastatic cancer.

The development of metastatic disease is a devastating event for cancer sufferers since, in many patients, it is likely to be the cause of their death. Primary cancers can usually be treated successfully with localised therapies including surgery and radiotherapy, but neither of these treatments is generally curable in the setting of distant metastases, unless a very limited number of secondary lesions are present. Given the fact that it is metastatic disease that leads to the demise of most patients with solid cancers, there is a major world-wide research effort to identify the genes, proteins and processes that regulate metastasis, to understand the contribution of host cells, including the major role of the immune system in metastasis, and to find targeted therapies against either the tumour cells or against tumour-promoting host cells.

The process of metastasis is complex, involving many genes and signaling pathways, both intrinsic to the tumour cells and those that influence the surrounding (host) tissues. Specific metastasis promoting and metastasis suppressing genes have been identified in tumour cells, where they regulate metastasis but have little impact on primary tumour growth, as reviewed by Roesley et al.[1] Therapies based on targeting these genes are being developed, since they are the initial drivers of metastatic disease. If we can block these genes we may indeed remove or reduce the risk of metastasis altogether.

However, many normal host cell lineages are also vital for the successful metastasis of a tumour, and provide additional opportunities for therapy. The circulatory systems, including the vasculature and the lymphatics, are essential to the growth of tumours and in addition, provide the avenues of escape for primary tumour cells and their carriage to distant tissues where secondary tumours can develop. Therapies are being developed to target both of these circulatory systems, with some anti-angiogenic therapies already in standard clinical use. Reviews of the contributions of these two circulatory systems are provided by Karnezis and Ramin for lymphatics and by Mellick et al for vasculature.[2,3]

As our preclinical models of metastatic disease improve to more closely reflect events that occur in patients, we have come to recognise the major contribution of various immune cell lineages to metastasis. While the initial response of the immune system is to attack the tumour cells, factors secreted by tumours subvert some immune cell lineages into tumour promoting cells, as described in the article by Edgington-Mitchell and Parker.[4] Hence the concept of targeting the immune system to avoid tumour progression to metastatic disease is gaining considerable momentum, with several anti-immune therapies in clinical trials.

Over the past few years, attention has been focused on the presence and significance of tumour cells in circulation. Technological advances have allowed researchers to detect very low numbers of these circulating tumour cells in blood, allowing their prognostic significance to be assessed, as reviewed by McInnes and Saunders.[5] The idea of analysing blood as a 'liquid biopsy' also extends to detection of cell-free DNA derived from the tumour cells and to exosomes secreted both from tumour cells and from host cells in response to the presence of the tumour. As reviewed by Wen et al,[6] exosomes are small microvesicles that contain proteins, lipids and RNA that can be transferred between cells. Evidence exists to show that tumour-derived exosomes can modulate the host to promote tumour growth and establish a more favourable metastatic site before the tumour cells even arrive. Initial investigations into the prognostic value of these small particles that can be isolated from the circulation are underway.

There is increasing realisation that many primary tumours have metastasised prior to diagnosis. Hence, our focus

needs to remain on effective therapies for established metastases, although it will also be important to prevent metastasis during therapy and from established metastatic lesions. It is remarkable that secondary disease may not become apparent for many years following successful treatment of the primary tumour. This latent metastasis is known as dormancy, whereby a solitary tumour cell or a micrometastasis can remain viable, but unable to expand into a clinically detectable lesion. It is proposed that eventually, a few of these dormant cells break free from this restraint, through mechanisms not at all well understood and hard to study experimentally. The concept of dormancy and its clinical relevance is reviewed by Chakrabarti and Anderson.[7]

The altered metabolism that occurs in cancer cells has become a major research focus in recent years and several genes involved in metabolism are now recognised to act as oncogenes. The analysis of metabolic pathways and genes that are altered in tumours offers a new therapeutic opportunity, as well as a means of monitoring tumour progression and response to therapy in patients. Pouliot and Denoyer review the key findings in this area and the use of positron emission tomography to image the changes in metabolism that occur in tumours during therapy.[8]

One of the challenges in treating metastatic cancer is the influence of the microenvironment in which the secondary tumour grows. It is well known that specific types of cancer metastasise preferentially to some tissues and not others. For example, breast, prostate and lung cancers, and melanomas, are more likely to home to bone than other types of cancer. In addition, some cancers metastasise to the brain, where the blood-brain barrier strongly influences our ability to treat these tumours. This concept of site-specific metastasis has led to the development of specific therapies for secondary tumours at different sites and also indicates very strongly the profound influence of the tumour microenvironment on the growth of metastases. Hossain and Dunstan discuss the unique microenvironment of the bone and how this allows for some specific therapy options, although they remain mainly palliative at this stage.[9]

A more general review of strategies to treat metastases at different sites by targeting the tumour microenvironment is presented by Quah et al,[10] bringing examples from clinical trials of a number of tumour types, including prostate, breast, lung and colorectal carcinomas, and melanomas both in the skin and eye. A number of therapies targetingstromal fibroblasts, infiltrating immune cells, blood vessels, signalling molecules, extracellular matrix and tissue oxygen levels have been tested, as described in their article.

Another major challenge for successful therapy of progressive cancer is the heterogeneity that develops between the primary and secondary tumours. It is likely that subpopulations of the primary tumour are able to metastasise and their response to therapy will be different to that of the primary tumour. Kutasovic et al discuss the evidence for heterogeneity in clinical samples and the consequences of this heterogeneity for therapy, using breast cancer as an example.[11] It is now apparent that tumour heterogeneity is a major cause of the intrinsic or acquired resistance to therapy.

The pace of metastasis research has increased in recent years, offering the potential of new therapies to combat progressive disease. Our better understanding of the molecular mechanisms and more clinically relevant animal models of metastatic disease will allow the development of therapies that provide a significant benefit for patients for whom current therapies provide only palliative relief.

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METASTASIS SUPPRESSORS AND THEIR ROLES IN CANCER

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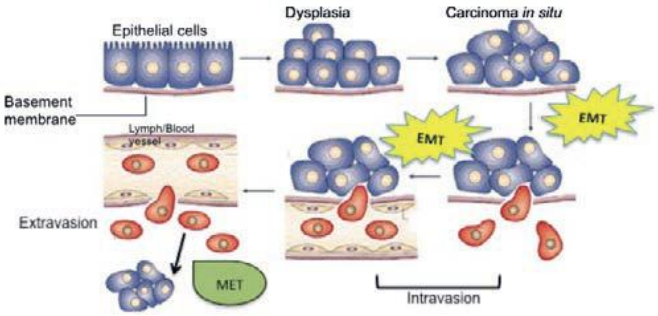
Abstract

Cancer arises from the deregulation of intracellular signaling pathways leading to uncontrolled cell proliferation and tumour formation. In many cases, cancer cells in the primary tumour disseminate and colonise distant tissues and organs to form secondary tumours by the process of metastasis. Metastasis is a complex multistep process, involving migration of cancer cells from the primary tumour, their systemic spread by the circulatory system, followed by the colonisation and growth of these cells into tumours at secondary sites. Metastatic tumours are responsible for the majority of cancer deaths. Understanding the mechanisms of metastasis is therefore crucial to understanding carcinogenesis, predicting the likelihood of primary cancer spread and devising new strategies for the treatment of metastatic cancer. Numerous pathways can affect metastasis and it is now clear that inactivation of members of the metastasis suppressor gene family plays a central role in this process in many human cancers. These genes suppress metastasis but not primary tumour growth. To date, over 20 metastasis suppressors have been discovered, which can act at various stages along the metastatic pathway. In this review we discuss the different mechanisms of action of selected metastasis suppressor genes to illustrate their diversity of action.

The development of cancer stems from cellular transformation and the ability of cancer cells to evade normal regulated processes. Cancer cells accumulate a series of defects in several regulatory processes, leading to tumourigenesis and malignancy. A number of key hallmarks are believed to be important during the development of cancer and malignancy, including self-sufficiency in growth signals, insensitivity to growth-inhibitory signals, ability to evade apoptosis, unlimited replicative potential and sustained angiogenesis. The final stages of cancer include the ability of cells to invade tissues and metastasise to distant tissues and organs.[1,2]

The majority of deaths among cancer patients are due to metastatic tumours rather than the primary tumour, due to impedance of the function of vital organs.[2] There has been a growing interest in the study of metastasis, to gain a deeper understanding of this process with the aim of improving cancer prognosis and treatment. Metastasis is a complex, multi-step process where cancer cells disseminate from the primary tumour, colonise distant tissues and organs and grow into secondary metastatic tumours. It is proposed that cells within a primary tumour can undergo a process termed the epithelial to mesenchymal transition (EMT), to become less adherent and more motile.[3,4] This aids in their ability to break through the basement membrane of an organ or tissue, allowing the cells to enter the vasculature by the process of intravasation and travel via the lymphatic or circulatory systems. The circulating tumour cells can get arrested on the lymphatic or blood vessel walls, before leaving the system and invading their new environment by the process of extravasation. For the cancer cells to proliferate and colonise the new secondary site, they are thought to undergo a mesenchymal to epithelial transition, losing their motility but leading to increased adhesion and proliferation (figure 1).[5] Metastasis is a very inefficient process, with only a small proportion of cancer cells acquiring the capacity to survive each step along the metastatic cascade. Metastatic cancer cells generally colonise specific tissues and organs that are permissive for their survival and growth. In 1889, Paget observed that breast cancer metastases preferentially colonise the liver rather than other organs such as the spleen, which is subject to a similar amount of circulation. He hypothesised that the tumour cells (seeds) are distributed equally across the body and only invade organs which provide a favourable environment (soil), facilitating their colonisation. This led to the 'seed and soil' theory to describe organ specificity of metastatic cancer cells.[6] Host-tumour interactions involving the reciprocal interaction between tumour cells and their surrounding micro-environment, inflammatory and other stromal cells, play an important role in determining which tissues and organs are colonised by cancer cells.[7-14] At a molecular level, numerous enzymes such as matrix metalloproteinases, cytokines, chemokines and growth factors are important for promoting remodelling of the extracellular matrix (ECM) and for facilitating cancer cell survival, proliferation and colonisation at the secondary site.[7-18]

Figure 1: Metastasis is a complex, multistep process. The metastatic cascade involves several steps. Normal epithelial cells are transformed into cancer cells in the primary tumour, then undergo an epithelial-to-mesenchymal transition (EMT) to become more motile. They can then, through the process of intravasation, break through the basal lamina and travel via the lymphatic or circulatory system. Upon reaching an appropriate secondary site, the cells can extravasate and colonise the new tissue, undergo a mesenchymal-to-epithelial transition (MET), losing their motility, continue to proliferate and form secondary metastatic tumours at the new tissue. Adapted from Kirton et al, 2010



Metastasis suppressor genes

Initial discovery

The discovery of tumour suppressor genes, such as the Retinoblastoma gene (Rb), which are mutated and inactivated in many human cancers leading to their

transformation and uncontrolled proliferation,[19] prompted the search for genes that may be involved in the regulation of metastasis. Early studies identified potential metastasis suppressor genes by loss of heterozygosity, comparative genomic hybridisation and karyotype analysis of chromosome abnormalities in human tumours. Chromosomes containing potential metastasis suppressor genes were then individually introduced into cells by microcell-mediated transfer. This method has been instrumental in identifying numerous metastasis suppressors.[20] Subsequently, positional cloning or differential gene expression studies were used to narrow down to a region within the chromosome and eventual identification of a specific gene.[21-24] To confirm its function as a metastasis suppressor, the gene is transfected into a competent cell line with low expression or activity of this gene. Various in vitro assays that measure phenotypes associated with metastasis, such as motility, invasion and colonisation abilities, are then evaluated. However, to validate a gene's metastasis suppressor function, studies must be completed to show that its expression reduces metastasis without affecting tumourigenicity in vivo.[25] To date, over 20 metastasis suppressor genes that act at various stages of the metastatic process have been identified (table 1).[26] We will discuss the roles of a selection of metastasis suppressors to highlight their diverse mechanisms of action.

Table 1: Metastasis suppressor genes.

Metastasis suppressor gene	Cancer cell type with suppressive activity	Function	Major roles in metastasis inhibition
BRMS1	Breast Melanoma Ovarian Non-small cell lung	Transcriptional repressor, complexes with histone deacetylase inhibits phosphoinositide signalling gap junction communication	Invasion, colonisation (induce anoikis)
Cadherin-11	Breast Osteosarcoma	Cell-to-cell and cell-to-matrix adhesion	Invasion
Caspase 8	Neuroblastoma	Induces apoptosis upon interaction with unliganded integrins	Colonisation (induce apoptosis)
CD44	Prostate	Transmembrane glycoprotein, binds to ECM components	Invasion
DCC	Prostate Oesophageal Squamous Pancreatic Colorectal	Caspase substrate Regulate MAPK signalling Cytoskeletal organisation	Colonisation (induce apoptosis)
DLC1	Breast Liver Gastric Ovarian	Rho GTPase-activating protein Regulates cytoskeletan	Migration, colonisation
DRG1	Breast Prostate Colon Pancreatic	Inhibits the expression of activating transcription factor 3	Invasion, colonisation
E-cadherin	Bladder Lung Breast Pancreas Gastric etc (multiple)	Cell-to-cell and cell-to-matrix adhesion	Invasion
GAS1	Melanoma	Inhibits cell cycle	Unknown
Gelsolin	Melanoma Ovarian	Actin-binding protein Cytoskeletal organisation	Migration, invasion, colonisation

Metastasis suppressor gene	Cancer cell type with suppressive activity	Function	Major roles in metastasis inhibition
HUNK	Breast	Protein kinase Cytoskeletal organisation	Migration, invasion, colonisation
KAI1	Lung Prostate Pancreatic Non-small cell lung Colon Colorectal Breast	Integrin interaction EGFR attenuation	EMT, colonisation
KISS1	Breast Melanoma Ovarian	Kisspeptin, G-protein coupled receptor ligand inhibits chemotaxis and activation of Akt	Colonisation (induce apoptosis)
KLF17	Breast	Transcriptional repressor	Invasion (EMT)
LSD1	Breast	Chromatin remodeller	Invasion
MKK4	Prostate Ovarian	Phosphorylates and activates JNK and p38	Colonisation (induce apoptosis)
MKK6/7	Prostate Ovarian	Phosphorylates and activates JNK and p38	Colonisation (induce apoptosis)
N-cadherin	Breast Melanoma	Cell-to-cell and cell-to-matrix adhesion	Invasion
NM23	Breast Melanoma Gastric Oral squamous	Histidine kinase, phosphorylates kinase suppressor of Ras Inihibits ERK phosphorylation and activation	EMT, Colonisation
OGR1	Prostate Ovarian	Regulates G-protein coupled receptor signalling	Migration
RhoGD12	Bladder	Regulates Rho, Negatively regulate Endothelin 1 and Neuromedin U expression	Migration
RKIP	Prostate Breast	Inhibits MEK, G-proteins and NFκB signalling	Angiogenesis, invasion, colonisation (induce apoptosis)
RRM1	Lung Liver	Induces PTEN expression Reduces phosphorylation of focal adhesion kinase (FAK)	Migration, invasion
SSeCKS	Prostate	Inhibits RhoA and Cdc42 Scaffold protein for PKC and PKA Regulates cytoskeletal organisation Downregulates Osteopontin and VEGF expression Upregulates Vasostatin	Angiogenesis, migration
TIMPs		Inhibits metalloproteinases and signaling	Angiongenesis, migration, invasion

NM23

Non-metastatic clone 23 (NM23) was the first metastasis suppressor gene identified.[27] Analysis of tumours from human hepatocellular carcinoma and gastric cancer demonstrated a negative correlation between expression of NM23 and metastasis.[28,29] Transfection of NM23 into metastatically competent breast,[30] melanoma,[31] gastric,[32] and oral squamous carcinoma cell lines,[33] resulted in reduced metastasis in vivo. Re-expression of NM23 induced a reduction in cell motility of human breast cancer cells and murine melanoma cells.[34] In early stage HD3 subline HT29 colon carcinoma cells, NM23 promotes transforming-growth factor (TGF-β)–induced adherence.[35] TGF-β has opposing effects on cells, depending on the stage of tumour progression. During the early stages, TGF-β acts as a tumour suppressor, while in the later stages, it promotes EMT and hence metastasis.[36] The product of this NM23 gene is a protein histidine-kinase and site-directed mutagenesis, demonstrating that its enzymatic activity is important for its function.[37,38] NM23 regulates the Ras/MAPK signaling pathway. Therefore, overexpression of NM23 in MDA-MB-435 breast cancer cells reduces mitogen-activated protein kinase (MAPK) activity.[39] NM23 co-precipitates with and phosphorylates the kinase suppressor of Ras on Serine 392, which is a binding site for the 14-3-3 kinase suppressor of Ras inhibitor.[40] Therefore, phosphorylation of kinase suppressor of Ras by NM23 contributes to reduced Ras/MAPK signaling. Numerous studies have demonstrated a correlation between tumour progression and deregulation of the Ras/MAPK signaling pathway. For example, increased expression and activity of MAPK is associated with lymph node metastases in breast cancer.[41] Increased activity of the Ras/MAPK signaling pathway can play several roles during tumorigenesis and metastasis, such as regulating apoptosis, cell migration and angiogenesis.[42] At a molecular level, this pathway can impinge on various molecules to regulate metastasis, such as increasing the production of matrix metallopeptidase-9,[43] and regulating the EMT.[44]

BRMS1

Microcell mediated transfer of chromosome 11 into the breast cancer cell line, MDA-MB-435, significantly reduced the metastatic potential of these cells in nude mice.[45] Further analysis identified the metastasis suppressor function to a novel gene termed, breast cancer metastasis suppressor 1 (BRMS1). Initial metastasis studies with MDA-MB-435 and MDA-MB-231 breast cancer cells expressing BRMS1 showed that although these cells were still locally invasive, there was a significant reduction in lymph node and lung metastases.[46] In addition to breast cancer, BRMS1 also reduces the metastasis of melanoma,[47] ovarian,[48] and non-small cell lung cancer cell lines.[49] BRMS1 regulates various aspects of cell behavior. One example is the regulation of homotypic gap junctions, which are involved in intercellular communication to regulate the ability of cells to detach from primary tumours and/or respond to signals during transportation or at the secondary site.[50] BRMS1 can also increase the susceptibility of cells to anoikis, which is programmed cell death induced by detachment from the extracellular matrix, thereby decreasing the likelihood of circulating cancer cells reaching and colonising secondary sites.[51]

BRMS1 is a protein of 246 amino acids and can regulate numerous cellular pathways.[46] Yeast two-hybrid screens identified the transcriptional regulators, retinoblastoma binding protein 1 and mammalian Sin3 as BRMS1 interacting proteins. These interactions were confirmed by co-immunoprecipitation studies of lysates from MDA-MB-231 breast cancer cells expressing BRMS1.[52] BRMS1 recruits the retinoblastoma binding protein 1/mammalian Sin3/histone deacetylase transcriptional repressor complex to repress transcription of various pro-metastatic genes such as osteopontin and urokinase-type plasminogen activator.[53,54] BRMS1 also reduces transcription of the epidermal growth factor receptor (EGFR) to decrease AKT signaling.[55] Microarray studies demonstrate that BRMS1 regulates the expression of numerous genes, such as those of the major histocompatibility complex and genes involved in protein localisation and secretion.[56] Therefore, BRMS1 metastasis suppressor function is at least in part mediated through regulation of the expression of different genes that play important roles in metastasis.

MKK4

The mitogen-activated protein kinase, kinase 4/stress-activated protein/Erk kinase 1 (MKK4/SEK1) gene, was identified as a metastasis suppressor following introduction of human chromosome 17 via microcell mediated transfer into the highly metastatic AT6.1 prostate cancer cell line.[57] When these cells were injected into mice, there was no difference in the size of the ensuing tumours, but a significant decrease in their metastatic ability to the lungs was observed. The region encoding the metastasis suppressor was later narrowed down to ~70 centiMorgan region of DNA,[57] and subsequently identified as MKK4.[22] Mice inoculated with ovarian cancer cells expressing MKK4 displayed a reduced number of metastases to the liver, small bowel, near the stomach and spleen, and prolonged their survival rate by 70%. The mean survival of the mice increased from 37 to 63 days.[58]

Loss of heterozygosity on Chromosome 17p has been observed frequently in human ovarian cancers, implicating MKK4 in its pathology.[59] Analysis of human clinical ovarian cancer samples by immunohistochemistry demonstrated a significant loss of MKK4 expression in metastases compared to the primary ovarian tumours, supporting the idea that this gene plays an important metastasis suppressor function in these tumours.[58] MKK4 acts upstream of the c-Jun NH2-terminal (JNK) and p38 kinase signalling pathways, which respond to stress stimuli.[60] In the presence of cellular stress such as irradiation, DNA damage or in response to proinflammatory cytokines, MKK4 is activated by upstream activators and becomes phosphorylated. MKK4 then phosphorylates and activates JNK and p38 kinases, which mediate downstream events.[61] The importance of the role of MKK4 as a kinase in the suppression of metastasis was demonstrated in studies where human ovarian cancer cells, SKOV3ip.1, expressing catalytically-inactive MKK4 mutant resulted in significantly more metastases in mice than cells expressing active MKK4.[62] Activation of the JNK and p38 pathways

typically leads to apoptosis.[63] Therefore, MKK4 at least in part, mediates its metastasis suppressor effects by inducing apoptosis, removing the ability of cancer cells to survive, proliferate, migrate and colonise new sites.

KAI1

Kang-Ai 1 (KAI1) was identified following initial microcell mediated transfer studies of chromosome 11 into the rat AT3.1 prostate cancer cell line. When these cells were injected into mice, it was found that the region p11.2-13 significantly suppressed the number of lung metastases.[64] The KAI1 gene was later identified when DNA fragments from chromosome 11p11.2-13 were used as probes to screen cDNA libraries obtained from both metastasis-suppressed and non-suppressed microcell hybrid AT6.1 cells.[23] The expression of KAI1 is deregulated in prostate,[65] pancreatic,[66] non-small cell lung,[67] colon,[68] colorectal,[69] and breast cancers.[70] KAI1 affects several cellular functions, such as migration and adhesion, which are often altered in cancer cells during metastasis. Therefore, studies using the stable colon cancer cell lines, BM314 with KAI1 knocked-down and DLD-1 cells overexpressing KAI1, were completed to assess these aspects of KAI1 function.[68] DLD-1 cells overexpressing KAI1 displayed reduced phagokinetic motility and migration through a filter coated with reconstituted basement membrane, a measure of invasiveness. The opposite effect was seen with cells expressing reduced KAI1. In addition, cells overexpressing KAI1 displayed a significant increase in binding to ECM components, such as fibronectin. Wound healing assays with fibronectin coated plates showed that knock-down of KAI1 in BM314 cells induced quicker migration on to the fibronectin-coated surface compared to control cells. Therefore, a major mechanism of KAI1 metastasis suppressor function is likely through its ability to reduce cancer cell migration and increased adhesion to the ECM.

KAI1 is a glycosylated protein of 46-60kDa containing peptide motifs, thereby placing it in the tetraspanin family that function as adaptors for large cell surface molecules.[71] Although the exact molecular mechanism behind the role of KAI1 as a metastasis suppressor remains to be fully defined, studies to date indicate that it can attenuate signaling of the EGFR pathway. Co-immuniprecipatetion studies showed that KAI1 associates with EGFR.[72] In wound healing assays, HB2 human mammary epithelial cells overexpressing KAI1 displayed reduced epidermal growth factor (EGF)-induced migration. Morphological differences were also observed following EGF-induced migration of cells overexpressing KAI1, with cells displaying fewer lamellipodial protrusions. Functional studies indicated that KAI1 promotes EGFR endocytosis, suggesting that this is the mechanism of KAI1 attenuation of EGFR signalling. EGFR signaling is a major pathway involved in promoting the proliferation of many cells and this pathway is deregulated in many cancer types.[73] In terms of metastasis, EGFR signaling is known to increase the production of matrix metalloproteinases-9 in breast cancer cells and enhance the invasiveness in prostate cancer cells.[74,75]

Future perspectives

The number of metastasis suppressor genes continues to increase. As already discussed, metastasis suppressors may regulate numerous aspects of cellular behaviour, such as apoptosis, anoikis, maintaining inter-cellular or cellular interactions with the surrounding ECM to regulate EMT. Tumour cells that undergo EMT and intravasion must be able to survive transport through the vasculature, extravasation and evade apoptosis at the new secondary site before establishing new colonies. Currently, over 20 metastasis suppressors that impinge on different aspects of the metastatic cascade have been identified (table 1). It is likely that as new genes in this family are discovered, novel mechanisms of metastasis suppression will be unveiled, providing new insights into this complex process.

Although our understanding of the biological mechanism of metastasis and the action of metastasis suppressors is increasing, significant challenges remain to translate this knowledge into a clinical setting for improved patient outcome. A major goal of clinicians is early detection of the cancer before metastasis occurs. Early detection is associated with better prognosis and treatment is less challenging when the cancer is localised to the primary site and has not metastasised. Metastasis suppressor genes may eventually be useful as prognostic markers to define the likelihood of primary tumour spread and response to therapy. For example, various cancers have shown high expression of metastasis suppressor genes such as NM23 and KAI1 in primary tumours, with a reduction in matched metastases.[65,76] Further clinical studies will be needed to determine if expression of these genes can predict outcome and thus provide utility for prognosis or therapeutic responses.

Apart from their prognostic potential, metastasis suppressors may provide new targets for cancer therapy. At this stage there are significant challenges in targeting metastasis suppressors as therapeutic targets, since it is envisaged that compounds would need to activate or restore their activity, as opposed to many anti-cancer compounds that bind and inhibit key molecules, oncogenes and pathways required for cancer cell survival. Nevertheless, anti-cancer drugs such as the histone-deacetylase inhibitor Vorinostat that has broad effects on the expression of many genes, demonstrates that compounds may be developed that activate tumour suppressor genes and pathways.[77-79] The development of compounds, that increase the expression or activity of metastasis suppressors, could open new possibilities for treatment of cancer.

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LYMPHATIC INTERACTIONS AND ROLES IN CANCER METASTASIS

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Abstract

Cancer remains a major cause of mortality, chiefly through metastatic spread of tumour cells to distant organs via the blood vascular or lymphatic circulations. The latter system has been more recently recognised to play a critical role in several normal physiological and pathological processes. The development of modern lymphatic markers and the discovery of protein growth factors that drive lymphatic vessel growth (lymphangiogenesis) have led to this enhanced understanding. Clinicians and researchers have begun to uncover the ways in which lymphatics are integral to immunity, interstitial fluid homeostasis and digestion, in addition to key interactions that occur between the lymphatics and other cells in disease states. Here we focus on some of these interactions, and the determinants that influence them, particularly those governing tumour spread. We highlight the altered characteristics of tumour lymphatics that may not only provide prognostic information, but also important diagnostic and therapeutic opportunities to treat these conditions. By understanding the tumour-lymphatic interface through emerging imaging techniques, refinements to existing clinical tools (such as sentinel node biopsy), and exploiting genetic and molecular advances in the field, it is hoped that novel therapeutic avenues may be developed to combat diseases such as lymphoedema and cancer metastasis.

Over 120,000 Australians are diagnosed annually with some form of solid malignancy, excluding the most common, non-melanoma skin cancer.[1] The chief cause of patient mortality attributable to these tumours is metastatic spread to vital organs such as brain, lung, liver and bone. Extensive research over previous decades focused on investigating and treating blood vessels forming within primary tumours to provide nutrients and oxygen to sustain the dysregulated growth of cancer cells.[2] Additionally, distant spread (haematogenous metastasis) may occur through these vessels.

In contrast, the lymphatic vascular system remained relatively ignored. Lymphatics however, play an important

role in several normal physiological and pathological processes.[3] While among the earliest of clinical observations in cancer (noted by Hippocrates himself),[4] dissemination to the loco-regional lymph nodes has been seen merely as a 'blunt marker' indicating aggressive tumour biology. During the 1990s, the advent of lymphatic markers facilitated histological identification of lymphatics in normal tissues.[4] Combined with the discovery of protein growth factors that can stimulate lymphatic vessel growth (lymphangiogenesis), this advance led to the development of experimental models examining the role of tumour lymphatics in disease.[5,6] In normal physiology, lymphatics are integral to immune surveillance, modulation of interstitial fluid balance and absorption and transportation of digested lipids.[3]

The lymphatic vasculature is now emerging as a key driver of metastasis and a promising therapeutic target in restricting tumour spread. Adaptations that promote tumour cell interaction with lymphatic endothelial cells (LECs) can enable cancer cells to utilise the lymphatics to enhance their spread.[7] Furthermore, lymphatic vessels may be functionally altered as a result of cancer treatment, such as radiation treatment or surgical lymph node clearance. This may often result in debilitating, long-term side effects that can remain with cancer survivors for the duration of their lives.[8]

Lymphatic vessels - structure, anatomy and developmental origins

Due to previous difficulties in identifying lymphatics in tissue, researchers traditionally ignored fundamental differences between the individual subtypes of lymphatic vessels. These distinct vessels have recently been recognised not only as morphologically and anatomically different, but also as displaying unique molecular profiles and varied responses to pro-lymphangiogenic growth signals.[9]

The lymphatic vascular system consists of a hierarchically-arranged vessel network that commences as capillary or initial lymphatics within the superficial layers of epithelial body surfaces,[4] interfacing with the outer environment, the dermis, respiratory, gastrointestinal and uro-genital systems. These vessels consist of the initial or capillary lymphatics that function to absorb fluids that bathe the interstitium, known as lymph. Therefore, initial lymphatics also function as the entry-point for antigens/pathogens by which to interact with the immune system.[10]

Following entry into the lymphatic system, fluid and cellular components are transported via the pre-collecting, then collecting lymphatic vessels, which contain valves within thicker walled vessels, and are located within the sub-epithelial layers of the body.[11] Collecting lymphatics are over 200 µm in diameter and have arterial-type mural intima, media and adventitia. [4] They traffic their contents toward loco-regional lymph node basins, where lymph is filtered for antigens/pathogens to which adaptive immune responses are generated, before returning the lymph fluid to the general circulation. Impeded absorptive function or restricted transportation function may result in accumulation of lymph in the interstitium, known as lymphoedema. [4] It has been recognised that the collecting lymphatic vessels are dynamic, and alterations in their caliber and rate of flow may be mediated by various external factors, such as nitric oxide,[12] autonomic regulation,[13] and prostaglandins.[11] In addition, tumour cells that exhibit similar molecular patterns as the elements or cells trafficked via the lymphatics, may utilise this surface profile to gain entry into and be transported along the lymphatics on the route toward tumour metastasis.[11,14]

Lymphatic interactions

Lymphangiogenesis and protein growth factors

In the embryo, lymphangiogenesis normally commences in response to the expression of 'master-switch' molecule Sox18 in the cardinal veins.[15] This polarisation of LECs to the lateral aspect of the anterior cardinal vein progresses to an out-pouching to envelop the first lymph node precursor, or anlagen, the jugulo-digastic lymph sac.[16] Post-developmentally, lymphangiogenesis occurs in response to pathological stimuli such as wound healing or increased interstitial fluid volumes.[9] Secreted protein growth factors, vascular endothelial growth factor (VEGF)-C,[17] and VEGF-D,[18] constitute a lymphangiogenic subset of the VEGF family of mitogenic proteins with specificity for endothelial cells. Predominantly driving proliferation, migration and other processes that promote neo-vessel formation,[4] or vessel remodeling,[11] VEGF-C and VEGF-D have become the focus of modern lymphatic research. More recently, several additional regulatory cues of the lymphatic system have begun to emerge.[10] VEGF-C and VEGF-D act primarily via LEC cell surface receptor-tyrosine kinase VEGFR-3, associated with neuropilin family co-receptors (NRP1 and NRP2).[4] VEGF-C and VEGF-D drive lymphangiogenic sprouting from nearby initial lymphatics, both within and around some primary solid tumours,[14,18] and also drive dilatation of the collecting lymphatic vessels that drain fluid from the area around the primary tumour to the draining lymph node basin.[11] Both of these 'forms' of lymphangiogenesis have been shown to contribute to cancer metastasis to the lymph nodes basin.[4,11,19]

Lymph fluid and lymphoedema

About 95% of the volume of the circulation is returned from the capillary bed by the venous system, while the remaining volume forms the lymph that bathes the interstitial tissues.[25] By filtering lymph through nodes, the body samples and monitors the interstitial space and processes any potential pathogens. Even minor impairments to this process may imbalance normal interstitial fluid homeostasis, leading to the accumulation of debilitating lymphoedema.[3]

Congenital lymphatic derangements or acquired defects of existing lymphatics due to surgery, radiotherapy or obstructive parasitic infection, may lead to lymphoedema and often result in significant debilitation, discomfort and potential complications for patients.[4] Providing clues into congenital lymphoedema, several mouse models of genetic modification have been observed to lead to lymphoedema or abnormal lymphatic development. These include genes encoding for: VEGF-C; its receptor VEGFR-3; transcription factors SOX18 and FOXC2; semaphorin receptor NRP-

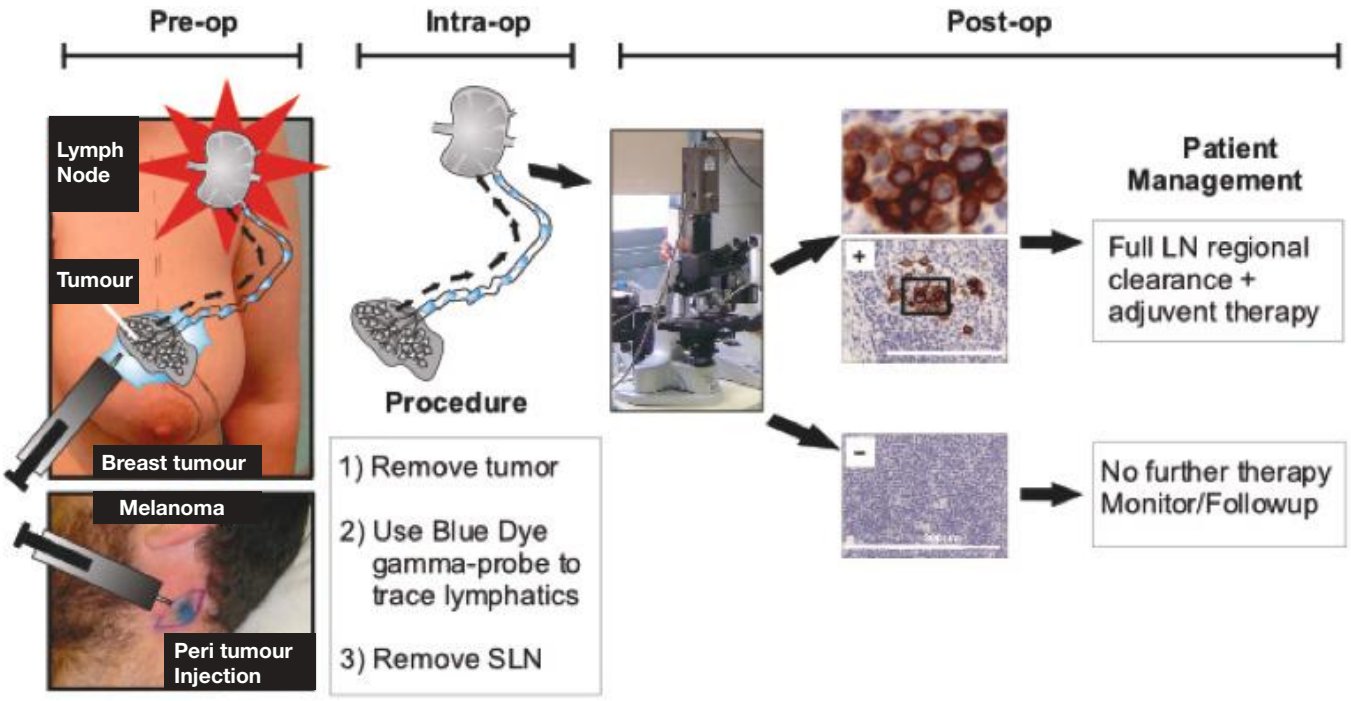
2; Angiopoietin-2; the transmembrane growth factor ephrinB2; integrin α9; Elk 3 (NET); podoplanin, Prox 1, and Lcp2 (SLP-76).[10] Several of these mutations are also implicated in known human lymphoedema, such as point-mutations in VEGFR-3, in which heterozygous mice show failed early vascular remodeling, lymphatic vessel hypoplasia and chylous ascites. The analogous human condition, autosomal-dominant hereditary lymphoedema, is Milroy disease.[26] The spontaneous missense mutations involving transcription factor SOX18, result in a phenotype, analogous to the human hypotrichosis-lymphoedema-telangiectasia syndrome (alopecia, thin skin, telangiectasia and lymphoedema).[15] Similarly, FOXC2 mutation is analogous to an autosomal dominant hereditary human lymphoedema known as lymphoedema distichiasis,[30] – a syndrome consisting of an accessory row of eyelashes, and lymphoedema. Finally, alterations in collagen-and-calcium binding epidermal growth factor domain 1 (CCBE1), recently identified to be critical in Zebrafish lymphangiogenesis,[10] were found to lead to lymphoedema-related syndromes originating from generalised lymphatic dysplasia in humans.[31]

LEC-immune cell interactions and cancer

Certain receptor-ligand relationships between tumour and host tissues, mediated by soluble factors that regulate haemopoetic cell migration (known as chemokines), may be 'hijacked' by cancer cells to facilitate localisation of and entry into lymphatic vessels, and tumour movement along them towards lymph nodes.[20] Further, tumour-endothelial interactions may be responsible for particular cancers displaying metastatic affinity towards specific tissues, and chemokine-ligand guided interactions may create a chemical gradient that predisposes circulating metastatic cells to home toward, and settle in certain distant organ tissues.[20] An example in lymphatic metastasis is CCL21/SLC, a ligand expressed on LECs for lymphocyte and dendritic cell signaling, and guidance towards lymph nodes via the CCR7 receptor.[29] Human melanoma and breast cancer cell lines expressing CCR7 have increased affinity for LECs and show increased lymph node metastasis in animal models.[22] The CXCR4-CXCL12 pathway is also a well-characterised T-lymphocyte signaling mechanism, and CXCL12 is expressed differentially between individual colorectal carcinomas, providing a prognostic indicator for local recurrence, metastases and overall survival.[20]

Similarly, adhesion assays using melanoma and breast carcinoma cell lines indicate that expression of adhesion molecules, which normally participate in immune cell traffic between interstitial and vascular compartments, may also be important in tumour influx or egress from the lympho-vascular space during metastasis.[4] One adhesion molecule, L-selectin, forms a ligand-receptor pair with mannose receptor, which is expressed on LECs.[23] This

Figure 1: Clinical pathway of sentinel lymph node biopsy.
Pre-operative injections of radio-isotope tracer and Patent Blue V dye allow intra-operative identification of the sentinel lymph node or nodes. These are the lymph nodes deemed to be the primary draining lymph node, or nodes on pre-operative imaging and/or intra-operative detection by visualisation of blue dye and/or detection of a radioactive count using a hand-held gamma-camera. The detected lymph nodes are removed, fixed and sectioned for review by a trained pathologist who identifies whether metastatic cells are present (meaning that, on the basis of evidence from large population-based studies, adjuvant treatment using chemo/radiotherapy may be warranted) or absent (indicating that only careful monitoring and follow-up are sufficient to detect any unexpected recurrence or spread of disease in these patients). Printed with permission: Ramin Shayan, Characterising lymphatic vessel subtypes and their role in cancer metastasis (2009). PhD Thesis, The University of Melbourne.



orchestrates intravasation of circulating lymphocytes from interstitial tissues into lymphatics, and subsequent lymph node homing – a process exploited by L-selectin-expressing tumour cells to promote metastasis – and a process inhibited in animal studies by administering anti-L-selectin monoclonal antibody.[23] Pro-inflammatory cytokines, and the resulting immune cell aggregations, may also stimulate additional lymphangiogenic growth factors, and so provide an indirect method of promoting lymphatic proliferation in the region of a tumour.[24]

Diagnostic/prognostication

Lymphatics and molecular signalling

Several varieties of solid human tumour may exploit the lymphatic system by actively inducing 'neo-lymphatics' to enhance tumour metastasis,[4] and both LVD and the lymphangiogenic growth factors have been shown to be prognostically-significant in these tumours.[4] For example, in malignant melanoma, both 'peri-tumoral' LVD, and expression of lymphangiogenic growth factors were important independent determinants of metastasis.[4,18] Similarly, analysis of head and neck squamous cell carcinoma, breast carcinoma, thyroid carcinoma and lung carcinoma samples,[32-35] revealed an association between VEGF-C expression and regional lymph node spread, which correlated with poor patient outcome. Similar conclusions were made relating to VEGF-C expression, nodal metastasis and poor survival in malignancies of the genito-urinary tract (cervical, ovarian and prostate carcinoma);[36-38] and malignancies of the gastrointestinal tract (gastro-oesophageal, pancreatic, gall-bladder and colorectal carcinoma).[37-42] Meanwhile, VEGF-D levels were a significant indicator of lymph node metastasis and overall prognosis in several studies involving breast cancer and colorectal carcinoma, in which VEGF-D expression was an independent prognostic indicator of poor disease-free and overall survival.[43]

In studies investigating both VEGF-C and VEGF-D expression, Onegawa et al. and Hu et al. found that that VEGF-C and VEGF-D expression together correlated with high LVD, nodal metastasis and poor overall patient survival,[43-45] indicating that these growth factors may be important novel markers of metastatic risk and poor patient outcomes. Using Cox multivariate regression analysis, VEGF-C, VEGF-D and VEGFR-3 were specifically predictive of metastasis, while VEGF-D and VEGFR-3 were independent indicators of poor prognosis.[45] Other authors found the likelihood of nodal metastasis was so low in gastric cancer patients expressing neither VEGF-C nor VEGF-D, that this could be taken as grounds to perform more conservative surgery.[46] Further, it has been suggested that detection of circulating VEGF-C or VEGF-C may be generally-useful biomarkers for lymph node metastasis and prognosis in gastrointestinal cancers.[47]

Surgical sampling - sentinel lymph node biopsy

The Sentinel Lymph Node 'SLN' theory, was popularised in melanoma patients as a method of sampling lymph nodes to which tumour cells may have metastasised.[4,48] The SLN Biopsy (SLNB) (figure 1) method has since been adapted for application to several other malignancies, in particular, skin squamous cell carcinomas, and carcinomas of the breast and gastrointestinal tract.[49-52] SLNB combines lymphatic injection techniques with dynamic studies of drainage patterns and enables accurate identification and surgical excision of SLN(s) (the first draining lymph nodes) - the node(s) most likely to harbour metastatic cancer cells.[4,48] This provides the node for histological examination, and the presence/absence of metastatic cells determines further patient management,[49] minimising unnecessary lymph node clearance in patients with non-metastatic tumours ('SLN-negative' patients) who would previously have automatically undergone lymph node clearance.[4,53] These patients are spared the morbidity of further lymphablation surgery, which can lead to side-effects such as lymphoedema.[53] In contrast, histologically-positive SLNs indicate a poor prognosis,[49] and detection of positive SLN(s) results in reclassification of the patient to a group with higher metastasis risk, identifying candidates in whom comprehensive surgical clearance of the regional lymph node basin containing the SLN is indicated, and making them eligible for systemic adjuvant treatment,[49,52] and thus potentially resulting in improved survival.[10] A pre-operative map of lymph node(s) draining a cancer is obtained using radiolabelled colloid injection,[51] and a gamma camera is used to show the drainage over time. Additionally, the intra-operative hand-held gamma-probe,[54] combined with on-table injection of lymphatic-specific patent blue V dye,[55] enables the reliable detection (detecting greater than 95% of SLNs), identification and sampling of the SLN intra-operatively.[54] The cutaneous lymphatic drainage pathways from the site of a primary tumour may be highly variable between patients, even within the same areas of the body.[54] Up to 30% of these tumours therefore defy clinical predictability based on regional node groups potentially involving multiple node fields.[56-59] Such cancers would be erroneously classified as SLN-negative using conventional methods in approximately 15-40% of patients with a melanoma on their limbs, trunk, or head and neck.[59]

Future directions for lymphatic imaging include developing accurate non-invasive methods for lymphatic and SLN assessment, such as high resolution magnetic resonance lymphangiography.[60] The incorporation of contrast materials such as gadodiamide have significantly improved the resolution and accuracy compared with traditional lymphoscintigraphy methods.[60,61] Also, utilising magnetic resonance technology, emerging nanoparticle technology using contrast substances such as the ultra-small superparamagnetic iron oxide nanoparticle, Ferumoxtran-10, has been trialled for the localisation and metastasis to SLN(s), on the basis of enhancement patterns.[62] Anatomically-based 3D computer models of the skin and lymph nodes, using data of thousands of cases of metastatic melanoma entered into spatial analysis software to create probability diagrams known as 'heat maps', has also proved of early prognostic value.[63] Adaptations of contrast studies using labelled antibodies to lymphatic vessels or lymph nodes, could also help analyse drainage patterns and abnormalities of lymphatic drainage.

Treatment approaches

Therapeutic approaches for the inhibition of receptor tyrosine kinases such as VEGFR-3, include monoclonal antibodies, small molecule inhibitors, peptide drugs and antisense techniques.[4,64] Folkman's vision of anti-angiogenesis as a cancer treatment has been realised with the release of a humanised anti-VEGF monoclonal antibody for the treatment of metastatic colorectal carcinoma (bevacizumab, Avastin).[64] Analogous to VEGF-A blockade to reduce tumour angiogenesis,[2,66] an approach to block VEGFR-3 ligands VEGF-C/VEGF-D appears to be promising for the inhibition of tumour lymphangiogenesis and lymphogenous metastasis.[67-69] Overall, experimental models demonstrating suppression of VEGFR-3 signaling have shown inhibition of both tumoural lymphangiogenesis, angiogenesis and metastatic spread.[68,70] The administration of soluble VEGFR-3-immunoglobulin fusion protein (VEGFR-3-Ig), which binds VEGF-C and blocks VEGFR-3 signaling, and intravenous recombinant adenoviruses expressing VEGFR-3-Ig,[68] have been examples of models that have achieved regression of tumour-induced lymphatic vessels. In addition, the interference with ligand-receptor interactions and the resulting inhibitory effect on lymphangiogenesis and metastasis produced by adeno-associated virus-delivered soluble VEGFR-3 decoy receptor, was dose-related in VEGF-C-secreting PC-3 and A375 tumour models.[71,72]

Interference with lymph node metastasis was also seen in animal models using neutralising anti-CCL21 antibodies,[22] and interference with CXCR4 signaling may present a further target in disease-directed therapy for colorectal carcinoma.[20]

Overall, the role of lymphatics in disease is becoming increasingly well understood. The next step will be elucidating the molecular intricacies of each different lymphatic subtype in disease models and exploring ways in which they may be utilised in diagnosis and therapeutics. Surgical treatment of metastasis has been limited to removal of an entire lymph node basin in response to a clinically obvious enlarged lymph node. More recently, the patients whom actually require this invasive procedure that can often result in chronic lymphoedema have been selected after detection of a 'positive' sentinel node – that is, a node shown to actually harbour metastatic cells. In other areas of the body, treatment of an established secondary tumor deposit has been limited to the excision of a mass deposit of cancer cells. The advent of molecular therapies that can target the growth factors that drive tumor lymphatics has provided potential new avenues. These include targeting the VEGF-C/VEGF-D-VEGFR3 axis including the protein growth factors themselves and their cognate receptors; or their co-receptors or small particles downstream of the receptor tyrosine kinases that are activated via this mechanisms. More recently, alternative signaling pathways that also contribute to lymphangiogenesis have been identified; as have molecular systems that guide interactions such as cellular adhesion and migration, which may in future also yield promising anti-metastatic targets.

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VASCULAR CONTRIBUTION TO METASTASIS

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Abstract

It has been 40 years since Folkman's seminal paper [Cancer Res 1974. 34:2109-13], proposing the presence of a tumour associated angiogenic factor, which could be targeted as an anticancer therapy. There are currently a handful of drugs in trial or use that have been marketed as targeting angiogenesis. Unfortunately, the most widely used of these, bevacizumab (Avastin™, Roche), has met with limited success clinically. For this reason and based on a calculation of cost benefit, bevacizumab is now only publically subsidised for use in a limited range of solid tumours. That the contribution of vasculature to malignancy remains poorly understood is increasingly clear. At the same time, the traditional view that vascularisation is a passive participant in the process of malignancy, and that endothelium merely provides a conduit by which tumour cells spread, is being replaced with an understanding that vasculature is a key player in the process of metastasis. Furthermore, the identification of non-traditional sources of vasculature has complicated our understanding of the tumour endothelium as a unique population that can be simply targeted as an anticancer therapy. The following review seeks to provide an up-to-date view of vascular contribution to metastasis and implications for new vasculature-targeted anticancer treatments.

At face value, the vascular contribution to metastasis is not self-evident. Many lesions that a patient may present with are vascular, despite being poorly malignant or benign. Consequently, the relationship between vascularity, tumour growth and malignancy remains controversial. In many instances, tumours can present with metastatic lesions, while the primary lesion remains relatively small, or undetected.[1] Conversely, relatively large and highly vascular breast lumps can also remain undetected and benign for many years. Yet despite these observations, vascularisation and spread are closely linked with clinical course in many solid tumours, including breast cancer.

While benign tumours can either be vascular or relatively avascular, almost all malignant tumours are vascular. Furthermore, while distal lymph node metastasis remains the main pathological indicator of grade, it is increasingly clear that spread via the lymphatics is a later event in metastasis.[2] This is supported by the detection of disseminated tumour cells in the peripheral blood and bone marrow of patients with early stage breast cancer.[2] Haematologic dissemination of tumour cells may therefore be considered the initial route for early spread of the tumour, although evidence for this to date remains circumstantial. Regardless, because of its important role in cancer spread, the role that vasculature plays in establishment and growth of metastasis at a secondary site has occupied a lot of research effort. It has been known for several years that drugs targeting vascular endothelial growth factor (VEGF) can enhance the anti-tumour effects of cytotoxic drugs. While the mechanism for this is uncertain, it is proposed that the effectiveness of combination therapies slows angiogenesis, leading to a normalisation of the vascular tree, and enhanced drug delivery.[3] However, many of these therapies are associated with the onset of adaptive resistance (a loss of response to therapy),[4] as well as a tumour environment that is selective for the development of highly aggressive tumour cells.

Understanding the role that vasculature plays in malignancy is key to the effective use of current therapies, and in the development of future effective therapies. To date, despite a sustained research effort over many years, only a handful of anti-angiogenic drugs are in clinical use.

Anti-angiogenic therapy

The Food and Drug Administration (FDA) in the US has approved several drugs that have antiangiogenic activity, including bevacizumab (Avastin®). Bevacizumab is the most widely used antiangiogenic therapy. It is designed to stop VEGF signalling and thus vascular growth. The FDA has approved bevacizumab to be used alone for, glioblastoma and in combination with other drugs for the treatment of: (i) metastatic colorectal cancer; (ii) non-small cell lung cancers; and (iii) renal cell cancer. Alone, bevacizumab is ineffective for the treatment of certain cancers, or is effective for a very short period of time as a result of adaptive resistance.[5] When combined with traditional chemotherapy (such as FOLFOX or FOLFIRI), bevacizumab provides colorectal cancer patients an average increase in survival of five months. Patients with advanced non-small-cell lung cancer have an average increase in survival of about two months.[6] These increases in survival in patients with highly advanced disease are significant, and it should be emphasised that they may translate into longer individual survival expectations when used in the actual target group.

The effectiveness of bevacizumab treatment in breast cancer has been more controversial. Although phase III trials of bevacizumab plus chemotherapy demonstrate modest effectiveness in metastatic breast cancer,

these trials used progression free survival as a measure of efficacy.[7] However, breast cancer populations are heterogeneous, and contain rare cell populations that are undetectable and capable of proliferating aggressively regardless of tumour size.[5,8] In contrast, clinical trials using overall survival fail to show clinical advantage to the use of bevacizumab. Furthermore, a significant proportion (2.5%) of patients on bevacizumab therapy will experience a fatal adverse event, resulting from haemorrhage. These include neutropenia with lethal infection, gastrointestinal tract perforation, pulmonary embolism and cardiovascular accident.[9] There are also a range of side-effects associated with bevacizumab therapy, including hypertension, cardiac toxicity, neutropenia, thromboembolisms, stroke, enhanced chemotherapy toxicity and impaired wound healing resulting in severe bleeding.[9,10] Finally, bevacizumab doubles the cost of chemotherapy to $100,000 USD per patient per year. For these reasons, as of December 2010, after only two years of use, the FDA withdrew bevacizumab treatment for metastatic breast cancer. The Therapeutic Goods Administration in Australia, and the National Health Service in the United Kingdom, have followed suit and do not provide government funding for bevacizumab treatment of malignant breast cancer.

Given the lack of effectiveness of bevacizumab in advanced breast cancer, but the clear advantage in other types of cancer, there remains a need for improved anti-angiogenic therapies, as well as the need to obtain a greater understanding of the underlying mechanisms supporting breast cancer angiogenesis, resistance and progression.

Mechanisms of neovascularisation

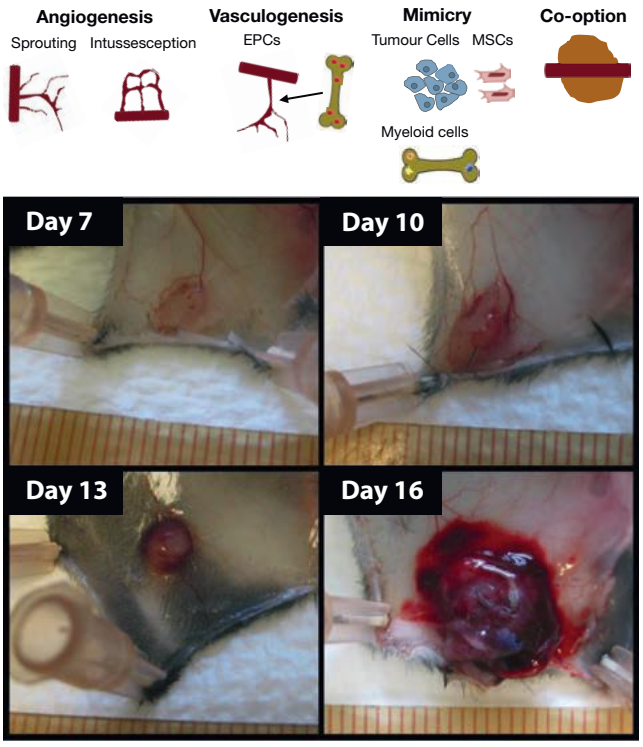
The classical model for tumour neovascularisation proposes that as a solid tumour grows, it first starts by co-opting pre-existing vessels, without vasculogenesis (de novo generation of vessels), and by recruiting pre-existing endothelial cells from surrounding tissues (angiogenesis) (figure 1). The transition from an avascular adenoma to a fully vascular, metastasising lesion, is referred to as the angiogenic switch, and its onset is associated with rapid tumour growth.[11] Fifteen years ago, Asahara put forward a further mechanism by which tumours recruited vasculature.[12] He proposed that a certain proportion of tumour vasculature was derived de novo from bone marrow adult stem cells. Since then, bone marrow derived endothelial progenitor cells (EPCs), with vasculogenic potential and capable of luminal incorporation into the vascular tree, have been identified as an alternate source of tumour vasculature.[13-16]

More recently, several post-classical mechanisms of neovascularisation have come to light, including tumour cells themselves,[17] myeloid cells and tissue resident mesenchymal stem cells (MSCs),[18] All of these populations have been found to acquire vascular markers and mimic endothelial cell biology, and therefore must be considered in the development of anti-angiogenic therapies. However, the role of vasculogenesis and vascular mimicry in the adult remains controversial because of the numbers of cells implicated in tumour pathology, and because of limitations in the tools available to study them.[19]

Vessel co-option

In vessel co-option, neither vasculogenesis nor angiogenesis play a role. Instead, the growing tumour simply incorporates existing vasculature from the host tissue bed.[20] Studies suggest that vessel co-option occurs in the initial stages of tumour growth.[21] Notably, tumour regrowth following anti-angiogenic therapy commonly involves vessel co-option, in addition to both angiogenesis and vasculogenesis.[22]

Figure 1: Upper, schematic representation of the process underlying tumour neovascularisation, showing sprouting/intussesception angiogenesis, co-option, vasculogenesis and tumour mimicry. Lower, murine breast tumours at day 7, day 10, day 13 and day 16, showing progressive neovascularisation with time.



Angiogenesis

Angiogenesis refers to the formation of new blood vessels from pre-existing vessels, and occurs concurrently with vasculogenesis in a rapidly vascularising tumour.[23] During angiogenesis, hypoxia drives vessel sprouting via VEGF mediated proliferation of endothelial cells, and basement membrane remodelling.[24] Sprouts invade surrounding tissues as a proliferating solid stalk, which is guided by a 'tip cell', possessing numerous filopodia that sense gradients of angiogenic molecules.[25] Formation of a functional lumen and integration into existing vascular structures follow. As a consequence, sprouting angiogenesis is slow to develop an organised structure and is dependent upon cell proliferation, as well as the activation of molecular pathways that support tissue invasion. Vessels formed in this way tend to be disorganised, leaky and disconnected from the existing vascular tree.[23]

Distinct from sprouting angiogenesis, intussusceptive angiogenesis is characterised by the formation of

transvascular tissue pillars across the vessel lumen.[26] Vessel walls join together, leading to the formation of a transverse endothelial bilayer, or pillar. The pillar then undergoes perforation, and dilatation. This is followed by branching and further development, including invasion of fibroblasts and pericytes, as well as the laying down of interstitial matrix.[27] As a consequence, intussusceptive angiogenesis leads to rapid neovascular branching (occurring in hours or even minutes), and does not require cell division. Once started, intussusception can dramatically increase vascular surface area, and unlike sprouting, allows for continuous blood during vessel formation. Intussusceptive angiogenesis has been demonstrated in a range of cancers, and is linked to regression following anti-cancer therapy.[28,29] While no single molecule has been implicated as a driver of intussusception, shear stress has been shown to play a role.[30]

Vasculogenesis

In embryology, vasculogenesis refers to the de novo formation of blood vessels from the differentiation of mesodermal precursor cells, referred to as endothelial precursor cells (EPCs).[31] There are two main theories for the origins of embryonic EPCs: (i) the idea that there exists a bipotential haemangioblast that comes directly from mesoderm and forms both the early vasculature as well as the haematopoietic system, and (ii) the proposition that there exists haemogenic endothelium (haemangioblast), an endothelial cell intermediate with haematopoietic potential which is not derived directly from mesoderm.[32-34] While studies suggest that the haemangioblast is the source of both haematopoetic cells and the majority of endothelium in embryos, the haemangioblast in adults remains undefined.

EPCs can collect both in normal and cancerous tissues, and contribute to de novo blood vessel growth.[13-16,35] Since they were proposed, observations have shown that circulating EPCs contribute to tumour angiogenesis.[14,36] However, because of their small number and because circulating vasculature shed from the tumours expresses many markers that are similar to EPCs, consensus as to their importance in human cancer biology has been difficult to arrive at.[37] In 2005, Peters et al demonstrated conclusively that EPCs were present in human cancer.[13] This was supported by studies in mice,[14-16] which showed that EPCs could be tracked from the bone marrow to luminal incorporation into tumour vascular and that they were present at the tumour periphery in the early stages of the angiogenic switch. In 2008, Gao et al [15] demonstrated that metastases also underwent an EPC dependent angiogenic switch. At the time, it was not self-evident that a metastasis, which had already undergone an angiogenic switch prior to spread, would have to undergo further changes to recruit vasculature to a secondary site. The fact that EPCs are not only participants in metastasis, but significant players in driving malignant spread, has made them important targets of anticancer therapy.

Vascular mimicry

In addition to EPCs, some aggressive cancers show a remarkable functional plasticity, exhibiting the phenomenon of vasculogenic, or vascular mimicry.[17] During vascular mimicry, cancer cells and/or cells of a non-endothelial lineage begin to express genes associated with angiogenesis and vasculogenesis, assuming some phenotypic traits of endothelial cells, including luminal incorporation. Vascular mimicry is driven by tumour hypoxia, and tumours displaying vascular mimicry exhibit a matrix-rich, vasculogenic-like network, lined with transendothelial tumour cells that have assumed the function of endothelial cells.[38] The earliest description of vascular mimicry was reported in melanoma by Maniotis,[39] who described vascular-like networks of tubular and non-tubular structures, which were rich in collagen, possessing a basement membrane lined with tumour cells co-expressing endothelial markers, and containing plasma and red blood cells. Further work has demonstrated similar structures in a range of aggressive tumour types, including carcinomas, sarcomas, glioblastomas and astrocytomas.[40-42] Tumour cells displaying vascular mimicry show phenotypic plasticity similar to embryonic stem cells, expressing key stem cell markers,[43] as well as expression of endothelial markers such as VE-cadherin, erythropoietin-producing hepatocellular carcinoma-A2, and extracellular matrix proteins (laminin V, fibronectin and collagen IV and VI), and down-regulation of genes that are cancer/epithelial cell specific.[44]

Functionally, the leaky vessels created via vascular mimicry provide an alternate perfusion route. Furthermore, channels of vascular mimicry may also connect to vessels, increasing the overall perfusion of the tumour, as well as providing a pathway for metastasis. Clinically vascular mimicry, although rare, is associated with poor prognosis, suggesting that it confers an advantage in tumour progression. Furthermore, as tumour cells displaying vascular mimicry lack the regulatory constraints on growth and differentiation displayed by normal endothelial cells, and are genetically unstable, they would be subject to the same propensity that cancer cells have to develop drug resistance.

In addition to tumour cells, there is a growing recognition that host derived cells with nonvascular lineages may also begin to express markers normally associated with endothelial cells. By far the best-studied bone marrow-derived tumour infiltrating cells contributing to angiogenesis, which may be candidates for myeloid vascular mimicry, are tumour-associated macrophages (TAMs).[45] As with EPCs, TAMs are recruited in response to tumour derived chemokines and growth factors.[46] TAMs produce pro-angiogenic molecules VEGF, interleukin-8, tumour necrosis factor-α and matrix metalloproteinase-9 (MMP-9).[47] Correspondingly, high numbers of TAMs often correlate with tumour vascularisation.[18,48] In addition, there is increasing evidence that TAMs start to express endothelial factors such as CD31 and they may contribute to tumour vasculature directly, although there is little evidence of luminal incorporation as endothelium and their role may be solely perivascular.

MSCs are found in many tissues, including bone marrow, and represent another heterogeneous stromal cell lineage that has been implicated in tumour angiogenesis and growth. Tissue resident MSCs play a role in the maintenance and regeneration of connective tissues through engraftment.[49,50] During cancer, significant

numbers of MSCs are recruited to the site of the primary tumour, where they play a role in invasion, metastasis and immunological evasion.[51,52] Non-bone marrow derived MSCs have recently been proposed as a source of vasculature in certain tumours.[53]

Dormancy and the endothelium

Studies in breast cancer metastasis have shown that dormant disseminated tumour cells reside within the lumen of microvasculature at common metastatic destinations such as the lung, bone marrow and brain.[54] Three-dimensional modelling of microvasculature in vitro has confirmed that the perivascular location of tumour cells is responsible for maintaining their quiescent state. It has also been shown that the protein thrombospondin-1 is secreted by endothelial cells, and can act to suppress tumour-cell growth at a secondary site.[54] Remarkably, this growth-suppressive microenvironment is found only around stable vasculature. Activated or sprouting tips of newly forming vessels actually have a growth-accelerating effect on tumour cells, through expression of pro-metastatic proteins, including periostin, tenascin-C, fibronectin and tumour growth factor-β1. Recent work by Wells et al. (University of Pittsburgh Medical Centre, US) has shown that transformed endothelial cells from the liver also confer a proliferative advantage to breast cancer cells, via the epidermal growth factor pathway.[55] While the role of endothelial cells in maintaining dormancy has yet to be shown in vivo, emerging lines of evidence such as these are compelling and provide the first tantalising glimpse of the importance of the vascular tree in regulating growth of secondary metastases.

Conclusion

Tumour neovascularisation is complex, involving both interlinked and distinct processes. Recruitment of pre-existing vasculature is characterised by complex architectural changes to the existing vascular tree. Furthermore, our lab and others,[14-16] have shown that EPCs and other bone marrow derived cells are significant drivers of angiogenesis and spread, and may be the main factor underlying development of adaptive resistance. It is also evident that other vasculogenic populations such as tumour cells themselves and cells of non-bone marrow/non-endothelial origin, are also important drivers of clinical course in cancer. The understanding that endothelial cells at the site of secondary metastasis can regulate growth and further spread directly, is a new revelation in cancer biology and has led to a greater appreciation of the role of the vasculature as drivers of malignancy and drug resistance. Targeting tumour angiogenesis is increasingly being used in anticancer strategies. These therapies are attractive, as endothelial cells from the body are not subject to the same selective pressure or genetic instability as tumour cells, and may be less likely to develop resistance. However, delivery of anti-angiogenic therapy treatment must be targeted and based on an understanding of the processes that underlie tumour vascular biology.

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DISPARATE FUNCTIONS OF MYELOID-DERIVED SUPPRESSOR CELLS IN CANCER METASTASIS

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Abstract

Myeloid-derived suppressor cells comprise a heterogeneous population of immature immune cells that expand during the course of cancer progression. These cells adopt an immunosuppressive phenotype that impairs the anti-tumour immune response through modulation of T cells, natural killer cells, dendritic cells and macrophages, as evidenced both in mouse models of cancer and patients. While much attention has been focused on the immunosuppressive roles of myeloid-derived suppressor cells, it is becoming increasingly clear that they can also promote tumour progression and metastasis via other immune-independent functions, including the regulation of angiogenesis and tumour invasiveness. Their arrival at metastatic sites prior to the arrival of tumour cells also contributes to the formation of a favourable pre-metastatic niche. This review will summarise the various roles for myeloid-derived suppressor cells, including new evidence that supports a role in promoting a favourable pre-metastatic environment for bone metastasis.

That cancer progression relies heavily on the interaction between malignant cells and host stromal cells within the tumour microenvironment, including fibroblasts, endothelial cells and immune cells, is now well accepted. Although initially thought to have anti-tumour roles, it is now well known that immune cells can also become influenced by tumour-derived and other microenvironmental factors and adopt a pro-tumorigenic, immunosuppressive phenotype. In the last three decades, much attention has been focused on the roles of myeloid-derived suppressor cells (MDSCs) in this process. Mostly known for their ability to suppress the anti-tumour immune response through

modulation of adaptive and innate immune activation, it is becoming increasingly clear that MDSCs have additional immune-independent pro-metastatic roles, including promoting angiogenesis and bone degradation, and that they play an integral part in the formation of a pre-metastatic niche.

MDSCs comprise a heterogeneous population of immature immune cells. In normal physiology, these cells differentiate into macrophages, dendritic cells, granulocytes and neutrophils. However, in cancer, tumour-derived factors such as vascular endothelial growth factor (VEGF), G-CSF, GM-CSF and the pro-inflammatory proteins S100A8 and S100A9 block this differentiation, leading to an expansion of immature myeloid cells in bone marrow, peripheral blood, spleen and tumour.[1-4] Murine MDSCs are most often characterised by co-expression of the cell surface markers CD11b and Gr-1, and they can be divided into subclasses based on differential expression of the Gr-1 epitopes Ly6G and Ly6C. Granulocytic MDSCs are $CD11b^+/Gr\text{-}1^+/Ly6C^{mid}/Ly6G^{high}$ while monocytic MDSCs are $CD11b^+/Gr\text{-}1^+/Ly6C^{high}/Ly6G^-$ (table 1).[2,5,6] In humans however, MDSCs are defined as $CD11b^+/CD14^-/CD33^+$ or $Lin^-/HLA\text{-}DR^-/CD33^+$.[1,2] Given the heterogeneity of these populations, it will be essential in the future to identify markers that further subdivide unique populations. At present, the most conclusive way to define an MDSC population is by demonstrating its functional ability to suppress anti-tumour immune responses.

Table 1: Summary of common cell surface markers for MDSCs.

Species	MDSC Markers
Mouse	$CD11b^+/Gr\text{-}1^+$ $CD11b^+/Gr\text{-}1^+/Ly6C^{mid}/Ly6G^{high}$ (Granulocytic) $CD11b^+/Gr\text{-}1^+/Ly6C^{high}/Ly6G^-$ (Monocytic)
Human	$CD11b^+/CD14^-/CD33^+$ $Lin^-/HLA^-/DR^-/CD33^+$ $CD14^-/Cd11c^{hi}/CD123^-$ (fibrocytes)[41]

Numerous studies have now demonstrated that MDSCs accumulate throughout disease progression. A small number of $CD11b^+/Gr\text{-}1^+$ cells are found in healthy naïve mice, however these cells are not true MDSCs because they do not exert immunosuppressive effects. During chronic inflammation and tumourigenesis however, these cells fail to differentiate and instead rapidly expand and adopt an immunosuppressive phenotype.[2,7] A correlation between MDSC accumulation in blood and metastatic progression has been observed in human patients with breast, colorectal, gastric, brain, prostate, pancreatic, oesophageal, liver and skin cancers.[8-13] For example, in the peripheral blood, MDSCs increase from an average frequency of 1.26% and 1.96% in healthy patients and early breast cancer patients respectively, up to 4.37% (and as high as 25%) in patients with advanced metastatic disease.[8] This correlation has been recapitulated in immunocompetent mouse models of cancer, where MDSC numbers in the spleen and peripheral blood correlate closely with tumour burden and metastatic potential.[14-17] One of the most exaggerated models of MDSC accumulation is the 4T1 murine model of metastatic breast cancer, in which MDSC levels can increase by >20-fold compared to naïve mice.[18] Although less pronounced, this accumulation is also observed in the MMTV-PyMT breast cancer model, with an increase in MDSC accumulation of approximately seven-fold in tumour bearing mice.[18] In general, MDSC accumulation is more pronounced in mouse models, since overall tumour and/or metastatic burden is often higher than in patients who have received therapeutic intervention.

Immunosuppressive roles for MDSCs

As suggested by their name, the most prominent role for MDSCs is their ability to suppress the anti-tumour immune response, as extensively reviewed elsewhere.[4,19,20] Both granulocytic and monocytic MDSCs can suppress the proliferation and activation of T cells. MDSCs perform this function through several mechanisms. They overexpress arginase-1, which converts L-arginine to ornithine and urea.[4] They also express high levels of the cystine transporter x_c^- for importing cystine and lack the ASC transporter required for exporting cysteine.[21] MDSCs therefore act as a sponge for arginine and cysteine, reducing the availability of amino acids that are essential for protein synthesis and proliferation of T cells. Additionally, granulocytic MDSCs produce high levels of reactive oxygen species and peroxynitrites, and monocytic MDSCs produce nitric oxide through expression of inducible nitric oxide synthase.[4,7,22] Peroxynitrites and nitric oxide impair DNA damage repair and protein synthesis and consequently lead to reduced T cell proliferation.

In addition to their effects on T cells, MDSCs interfere with anti-tumour responses by decreasing the prevalence and function of natural killer cells through TGFβ, IL-1β, or natural killer p30-dependent mechanisms.[23-25] They are also able to dampen the immune response by triggering the activation and expansion of another immune suppressive population, regulatory T (T_{Reg}) cells.[26-28] Furthermore, MDSCs can function to skew the polarisation of macrophages to a pro-inflammatory and tumour promoting M2 phenotype,[29] and impair the ability of dendritic cells to activate T cells.[30]

These multiple suppressive functions are likely to have important implications therapeutically. A number of therapeutic approaches for cancer rely on immune activation and therefore may not be efficacious in an immune-suppressed environment populated by MDSCs. A recent strategy has focused on improving the anti-tumour immune response by combining immunotherapies with agents that manipulate the function of MDSCs, either by eliminating them, deactivating them, blocking their formation or forcing their differentiation, as extensively reviewed elsewhere.[31,32] One agent trialled in the clinic is all-trans retinoic acid (ATRA), which functions to promote the maturation of MDSCs into mature myeloid cells such as macrophages, dendritic cells and granulocytes. In renal cell and small cell lung cancer patients, ATRA improved

anti-tumour immune effects in combination with vaccine therapy.[33,34] However, a new study in murine breast cancer models suggests that ATRA promotes the differentiation of MDSCs into CD11b^{+}/Gr-1^{-}/F4/80^{+} macrophages that are even more immunosuppressive than their precursors.[18] This increase in T cell-suppressive ability was associated with enhanced metastatic outgrowth in the lungs of ATRA-treated mice, suggesting that extreme caution should be taken when going forward with this therapeutic approach. Therapies such as 5-fluorouracil or gemcitabine that deplete MDSCs without inducing differentiation, might be more efficacious at preventing tumour progression.[32] In order to improve the outcome of combination therapies, more information is needed regarding the diverse functions of MDSCs, both within the primary tumour and in circulation. This will ensure the educated design of clinical trials that use combination therapies in the right setting based on mechanisms that maximise the likelihood of synergy.

Pro-angiogenic and pro-invasive roles for MDSCs

In addition to their presence in bone marrow, blood and spleen, MDSCs have been shown to infiltrate into the tumour microenvironment, where they concentrate at the periphery of the tumour and can exert effects on tumour growth.[16,18] MDSCs can mediate reduced sensitivity to anti-VEGF therapy,[35,36] suggesting an important role in promoting angiogenesis. In fact, it has been shown in models of colorectal cancer and Lewis lung carcinoma that MDSCs not only promote angiogenesis and tumour growth, but actually directly incorporate into the vasculature and express endothelial markers.[15] In this study, enhanced tumour growth and angiogenesis were reversed using matrix metalloprotease-9 (MMP9)-deficient MDSCs. Considering MMP9 has been implicated in VEGF activation,[37] this study suggests that apart from direct differentiation into endothelial cells, MDSCs are likely to be critical for promoting angiogenesis within the tumour microenvironment via production of MMP9.[15] This was supported in studies using mice lacking the MMP inhibitor TIMP2, whereby accumulation of MDSCs with high MMP activity increased angiogenesis and tumour growth rate in the Lewis lung carcinoma model.[38] Additionally, in the 4T1 breast cancer model, co-culture of tumour cells with MDSCs from late-stage tumour bearing mice enhanced tumour cell invasion in an MMP-dependent manner, and this was associated with enhanced metastasis to lung.[16]

The evidence linking MDSCs to angiogenesis is not limited to their expression of MMPs. Other potential promoters of MDSC-induced angiogenesis include regulator of G protein signaling-2 (Rgs2),[39] and Stat3, which governs MDSC-induced angiogenesis through upregulation of angiogenic genes such as VEGF, bFGF, IL-1β, MMP, CCL2 and CXCL2.[40] Human studies that support this pre-angiogenic role of MDSCs are lacking, however recent work in patients with metastatic paediatric sarcomas has reported a novel subset of human MDSCs called fibrocytes (CD14^{-}/Cd11^{chi}/CD123^{-}) that, apart from their immune suppressive activity, stimulate vessel growth in tube formation assays.[41]

Role of MDSCs in the pre-metastatic niche

In addition to their roles in promoting local invasion and angiogenesis within the primary tumour microenvironment, myeloid-derived suppressor cells have also been implicated in the formation of a pre-metastatic niche. The concept of a pre-metastatic niche involves tumour-induced changes to distant organs that make them well suited to support impending metastatic outgrowth. In 2005, Lyden and colleagues first described early events that initiate a pre-metastatic niche, whereby recruitment of hematopoietic progenitor cells into the lung promoted tumour cell recruitment and degradation of the extracellular matrix.[42] Studies in the last decade have focused on MDSCs as a subset of bone marrow-derived cells that is critical for establishing this favourable environment due to their roles in both angiogenesis and immunosuppression.

Hypoxia, or low oxygen levels, in the primary tumour has been linked with metastatic progression and is associated with the formation of a pre-metastatic niche at distant sites.[43] It has been demonstrated in mouse models of metastasis that hypoxia inducible factor (HIF)-dependent lysyl oxidase (LOX) secreted by hypoxic tumour cells, can remodel the extracellular matrix in secondary sites to promote the adhesion of CD11b+ bone marrow-derived cells.[43] In support of this, pre-injection of mice with conditioned media from hypoxic breast tumour cells prior to intravenous injection of tumour cells resulted in clustering of granulocytic MDSCs at the terminal bronchioles of the lung and increased lung colonisation, a phenomenon that was not observed using the normoxic conditioned media counterpart.[44] In this study, enhanced metastasis was associated with immune suppression, whereby NK cells had reduced cytotoxic activity.

MDSC accumulation in pre-metastatic lungs is also observed in the 4T1 mammary tumour model, where they have been associated with immune suppression via inhibition of interferon-γ (IFN-γ) production.[45] In this study, pre-metastatic lungs harbouring MDSCs had increased levels of growth factors, Th2 cytokines (IL-4, IL-5, IL-9 and IL-10) and inflammatory cytokines (IL-1β, SDF-1, MCP-1), which functioned collectively to create a proliferative, immunosuppressive and inflammatory environment optimal for tumour cell growth. Additionally, the vasculature of the pre-metastatic lung was more disorganised and leaky than the normal lung and more amenable to tumour cell extravasation and metastasis.[45] This confirmed an important role for MDSCs in vascular remodelling of the pre-metastatic niche. The evidence for a pre-metastatic niche extends beyond the lung in this model. MDSCs also infiltrate into the brain prior to the arrival of 4T1 tumour cells, where they have been implicated in tumour recruitment to the metastatic site.[46]

Role of MDSCs in bone metastasis

Apart from their role in supporting a pre-metastatic niche in soft tissues, recent evidence in breast cancer models suggests that MDSCs also function to promote a favourable microenvironment in bone, a common site of metastasis in patients. When MDSCs from tumour-bearing

mice were co-injected with MDA-MB-231 mammary tumour cells into the fat pad of nude mice, there was a 30% decrease in trabecular volume in femurs compared to tumour cells co-injected with naïve myeloid cells.[47] This phenomenon was observed prior to the outgrowth of bone metastases, suggesting that bone loss due to MDSCs may be integral in establishing a pre-metastatic environment in bone. To further confirm this, mice were injected intra-tibially with tumour MDSCs or naïve myeloid cells just prior to cardiac injection with MDA-MB-231 cells. Tumour MDSCs enhanced bone tumour growth and this was associated with enhanced bone degradation and an increased osteoclast activity.

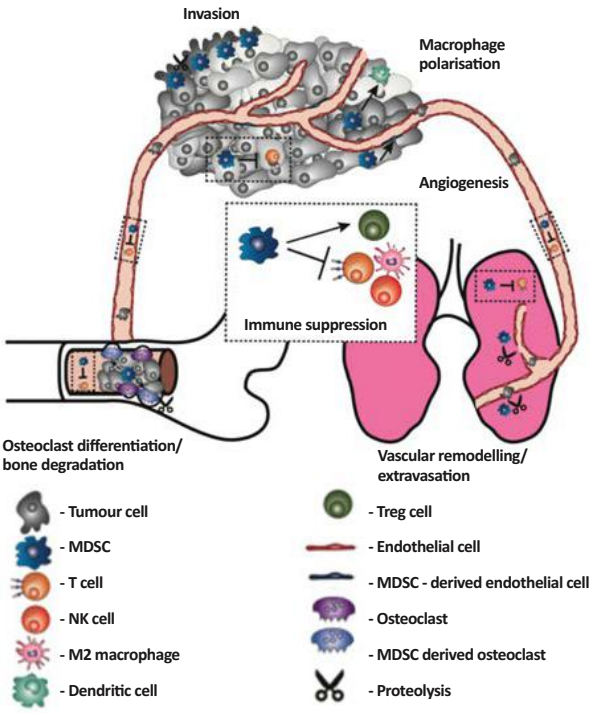
Surprisingly, when MDSCs were isolated from the bone marrow of tumour-bearing mice and cultured on dentine slices in the presence of RANK ligand, the cells differentiated into tartrate-resistant acid phosphatase positive ($TRAP^+$) osteoclasts with bone-degrading capabilities. This was confirmed *in vivo*, where GFP^+ MDSCs became $TRAP^+$ after intratibial injection, providing the first evidence that MDSCs can differentiate into osteoclasts in the bone microenvironment.

Other groups demonstrated that this phenomenon also exists in the 4T1 breast cancer model,[48] and in multiple myeloma.[49] In the 4T1 model, MDSCs that were isolated from the bone marrow of mice bearing bone metastases were able to differentiate into $TRAP^+$ osteoclasts, whereas those isolated from the metastatic lung, lymph node, spleen or blood of tumour bearing mice could not. This highlighted an importance for the bone microenvironment in this process. MDSC-derived osteoclasts also expressed other osteoclast markers such as cathepsin K, MMP9 and carbonic anhydrase-2, and were capable of resorption of bovine cortical bone slices in vitro and of tibias in vivo.[49] High levels of nitric oxide were shown to be critical for the differentiation of MDSCs to osteoclasts, as treatment of mice with 1400W, an inhibitor for inducible nitric oxide synthase, attenuated bone damage caused by MDSCs. Considering recent work by Kang and colleagues has revealed that metastatic outgrowth of disseminated breast tumour cells in bone is dependent on osteoclast activation,[50] MDSCs may represent an important subset of osteoclast progenitors that is crucial in the establishment of a pre-metastatic niche in bone.

Conclusion

We have provided a brief overview of the diverse functions of MDSCs in tumour growth and metastasis (figure 1). Most of the work to date has focused on the immune suppressive nature of these cells and their ability to extinguish the anti-tumour immune response through modulation of T cell proliferation and activation. There is now clear evidence however, that these cells have other functions in promoting angiogenesis and providing a favourable microenvironment for outgrowth in common metastatic sites such as lung and bone. Additionally, MDSCs have varying roles depending on the microenvironment from which they are derived. Therefore, further characterisation of specific markers that predict functional characteristics of MDSCs is necessary. Among many other factors, the secondary roles for MDSCs seem to be governed in large part by proteolytic enzymes such as MMPs, which are highly expressed in these cells and whose activity is critical for the cell signalling, matrix degradation and bone resorption necessary to promote metastasis. As the understanding of these diverse MDSC subtypes and functions continues to expand, therapies targeting MDSCs in combination with immunotherapy will continue to improve.

Figure 1: Diverse roles for myeloid-derived suppressor cells in cancer metastasis. MDSCs compromise the anti-tumour immune response through suppression of T cells, dendritic cells, and natural killer cells. Their stimulation of T_{REG} activation can also diminish the anti-tumour response. While much attention has been focused on these immune suppressive roles for MDSCs, they function in various other ways to promote metastasis. At the site of the primary tumour, MDSCs stimulate angiogenesis and can even become incorporated into the endothelium. They promote local invasion of tumour cells and can also polarise macrophages towards an M2 phenotype, which further augments angiogenic and invasive potential. At distant sites, MDSCs function to create an environment that is immune suppressed and primed for metastatic outgrowth. In lung, this includes remodelling the vasculature to support tumour cell extravasation. In the bone, MDSCs can differentiate into osteoclasts that are capable of degrading bone. Many of these non-immunosuppressive roles for MDSCs rely on proteases such as matrix metalloproteases, and in the case of bone degradation, cathepsin K.



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METASTATIC BREAST CANCER AND CIRCULATING TUMOUR CELLS

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Abstract

Circulating tumour cells – often referred to as a 'liquid biopsy' for cancer – can be found in a large proportion of patients with breast cancer that has spread to other organs. Changes in circulating tumour cell levels in metastatic breast cancer patients receiving chemotherapy have been shown to correlate with survival. Consequently, they have been shown to be an independently important way of predicting both the course of the metastatic disease, which will ultimately prove to be fatal, and the magnitude of response to systemic therapy. Circulating tumour cell research will not only elucidate the metastatic process but will also provide a platform for development of new cancer treatment regimens and drug targets. In this article, we will show how circulating tumour cell analysis is currently used in clinical practice, in clinical trials and the challenges that remain both in detecting these rare cells in the blood and in unravelling their molecular signatures, which may differ considerably from the primary cancer. As the isolation and characterisation of circulating tumour cells steadily improves, new metastatic breast cancer treatments will be developed, old regimes refined and patients will ultimately benefit.

Breast cancer, the most common cancer affecting women in Australia, claims the lives of approximately seven women every day in Australia.[1] Deaths due to breast cancer are caused by metastatic spread of the tumour from the primary site to other parts of the body. Although some women who will ultimately have metastatic breast cancer present with it at time of diagnosis, the majority have localised disease at diagnosis. An Australian study found approximately one in 20 women with localised node-negative disease and one in six women with regional disease at diagnosis went on to develop metastatic breast cancer within five years of diagnosis.[2]

Metastatic breast cancer is a heterogeneous disease that can be confined to one site, to one organ (most commonly bone) or display diffuse and multiple organ involvement. Current identification of metastatic breast cancer relies on clinical manifestations of the metastases, biopsy results, imaging tests and serum tumour markers. Although metastatic breast cancer is considered incurable, the introduction of novel therapies and drug combination regimes (see Madigan et al, this issue) has led to considerable prognostic improvement for most patients. Metastatic breast cancer is treated with systemic therapy (chemotherapy, biological therapies and/or endocrine therapy), often local palliative radiation and less commonly palliative surgery.[3] The choice of treatment depends on: the type of primary cancer; receptor status (hormones; oestrogen (ER), progesterone and human epidermal growth factor receptor 2 (Her2)); size and location of metastases (visceral versus nonvisceral); patient age, comorbidities and preferences; and previous treatments and response.[3]

Role of circulating tumour cells and epithelial to mesenchymal transition in cancer metastasis

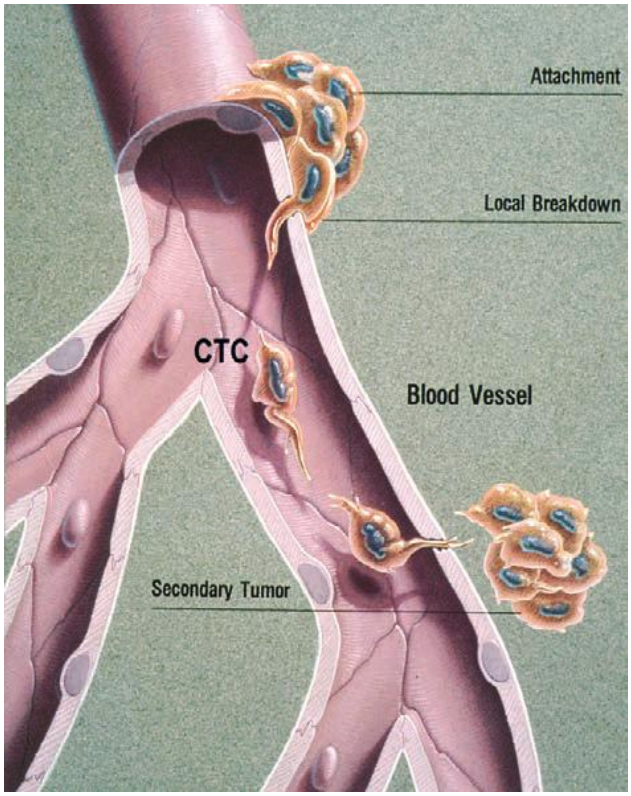
The mechanisms of metastasis have been rigorously debated since 1874, when British surgeon Campbell Greig De Morgan postulated that breast cancer arose locally, spread to lymph nodes and thereafter to other organs.[4] We now understand cancer spreads via growth in situ, and via blood and lymphatics.[5] Furthermore, breast cancer is known to not only spread from breast to lymph nodes and other organs, but also from metastatic lesions, re-seeding breast tissue and distant sites.[6] The tumour cells are called circulating tumour cells (CTC) when found in blood, and disseminated tumour cells when located in the bone marrow. Depending on the method of isolation and the tumour burden, these cells can be found in up to 50-70% of women with breast cancer which has spread.[7]

Increasingly, experimental data show that CTCs have undergone epithelial-mesenchymal transition (EMT), a transient process by which fixed, rigid epithelial cancer cells lose their apical-basal polarity and transition to an intermediary elongated (fibroblast-like), motile, mesenchymal cell, allowing escape from the primary tumour site, resisting apoptosis (programmed cell death) and allowing transportation via the blood circulatory system to establish metastases (figure 1).[8-12] The EMT mechanism was originally recognised in embryogenesis and wound healing.[13]

Isolation, characterisation and analysis of CTCs and DTCs from metastatic breast cancer patients are important for developing deeper understanding of metastatic spread and may lead to improved treatments. Analysis of CTCs is

favoured over that of DTCs due the invasiveness of bone marrow sampling required for DTC collection.[14] This article will therefore focus on the isolation and analysis of CTCs in metastatic breast cancer.

Figure 1: A diagram of the transient process by which fixed, rigid epithelial cancer cells lose their epithelial characteristics, escape from the primary tumour site (local breakdown) to become circulating tumour cells that are then transported by the blood circulatory system to other parts of the body where they may establish secondary tumours also known as metastases. This figure is adapted from the public domain illustration (ID 2446) created by Jane Hurd and the National Cancer Institute.



Circulating tumour cell characterisation and isolation

CTCs were first observed and described in 1869 by the Australian physician Thomas Ashworth.[15] CTCs are, in most patients, extremely rare (~1-10 CTCs to 10^6-10^9 normal blood cells),[16] and are difficult to detect with immunohistochemistry, however the development of immunomagnetic capture techniques, allowing enrichment of tumour cells, has made this easier.[17,18] CellSearch (Veridex, US), a semi-automated instrument with an immunomagnetic bead capture system based on epithelial cell adhesion molecule (EpCAM), followed by immunofluorescence analysis,[19] has become the most commonly-used CTC assessment method for epithelial cancers such as breast cancer, gaining Food and Drug Administration approval in the US in 2004 for metastatic breast cancer. Its use in Australia outside a research setting is limited by the very small number of machines available, the lack of Therapeutic Goods Administration approval, emergence of evidence of EMT in CTCs which may cloud its efficiency, and still poorly understood clinical utility. Other CTC immunomagnetic isolation methodologies include the Isoflux system,[20] AdnaTest,[21] CTC-chip,[22] Herringbone-Chip,[23] and Magsweeper,[24] and methodologies which utilise CTC physical properties (isolation by size of epithelial cells),[25] Dean Flow Fractionation,[26] microtube device,[27] and filter based technique or techniques,[28] using a combination of immunological and physical properties to isolate cells (^{pos}CTC-iChip).[29] Although comparisons of CellSearch with the other CTC isolations methods have reported discordance in detection rates with some methods reported to be more sensitive or specific,[20,30,31] CellSearch remains the predominant CTC isolation method in clinical trials due to the FDA approval and depth of experience.

In 2004, Cristofanilli et al.[32] using the CellSearch system, conducted a landmark study enumerating baseline CTCs of patients with metastatic breast cancer prior to the commencement of a new line treatment and at the first follow-up visit, and determined that a CTC baseline of 5 per 7.5 mL whole blood distinguished patients with a slow disease progression from those with rapid disease progression.[32] Subsequent studies reported ≥5 CTCs correlate better with overall survival than radiological changes.[33] This has been confirmed by the recently presented SWOG S0500 randomised trial, which confirmed patients whose CTC count was low at start of treatment for metastatic breast cancer had a better survival, and for those whose count was high and remained elevated three weeks into chemotherapy, survival was worse.[34]

Circulating tumour cells as a guide to treatment selection

It is hoped that monitoring CTC levels and characteristics can be useful in deciding when to change treatment due to progressive disease and potentially to help decide initial treatment for metastatic breast cancer.

Changes in CTC levels in metastatic breast cancer patients receiving chemotherapy correlates with survival.[35] Yet changing chemotherapy regimen in response to CTC count does not appear to improve overall survival or time to progression, as shown in a number of studies including the SWOG S0500 study. In this study, CTC count was prognostic, but in those whose CTC levels remained high after a cycle of chemotherapy, changing treatment did not improve survival.[34] Although chemotherapy can be useful in metastatic breast cancer patients, this study suggests other more effective treatment options are needed. This is still being studied in patients on third line chemotherapy in the CirCé01 trial (France, NCT01349842). The randomised CirCé01 trial will enumerate CTCs before and after the first dose of a third line of chemotherapy. If the CTC values do not respond to treatment, then a subsequent fourth and fifth line of chemotherapy will be tested in the same manner. In this instance, CTCs are being used to tailor individual treatment and reduce the usage of costly, toxic, ineffective chemotherapeutic agents during palliative care.[36]

The potential of using changes in CTC levels to guide non-chemotherapeutic treatments is also being explored. Minimal survival gains with first line endocrine therapy alone are seen in patients with ≥5 CTCs/7.5 mL, regardless of tumour hormone receptor status.[37] Patient treatment selection on the basis of CTC baseline is currently being examined in the STIC CTC Metabreast clinical trial (France, NCT01710605), which will stratify treatment of hormone sensitive, Her2- metastatic breast cancer patients based on CTC count to chemo- or endocrine therapy.

A number of studies have reported discordance between tumour and CTC hormone receptor and Her2 receptor status, with hormone positive primary tumours and negative CTCs and vice versa,[38-41] and this may signal altered treatment response in metastatic breast cancer.[42] Trials currently underway evaluating such discordance include those using Lapatinib in the DETECT III trial (Germany, NCT01619111), Herceptin in the TREAT-CTC trial (European Union, NCT01548677) or Trastuzumab-emtansine (T-DM1) in the T-DM1 trial (France, NCT01975142) and a Dana-Farber trial assessing Trastuzumab and Vinorelbine (US, NCT01185509).

There is to date no methodology to identify the endocrine resistant metastatic breast cancer patients who would benefit from a switch to chemotherapy treatment as a first line treatment. The COMETI PD clinical trial (US, NCT01701050) seeks to use an algorithm, the CTC-Endocrine Therapy Index (CTC-ETI), which incorporates CTC counts and measures of CTC endocrine sensitivity (ER and B-cell lymphoma 2 (Bcl2)) and resistance to chemotherapeutics (Her2 and Ki67) to identify ER+, Her2+ metastatic breast cancer patients who would benefit from being treated with chemotherapy as a first line therapy.

Circulating tumour cells challenges

While the analysis of CTCs is demonstrating great promise for personalised medicine, there are many challenges. The dynamic processes by which cancer cells undergo continuous evolution means structuring cancer treatment on the status of the primary tumour, or even a single metastatic biopsy, is inherently flawed. The feasibility of performing several biopsies on a patient in order to keep abreast of an ever-changing cancer cell population is difficult. CTC analysis offers a less invasive 'liquid biopsy', however heterogeneity is also evident in the CTC population. The challenge is how to establish an efficient method to isolate this rare and heterogeneous population and characterise it effectively. Reliance on the EpCAM marker and cytokeratin expression, the basis of CellSearch for the isolation of CTCs from blood is problematic – many CTCs show low EpCAM expression and may have undergone EMT,[43] yet other isolation methodologies are not fully validated. There is therefore an evident need for an isolation methodology that can recognise CTCs that have undergone EMT and exhibit few epithelial characteristics and more pronounced mesenchymal traits. In addition, the clinical relevance of circulating tumour masses (CTM), clusters of CTCs which may also incorporate accessory host cells,[44] needs to be examined and incorporated into isolation methodology. Several studies hypothesise that the CTM environment enhances CTC survival in the bloodstream and is therefore an important part of the metastatic process.[44] Importantly, interaction of tumour cells with platelets, which are integral to CTM, has been shown to induce EMT in the tumour cells.[45]

A novel CTC capture using mesenchymal-marker based ferrofluids (N-cadherin or O-cadherin) has been developed at Duke University, in acknowledgement of the EMT process. This system will be evaluated in a clinical trial on metastatic castration-resistant prostate cancer and metastatic breast cancer patients (US, NCT02025413).

In addition to CTCs which have undergone EMT, subpopulations of CTC with stem cell characteristics may not be adequately detected by current methods,[46] and may indeed be very important in initiating and sustaining the metastatic process.[47] We are on the brink of understanding how these cells form, circulate and contribute to metastasis and studies elucidating the importance of CTMs and stem cells, culturing CTCs and analysing single cells for genomic and other alterations, will further advance our understanding of how these cells contribute to disease.

Conclusion

CTC analysis – the 'liquid biopsy' for cancer – has been shown to be an important prognostic and potentially predictive marker in metastatic breast cancer. However, their promise is yet to be fulfilled, with research needed to elucidate CTC heterogeneity and with ongoing clinical trials trying to establish how best to use CTCs to guide treatment. Evidently, we need to develop better ways to enumerate and characterise these rare but fascinating insights into the metastatic process.

We currently do not recommend the use of CTCs in Australia outside a clinical trial, but encourage further work in the area of technology development, enhancing understanding of the underlying biology of CTCs, and the metastatic process. The ultimate goal is to translate CTC knowledge and advances into better care of our patients with metastatic breast cancer.

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EXOSOMES IN CANCER METASTASIS: NOVEL TARGETS FOR DIAGNOSIS AND THERAPY?

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Abstract

Tumour cells secrete small membrane vesicles (30-100nm) termed exosomes that are derived from late endosomal compartments of cells. Exosomes are increasingly being recognised for their role in intercellular communication in the tumour microenvironment. Exosomes contain an array of proteins, messenger RNA (mRNA) and micro-RNA (miRNA), which are transferred from donor to target cells, thereby mediating local and systemic cell communication. Here, we review the functional role tumour-derived exosomes play in controlling tumour progression and its metastasis, as well as the potential to use exosomes in the diagnosis and targeting of cancers.

The cross-talk between cancer cells and their surrounding stroma is essential in regulating tumour progression and systemic spread. While tumour cells are classically known to communicate via direct cell-to-cell contact and the secretion of soluble factors, alternative novel mechanisms have recently emerged. Evidence suggests that small membrane vesicles, termed exosomes, contribute to the intercellular cross-talk and subsequent reprogramming of the tumour microenvironment.[1] Exosomes are microvesicles of endocytic origin (from inside the cell) with a size of 30-100nm that are released under both physiological and pathological conditions. Originally described as a mechanism for the removal of redundant molecules from reticulocytes (immature red blood cells), it is now clear that exosomes have a much more significant biological role.[1]

Exosomes are generated from secretory multivesicular bodies (MVB; late endosomes) that fuse with the plasma membrane for release into the extracellular environment. The release of exosomes has been observed in various cell types, including immune cells, epithelial cells, fibroblasts and various cancer cells.[2-5] They contain lipids, proteins, messenger RNA (mRNA) and micro-RNA (miRNA), which are transferred from donor to target cells.[1,6] In addition to canonical proteins commonly found in most exosomes, exosomes contain cell-type specific content in the form of biological molecules that can have significant impact on the phenotype of recipient cells (figure 1).[7] Indeed, tumour-derived exosomes can transport various biological molecules that are postulated to assist cancer progression and epigenetic reprogramming to increase oncogenic potency of cancer cells.[7] Importantly, the concentration of exosomes is often increased in the blood of cancer patients compared to normal controls,[8-10] and while the mechanisms for this observation have not been fully elucidated, common microenvironmental cues in tumours, such as hypoxia and low pH, have been implicated in exosome secretion.[11,12]

Functional role of exosomes in tumour progression

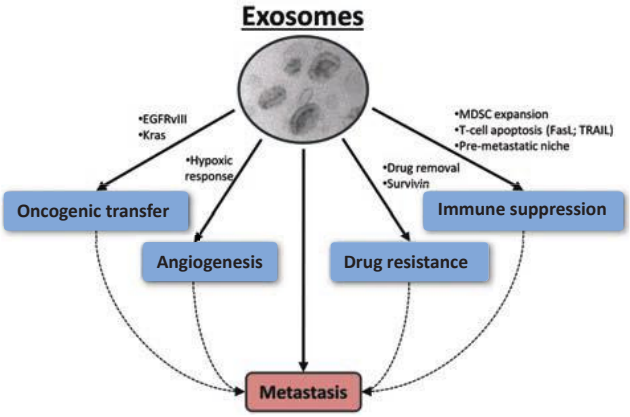
The tumour microenvironment is a complex system intimately linked by many cell types, including cancer cells, vascular cells, leukocytes, antigen-presenting cells and fibroblasts.[13] The tissue milieu is integral in determining the functionality, physiology and spread (metastasis) of cancer. Progression of the primary tumour is often characterised by the accumulation of genetic change, vascular growth (neo-angiogenesis), increased proliferation and subsequent invasion/metastasis to distant organs. Increasing evidence suggests that the rich array of proteomic and genomic information carried by tumour-derived exosomes is a novel mechanism by which cancer cells modify surrounding stroma and malignant cell behaviour.[7] Exosomes can affect signalling processes involved in neo-angiogenesis,[14] and immune suppression,[15] and induce drug resistance and oncogenic transfer.[16-18] Moreover, the ability of exosomes to induce systemic changes is thought to promote metastatic dissemination, which accounts for a majority of patient deaths,[19] as discussed later.

Oncogenic transfer

Recent findings indicate that exosomes shuttle both mRNA and miRNAs,[20-22] suggesting their involvement in the exchange of genetic information to recipient cells. This was first demonstrated by Valadi et al. showing that exosomes from mouse mast cells shuttle RNAs that can be transferred to human mast cells.[22] Importantly, mRNA transcripts from donor exosomes may be translated into proteins in recipient mast cells, indicating that transferred mRNAs have functional consequences. Similarly, lung-derived exosomes can transfer lung-specific mRNA to marrow cells.[23] Analysis of the mRNA profile and proteome of marrow cells after internalisation of the lung-derived exosomes were vastly different compared to untreated cells, further suggesting that transferrable mRNAs are functional.[23] Moreover, endothelial cells exposed to

exosomes containing GFP mRNA transcripts subsequently produce GFP protein after their uptake, indicating the active translation of transcripts delivered by exosomes.

Figure 1: Exosomes are small vesicles released by cells into the extracellular milieu. Exosomes can carry signalling molecules that have diverse roles in promoting the growth and metastasis of tumours.



The transfer of oncogenic proteins by exosomes has also been reported (figure 1).[17] Exosome transfer in glioma cells has recently been demonstrated to enhance tumorigenesis through delivery of a mutant epidermal growth factor receptor (EGFRvIII) isoform, resulting in increased expression of anti-apoptotic genes and enhanced proliferation.[17] Similarly, colon cancer cells with a mutant form of KRAS are capable of enhancing the three-dimensional growth of wild-type KRAS colon cells via exosomal transfer of mutant KRAS to the wild-type cells. Additionally, non-metastatic melanoma cells can be induced to become more metastatic by the uptake of exosomes derived from a highly metastatic melanoma cell line.[24] However, whether this change in metastatic potential is permanent remains unclear.

Angiogenesis

Angiogenesis is a physiological process involving the growth of blood vessels. In a cancer context, neo-angiogenesis is necessary to overcome hypoxia and for continued tumour growth.[25] Indeed, a pro-angiogenic tumour profile is closely associated with poor patient survival.[26,27] Recent studies have demonstrated that exosomes are a key mediator of hypoxia-dependent intercellular signalling between malignant and vascular cells to exert a pro-angiogenic response (figure 1).[28-30] Exosomes derived from glioblastoma cells grown under hypoxic conditions significantly induced microvascular sprouting ex vivo, and enhanced vascularisation of tumours in xenograft models leading to accelerated tumour growth.[14] Interestingly, these exosomes were enriched for several hypoxia-associated proteins, some of which are predictive of poor prognosis in glioma patients. Similarly, exosomes isolated from hypoxic squamous carcinoma cells enhanced angiogenic and metastatic potential by reducing blood vessel branching, and cell-extracellular matrix adhesion.[29] Furthermore, tumour-derived exosome secretion can also be enhanced by low pH.[12] Given this, exosomes may serve as novel biomarkers in which to ascertain low oxygen tension, and therefore potential of disease progression in patients with solid malignancies.

Exosomes and immuno-suppressive mechanisms

Studies have shown that tumour-derived exosomes can suppress specific T-cell immunity and induce innate immune cells towards a pro-tumour phenotype.[15,19] Exosomes derived from human colorectal and melanoma cells impair the differentiation of peripheral blood monocytes to functional dendritic cells, instead skewing them towards the phenotype of myeloid-derived suppressor cells (MDSCs).[31] MDSCs are immature myeloid cells with various immunosuppressive functions, including the suppression of T-cell immune responses.[32] Exosomes isolated from tumours of a breast carcinoma model promoted the accumulation of MDSCs via an exosomal prostaglandin E2 and TGF-beta mediated pathway,[15] which enhanced tumour growth. This is important, as numerous studies have found that increases in MDSC populations correlate with poor prognosis and overall survival in cancer patients.[32]

The immunosuppressive role of tumour-derived exosomes is not solely limited to prompting MDSC differentiation, but also the induction of apoptosis in recipient T cells. Various cancer cell types are described to secrete exosomes capable of inducing apoptosis in activated T cells by the transfer of the death ligands FasL and TRAIL.[33,34] For example, exosomes isolated from sera of patients with ovarian cancer are enriched with FasL and can suppress the CD3-zeta chain of T-cells to induce T cell apoptosis.[34] Furthermore, the pre-treatment of mice with exosomes derived from mammary tumour cells was shown to accelerate tumour growth, an effect that was due to the suppression of IL-2-mediated activation of NK cells and their cytotoxic response to tumour cells.[35] Taken together, the data suggests exosomes are a major contributor in the immunosuppressive tumour microenvironment (figure 1).

Exosomes and resistance to anti-cancer therapies

Exosomes have a role in the development of drug resistance to current anti-cancer therapies. Multi-drug resistance pumps that are commonly associated with drug resistance of tumours in vivo, are capable of being transported between cells via exosomal communication.[36] Furthermore, various anti-cancer therapies, including radiation and certain cytotoxic drugs, enhance exosome secretion by cancer cells.[37,38] Not only does the rate of exosome secretion increase, proteomic studies have also revealed that exosomes derived from cells exposed to anti-cancer therapies are enriched with anti-apoptotic proteins. For example, in response to radiation, HeLa cell exosomes are enriched in the protein Survivin,[9] which is known to play a role in the suppression of cell death and regulation of mitosis. This suggests exosomes can be utilised by cancers as a form of self-protection. Exosomes may also be capable of directly reducing the efficacy of cytotoxic drugs by removing the drug from the cell before it has been capable of inducing any cytotoxicity.[39] This phenomena

has been shown in cisplatin-resistant cancer cells, where cisplatin is enriched within the exosome compared to the cytoplasm of parental cells.[39] Moreover, exosomes may be capable of sequestering targeted antibody therapeutics. For example, HER2-enriched exosomes, derived from HER2-overexpressing breast carcinoma cell lines, were shown to sequester and abolish the therapeutic activity of Trastuzumab.[18] To this end, exosomes may be capable of carrying out a multi-faceted role in allowing cancer cells to evade the effects of current therapies (figure 1), and supports the concept that exosome secretion is an essential component enabling cancer cell survival under stressful conditions.

Functional role of exosomes in tumour metastasis and pre-metastatic niche establishment

Metastatic disease is responsible for over 90% of cancer-related deaths, and very few therapies have proven successful in the clinic for treating patients with metastatic deposits.[40] Therefore, we need to understand the underlying mechanisms of metastasis in order to generate novel, effective therapies for patients with advanced, aggressive cancers. The metastatic process comprises a number of sequential events that tumour cells are required to accomplish in order to successfully disseminate and implant in secondary organs. This complex multi-step process is known as the metastatic cascade, a series of systematic steps involving local invasion, survival and evasion of immune responses, intravasation into the circulation, extravasation at secondary organs, and finally proliferation of macroscopic metastatic tumour deposits.[41] The seeding of cancer cells at secondary organs is not random, a concept originally recognised over 100 years ago with Paget's 'seed and soil' hypothesis.[41] Accumulating evidence highlights that primary tumours can secrete factors capable of priming distant tissues for the arrival of cancer cells, thereby creating a supportive microenvironment termed a pre-metastatic niche.[42-44]

For both processes, the metastatic cascade and pre-metastatic niche formation, exosomes have been reported to be critical (figure 1).[19] Enhanced aggressiveness due to exosomal communication has been demonstrated with prostate and lung cancer. Exosomes secreted from these cancer cells can modify the phenotype of stromal cells by up-regulating MMP-9, leading to increased angiogenesis, motility and enhanced resistance to apoptosis.[45] Furthermore, exosomes from melanoma cell lines are capable of increasing pre-metastatic niche formation and metastatic burden in mice.[19]

Exosome mediated promotion of metastasis is not solely limited to tumour-secreted exosomes. Stromal cells are also capable of enhancing metastasis, demonstrating the complex nature of the communication between normal and malignant cells. In vitro findings have shown that exosomes from untransformed stromal cells increase the motility and invasiveness of several cancer cell lines,[46] with mesenchymal stem cell exosomes shown to promote migration of MCF7 breast cancer cells through upregulation of WNT signalling.[47] Human primary cancer associated fibroblasts also increase motility and metastasis of several breast cancer cell lines by secreting CD81 positive exosomes.[48] Moreover, exosomes from activated T-cells are capable of promoting invasion, and have been demonstrated to enhance the migration of murine B16 melanoma cells to the lung.[49]

Relevance of exosomes in clinical diagnosis and cancer therapy

Exosomes as biomarkers in diagnosis

Currently, there is a large unmet need to develop non-invasive and informative diagnostic markers for a variety of solid malignancies. The proteomic and RNA information contained in tumour-derived exosomes has generated significant interest for the use of exosomes as a non-invasive diagnostic tool. As exosome isolation techniques are now well established, and because exosomes are stable in bodily fluids, including serum, urine and saliva, they demonstrate great potential as reliable biomarkers of disease progression.[1] Given that exosomes may provide molecular signatures of their cell of origin, proteomic and RNA analysis may also provide an efficient means to determine oncogenic mutations. Moreover, exosomes derived from patients may prove useful in understanding the progression and treatment options for the disease. This has already been demonstrated with exosomes isolated from melanoma patients, which exhibited high protein content and elevated expression of TYRP2, VLA 4 and HSP70, proteins that were enriched in patients with a poor prognosis.[19] Not surprisingly, several commercial companies are developing exosome-based diagnostic tests to assist in determining diagnosis, drug response and prognosis in cancer patients, which are currently undergoing clinical validation.[1]

Exosomes as therapeutic targets

Given that tumour-derived exosomes are capable of reprogramming tissue microenvironments to support tumour progression, it may be beneficial to selectively deplete exosomes in circulation. Conversely, some researchers believe exosomes may be used as a potential vaccine for cancer immunotherapy. For example, tumour-derived exosomes transduced to express MHC class II molecules possess enhanced capability of immune stimulation and can subsequently reduce tumour progression in immunised mice.[50] Using a similar approach, several studies have reported the potential use of dendritic cell-derived exosomes for cancer immunotherapy.[51,52] Dendritic cell-derived exosomes were generated to contain functional MHC peptide complexes and further processed by the attachment of tumour antigens. These processed exosomes were shown in several phase I studies to be capable of inducing T-cell immune responses and tumour regression.[51-53] However, the efficacy of these approaches may be limited, given the immunosuppressive effects of tumour-derived exosomes in vitro and in vivo.

A more promising method may be to deplete exosome numbers with drugs such as dimethyl amiloride and its analog amiloride, which blocks H^+/Na^+ and Na^+/Ca^{2+} channels associated with exosome secretion. Dimethyl

amiloride was shown to mitigate the immunosuppressive effect of exosomes and enhance the chemotherapeutic drug, cyclophosphamide.[54] This effect was mirrored in patients with colorectal cancer, where the administration of amiloride to treat hypertension also inhibited exosome formation, and reduced immune suppressive functions.[54] Exosome secretion can also be inhibited by sphingomyelinase inhibitors (e.g. GW4869), which prevent the formation of exosomes through depletion of ceramide.[55] This possibility is supported by work showing that GW4869 administered to Lewis Lung carcinoma bearing mice had significantly fewer lung metastases.[56] These approaches suggest that the further development of inhibitors specific for exosome secretion may be warranted.

Recently, novel approaches to remove circulating tumour-derived exosomes have been proposed. These involve the use of extracorporeal hemofiltration devices,[57] a process that would avoid the risk of drug toxicity associated with pharmacological approaches. Using this approach, patient blood is passed through porous fibres that contain affinity-capture moieties for exosomes, which are selectively absorbed and depleted from the blood. The safety and efficacy of these devices has already been demonstrated in other disease settings. For example, viral removal in patients infected with hepatitis C has demonstrated extracorporeal hemofiltration to be well tolerated and effective at reducing viral load.[57] These systems are currently being evaluated for their efficacy in capturing tumour-derived exosomes present in biological fluids of cancer patients. However, the biological impact of depleting exosomes derived from non-malignant cells remains unclear.

Future directions

Given that tumour-derived exosomes display unique proteomic and RNA content, together with established methods of recovery from a range of body fluids, they represent a class of novel targets for biomarker analysis and therapeutic intervention. Importantly, questions remain on how informative exosomes can be in detailing the aggressiveness and clinical response in patients undergoing therapy. The potential of exosome depletion as a therapeutic adjunct in cancer patients also poses exciting possibilities for future clinical therapies. However, these approaches are still in their infancy, and more research is required to fully understand the role exosomes play in tumour progression, and their potential efficacy in the diagnosis and treatment of cancer patients.

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CURRENT INSIGHTS INTO CLINICAL DORMANCY AND METASTASIS

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Abstract

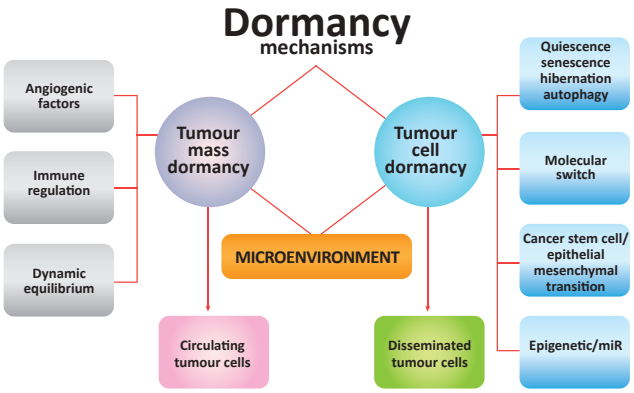
Survival after breast cancer diagnosis and treatment has improved markedly, however recurrence is still a treatment challenge for clinicians. The underlying cause for late recurrence after a long disease-free period is still unknown. It is known that undetectable metastases exist in two states, with different mechanisms playing a role in each dormancy state. In tumour mass dormancy, extrinsic factors such as angiogenesis and immune surveillance are in equilibrium with the tumour cells to maintain dormancy. In tumour cell dormancy, mechanisms intrinsic to the isolated tumour cells can dictate a dormant cellular state. The process of senescence, or pathways leading to cell cycle arrest, may be the key to unlocking the mystery behind these tumour cell deposits. With greater knowledge of the mechanisms that control undetectable disseminated disease, we have the opportunity to target these pathways to enable therapeutic strategies against metastatic disease.

Survival after a cancer diagnosis has improved markedly with the advancement of modern therapies, leading to a shift in the paradigm of cancer epidemiology towards chronic disease. With greater longevity, there is a new challenge in treating patients with metastatic recurrence long after successful treatment of the primary tumour.[1]

The disease-free period leading to recurrence long after the expected timeframe is defined as clinical dormancy and is a feature of several types of cancers, including follicular thyroid cancer, prostate cancer, B-cell lymphoma, melanoma and breast cancer. While there has been research exploring the mechanisms of primary tumour dormancy, the aim of this review is to summarise what is known about metastatic dormancy.

Metastases are a significant cause of patient demise, and are still a treatment challenge for clinicians. Current therapies are not very effective in treating recurrent disease, and technologies in use today limit our evaluation of interval disease. With increasing evidence to support the occurrence of metastasis as an early event in tumourigenesis,[2] there has been a focus on early detection and monitoring and in particular, on isolating and characterising circulating tumour cells (CTCs) and disseminated tumour cells (DTCs). While many cells may be released from the primary tumour from early stage disease onwards, only a very low percentage will form macrometastases. The presence of these cells can be misleading as a prognostic indicator, and can potentially result in overtreatment of the patient.[3] Nonetheless, CTC and DTC may provide the pool from which clinically dormant disease progresses to advanced disease, and further characterisation of the biological pathways involved with protracted metastasis and the metastatic potential of these cells is therefore imperative for our understanding of clinical dormancy and for the development of more effective therapies.

Figure 1: A theoretical model for the mechanisms involved in dormancy.



MODELLING DORMANCY MECHANISMS
Conceptually Tumour Mass Dormancy is regulated by external mechanisms. Evidence that Tumour Mass Dormancy, is the presence of circulating tumour cells. With Tumour Cell Dormancy, intrinsic cellular features may dictate dormancy in disseminated tumour cells.

Classifying dormancy

To understand minimal residual disease in patients, we need to explore the possible explanations for the existence of CTCs and DTCs. A proposed model to understand the biology of our clinical observations is the theory of tumour mass dormancy and tumour cell dormancy (see figure 1). Biologically, macrometastasis (defined as tumour deposits >2mm in size) is not associated with clinical dormancy and is characterised by more rapid progression to advanced disease with a continuing increase in overall tumour burden. The presence of micrometastatic disease (0.2-2mm deposits) is associated with tumour mass dormancy, while isolated tumour cells (<0.2mm in size) and DTCs are seen in association with tumour cell dormancy.

Tumour mass dormancy

Tumour mass dormancy refers to a state in which a clinically undetectable tumour mass is held in homeostasis by external mechanisms. Although we cannot directly detect tumour mass dormancy in patients, we can predict its existence from the presence of CTCs long after the treatment and removal of the primary tumour.[4] Current limitations of our understanding of CTCs are due in part to suboptimal analytical tools, often capturing only a small fraction of the CTC population when using capture antibodies that bind to EpCAM, HER2 and/or EGFR. Another limitation is our poor understanding of the biology of these micrometastases and thus the unmet promise of clinical utility. To date, only numbers of CTCs greater than 5 per 7.5 ml blood in breast cancer are predictive of outcome and response to therapy,[5] indicating significant clinical and biological heterogeneity in the majority of patients with low to moderate numbers of CTCs.[6]

The proposed mechanisms behind tumour mass dormancy are well described. Once tumour cells grow beyond a size that tissue vasculature can support, neovascularisation is required to enable growth. Limitations in factors involved in angiogenesis prevent the micrometastasis from expanding. A successful activation of angiogenesis, known as the angiogenic switch, contributes to the expansion of the micrometastasis into a macrometastasis.[7] Immunosurveillance is another mechanism that keeps the overall tumour burden in check, by removing tumour cells that induce an immune response. In addition, the micrometastasis is in equilibrium between cell death and cell renewal, the end result being a dynamic but dormant tumour mass. Any disruption in this delicate balance can favour a growth phase, with subsequent development of a macrometastasis.

Tumour cell dormancy

Pre-clinical modelling of tumour cell dormancy has been challenging due to limitations in current technology and to a lack of highly specific tumour cell dormancy biomarkers. In vivo, dormant tumour cells have been observed long after treatment of the initial primary tumour. As yet, we are still uncertain about the molecular events that result in their presence in tissues, their sustained viability over many years and the mechanisms by which they maintain their clinical indolence. What we do know is that current chemotherapeutic treatments are not effective at killing these cells. It is imperative therefore, to understand the pathways driving dormancy, to enable us to manipulate these pathways for future targeted therapy.

As outlined below, DTCs may develop mechanisms to allow them to survive for long periods in tissues such as bone marrow. Though we are unable to study DTCs

directly, it is predicted that their stem cell properties and their transition from an epithelial state to a mesenchymal state, known as epithelial to mesenchymal transition, may explain their continued existence. However, it is not only their cellular properties that determine the dormant phenotype in DTCs. There is a dynamic equilibrium between the microenvironment and intracellular and genetic processes of the tumour cells that give rise to the prolonged viability of these dormant cells.

The epithelial to mesenchymal transition has been widely accepted as a model to describe the escape of tumour cells from the primary mass and is predicated on the ability of individual cells to reversibly change their phenotype from epithelial to mesenchymal. CTCs are evidence of this theory, as these cells can exhibit both epithelial and mesenchymal markers. Moreover, the reverse process, mesenchymal to epithelial transition, is required to enable proliferation at the secondary site. While it is not known what may trigger DTCs to revert to the proliferative epithelial cell state, targeting these mechanisms may provide a therapeutic avenue.[8]

Cancer stem cells have multiple properties that may be applicable to DTCs and their ability to survive in vivo. They are slow cycling cells, they are immuno-evasive and they are resistant to conventional chemotherapy and radiotherapy. They express high levels of the ATP-binding cassette transporter proteins that efflux many chemotherapeutic drugs and they are inherently resistant to reactive oxygen species, thus rendering radiotherapy redundant.[9] These are properties required by an isolated tumour cell to survive in an otherwise unfavourable environment.

Senescence and related changes

Cells that exit the cell cycle after stressful stimuli in a seemingly irreversible capacity become senescent. Many cellular stresses can induce senescence, which is identifiable by a multitude of morphological cellular features, most commonly the expansion of lysosomal beta-galactosidase levels.[10] Senescence induction and maintenance are primarily mediated by the Arf/p53/p21 and p16/pRb tumour suppressor pathways, and also in response to progressive telomere shortening.[11] Senescence may be partially rescued by tumour suppressor genes.[12,13] Therefore, it is plausible that dormant cells may exist in a senescent-like state, still able to re-enter the cell cycle upon appropriate stimulation.

Quiescence is the reversible exiting of a cell from the cell cycle into a G_0 arrested state. Factors that dictate cell cycle arrest or quiescence include the cyclins, p27 and p21, and this is the favoured explanation for the longevity in vivo of DTCs.

Hibernation has been explored as a mechanism to explain dormancy in the haematopoietic stem cell model. Haematopoietic stem cells in their bone marrow niche may mimic DTC in secondary sites. Cells in hibernation have the salient feature of altered lipid raft morphology through the PI3K-Akt-FoxO pathway, where the end products can block cell cycle progression.[14] These lipid rafts act as an intermediary platform where cytokine signalling, membrane trafficking and cytoskeleton organisation are controlled. Several molecular mechanisms have been implicated in the haematopoietic stem cell hibernation theory e.g. Ang-1–Tie-2, Notch ligand–Notch signal.[15-17] Some of these pathways even cross over to other known cellular mechanisms, for example, the cell cycle regulator molecule p21, also active in quiescence and senescence.

Macroautophagy has been shown to be a mechanism for dormancy in primary tumours.[18] In macroautophagy, cells undergo a bulk degradation of their intracellular organelles until favourable conditions return to allow proliferation to resume. The same phenomena may be replicated in DTCs as a survival mechanism. Cells that undergo macroautophagy in vitro switch to an apoptotic pathway. However in vivo, autophagic cells can persist in a dormant but viable state for prolonged periods.

Other mechanisms

Changes in genetic regulators may also play a part in the dormancy mystery. Methylation and histone deacetylation are important in regulating tumour promoters and suppressors. Potentially we can utilise knowledge from these known mechanisms to explain cellular interactions in clinically indolent disease. MicroRNAs are small non-coding RNAs that can regulate genes at both transcriptional and post-transcriptional levels. There is a growing number of dormancy associated microRNAs that have been shown to play a governing role in primary tumour dormancy, and their roles can be extrapolated to metastatic mechanisms as well. A single microRNA is able to alter the expression of multiple genes, and can play a key role in explaining how DTCs are regulated. Several microRNAs, including microRNA-16 and -19, are consistently found to regulate dormancy.[19]

Interactions with the extracellular matrix are integral for the survival, growth, proliferation and invasion of tumour cells. Extracellular matrix of distant potential sites of metastasis may also affect DTC biology. Evidence of this in breast cancer is seen with BMP7, which is present in bone marrow and has been shown to induce tumour cell dormancy. Also, increased TGF-β in the microenvironment along with the stimulatory effects of mitogenic cytokines, may regulate the balance between dormancy and proliferation. Adhesion of tumour cells to the extracellular matrix is also required to enable growth, and inhibition of adhesion renders highly metastatic cells dormant.[20]

A key concept in dormancy is the 'switch' from solitary tumour cells that may have survived for long periods, to an activated state resulting in the onset of clinically apparent metastasis. There are many proposed mechanisms for this switch, including activation of the endoplasmic reticulum stress signalling pathways that induce dormancy via p38/MAPK signalling. Alterations in the ERK/p38 activity ratio define primary tumour behaviour. A low ERK/p38 ratio correlates with slow tumour growth and a high activity ratio induces tumour proliferation.[21] This concept may be applicable in the metastasis context as well. Another proposed mechanism involves TGF-β2 signalling through TGF-β-RIII, resulting in p27-induced dormancy signalling.[22]

Dormant cells as a target

Historically, the process of metastasis has been hypothesised as a linear growth pattern that should follow the behaviour of the primary tumour, and even somehow resemble the primary tumour. Both mathematical modelling and scientific studies have refuted this concept, and it now seems that there is a spectrum of behaviours of metastatic cells, possibly related to the timing at which these cells escape from the primary site. Cells undergo many changes to enable lodgement at a distant site, undetected by the surveillance systems of the host. Most studies of dormancy explore primary tumour dormancy as opposed to spontaneous metastatic dormancy, although the concepts can relate to both scenarios.

Dormant metastases are very difficult to study, and indirect methods are needed to further our understanding. The dormancy phenotype may be a culmination of multiple pathways and multiple phenotypes. Targeting dormant cells at the molecular level will be the next step in minimising recurrence rates and avoiding the corresponding clinical consequences.

Therapy development will depend on knowledge of the mechanisms that regulate dormancy. Using the proposed mechanisms discussed, there are two options for a therapeutic strategy against these evasive cells. Firstly, we can aim to impose dormancy on already disseminated tumour cells, forcing these cells to remain quiescent for the life of the host. Dormancy imposing therapies would need to be non-cytotoxic and applied chronically, aiming to prevent the switch from dormant disease to overt secondary growth. Alternatively, we could consider the opposite by preventing these cells from entering dormancy, so they become more sensitive to current therapies. A dormancy breaking treatment would then be used as an adjunct to current therapies. Both of these strategies require further understanding of the processes that control the dormancy of metastatic cells, an area of research being actively pursued by several groups, but still in its infancy.

Late recurrence is thought to be due to the survival of clinically undetectable metastases in patients. These are exceedingly difficult to study due to limitations in detection and understanding of their biology. Current hypotheses, as outlined above, need to be tested in relevant preclinical models. With increasing frequency of late recurrence, we must develop novel strategies to combat these dormant repositories of cells, both at the tumour mass level and at the tumour cell level. It is likely that we need to use a combination of mechanisms to target dormancy effectively.

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MOLECULAR IMAGING OF METABOLISM IN CANCER METASTASIS

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Abstract

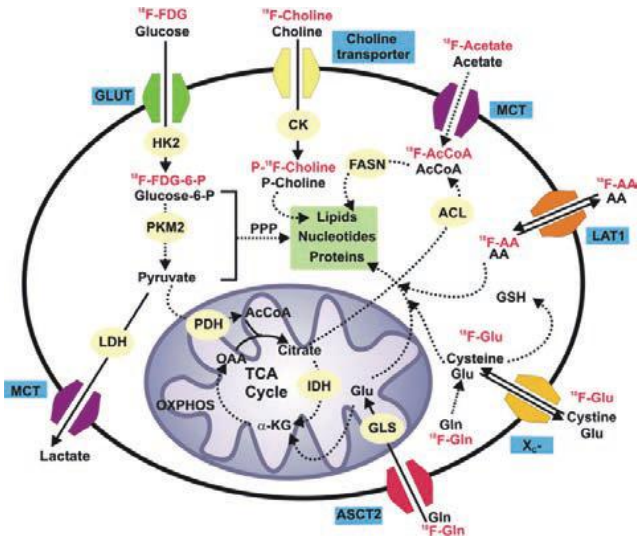
Cancer progression is characterised by extensive metabolic reprogramming. Renewed enthusiasm in this field has been sparked in part by the realisation that metabolic pathways, oncogenes and tumour suppressors are intimately linked and regulate tumour growth and metastasis through complex reciprocal interactions. The identification of key pathways and enzymes regulating metabolism in cancer cells provides new opportunities for cancer therapy. This has motivated the development of several specific inhibitors targeting metabolic pathways and their therapeutic evaluation in pre-clinical models or in cancer patients. The unravelling of metabolic pathways associated with cancer progression has also highlighted the extensive metabolic heterogeneity that exists between, and within, each cancer type as well as between metastatic sites. The translation of these findings into personalised therapy remains a considerable challenge. To this end, the use of positron emission tomography to non-invasively visualise tumour metabolism is likely to facilitate the implementation of and assessment of new targeted therapies. Here, we briefly review the key metabolic changes associated with cancer progression and discuss recent advances in the field of positron emission tomography for metabolic imaging of cancer and their potential to improve the clinical management of cancer patients.

Extensive metabolic reprogramming — a 'hallmark of cancer' — contributes to progression and metastasis.[1,2] Cancer cells often display a high rate of glucose consumption, even under oxygen-rich conditions, a process termed aerobic glycolysis or 'Warburg effect'.[3] Warburg postulated that increased energy production by aerobic glycolysis in cancer cells was a consequence of impaired mitochondrial oxidative metabolism.[3,4] However, it is now evident that tumours rarely exhibit mitochondrial defects and that most cancer cells still rely on oxidative phosphorylation to produce the majority of their energy, although this varies considerably between cancer types and sites of metastasis.[5-8] Moreover, aerobic glycolysis is not exclusive to tumour cells, but is also a common feature of proliferative normal cells.[9-11] The current view is that the primary function of elevated aerobic glycolysis is to generate biomass by diverting glycolytic intermediates towards the biosynthesis of macromolecules (nucleotides, lipids, proteins) required for rapidly proliferating tumour cells (figure 1).[12] An alternative model of cancer metabolism called the 'reverse Warburg effect' has been described more recently. In this model, glycolytic stromal cells under oxidative stress generate lactate, ketone bodies, glutamine and fatty acids that are taken up by metastatic tumour cells to generate energy through the oxidative mitochondrial metabolism.[13-15] While this phenomenon may have relevance to the anti-tumour effect of natural antioxidants,[16] more work is required to clarify how it can be targeted in metastatic disease.

Increased amino acid consumption resulting from overexpression of cell surface transporters in tumour cells provides an alternative to glucose.[17-19] Most notably, glutamine is utilised as a source of nitrogen and carbon to generate biosynthetic components and for mitochondrial metabolism, essential for growth, proliferation and survival (figure 1). This feature has been exploited for tumour imaging by positron emission tomography (PET) as described below.[19, 20] Alterations in lipid biosynthetic pathways also have been described in cancer.[21,22] Unlike most normal cells, tumour cells reactivate de novo lipid synthesis.[23,24] Fatty acid synthesis contributes to many aspects of transformation, including survival under oxidative and energy stress, maintenance of high glycolytic rate, growth and proliferation (figure 1).[22] Among other alterations, the expression of fatty acid synthase, an enzyme essential for fatty acid synthesis, is increased in several cancers including breast and prostate tumours.[25,26]

Perhaps the most significant development in recent years is the realisation that metabolic reprogramming is intimately linked to oncogenic signalling.[2,27,28] Activation of oncogenic pathways (PI3 kinase/AKT, Ras, Src, B-Raf and Myc), or the loss of tumour suppressors (p53 or PTEN), increases glycolysis by upregulating the expression of glucose transporters and/or several of the glycolytic enzymes.[2,29-31] Myc promotes mitochondrial metabolism of glutamine by increasing the expression of ASCT2 transporters and glutaminases.[12] Induction of the transcription factor HIF-1 under hypoxia leads to increased expression of fatty acid synthase, thereby promoting lipogenesis for

Figure 1: Schematic of key metabolic pathways in cancer cells. Alterations in glycolysis, through the overexpression of glucose transporters and several glycolytic enzymes, lead to an increased energy production and to the redirection of intermediate metabolites towards the pentose phosphate pathway for the synthesis of nucleotides, proteins and lipids. Metabolic reprogramming also involves alterations in lipid metabolism, mainly due to the increased expression/activity of enzymes responsible for de novo fatty acid synthesis. Enhanced accumulation of amino acids is also essential for cancer cell proliferation. Glutamine transfers its nitrogens to intermediate metabolites of nucleotide and protein synthesis. Its deamination by glutaminases produces glutamate that is further processed to form glutathione, the major cellular antioxidant and α-ketoglutarate to replenish the TCA cycle (anaplerosis) for energy production and fatty acid synthesis. Many of the enzymes (round yellow boxes) and transporters (square blue boxes) regulating metabolism are modulated by oncogenic signalling (e.g. Myc, Ras, p53) and are potential targets for therapy and molecular imaging. PET-probes used in the clinic or still in pre-clinical development are shown in red. Plain arrows denote a one-step reaction while dashed arrows denote a multi-step reaction. HK2 = hexokinase 2; PKM2 = pyruvate kinase M2; LDH = lactate dehydrogenase; PDH = pyruvate dehydrogenase; IDH = isocitrate dehydrogenase; GLS = glutaminase; ACL = ATP-citrate lyase; FASN = fatty acid synthase; CK = choline kinase; GLUT = glucose transporters; MCT = monocarboxylate transporters; LAT1 = system L amino acid transporters; ASCT2 = systeme ASCT glutamine transporters; X_C^- = system X_C^- glutamate transporters; ^{18}F-FDG-6-P = ^{18}F-FDG-6-phosphate; Glucose-6-P = glucose-6-phosphate; PPP = pentose phosphate pathway; AcCoA = acetyl CoA; α-KG = α-ketoglutarate; OAA = oxaloacetate; OXPHOS = oxidative phosphorylation; TCA cycle = tricarboxylic acid cycle; Gln = glutamine; ^{18}F-Gln = ^{18}F-labelled glutamine analogues; Glu = glutamate; ^{18}F-Glu = ^{18}F-labelled glutamate analogues; GSH = glutathione; AA = amino acids; ^{18}F-AA = ^{18}F-labelled amino acid analogues; P-choline = phospho-choline.



membrane formation and energy storage.[22] PI3 kinase/AKT signalling can activate ATP-citrate lyase, which converts cytoplasmic citrate into acetyl-CoA to promote lipid synthesis.[2] Interestingly, recent evidence indicates that interactions between metabolic and oncogenic pathways are bi-directional. For example, high glucose concentration or overexpression of FASN is sufficient to trigger the activation of oncogenic pathways and induce a malignant-like phenotype in mammary epithelial cells.[32,33] The characterisation of cooperative interactions between metabolic and oncogenic has identified several key molecular targets for diagnostic imaging and/or therapy (figure 1) and prompted the development of several metabolic inhibitors, many of which are already under pre-clinical evaluation.[31,34]

Metabolic heterogeneity and metastasis

The metabolic activity of tumours is greatly influenced by their microenvironment. This implies not only that tumours from different tissues display different metabolic profiles, but also that the metabolic activity of metastases from the same tumour may differ depending on the site of metastatic lesions. Evidence for this is emerging. Facilitative sugar transporters (GLUTs) that mediate transport of glucose (and other sugars) across the plasma membrane, are often deregulated in cancer and their expression is often correlated with poor prognosis.[29,35-37] However, the extent to which specific transporters are upregulated depends on the tumour type and is influenced by various extrinsic factors such as glucose concentration, inflammation and the specific microenvironment of the primary tumour or metastasis.[38-40] For instance, primary lung cancer is associated with increased GLUT1 expression compared to normal lung tissue, whereas liver metastases show enhanced GLUT3 and GLUT5 expression.[39] A study examining the expression pattern of sugar transporters in over 200 tumour samples by immunohistochemistry revealed a strong upregulation of GLUT1, GLUT2 and GLUT5 in breast cancer compared to normal breast tissues.[38] Increased expression of the high affinity fructose transporter, GLUT5, indicates that some breast tumours may utilise fructose as an energy source in addition to glucose. These findings may have important diagnostic (e.g. PET-imaging of fructose analogues) and therapeutic implications as suggested by the authors.[38] In contrast to breast and lung tumours, prostate tumours show lack or decreased expression of most GLUTs, an observation consistent with their greater reliance on fatty acid synthesis and glutaminolysis for growth and the poor imaging sensitivity of ^{18}F-FDG in these tumours.[20,38,41]

The link between metabolism and metastatic progression extends beyond elevated sugar transporter expression. Immunohistochemical and differential expression analysis of metabolic genes in metastatic pancreatic cancers revealed increased expression of several genes involved in aerobic glycolysis in primary tumours and site-specific overexpression of GLUT1, pyruvate kinase M2 and hexokinase-2 proteins in metastatic lesions.[8] Similarly, unique alterations in multiple metabolic pathways have been identified in breast cancer brain metastatic variant cells compared to bone metastatic variant cells.[42] Brain metastatic variants were characterised by increased expression of enzymes involved in glycolysis, TCA cycle, and oxidative phosphorylation pathways in addition to the activation of the pentose phosphate pathway and the glutathione system. The authors proposed that these

changes could reflect a predisposition or bioenergetics adaptation of tumour cells to the brain microenvironment that contribute to their survival and growth in brain. It is not clear from this study whether this metabolic reprogramming is characteristic of all or only a subtype of brain-metastatic tumours. Breast cancer is a heterogeneous disease, with at least two molecular subtypes having high affinity for brain, the triple negative and HER2^{+ve} subtypes.[43] Others have reported that HER2^{+ve} tumours are more glutaminolytic, whereas triple negative tumours are more glycolytic or 'Warburg-like'.[44,45]

A similar association between increased lipid metabolism and metastasis has been reported.[46-49] Lipid accumulation in brain metastatic lesions from melanoma, lung, colorectal and breast tumours was correlated with areas of necrosis.[46] In another study using a pre-clinical model of osteosarcoma metastasis to lung, increased lipid metabolism was correlated with the development of metastatic lesions.[47] Interestingly, ovarian cancers, which have a high propensity for metastasis to the adipocyte-rich omentum, can coerce adipocytes to sustain their bioenergetics requirements and rapid growth in this tissue via direct transfer of lipids from adipocytes to ovarian cancer cells.[49] Similar mechanisms may operate in metastatic endometrial, prostate and breast tumours.[50-52] Collectively, these studies highlight the metabolic heterogeneity between tumours and metastases, which provides tremendous diagnostic and therapeutic opportunities. However, if the goal is to translate these findings into personalised therapies, a major challenge ahead will be to define the metabolic profiles specific to each tumour type and their site-specific metastases.

Molecular imaging of tumour metabolism

Metabolic imaging non-invasively measures the functional state of tumours and metastases, and provides a mean by which to rapidly evaluate tumour response to therapy, resistance to a given treatment or even to predict treatment response. Thus, imaging of metabolism has high potential to improve the clinical management of patients. Several imaging modalities that take advantage of the increased metabolic activity observed in cancer cells have been implemented in the clinic or are in pre-clinical development. These include PET, single photon emission computerised tomography and magnetic resonance spectroscopy. The clinical use of single photon emission computerised tomography and magnetic resonance spectroscopy in cancer patients has been reviewed elsewhere.[20,53] Here, we focus on the clinical applications and limitations of current radiotracers and recent advances in the field of PET imaging.

Imaging of glucose metabolism

The first and most commonly used probe developed for imaging tumour metabolism is the glucose analogue fluorodeoxyglucose radiolabelled with the positron emitter fluor-18 (^{18}F-FDG). The transport of ^{18}F-FDG via glucose transporters directly reflects the cellular use of glucose. However, unlike glucose, phosphorylation of ^{18}F-FDG by hexokinase produces ^{18}F-FDG-6 phosphate, which cannot be further metabolised and therefore is trapped and accumulates in cells. Clinical studies on lymphomas and solid tumours have demonstrated the prognostic value of ^{18}F-FDG for the early assessment of tumour response to conventional therapies.[20,54] Therapy-induced changes in glycolysis occur as early as a few hours following treatment, and well before any detectable changes in tumour size. Therefore, ^{18}F-FDG uptake informs on the suitability of a chosen therapeutic intervention and allows rapid identification of non-responders who could benefit from alternative interventions. Thus, ^{18}F-FDG is increasingly utilised as a non-invasive marker in clinical studies, validating the efficacy of new therapies for which no reliable biomarkers are currently available (e.g. B-Raf inhibitors for melanoma, c-Kit inhibitors for gastrointestinal stromal tumours and EGFR inhibitors for non-small cell lung cancers).[55-57] Similarly, ^{18}F-FDG could be a valuable marker of efficacy for new drugs targeting glycolysis or mitochondrial metabolism.

However, ^{18}F-FDG suffers from some limitations. Its sensitivity is low for the detection of micrometastases from breast cancer and melanoma.[41] Moreover, due to the high glycolytic activity in the brain, accumulation of ^{18}F-FDG hinders the detection of gliomas or metastases in this organ.[20] Areas of inflammation are also ^{18}F-FDG avid. Therefore, despite the rapid therapy-induced changes in glucose metabolism, response to chemo or radiotherapy is usually monitored four to six weeks after the end of treatment to avoid false-positives resulting from therapy-induced inflammation.[41] In addition, ^{18}F-FDG is only weakly taken up and is a poor marker of metabolic activity in tumours that rely more heavily on fatty acid synthesis and glutaminolysis to grow (e.g. prostate carcinoma).[20,41]

Imaging of lipid metabolism

Changes in lipid metabolism provide an alternative for molecular imaging of tumours that are difficult to visualise with ^{18}F-FDG, such as gliomas, prostate and breast cancers.[41] Most commonly used markers of phospholipid biosynthesis are ^{11}C- and ^{18}F-choline and analogues. The specificity of choline-based tracers for cancer cells is the consequence of increased transport and phosphorylation of choline due to an increased expression of choline kinase in transformed cells.[58-60] Their low urinary excretion makes these tracers more effective than ^{18}F-FDG for identifying patients with recurrent prostate cancer.[20,61] ^{11}C-acetate was first developed to image oxidative metabolism of the myocardium. ^{11}C-acetate enters cells through monocarboxilic acid transporters before being converted to ^{11}C-acetyl-CoA by acetyl-CoA synthetase. In the myocardium, ^{11}C-acetyl-CoA is used by the TCA cycle and then released from the cells as $^{11}C\text{-}O_2$. In contrast, cancer cells metabolise ^{11}C-acetyl-CoA predominantly into lipids for membrane biosynthesis and cellular energy production.[62] Similar to choline, acetate analogues (^{11}C or ^{18}F) accumulate more efficiently than ^{18}F-FDG in prostate tumours and are useful tracers for detection of disease recurrences. Recently, ^{11}C-acetate has shown potential as a non-invasive biomarker to monitor therapy response to orlistat, a fatty acid synthase inhibitor.[62]

Imaging of amino acid metabolism

Cancer cells increase their amino acid consumption to accommodate their biosynthetic needs. Hence, amino acid-based tracers are potential alternatives to ^{18}F-FDG. Amino acids are internalised via plasma membrane transporters, including systems L (leucine-preferring, LAT), A (alanine-preferring), ASCT (alanine-serine-cysteine-preferring) and X_C^- (cytine/glutamate exchange transporter). Increased expression of LAT1, ASCT2 and X_C^- have been associated with cancer progression and poor prognosis in gliomas, lung, prostate and colon carcinomas, leading to the suggestion that labelled amino acid analogues could be valuable non-invasive prognostic markers for these malignancies.[17, 63] Research over the past decades has focused mainly on LAT1 substrates.[63,64] These have the advantage of crossing the blood brain barrier and are weakly retained in the normal brain, and thus provide an accurate visualisation of brain lesions.[63] However, the clinical use of amino acid-based tracers for imaging extracranial solid tumours is limited by their relatively low specificity compared to ^{18}F-FDG.

Glutaminolysis metabolism is increasingly recognised as a promising target for cancer therapy.[18] Surprisingly, glutamine-based tracers are still in their early phase of development.[65, 66] This stems in part from the fact that ^{11}C- and ^{13}N- labelled glutamine have short half-lives, are rapidly metabolised, and their metabolites are excreted from the cells, making their use as imaging agents difficult. In addition, labelling glutamine/glutamate with the longer-lived isotope ^{18}F is technically challenging. This has been addressed in part with the development of the glutamine analogue ^{18}F-(*2S,4R*)4-Fluoroglutamine (^{18}F-4FGLN) which has excellent tumour targeting properties in preclinical models of glioblastomas and mammary tumours.[65,67] While ^{18}F-4FGLN has yet to be tested in the clinic, pre-clinical data suggest that it could be useful in patients with tumours that are addicted to glutamine and sensitive to therapies targeting glutamine metabolism, such as tumours carrying N-Myc amplification or HER2^{+ve} breast cancer.[45,68]

Labelled glutamate analogues, including (4S)-4-(3-^{18}F-fluoropropyl)-L-glutamate (^{18}F-FSPG or BAY 94-9392) and ^{18}F-(*2S,4R*)4-Fluoroglutamate (^{18}F-4FGLU) have also been evaluated for PET imaging.[66,69] ^{18}F-FSPG was well tolerated in patients and had a similar tumour-to-background ratio to ^{18}F-FDG in a pre-clinical model of hepatocellular carcinoma, but unlike ^{18}F-FDG, exhibited low accumulation in inflammatory lesions.[69,70] Comparison of the glutamine analogue ^{18}F-4FGLN and its glutamate counterpart ^{18}F-4FGLU, showed that both tracers have potential as tumour imaging agents for glioblastomas and prostate cancers.[66] However, while glutamine and glutamate belong to the same metabolic pathway, ^{18}F-4FGLN is taken up by ASCT2 transporters, rapidly converted to various metabolites and a large proportion is incorporated into proteins.[66] In contrast, ^{18}F-4FGLU enters cells via system X_C^- and is not metabolised nor incorporated into the protein fraction.[66] Therefore, ^{18}F-glutamine analogues could be used to identify tumours that consume glutamine as an energy source and ^{18}F-glutamate analogues to assess detoxification potential mediated by glutathione, synthesised from glutamate.[65,66,69] One possible limitation of ^{18}F-4FGLN and ^{18}F-4FGLU is their low penetration through the blood brain barrier.[66] Whether this will impede detection of brain lesions remains to be investigated in pre-clinical models of brain tumours and in patients.

Conclusion and perspectives

Metabolic alterations in cancer provide exciting new therapeutic opportunities for this often fatal disease. PET imaging is an integral part of cancer diagnosis and treatment. Advances with the design of new radiotracers will contribute to the clinical translation of anti-metabolic drugs currently in development. However, the apparent metabolic heterogeneity of tumours and metastases poses a significant challenge with regard to the selection of best treatment and imaging modalities for specific tumour type and metastatic site. While ^{18}F-FDG remains the gold standard for imaging of glycolytic tumours, other radiotracers are required to overcome some of its limitations, particularly in tumours that rely on alternative metabolic pathways for growth. The most promising new tracers currently in development are glutamine and glutamate analogues. Whether these tracers, alone or in combination with ^{18}F-FDG, could provide more accurate detection of metastatic spread should be further investigated. Currently, most radiotracers only reflect the accumulation of nutrients due to increased transporter expression. Since many metabolic alterations involve changes in the expression or activity of metabolic enzymes, efforts should be put towards developing more specific probes for these enzymes.

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BONE MICROENVIRONMENT AND ITS ROLE IN BONE METASTASIS

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Abstract

Bone is a common site for cancer metastasis. The development of bone metastases represents a serious progression in a patient with cancer, leading to treatment goals changing from curative to palliative. Cancers that have metastasised to the bone are resistant to therapies and lead to destructive and painful changes in bone structure. Here we describe the multiple steps in metastasis and the varying processes that drive tissue selectiveness. There is an emphasis on the basis of the fertile 'soil' for cancer growth that is provided by the bone microenvironment. The basic changes in host/cancer cell interactions as a metastatic cancer cell invades the bone, escapes dormancy to grow to a micro metastasis, induces angiogenesis and hijacks the normal physiological processes of bone remodelling to fuel its own growth are outlined. Steps in the process of metastasis that offer potential as therapeutic targets are briefly discussed.

Bone is a common site for the metastasis of solid cancers, particularly of breast cancer and prostate cancer, which are common cancers in women and men respectively. In advanced breast and prostate cancer, 70 to 80% of patients are found to have bone metastases. Once breast and prostate cancer invade bone, they have the ability to profoundly influence bone cells in their environment, resulting in predominantly destructive lytic lesions in breast cancer and painful osteosclerotic lesions in prostate cancer. In both diseases, the identification of bone metastases is usually associated with the change of clinical goals from curative to palliative, due to the resistance of disseminated skeletal metastases to current therapies.[1-3] The target tissue specificity of the metastatic process is indicative of the importance of the micro-environment the target tissues provide. This observation of cancer selectiveness for particular tissues has given rise to the seed (cancer cell) and soil (target tissue) analogy first suggested by James Paget in the 19th century.[4]

Steps in metastasis to bone

Intravasation

Metastasis of cancer cells is not a simple process and requires the successful completion of multiple steps. The first step of metastasis requires escape from the primary tumour. To escape from the primary tumour there are changes in cancer cell behaviour required. These include loss of cell – cell adhesion, loss of responsiveness to tumour chemo-attractive signals, and gain or maintenance of responsiveness to extra-tumoural chemo-attractive signals. Development of the capability to migrate through tissues is required to enable single cancer cells to escape from the primary tumour mass or local lymphatic tissues into blood vessels – a process called intravasation. These attributes then lay the foundation for escaping the blood vessel and establishment of these cells in a target tissue.[5-8] There is also evidence that prior to metastasis occurring, the primary tumour can act to condition or prime target tissues for metastasis to make them receptive to colonisation of cancer cells once they enter the circulation.[9] (See also articles in this issue from Moeller, Parker)

Extravasation

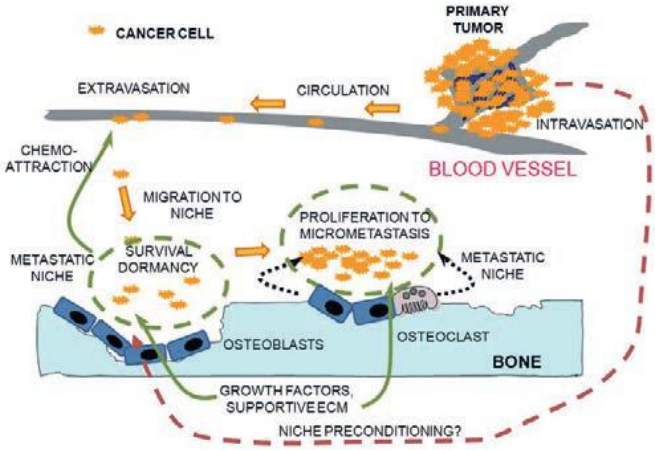
Once cancer cells have entered the circulation, their distribution throughout the body is initially a passive process dependent on the anatomic proximity to the primary cancer and the relative blood perfusion rate of the various tissues.[1] To establish in target tissues, the circulating cancer cells must escape the blood vessel that carries them by adhering to a blood vessel wall and migrating from the vessel into the surrounding tissues, a process called extravasation.[7] The local tissue microenvironment can influence extravasation via the nature of the vascular structure, with escape from the blood vessels in bone marrow likely to be enhanced by the thin-walled sinusoidal blood vessels present in bone. Additionally the presence of chemo-attractive agents within a tissue and diffusing into blood vessels may drive extravasation. Thus initial vascular deposition of cancer cells in a tissue may be random or may reflect active targeting (or both). Solid tumour cells tend to be large relative to haematopoietic cells. Intracardiac injection of breast and prostate cancer cells typically shows an initial rapid clearance of cancer cells from the blood and fairly broad distribution of cells in tissues, approximately consistent with the organ perfusion rate, supporting the concept of passive clearance of cancer cells from the blood and into tissues.[1] However, it is known that bone contains cytokines and growth factors that are chemo-attractive to cancer cells such as transforming growth factor beta (TGF),[8] and CXC12 (also known as SDF1),[10] for which the receptors are found on breast and cancer cells, and so there remains the possibility that there is also active homing of cancer cells to particular tissues including bone. This certainly occurs with haematopoietic sourced cancers such as multiple myeloma, however

the cells in these cancers are much smaller and arise from known cell types that naturally home to the bone marrow.[11] The presence of cancer cells in tissues non-receptive to metastasis rapidly reduces after intracardiac systemic injection, indicating that failure to survive and clearance from the body is the most common destiny for most cancer cells entering the vascular system.[12]

Targeting to the metastatic niche in bone and dormancy

Once cancer cells have been immobilised in blood vessels within the bone, there is the potential that chemo-attractive signals and tissue adhesion molecules specific to a target tissue, such as bone, can drive extravasation, enabling metastatic cancer cells to enter a microenvironment conducive to their survival. It is apparent that very few cancer cells escaping from a tumour are responsible for giving rise to a secondary tumour. Many cancer cells entering the circulation do not survive and disappear completely. Others escape from the vasculature but remain as single cells, identifiable in tissues, but remaining as single cells even years after a primary tumour has been removed, surviving in a state of apparently permanent dormancy.[7] The initial establishment of a cancer metastasis in bone depends on the presence of a microenvironment which induces the cancer cells to extravasate, survive and escape dormancy. These requirements probably are dependent on the nature of the environment in which the cancer cells find themselves, with bone providing a particularly fertile 'soil'. The rarity of all these events occurring is indicated by the initial small number of metastases observed in patients and after intracardiac of breast and prostate cancer cell injection of mice, which has given rise to the concept of the presence of a metastastic niche within bone.[13,14] It is known that there are particular niches within bone for both haematopoietic and mesenchymal stem cells. These appear to be closely dependent on the presence of a bone surface and osteoblasts, the bone lining cells which are able to synthesise bone (figure 1).

Figure 1: Initial steps in cancer metastasis to bone. Prior to metastasis, the primary cancer may condition the bone tissues to receive cancer cells (dashed line). Cells escape the primary tumour by extravasation into a blood vessel, which involves adherence to a blood vessel wall, invasion into the surrounding tissues and migration to a receptive niche. These cells may be initially dormant, but can be triggered by signals (dotted line) to proliferate and form micro metastases.



It is thought that important components of the niche are the expression of chemo-attractive signals that retain cells in the niche, the expression of cell surface adhesion proteins such as integrins on both the cancer and niche cells, and the presence of extracellular matrix proteins with ability to signal to cells through the presence of surface signals such as RGD domains (arginine-gycine-asprtate). Another important factor is likely to be the expression in the niche of various growth factors and chemokines.

How the metastatic niche maintains the survival of cancer cells and at some point allows their escape for dormancy is not known, but may be dependent on the varying expression of growth factors and cytokines which cycle during the normal periodic remodelling of bone, with migration of bone remodelling units across the surface of bone participating in a process that removes and rebuilds the skeleton in a seven to ten year cycle.[15] Bone tissue itself contains significant amounts of a wide range of growth factors that are released during the bone resorptive phase of this process, including many that are potentially able to act as growth factors for cancer cells able to drive their proliferation and migration. These include TGF beta, IGF1, fibroblast growth factors and bone morphogenetic proteins.[16] It has been demonstrated that increasing background rates of bone remodelling through calcium deficiency, vitamin D deficiency or by ovariectomy could each increase the growth rate of metastatic tumours in bone,[17-20] while reduction of bone remodelling inhibits the ability of tumours to grow in bone.[21]

Angiogenesis

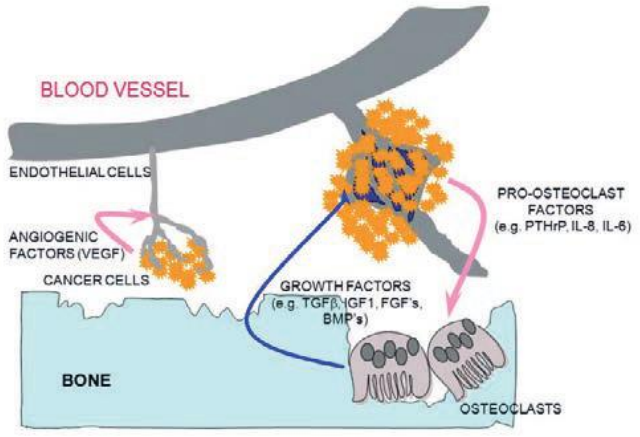
It is likely that the initial factors driving bone metastasis establishment are reliant on the pre-existing bone microenvironment into which the invading cancer cell migrates. However, as the cancer cells proliferate and form micro-metastases, they develop more and more ability to modulate the microenvironment in which they find themselves. In some patients, small cancer foci or micro metastases can be observed, in which initial proliferation of cancer cells occurred but progression has been inhibited. The development of a capability to induce neo-angiogenesis becomes essential for progression when a tumour reaches about 1mm in diameter, as its further growth is then impaired unless blood vessel invasion of the tumour can occur to provide the necessary nutrient supplies and waste removal. At this point, further growth becomes dependent on development of a vascular supply for the tumour, which can be achieved if the cancer cells are able to produce angiogenic signals that drive the vascularisation of the growing tumour mass.[22] The elevated expression of VEGF by breast cancer cells is associated with poor prognosis (see figure 2).[23]

Hijacking host regulator systems

As tumours grow further, their ability to modulate the signalling in host tissues to support their own further growth increases. The metastatic tumours now demonstrate the ability to mimic the regulation of normal bone tissue processes and so to hijack normal signalling processes in bone to induce increased bone resorption by host tissue osteoclasts. This has the potential to initiate self-amplifying cycles through the osteoclast mediated release from bone

of growth factors able to further expand tumour growth. The initial cycle described by Mundy and colleagues,[6] was termed a 'vicious' cycle in bone metastasis. In this cycle, they identified the ability of breast cancer cells in bone to secrete parathyroid hormone-related protein (PTHrP), to induce the formation of osteoclasts via increased local production of the osteoclast inducing cytokine, receptor activator of NF kappa B ligand (RANKL), by cells of the osteoblast lineage.[7,16,24] They were also able to identify the release of TGF from the bone matrix and its activation by the acid conditions produced by osteoclasts within the resorption sealed space between the osteoclast and the bone surface. TGF beta could then be demonstrated to increase cancer cell proliferation. Thus a vicious cycle was developed, in which cancer cells were able to cause osteoclastic bone resorption of the surrounding bone, both removing the physical limits on tumour growth and providing a source of growth factors to drive further cancer cell proliferation and PTHrP production, and thus more bone resorption and so on (see figure 2).

Figure 2: Progression of micro metastases into larger vascularised tumours regulating their own environment. To grow beyond micro metastases, cancer cells must induce neo-angiogenesis. As tumours grow further, they can produce pro-resorptive factors to drive bone resorption, thus releasing growth factors in a cyclic process driving more resorption and thus more growth.



Since this initial description of the vicious cycle, it has become apparent that additional amplification loops and intermediates are active in this cycle. In addition to TGF, other growth factors such as IGF1, endothelin-1 and fibroblast growth factor 2 also may contribute to cancer cell proliferation. Similarly, other cytokines secreted by cancer cells, such as IL-8,[25] and MIP -1alpha,[26] can drive increased bone resorption. Recently, a parallel amplification loop was identified in which tumour secretion of interleukin-6 (IL-6) was found to be induced by RANKL secreted by cells of the osteoblast lineage. In turn, IL-6 is known to be able to increase RANKL production by bone cells to further increase bone resorptive activity. Interestingly, IL-6 secreted by the tumour was also able to increase tumour RANK expression, further sensitising the tumour to the actions of RANKL. Knockdown of RANK or IL-6 in the cancer cells was able to reduce tumour growth in the bone, but not in the mammary gland, again emphasising the importance of cancer cell/bone cell interactions in driving bone metastasis.[27] This parallel loop supplements the actions of the vicious cycle to further increase bone resorption. It is apparent that resorption is a primary process driving tumour growth and that there are multiple pathways by which the tumour cells are able to modulate bone resorption to fuel their own growth.

In the final stages of metastatic cancer, the seriousness of the disease increases and the tumour, through its local effects, begins to impact the whole skeletal element in which it resides, frequently inducing bone pain, pathologic fracture and nerve compression.[2]

Therapeutic opportunities

The prevention or control of metastatic disease remains an area of significant unmet medical need. There are many potential steps, as outlined in this review, which provide potential targets for the prevention of metastasis or of the adverse effects. Ideally, the prevention of the development of actively growing metastases would be the most effective therapeutic approach. Therapies directed against intravasation, extravasation and tissue invasion represent a possible strategy. However, by the time primary tumours have been identified and removed as a source of metastasis, many cancer cells are likely to be already resident in the patient's tissues.

The metastatic niche also represents a valid target whose disruption could impair the survival of cancer cells in the metastatic target tissue, or prolong cancer cell dormancy. Arresting the transformation of dormant cancer cells to rapidly proliferating cells represents a compelling target for developing new therapies, as often patients show no evidence of tumours after primary tumour removal, but relapse with metastatic disease sometimes years later.

The lack of knowledge of the requirements for achieving cancer survival through dormancy, and of the nature of signals that initiate escape from dormancy, has limited progress in this area.[28] Another approach would be to change the bone environment to make it less supportive of bone metastasis. There is considerable mouse model evidence that increased bone remodelling makes the bone a more supportive place for cancer metastasis, while reducing bone remodelling has the opposite effect.[17-21] Initial treatment to reduce bone remodelling would be to correct common causes of high bone remodelling, such as calcium and vitamin deficiency,[29] with the latter particularly common in women at the time of breast cancer diagnosis.[30] These can each be readily diagnosed and addressed by providing oral supplements. As described in more detail below, the bone remodelling rate can also be reduced pharmacologically with bisphosphonate or anti-RANKL (denosumab) therapies.[31]

Inhibiting tumour angiogenesis is a highly promising treatment paradigm for metastatic disease, and while some initial approaches have proved somewhat disappointing, especially in terms of overall survival, much research activity is directed to this strategy.[32]

Inhibiting the development of vicious cycles within bone has proved an effective palliative strategy for prostate and breast cancer. The most developed and effective approach has been to target osteoclast activity, either through inhibition of osteoclast function with bisphosphonate treatment, or by preventing osteoclast formation with denosumab treatments.[32,33] Both of these strategies significantly reduce the incidence of skeletally related events in clinical trials. Therapies targeting other components of vicious cycles, such as PTHrP, are also showing some promise.[34] However, it appears that osteoclastic bone resorption is a fundamental mediator of the cycles so far identified, and may prove to be the most effective point of intervention. Multiple potential mediators have been implicated for pro-resorptive and cancer cell proliferative effects, and thus targeting single candidates may have only limited effects. Interestingly, there is some limited evidence that blocking bone resorption can delay the development of bone metastatic disease in prostate cancer patients,[35] and can increase patient survival in breast cancer patients.[36, 37]

In summary, the development of bone metastases is common in both breast and prostate cancer due to the fertile soil that the bone microenvironment provides for these cancer cell types. Once in bone, the tumours can lie dormant or be activated to proliferate and eventually produce destructive and painful metastatic lesions. This is a multi-step process with many potential points of therapeutic intervention, but therapies remain limited and primarily palliative in nature.

Acknowledgements

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EMERGING STRATEGIES FOR THERAPEUTIC TARGETING OF THE TUMOUR MICROENVIRONMENT

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Abstract

Metastatic cancers are often resistant to conventional chemotherapy and radiotherapy, reflecting an unmet need for novel therapeutic approaches to inhibit metastasis. Growing evidence highlights the importance of targeting not only the tumour but also its 'normal' microenvironment - the milieu which surrounds the tumour, including stromal fibroblasts, infiltrating immune cells, blood vessels, signalling molecules, extracellular matrix and tissue oxygen. The microenvironment varies between different organs, presumably explaining the propensity of certain cancers to spread to particular sites. This review summarises current and emerging therapies targeting the metastatic microenvironment in human tumours. The role of the tumour microenvironment in the establishment and support of metastatic disease is increasingly evident. Novel therapeutic strategies targeting the microenvironment encompassing the complex interactions between tumour cells and surrounds now need to be incorporated into clinical trials with appropriate biomarker endpoints.

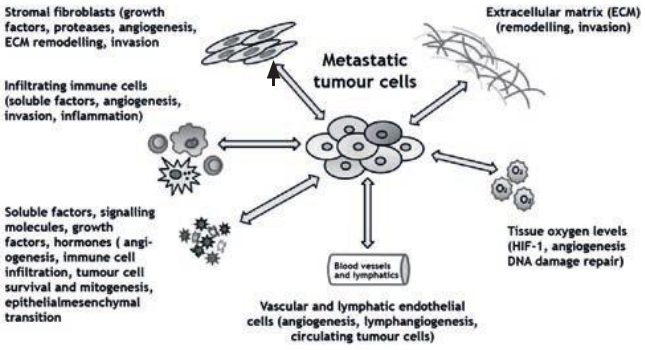
Metastasis and the tumour microenvironment

Cancer metastases account for over 90% of mortality in cancer patients.[1] As such research into the causes of metastases has the potential to yield novel therapeutic targets. Recent research into the oncogenic role of the tumour microenvironment has begun to reveal new insights, triggering the evolution of a paradigm shift in the way we understand and treat metastatic cancers.

Metastasis occurs when a cancer cell undergoes changes allowing extravasation and colonisation at a distant location to form one or more secondary tumour clones. We envisage metastasis as a complex and multistep process - the metastatic cascade - involving loss of tumour cellular adhesion, local tissue invasion, extravasation and survival in the circulation, movement into new tissue and eventually colonisation of a distant site.[2-4] In theory, any step(s) in the metastatic cascade could be targeted, however certain steps can occur prior to the clinical manifestation of the metastatic disease prior to clinical presentation of overt metastatic disease, potentially rendering palliative treatments targeting the later steps ineffective.[5] Targeting the site of metastatic colonisation may thus prove to be an important strategy in preventing the emergence and/or progression of metastatic disease.

The tumour microenvironment encompasses the milieu surrounding a tumour cell, including stromal fibroblasts, blood and lymphatic vessels, infiltrating immune cells, signalling molecules, tissue oxygen and components of the extracellular matrix (figure 1).[6] The tumour microenvironment varies between different organs,[3] which may explain the tendency of certain primary cancers to metastasise to particular sites.[6] Cancer cells can communicate with and alter their microenvironment, establishing molecular changes that encourage the metastatic colonisation of cancer cells. For example, in a study that grafted cancer cells into mice with either normal fibroblasts, or cancer-associated fibroblasts, cancer proliferation was only apparent in mice with the cancer-associated fibroblasts.[7] Therapeutic targeting of the tumour microenvironment is thus an increasingly attractive strategy to prevent or treat metastatic cancer. Furthermore, the genetic stability of the microenvironment compared to the metastatic tumour itself makes it a favourable target for minimising resistance.[6] What still remains unclear however, is how the tumour microenvironment - 'normal' as this is widely assumed to be - may be both selectively, yet tolerably, targeted.

Figure 1: Schematic diagram showing the milieu surrounding tumour cells, including stromal fibroblasts, blood and lymphatic endothelium, infiltrating immune cells (including T-lymphocytes, macrophages and dendritic cells), soluble factors, signalling molecules and growth factors, tissue oxygen levels, and the extracellular matrix.



Cells in the microenvironment may communicate both positive and negative signals to the tumour cells, resulting in establishment of a pro- or anti-tumour microenvironment respectively. Proteins derived from exosomes released by melanoma cells for example, can enhance the metastatic microenvironment via promotion of pro-inflammatory and pro-angiogenic properties of bone-marrow derived progenitor cells, providing a metastatic niche.[8] Similar events can lead to upregulation of factors in sentinel lymph nodes that potentiate extracellular matrix production and migration of melanoma cells to these.[9] Further to this, it has been suggested that 're-education' of stromal cells to provide an anti-tumour environment may be a more effective strategy than non-selectively ablating tumour-associated stromal cells in controlling metastatic disease.[6] In this review we describe some current and emerging approaches to targeting the metastatic microenvironment for several cancers (table 1).

Table 1: Examples of potential metastatic cancer microenvironment targets and strategies for current and emerging therapies.

Microenvironment targets	Strategy (example)
Bone osteoblasts and osteoclasts	Inhibit pathological bone formation and bone resorption (for example, antibodies to RANK-L)
Immune cells	Promote T-cell activation and proliferation, enhance anti-tumour immune responses (for example, CTLA4 antibody ipilimumab; sipuleucel-T)
Tumour-associated macrophages (TAMs)	Inhibit monocyte recruitment to metastatic sites and macrophage differentiation (for example, inhibit chemokines such as CCL2, CSF-1R)
Vascular endothelium	Inhibit new vessel growth induced by VEGF (antibodies to VEGF and receptors, for example, bevacizumab, aflibercept)
Tumour hypoxia	↑ tissue oxygen levels, inhibit hypoxia-induced signals for angiogenesis (e.g. HIF-1a inhibitors)
Cancer-associated fibroblasts (CAFs)	Regulate production of growth factors and ECM proteins (for example, FGF and PDGF pathway inhibitors)
Extracellular matrix (ECM)	Inhibit ECM degradation (for example, antibodies inhibiting proteases including MMPs)
Signalling molecules, growth factors and soluble factors, cytokines	Inhibit kinases and kinase receptor activity (for example, regorafenib inhibiting receptor tyrosine kinases, with effects on VEGF, PDGF signalling pathways for example)

Prostate cancer

Prostate cancer, the most common non-skin cancer in Australian men, preferentially metastasises to bone, often resulting in abnormal bone formation (osteosclerosis), fractures, pain and neural compression.[10] Newly diagnosed prostate cancers tend to be dependent on stimulation via the androgen receptor for survival and proliferation. Medical prostate cancer therapy therefore first involves targeting the cancer cells via androgen deprivation therapy such as orchiectomy or pharmacological castration, for example using gonadotropin-releasing hormone agonists or antagonists.[11] Although androgen deprivation therapy tends to be initially effective, most prostate cancers relapse within a few months or years, progressing to castrate-resistant prostate cancer. Chemotherapy (for example, using docetaxel or cabazitaxel) remains a key treatment for castrate-resistant prostate cancer.[12] However, treatment-induced damage to the tumour microenvironment may swiftly promote resistance to standard chemotherapy,[13] for example, by inducing tumour cell epithelial-mesenchymal transition,[14] with associated changes in gene expression profile.

Targeting cells in the bone microenvironment is also useful in the treatment of metastatic castrate-resistant prostate cancer. Osteoblasts in bone express the protein RANK-L, which is responsible for the activation of osteoclasts. In prostate cancer, RANK-L is upregulated in osteoblasts, osteocytes and fibroblasts in bone in response to tumour-secreted growth factors, leading to release of growth factors from bone matrix that may promote the development of skeletal metastases.[15] Denosumab, a monoclonal antibody against RANK-L, appeared to inhibit castrate-resistant prostate cancer metastasis to bone in a phase III trial.[15] Radium-223, an alpha-emitting calcium mimetic radioisotope that selectively binds the hydroxyapatite moiety of the osteoblastic microenvironment, also unexpectedly prolongs survival compared to placebo, consistent with a changing paradigm of how direct modulation of the metastatic microenvironment may impact on disease natural history.[16]

Immune cells in the microenvironment are important potential therapeutic targets. The host immune response can induce tumour suppression, but this response is generally ineffective due to local secretion of immunosuppressive agents.[17] For example, T-lymphocytes express the membrane protein CTLA-4, which when activated, downregulates activity of the immune system. Ipilimumab, a monoclonal antibody to CTLA-4, promotes T-cell activation, allowing the immune system to destroy cancer cells.[17,18] Preliminary studies of ipilimumab have demonstrated anti-tumour activity in patients with metastatic castrate-resistant prostate cancer.[18,19] Immune cells are similarly targeted by sipuleucel-T therapy, an ex vivo autologous cellular immunotherapy derived from a patient's own peripheral leukocytes.[20] The extracted leukocytes are incubated with a fusion protein consisting of a prostate cancer antigen recombinantly linked to granulocyte-macrophage colony stimulating factor. Following this ex vivo activation, the blood product is reinfused into the patient to elicit an immune response against the prostate cancer cells.[20] Intriguingly, despite relatively modest tumourolytic activity and prostate specific antigen (PSA) response rates, sipuleucel-T treatment has been associated with clear improvements in overall survival, and is the first successful vaccination-based approach for prostate cancer.[20]

Breast cancer

Breast cancer is the most common cancer in Australian women, excluding non-melanoma skin cancer, and is the second leading cause of cancer-related death in Australian women. Three main receptors are used to classify breast cancers: the oestrogen receptor (ER); the progesterone receptor (PR); and human epidermal growth factor receptor (EGFR) type 2 (HER2).[21] The pattern of metastatic spread in breast cancer is dependent on the receptors present on the cancer cells,[22] with bone being the most common site of metastases.[23]

ER- and PR-positive cancer cells are typically dependent on hormonal signalling for growth in metastatic sites ab initio, and adjuvant hormonal therapies are effective in reducing the frequency of micrometastatic spread of these breast cancers.[24] ER-positive lobular carcinomas, which do not express the epithelial-stromal adhesion factor E-cadherin, metastasise more often to serosal surfaces (for example, pleura and peritoneum) than do invasive ductal cancers.[25]

The survival benefits of adjuvant cytotoxic therapy, particularly in ER-negative premenopausal patients with lymph node metastases,[26] have been associated with indicators of stromal toxicity such as neutropaenia,[27] consistent with a therapeutic role for paracrine loop disruption. The small but significant survival benefit associated with post-mastectomy radiotherapy, suggests further that the cytotoxic modulation of the local peritumoral microenvironment may improve not only local recurrence rates, but also frequencies of distant relapse.[28]

Chemotherapy combined with adjuvant anti-HER2 humanised monoclonal antibody, significantly improves clinical outcomes and survival for patients with HER2-positive metastatic breast cancer.[29,30] Metastatic disease often progresses and resistance to HER2 antibody can be encountered over time; alternative therapies and targets are thus being explored. Recent studies have used chemotherapy with adjuvant combinations of HER2 antibodies (trastuzumab and pertuzumab) that target different regions of the receptor and found clinical efficacy.[31] Dual inhibitors of EGFR, HER2 and HER4 (such as lapatinib, afatinib and neratinib) are also being trialled for metastatic breast cancer.[32]

Approximately 10% to 15% of women with metastatic breast cancer will develop brain metastases, patients with HER2-positive or triple-negative breast cancer being more susceptible. Conventional systemic therapies are limited in this context related to blood-brain barrier (BRB) permeability. Novel approaches are currently being explored including BRB permeable cytotoxic agents, physical disruption of the BRB, and HER2-directed therapies.[33]

Bone metastases in breast cancer tend to result in abnormal bone resorption (osteolysis), predisposing to pathological fractures, in contrast to the bone formation (osteosclerosis) noted above for observed for metastatic prostate cancer. Bisphosphonates or denosumab are used concurrently to reduce metastatic bone complications and/or to counteract the osteoporotic effects of long-term aromatase-inhibitory hormonal therapy.[34] Interestingly, the bone metastases microenvironment may induce HER2 expression, regulated by the RANK-L protein discussed above, with potential for stimulating breast cancer stem cells and bone micrometastasis.[35,36]

Hypoxic conditions, such as may be found in bone metastases, can lead to production of vascular endothelial growth factor (VEGF), and tumour-related vessel growth. However, despite this plausible rationale for targeted therapy, adjuvant use of bevacizumab (VEGF antibody) with chemotherapy for breast cancer metastases does not increase overall survival,[37] is associated with increased adverse outcomes, and is not indicated for metastatic breast cancer. This lack of preventative adjuvant efficacy is also true of small-molecule kinase-inhibitory anti-angiogenic drugs such as sorafenib and sunitinib.[38,39]

There is also evidence for reduced rates of breast cancer incidence and post-primary recurrence, associated with non-pharmacological modulation of the normal metabolic microenvironment by interventions such as weight loss and/or physical activity.[40,41] These interventions appear to work via several physiological microenvironment-related processes, including altering the circulating hormone levels, and regulating various growth factor and/or apoptosis-related signalling pathways.[42]

Colorectal cancer

Colorectal cancer is the second most commonly diagnosed cancer in Australia, and often metastasises to multiple sites, including the liver.[43] Bevacizumab is of clinical value when used together with chemotherapy in the palliative, but not adjuvant, setting for metastatic colorectal cancer.[44,45] Similarly, antibodies to the epidermal growth factor receptor (EGFR) pathway, such as cetuximab or panitumumab, are effective as tumourolytic drugs for cancers that lack constitutively activating KRAS mutations (wild-type KRAS tumours).[46,47] However, EGFR overexpression or amplification in colorectal cancer biopsies is not consistently predictive of cetuximab efficacy.[48,49] Several gene polymorphisms have been identified as positive outcome predictors in patients with wild-type KRAS tumours that are not cetuximab-responsive.[49,50] Early onset of skin toxicity (within two weeks of starting treatment) predicts a better response to cetuximab in colorectal cancer patients.[51] Although the mechanism for the severe skin toxicity has not been clearly identified, cytokine regulatory changes in skin (and tumour) microenvironment related to EGFR inhibition have been suggested.[52]

Regorafenib, an oral small molecule multi-kinase inhibitor, can inhibit a wide range of stromal, angiogenic and tumour-related receptor tyrosine kinases that may be important for the metastatic cascade, including promotion of new tumour vessels and lymphatic vessel formation. Recent studies show clinical benefits (prolonged survival) of regorafenib for patients with progressive metastatic colorectal cancer who have been treated previously with standard cytotoxic agents and targeted therapies.[53] Preclinical observations discussed above, suggested that

regorafenib regulates processes important in the tumour microenvironment, although the mechanisms involved clinically are not fully understood.

Lung cancer

Lung cancer is the leading cause of cancer death in Australian men and women. Palliative systemic therapy for lung cancer includes anti-mitotic compounds such as cisplatin, carboplatin or taxanes.[54] Molecular drug targets with demonstrated efficacy include the EGFR pathway (erlotinib/gefitinib), and the VEGF pathway.

Hypoxic conditions in the cancer microenvironment can increase the risk of metastasis,[55] making hypoxia and its metabolic responses potential therapeutic targets. Hypoxia stabilises the hypoxia-induced factor (HIF) family of proteins, which bind to HIF-response elements, upregulating proteins involved in angiogenesis (in particular, VEGF) and tumour invasion.[55,56] HIF-1 is overexpressed in 60% of non-small cell lung cancers, and is predictive of a poor overall survival.[57] Targeting HIF family proteins could potentially reduce production of downstream proteins such as VEGF, important in tumour growth and the metastatic cascade. A study investigating the effects of PX-478, a small molecule inhibitor of HIF-1 protein synthesis, found reduced tumour volume and metastatic spread in a mouse model of small-cell lung cancer.[58] Phase I clinical trials have found that oral PX-478 is well tolerated, and further trials are now needed to evaluate its effectiveness in a clinical setting.[59]

Hypoxia can also induce tumour resistance to radiotherapy via the action of antioxidants which repair the radiation-induced DNA strand breaks.[60,61] This is relevant to management of cerebral metastases, which often involves whole brain radiotherapy. Efaproxiral, a drug that allosterically modifies haemoglobin by reducing its oxygen binding affinity, may facilitate the release of oxygen to tissues, mitigating radiotherapy resistance in hypoxic tumours.[62] However, phase III clinical trials investigating whole brain radiotherapy and adjuvant efaproxiral for the treatment of brain metastases from lung cancer, have not, to date, found an improvement in overall survival compared to treatment with whole brain radiotherapy alone.[63]

Melanoma

Cutaneous melanoma is the fourth most common cancer in Australia, accounting for 3% of all skin cancers, but 75% of skin cancer-related deaths. Melanoma cells can spread throughout the body at early stages and the prognosis in metastatic disease remains poor. Recent studies targeting the BRAF/MEK pathway via inhibitors of the mutated BRAF kinase in patients with metastatic cutaneous melanoma have been associated with dramatic responses, and modestly improved overall survival, including those with brain metastases.[64,65] Immunotherapy with the T-cell-activating CTLA4 antagonist ipilimumab, causes fewer responses but does seem to lead to greater survival gains,[66] emphasising the importance of 'off-target' effects (in this case, immune regulation) in modulating tumour natural history.

Uveal melanoma (affecting the inner vascular pigmented layer of the eye) is the most common primary eye tumour in adults,[67] and as with cutaneous melanoma, metastatic disease is associated with a poor prognosis. Intriguingly, liver metastases are seen in 95% of uveal melanoma patients with metastatic disease,[68] suggesting possible therapeutic targets within the liver microenvironment. Immunotherapy using ipilimumab is currently being trialled for patients with metastatic uveal melanoma.

Metastatic disease in both cutaneous and uveal melanoma shows poor responses to conventional chemotherapeutics such as dacarbazine,[69] indicating a need for new treatment strategies targeting the tumour-host interface

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INTRATUMOUR HETEROGENEITY IN THE PROGRESSION TO BREAST CANCER METASTASIS

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Abstract

Unlike the great advances that have been made in reducing breast cancer mortality through the multidisciplinary treatment of primary disease, much less is understood about the natural history of metastatic disease, despite this being the single most significant predictor of poor outcome for patients. Currently, much of treatment decision-making concerning a patient with metastatic disease is based on the biological characteristics of their primary tumour. There is a growing appreciation, however, that metastases arise from clonal subpopulations of cells from a primary tumour that may be genetically and phenotypically heterogeneous. The metastases may also undergo successive rounds of clonal expansion and adaptation in response to selective pressures endured in the foreign microenvironment of new tissues and treatment. In fact, cancer progression and colonisation is likely to be underpinned by a collective cooperation between cellular, genomic and microenvironmental factors that generate diversity to facilitate treatment resistance and metastatic capability. The extent and overall clinical significance of this diversity in metastatic progression is still unclear, owing to the scarcity of samples of metastases that are available for molecular analysis.

Breast cancer is extremely complex, encompassing a wide variety of entities with respect to radiological appearance, histological and molecular subtypes, therapeutic options and responses to treatment and clinical outcomes. Pathologists have recognised the histological diversity for many years and in the recent *World Health Organisation Classification of Tumours of the Breast*, there are at least 19 different morphological subtypes of invasive breast cancer.[1] Invasive carcinoma no special type (previously called invasive ductal carcinoma NST) is the most frequent type. This classification term is inherently wide ranging, as these tumours do not demonstrate specific morphological features to be classified as a 'special' subtype (e.g. invasive lobular carcinoma). The special subtypes are generally more homogeneous and some convey prognostic information. For example, tubular and mucinous carcinomas are associated with favourable outcomes, whereas metaplastic carcinomas have an aggressive clinical course.[1] Tumours are further subdivided according to histological grade and phenotype (e.g. the status of oestrogen (ER) and progesterone (PR) receptor proteins and Human epidermal growth factor receptor 2 (HER2) amplification) to determine the most appropriate therapeutic options. The implementation of adjunct diagnostic tests including biomarker expression (e.g. EGFR, Ki67 and E-cadherin), gene expression profiles or DNA mutation analyses, will provide incremental improvements in patient outcomes by refining existing classification systems and treatment decision-making.[2-8]

One of the most exciting developments in this arena relates to the recent advances in massively parallel sequencing (next generation sequencing) technology that allow scientists and clinicians to delve deeper into the genome of a tumour in order to gain a greater understanding of the complex genetic mechanisms that drive tumour growth and progression. It is now possible to sequence multiple genes to entire genomes in clinically relevant time scales and at reasonably low cost (the Human Genome project cost $3 billion and took 13 years to complete). The diagnostic utility of this technology is now within reach, particularly with the development of assays for sequencing disease-specific panels of genes. In cancer, this enables important driver gene mutations to be rapidly identified, many of which are considered 'actionable' in that therapies targeting the mutated gene are already available. Sequencing the cancer genome to a high coverage (deep sequencing) also enables rare, low frequency variants to be discovered, which would not have been previously detected by traditional sequencing methods such as Sanger sequencing. These developments are revealing important insights into intratumour heterogeneity and the clonal progression of disease to metastasis.

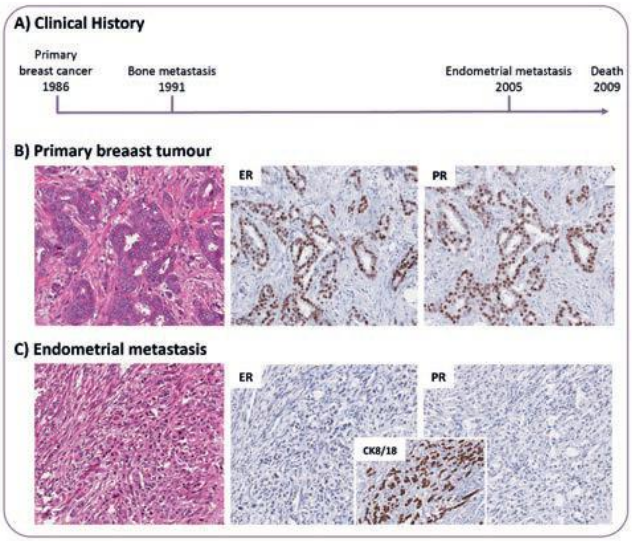
Metastatic breast cancer

By comparison with the primary tumours, there is a far more limited understanding of the complexity of both metastatic progression and metastatic lesions, despite this being the final and often fatal stage of tumourigenesis. The analysis of historical autopsy series of patients who died of metastatic breast cancer and the collection and analysis of primary and matched metastatic samples from the same individuals has and will continue to make

important mechanistic and observational contributions to our knowledge. For example, as far back as 1889, Stephen Paget considered that dissemination was non-random and that the biological characteristics of certain tumours meant they were predisposed to colonise specific distant tissues in which the microenvironmental conditions were most appropriate (the famous 'seed and soil' theory).[9] James Ewing countered this idea, suggesting that tumour cells simply seed distant sites according to the mechanical flow of the blood supply/lymphatics.[10,11] Elements of both of these theories are accepted in breast cancer, where the most common sites of metastatic involvement are regional axillary lymph nodes, lungs, liver, bone and brain. The particular distribution and latency of dissemination to these and other organs is influenced by the histological type, grade and phenotype of the primary tumour. For instance, invasive ductal carcinoma and invasive lobular carcinoma colonise distant tissues with different frequencies. Invasive ductal carcinoma preferentially seed lung and brain metastases and invasive lobular carcinomas preferentially spread to the gastrointestinal tract, gynaecological organs and peritoneum.[12-14] In general, low grade, ER positive tumours have a longer latency and a different pattern of spread compared with ER negative tumours (e.g. bone versus lung, liver and brain).[15,16] Furthermore, pioneering work by the Massague group using a combination of animal models and clinical samples, has demonstrated that specific gene expression programs in subpopulations of cells of the primary tumour mediate tissue specific metastatic spread to bone, lung or brain.[17-20]

A number of conceptual models prevail in research regarding the complex evolution of metastatic disease.[19] In general, histological, phenotypic or molecular (gene expression and genomic profiles) features of the primary tumour are reflected in the resulting metastases, supporting the clonal nature of progression.[21-27] Thus for the most part, the molecular factors defining the salient characteristics of a primary tumour are stably maintained during progression, and this tends to support the 'linear' model of metastatic progression.[28] The linear model implies that metastatic capability is acquired late in development after successive rounds of mutation and clonal selection and establishment of the primary tumour. This is in contrast to the 'parallel' model of progression, in which tumour cells can be shed from the primary tumour site early and at any time during its development. In this model, the primary tumour and the metastasis evolve in 'parallel', giving rise to increased biological variance.[28] In simple terms, the distinction between these two pathways of progression has clinical implications since they infer that a metastasis should be treated either as if it has the same biological properties as the primary tumour (linear model), or it is different and thus should be biopsied to guide treatment (parallel model). In reality, progression of some tumours is monoclonal, while in others it is polyclonal, and in both there may be discordant features between the primary tumour and the resulting metastases (figure 1), indicating that tumour cells have an inherent ability to evolve and adapt to the selective pressures they undergo, whether they leave the primary tumour early or late.

Figure 1: A clinical case to illustrate the complexity of metastatic progression. This case illustrates not only the long latency of metastatic progression for an ER positive primary tumour, but also the diversity of disease during the evolution of the metastatic clone, involving a change in the morphological growth pattern and down regulation of hormone receptors, possibly in response to exposure to endocrine-based therapy. The key episodes in the clinical history of a breast cancer patient are outlined (A). The patient was initially diagnosed with a primary breast carcinoma in 1986 and died 23 years later in 2009 of metastatic disease. The primary tumour was an invasive ductal carcinoma NST that was positive for oestrogen (ER) and progesterone (PR) receptors (B) and was HER2 negative (not shown). The first evidence of metastatic progression was in 1991 with the diagnosis of a bone metastasis. The patient underwent prolonged chemotherapy and radiation therapy at various stages throughout the disease course, and was given Tamoxifen before switching to an aromatase inhibitor prior to the development of an endometrial metastasis, which was diagnosed in 2005. The endometrial metastasis (C) was ER and PR negative (positive staining for the epithelial marker CK8/18 is shown in the inset to demonstrate the carcinoma cells).



Morphological and phenotypic complexity

Intratumour heterogeneity within a primary tumour, in the context of cellular or phenotypic plasticity or clonal heterogeneity, has important implications for disease progression and patient outcomes. Breast carcinomas, exhibiting mixed growth patterns reflecting different histological subtypes, are perhaps the most obvious examples of primary tumour heterogeneity. Mixed tumours evolve either as independent tumours in collision, or from a common clonal population that diverges following ongoing genetic instability or cellular plasticity. Mixed invasive carcinoma of no special type (NST) and invasive lobular carcinoma account for 3-5% of all breast cancers and are composed of both ductal and lobular histological components.[29] The morphology of resulting lymph node metastases is more likely to reflect the predominant histological component of the primary tumour,[29] but either

or both components may disseminate. The diffuse growth pattern of lobular cells may be more efficient at colonising some anatomical tissues, such as the peritoneum or ovary.[12,14,30,31] Metaplastic carcinomas account for up to 5% of all breast cancers and are characterised by metaplastic transformation to squamous and/or mesenchymal components.[1] Consistent with this, tumours show striking phenotypic plasticity with heterogeneous staining patterns of markers for: i) luminal and basal/myoepithelial cell differentiation (CK19 and basal markers such as CK5/6, CK14, p63, CD10); ii) stem cell-like characteristics ($CD44^{+}/CD24^{-/low}$); and iii) mixed epithelial to mesenchymal plasticity,[32] (including down regulation of E-cadherin). This inherent 'plastic' nature likely contributes to their very aggressive clinical course,[33] providing cellular capabilities to adapt, evade treatment and disseminate.

Phenotypic and genomic analysis of primary breast carcinomas and their matched metastases highlight how heterogeneous progression can be. Studies limited to analysing isolated metastases are complemented by the analysis of autopsy series of patients who have died of metastatic disease, where a more comprehensive analysis of multiple metastases can be undertaken. Key findings related to therapeutic targets in breast cancer demonstrate that ER and PR are frequently discordant and most notably down-regulated during metastatic progression (figure 1).[24,30,34] Such down-regulation may vary between different metastases within the same patient and be non-randomly associated with colonisation of specific tissues, such as lung, bone and liver.[24] HER2 overexpression, determined by the amplification of the ERBB2/HER2 gene, is generally more stable during progression, yet discordance has been reported in approximately 10% of cases.[35] There are a number of explanations for this phenomenon, including the fact that expression of hormone receptors are dynamically controlled and hence may be readily down regulated during the emergence of tumour clones that are resistant to endocrine-related therapy (figure 1). Metastases may also arise from primary tumours that are themselves heterogeneous. For instance, they may exhibit non-uniform expression (e.g. ER positivity in 30% of cells) or be clonally diverse (e.g. ERBB2/HER2 amplification occurs in a tumour subclone).[34] An integrative analysis of genomic (patterns of common amplification e.g. 8q24, 11q13) and phenotypic (status of CD24 and CD44 expression) heterogeneity at a single cell resolution reveals significant heterogeneity between primary tumours and distant metastases, and even between neighbouring metastatic cells within the same metastatic foci.[36]

As cells metastasise, they must also adapt to survive and meet the physiological requirements of the new local microenvironment, where growth signals may be quite different to the breast tissue of origin. The brain is a foreign environment for breast cancer cells, yet some breast cancers, and cancers from other sites readily colonise brain tissue. Some breast and lung carcinomas have been shown to up-regulate HER3 signaling during colonisation as a possible mechanism of growth and survival in the brain. This adaptive response is likely due to the abundance of the HER3 ligand neuregulin, which is expressed by multiple cell types in the brain.[37, 38] It has also been shown that some breast cancer metastases in the brain adopt a GABAergic phenotype similar to that displayed by neurons, as a potential adaptive response to enhance survival in this new microenvironment.[39] Understanding mechanisms of colonisation and adaptive responses of tumour cells will be pivotal in trying to both prevent colonisation of specific distant organs and develop new metastatic site-specific therapeutics.

Genomic diversity in metastatic progression

All cancers are characterised by the acquisition of somatic mutations that accumulate over the lifetime of the tumour.[7] The pattern of mutations is extremely diverse among individual tumours, prompting large-scale initiatives to catalogue this diversity using molecular profiling (of gene expression and DNA copy number) and deep sequencing to better understand the evolution of the cancer genome during tumour development and progression, and to identify key 'driver' alterations for therapeutic targeting.[2,3,8] Driver mutations confer selective growth and survival advantages to the tumour cell lineage and make a significant contribution to clonal diversity, treatment resistance and metastatic progression. Typically, critical driver mutations (mutation of TP53 and PIK3CA, amplification of MYC, CCND1, ERRB2/HER2) occur within the early phases of tumour growth and are thus present in the majority of cells of the primary tumour.[40] The late acquisition of new driver mutations or amplifications may further drive clonal diversity in a primary tumour or a metastasis,[24, 26, 36, 40, 41] and these alterations may facilitate dissemination and/or treatment resistance.[23,25,35,41,42]

Elegant massively parallel sequencing and copy number profiling studies of breast,[27,40,42,43] renal cell,[44] prostate,[22] colorectal,[45] and pancreatic[23,46,] carcinomas,[22,47] as well as leukaemias,[47] exemplify these findings at nucleotide resolution and demonstrate clonal evolution and metastatic progression can be very complex in some instances, but monoclonal in others. Multiple sampling from spatially defined tumour regions or multiple metastatic biopsies, and phylogenetic relationship modelling based on mutation clonal frequencies, clearly delineates significant subclonal diversity. The trunk of the phylogenetic tree represents founder mutations that are responsible for the initiation and establishment of the tumour, and these mutations are present within all resulting subclones. In fact, many of the mutations occur in the primary tumour. The branches of the tree represent the evolution of distinct subclonal populations that arise due to the acquisition of 'progressor' somatic mutations in specific lineages, some of which progress to metastases in different organs. The data imply that different subclones within a primary tumour have developed metastatic capability. As a consequence, this yields genomic diversity in metastatic samples from the same patient. Further clonal evolution can occur at the metastatic sites involving the alteration of key driver genes such as KRAS, MYC and CCNE1.[41,46] Interestingly, distinct and spatially separated inactivating mutations within the same tumour suppressor genes (e.g. SETD2 and PTEN) were observed in the same patient, suggesting the evolution and selection of separate clones with convergent phenotypic characteristics.[44]

There are now several important examples in the literature where the selective pressure of chemotherapy or targeted therapy specifically drives the evolution of treatment resistant subclones. Treatment is typically directed towards the phenotype or genotype of the largest clonal population and can cause a massive reduction in malignant cell numbers, but may in time lead to the expansion of a low frequency, chemotherapy resistant clone by eliminating competition for growth. This has been demonstrated in multiple myeloma and acute myeloid leukaemia, where chemotherapy has been shown to contribute to increased tumour diversity and a greater rate of tumour evolution.[47] In breast cancer, activating mutations in the ESR1 gene, coding for ER alpha, represents a mechanism of resistance to prolonged endocrine therapy in hormone receptor positive breast cancer and the resulting development of metastatic disease.[48,49] Fascinating insights have also come from breast and ovarian cancer patients with germline BRCA1 or BRCA2 mutations and the development of resistance to targeted treatment with PARP inhibitors or the use of platinum-based chemotherapy. PARP inhibitor treatment works through the concept of synthetic lethality, whereby the combined inactivation of two genes causes cell death, whereas the cells remain viable with inactivation of either gene alone. In this context, BRCA1 or BRCA2 inactivation through germline mutation is lethal when combined with PARP inhibitors, since two related and key DNA repair pathways are disrupted. Remarkably, tumour cells can acquire resistance to treatment by restoring the wild type BRCA1 or BRCA2 gene via mutation reversion, leading to re-establishment of functional homologous recombination and tumour progression.[50]

Conclusion and clinical challenges

A considerable challenge with treating breast cancer patients lies in the diversity of disease, with respect to subtypes, treatment responses and outcomes. The inherent nature of some individual tumours to exhibit diversity, compounds this complexity and plays a significant role in the development of treatment resistance and metastatic progression (figure 1). An understanding of both the basis and the extent of genomic and phenotypic diversity would therefore be extremely valuable in helping to determine guidelines for the clinical management of metastatic disease, including predicting sites of relapse, identifying mechanisms of treatment resistance and defining the most appropriate treatment options.

When tumours progress after treatment, taking a biopsy of the metastatic deposit to guide treatment decisions is not routine, and so clinical decisions are made based on predictive biomarkers performed on the primary tumour specimen. From the clinical point of view, it is not always practical or even possible to biopsy metastases, in which case there is little choice but to be guided by the biological features of the primary tumour. Taking evidence from the detailed genomic studies illustrating the clonal heterogeneity of tumour development and progression, it might be most fruitful to target the early driver mutations found in the 'trunk' of the phylogenetic evolutionary tree, since these alterations are likely to be consistent in all resulting subclones that may develop. Where biopsy of metastatic deposits is possible, it may help delineate treatment choices. In a recent multicentre clinical trial, biopsies of accessible breast cancer metastases were obtained from 407 patients, and of these, 283 were analysed to identify potentially targetable genomic alterations using DNA copy number profiling and Sanger sequencing for hot spot mutations in PIK3CA and AKT51. A potentially targetable alteration was identified in 46% of patients and 13% of the cohort received targeted therapy based on their molecular results.[51] This study has demonstrated the feasibility of performing molecular testing on metastatic biopsy samples for the development of personalised medicine.

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A SOUTH AUSTRALIAN CANCER ATLAS SHOWS IMPORTANT VARIATIONS IN CANCER RISK AND OUTCOMES, BUT CAN BETTER USE BE MADE OF AUSTRALIAN DATA TO SUPPORT THE WORK OF CANCER COUNCILS?

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Abstract

Cancer Council SA commissioned the production of *An Atlas of Cancer in South Australia* by the Public Health Information Development Unit of the University of Adelaide, to identify areas where primary and secondary preventive programs might be better directed to improve cancer outcomes in rural South Australia. The Atlas illustrated the benefit of using data from multiple sources together to highlight inequalities in cancer risk in regional and remote compared with metropolitan areas. Differences in survival were also presented, including important ones requiring immediate attention, but in most instances the differences were small and suggestive of reasonably equitable access to critical services. Based on Atlas data, we have made recommendations regarding cancer-control initiatives needed to reduce inequalities in cancer risk and outcomes in South Australia, particularly in high risk populations such as Aboriginal and Torres Strait Islander people. Acquisition of data for the Atlas proved to be a slow and difficult process. There was good support from many data custodians, but also major barriers, including some that proved insurmountable within the two-year period of the project. Major differences existed in data access and approval processes, and in resource availability to extract data. There is a pressing need to improve data governance arrangements to increase access to existing Australian data to guide cancer-control initiatives.

Background

A quarter of the South Australian population resides outside of metropolitan Adelaide and the local Adelaide hills.[1] South Australia has a highly centralised distribution of cancer treatment facilities, with all radiotherapy services and most chemotherapy services provided in metropolitan Adelaide. A key aspect of the Cancer Council SA Strategic Plan for 2012-2015 is to "*identify areas in secondary prevention ... where investment by Cancer Council SA would be productive to improve cancer outcomes in rural South Australia*". To advance this strategy, and to guide primary prevention and therapeutic initiatives, Cancer Council SA commissioned an atlas from the Public Health Information Development Unit of the University of Adelaide to show inequalities between metropolitan and country residents of South Australia in cancer risk factors, cancer incidence and outcomes.

The final report, *An Atlas of Cancer in South Australia* (referred to in this paper as 'the Atlas'), provides an overview of patterns of cancer and cancer risk factors, and includes a focus on rural and remote communities, residents of areas of socioeconomic disadvantage and Aboriginal and Torres Strait Islander people.[2]

The purpose of this paper is: (1) to outline key observations from the Atlas and demonstrate the benefit of using combination data from multiple sources, such as health surveys, screening registers, vaccination registries, cancer registries and treatment databases, for population assessment; and (2) to report on the data retrieval process for the Atlas. Data from different sources are frequently reported independently in Australia, but they have complementary qualities and when viewed together, they can provide a better overview of service needs than when considered independently.

Atlas methodology

The methodology included a review of scientific literature, both journal publications and 'grey literature' reports that were known to Cancer Council SA and Public Health Information Development Unit. The purpose was to describe what already was known about geographic differences in cancer incidence and mortality, risk-factor prevalence, screening uptake and case survivals in rural South Australia, low socio-economic areas and Aboriginal people. Methodological details are provided in the Atlas.[2] Risk data and cancer rates were age-standardised, using conventional direct standardisation methods and the Australian 2001 reference population, to facilitate comparisons.

The Atlas used data from population surveys, the cancer registry, screening registers and administrative databases to describe geographic differences in risk factor prevalence, screening participation and outcomes, cancer incidence and mortality, descriptors of cancer stage at diagnosis, five-year relative survivals by statistical local area, remoteness of residence from Adelaide, and extent of socioeconomic disadvantage of residential area.[2]

Key observations

Cancer is responsible for about 28% of South Australian deaths and is one of the largest contributors to disease-related morbidity and mortality.[4] About one in three South Australians diagnosed with cancer resides in a country region rather than metropolitan Adelaide.[5]

There are inequalities in cancer risk, cancer rates and cancer survival for regional and remote populations compared with metropolitan Adelaide residents, although the Atlas showed the differences in survival to be generally quite small and suggestive of reasonable state-wide access to critical services.[2] Cancer risk factors more common in regional and remote than metropolitan areas included excess sun exposure, tobacco smoking, high-risk alcohol consumption and excess body weight.[2] Areas of socioeconomic disadvantage and long travelling distances to screening and specialist cancer services were also evident in country South Australia.[2] Treatment for cancer is complex and multidisciplinary in nature, with a need for specialist centres, which can complicate access for many regional and remote residents where population numbers are insufficient to support specialist centres.[2]

The incidence of cancer types with lower prospects of survival, such as cancers of the lung, stomach, pharynx/oesophagus, liver, pancreas and unknown organ site, is elevated in Aboriginal and Torres Strait Islander people, which would contribute to geographic differences seen in South Australia. However, the recording of Aboriginal and Torres Strait Islander status in South Australian and other cancer registries has been unreliable, such that differences by ethnicity remain poorly defined.[2,6]

Risk factors

General overview

Compared with the metropolitan Adelaide population, residents of remote populations have elevated rates of tobacco smoking, alcohol consumption, obesity, low physical activity and excess sun exposure.[2] Data from the National Health Survey (2007-2008) were used to identify patterns in tobacco smoking, alcohol consumption, body weight and physical activity. SA Health Omnibus Study data were used to provide information on excess sun exposure.[2]

Tobacco smoking and alcohol consumption

The prevalence of smoking was higher in metropolitan areas of high socioeconomic disadvantage and non-metropolitan areas than in other metropolitan areas, with statistically significant differences ($p<0.05$) in age-standardised rates of greater than 10% reported.[2] Of males aged 18 years and over living in non-metropolitan areas, survey data indicated that 26.5% were current smokers, compared with 23.6% in metropolitan Adelaide (Rate Ratio (RR) = 1.12).[2] The corresponding figures for females were 20.8% and 16.5% respectively (RR = 1.26).[2] The percentages of adults reporting alcohol consumption at dangerous levels were relatively low, averaging 4.7% overall, but the percentage for regional and remote areas of 5.9% was 1.4 times higher than the corresponding 4.3% for metropolitan Adelaide.[2]

Body weight and physical activity

The geographical distribution of overweight and obesity differed between males and females.[2] The proportions of males found to be overweight or obese were similar in Adelaide and non-metropolitan areas, with 37.6% classified as overweight and 16.8% as obese. Atlas maps for males for metropolitan areas showed distinct patterns, with overweight concentrating in areas of higher socioeconomic (SES) status and obesity concentrating in lower SES areas.

The estimated proportions of females who were overweight or obese were also similar in metropolitan and non-metropolitan areas. The obesity rate in females (17.4%) was comparable to the male rate, whereas the proportion of women who were overweight (26.5%) was about a third lower than for males. Atlas maps showed a concentration of overweight and obesity in females in lower SES areas. The distribution of overweight and obesity in females was more variable than in males, demonstrating a weaker association with socioeconomic disadvantage than seen for males.[2]

Consistent with the distribution of excess body weight, the proportion of residents aged 15 years and over who were physically inactive was higher in country areas than in metropolitan Adelaide.[2] High levels of physical inactivity were also strongly associated with socioeconomic disadvantage.[2]

Excess sun exposure

The annual South Australian Health Omnibus Survey was used to describe excess sun exposure. Survey participants were asked if they had been sunburnt in the previous summer and whether they were regularly compliant with the five sun protection behaviours (i.e. wearing a hat, SPF30+ sunscreen, clothes that covered all of the arms and legs, and sunglasses, and using shade).

There was little variation reported between metropolitan and regional areas regarding sunburn experience and engagement in sun protection behaviours, but there was a difference in sun exposure for populations in very remote areas.[2] Residents of very remote areas were reported to be three times more compliant with recommended sun protection behaviours, but nonetheless reported a sunburn rate more than 50% higher than for other South Australians.[2] It is possible that very remote residents, while taking additional measures to mitigate increased risk of excess sun exposure in country living, may need to intensify these measures to nullify their increased risk.[2]

Screening

There are three national population-based cancer screening programs in Australia, namely, BreastScreen, the National Cervical Screening Program, and the National Bowel Cancer Screening Program.

Breast screening

The BreastScreen SA program was introduced in 1991 as a joint Commonwealth/SA Government initiative.[7] Approximately two thirds of 50-69 year-old SA women were attending for biennial screening around 2000, but there was a reduction of about 12% in screening in absolute terms in both non-metropolitan and metropolitan areas from 2002 to 2009.[2] This reflected a growth in size of the screening cohort that outstripped service capacity.[7]

The Atlas showed an association between lower screening participation and lower SES area.[2] Also, the decline in screening participation was largest for women in the lowest SES areas, which widened the gap in participation between the lowest and highest SES areas from 9% to 13% in absolute terms.[2] A weak positive correlation between screening participation and breast cancer incidence was also evident, which may reflect increases in the detection of breast cancer cases from screening, and associated lead-time and related effects, rather than true elevations in incidence.[2, 10]

Cervical screening

The National Cervical Screening program was also introduced in 1991.[2] Screening participation rates were mapped in the Atlas. Participation by women aged 20-69 years declined by about 6% in absolute terms across South Australia between 2002 and 2009 to 60.7%. Rates were similar for Adelaide and non-metropolitan areas in general, but appreciably lower for women in very remote regions. As with breast cancer screening, the decline in participation was most marked among residents of the most disadvantaged areas.[2]

Bowel screening

The National Bowel Cancer Screening Program was introduced in 2006, whereby invitations for Faecal Occult Blood Test (FOBT) screening were sent to Australians turning 55 and 65 years. The program subsequently was extended to include those turning 50 years, and then 60 years, and further extensions are planned. In 2010, participation rates were similar for Adelaide and non-metropolitan areas, but lower in very remote areas. Females had a higher participation rate than males. There was a pronounced, positive correlation of participation with higher SES area both for males (r=0.64) and females (r=0.71).[2]

Incidence

South Australian cancer incidence (all cancer types combined) is high by world standards, but comparable to that for Australia overall and other developed countries, including the United States.[2] The age-standardised incidence rate for South Australia in 2008 was 601 invasive cancers (all types) per 100,000 for males and 404 per 100,000 for females.[2,4] Incidence rates increased between 2003 and 2008 for both men and women. Incidence rates increased for those cancers targeted in population-based screening and allied early detection activity, including breast, bowel and prostate cancers, and for those likely to be more detectable through advances in ultrasound imaging, MRI, PET and other diagnostic technology, such as thyroid and kidney cancers.[4]

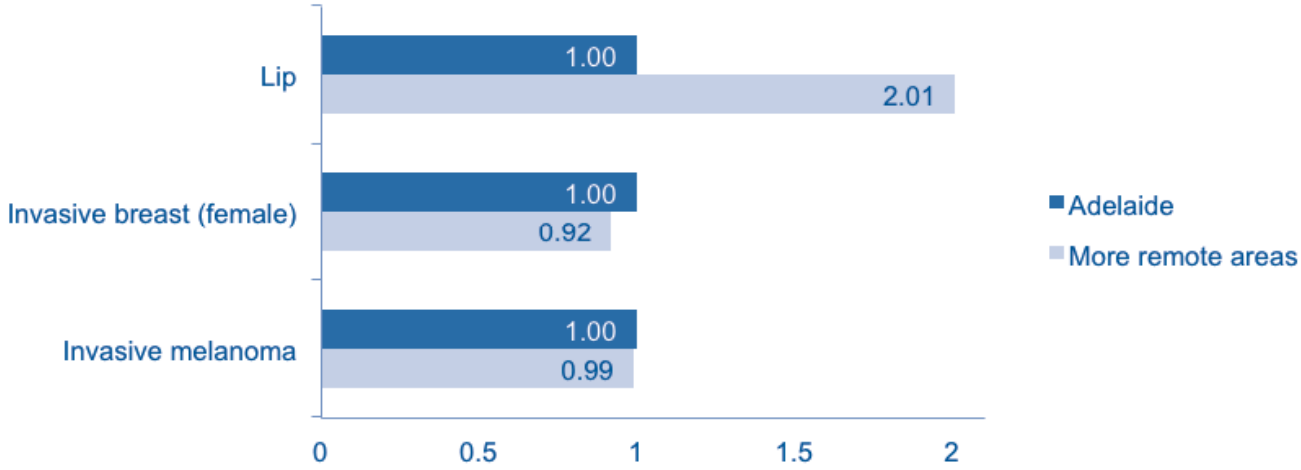
For all cancers combined, a 1.1% annual increase in incidence occurred for males from 2004-2008, largely attributable to increases in prostate cancer detection, while a 0.6% annual increase occurred for females, largely due to small increases in bowel and lung cancer diagnoses.[2,4] The Atlas showed that increases in cancer incidence were evenly distributed across socioeconomic quintiles, both in metropolitan and non-metropolitan areas.[2]

The Atlas showed rural residents to have similar age-standardised incidence rates to metropolitan residents for all types of cancer except lip cancer, where incidence rates in more remote areas were nearly twice those in metropolitan areas (figure 1).[2] This observation is consistent with data published by the SA Cancer Registry since 1980.[4,5] Lip cancers mostly occur on the outer vermilion border of the lower lip and their higher incidence in non-metropolitan areas is attributed to excess sun exposure.[5] The incidence of lip cancer is often high in populations with a high incidence of non-melanoma skin cancers (NMSC), including basal and squamous cell carcinomas, but NMSC is not recorded in the SA Cancer Registry (or most other Australian registries).[4,8-11] Because of the high incidence of lip cancer in rural areas, it is expected that the rates of NMSC would also be high in those areas. While NMSC and lip cancers rarely cause death, they place a large burden on the health system.[4,10]

Conversely, the Atlas showed that the incidence of invasive female breast cancer was about 8% lower in more remote than metropolitan areas.[2] This difference is thought to be due in part to differences in reproductive history, with younger age at first pregnancy and higher parity occurring more frequently in more remote areas.[2] Differences in use of hormone replacement therapy may also contribute, but the reasons for these differences require further investigation.

In metropolitan Adelaide, breast cancer incidence is highest for women in the highest SES quintile (least disadvantaged) and lowest for women in the lowest SES quintile (most disadvantaged). The incidence of female breast cancer increased by approximately 40% in metropolitan and non-metropolitan areas between 1986 and 2008.[2] This coincided with the introduction of the BreastScreen program, but the extent to which the increase reflected lead time effects of screening, a potential contribution from over-diagnosis, changes in diagnostic practices, and real increases in incidence due to changes in reproductive and related lifestyle factors is unclear.[7] It is evident that mammography screening of 50-69 year-olds has reduced breast cancer mortality in Australia and South Australia specifically, by around 40%.[2,7]

Figure 1: Mean annual age-standardised incidence; South Australia, 1995-2008. Note: Age-standardised to Australian population 2001; Adelaide incidence set at 1.00.



In contrast to the increase in the incidence for all invasive cancers collectively between 1986 and 2008, rates of lung cancer in men aged 20 years and over declined by nearly 10% in metropolitan Adelaide.[2] This reduction was not seen in non-metropolitan areas where the incidence increased marginally, and with particularly high incidence rates occurring in very remote areas. The incidence of lung cancer among women increased substantially, by about 48% over the same period in metropolitan and non-metropolitan areas, with the largest increase seen in the lowest SES quintile.[2]

Aboriginal and Torres Strait Islander people comprise approximately 2% of the South Australian population, with over 50% residing in rural and remote areas. Overall, Aboriginal and Torres Strait Islander people experience higher incidence rates than other South Australians of more life-threatening cancer types, including cancers of the lung, stomach, pharynx/oesophagus, liver, pancreas and unknown organ sites.[6] Cervical cancer incidence is also elevated in Aboriginal and Torres Strait Islander people.[2] This cancer pattern points to high levels of modifiable risk factors in the Aboriginal population, including smoking, excess alcohol consumption and poor diet, as well as higher levels of HPV, hepatitis B and helicobacter pylori infections, which could be targeted in cancer control interventions.[2,10] It is important though that the broader social determinants of these cancers are addressed, since these would underpin most of the risk-factor elevations.

Cancer stage

The SA cancer registry does not routinely collect stage of progression of cancer at diagnosis, although tumour size and thickness are collected for breast cancers and melanomas respectively.

The Atlas showed that the percentage of invasive breast cancers classified as large (i.e. greater than 30mm in diameter) was higher in more remote areas than in Adelaide for females aged 40-49 years and 70 years and over, but not for females in the BreastScreen SA target screening group aged 50-69 years.[2] This may reflect effects of BreastScreen SA in reducing socio-demographic inequality in its target population. Collation and analysis of quality data and more intensive research is required to determine the effectiveness of screening in various age groups outside the recommended target. There is a need to focus on the promotion of early breast cancer detection in the more remote areas, especially for Aboriginal and Torres Strait Islander females, who are diagnosed at a more advanced stage and experience lower survival from breast cancer than other South Australian females.[2,10]

Similarly, the Atlas showed that the percentage of invasive melanomas classified as thick (i.e. thickness greater than 1.5mm) at time of diagnosis was higher in non-metropolitan regions. Thickness was found to be larger in more remote areas in all age groups, with a statistically significant difference applying in the 50-59 and 60-69 year old age groups.[2] These findings, along with increased lip cancer rates, highlight the need for priority to be given to rural and remote areas in early skin cancer detection programs and alongside the promotion of sun protection.

Survival

Advances in screening, diagnostic technologies, treatment and service delivery are leading to improved cancer survival in Australia and other developed countries.[2] Significant all-cancer survival gains have been realised over the past two decades, although an unacceptably high case fatality persists in the Aboriginal and Torres Strait Islander populations.[6]

Survival from cancer in South Australia is high by world standards, with a five-year survival for all cancers collectively of 61%.[2] This is comparable to the leading United States figure of 63%, and is much higher than the 48% survival in Europe.[2]

The Atlas showed that case survivals for all cancers collectively tended to be lower for residents of more remote areas than Adelaide residents. The difference, although

reaching statistical significance ($p<0.05$), was small (i.e. a five-year survival of 62% compared with 64%).[2] It could reflect more advanced stages at diagnosis for some cancers, as well as differences in treatment management and timely access to services. Further research is needed to explore survival differences across South Australia and reasons for differences. Although the survival differences reported in the Atlas were generally small, attention should be given to strengthening regional service access and referral patterns so that these differences do not increase. The numbers of health care practitioners per capita decrease with degree of remoteness, limiting opportunities for country residents to gain professional health advice and referrals for specialised oncology care.[2]

There are inadequate data to accurately assess cancer outcomes in Aboriginal and Torres Strait Islander people due to incomplete documentation of ethnicity status.[6] Nonetheless, available data suggest that Aboriginal and Torres Strait Islander residents have lower cancer survival than other South Australians for all cancers combined, but possibly not lung cancer, for which there may be a small survival advantage (figure 2).[6] Survival from cancer is particularly low in Aboriginal communities in very remote areas (e.g. the Far North and Far West).[6] Aboriginal and Torres Strait Islander people experience more lethal types of cancers.[6] They are also diagnosed at more advanced stages, have higher rates of accompanying diabetes and other co-morbidities, and often experience poorer access to specialist treatment services.[6]

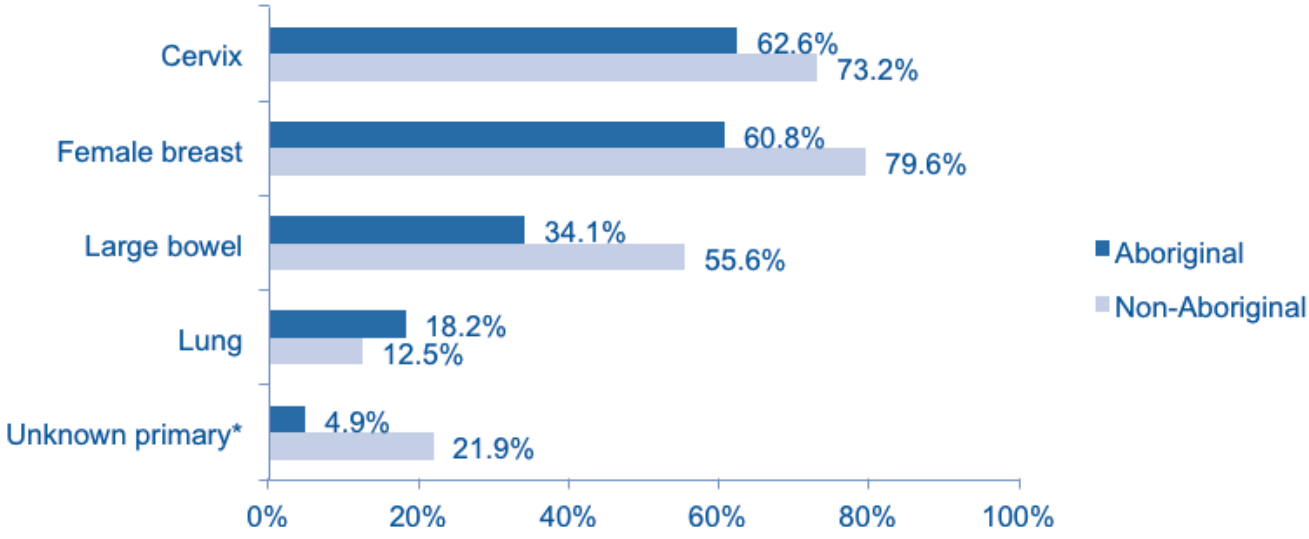
Data retrieval process

Australia has comprehensive cancer-related data, but we were aware at the beginning of the Atlas project of access barriers and were uncertain whether these barriers would prevent sufficient data access in the two-year project time frame.[3] Many data custodians were involved across multiple jurisdictions and data custodian organisations. Examples include the Australian Bureau of Statistics, Commonwealth Department of Health, Commonwealth Department of Human Services, Australian Institute of Health and Welfare, and South Australian Department of Health and Ageing. The data sources covered cancer and cancer screening registries administered by government departments and contracting agencies, plus university-based and other data holdings. Because we wished to test the utility of using multiple data sources together for Cancer Council service planning and evaluation, we wished to see whether retrieving these data was straightforward.

Data retrieval proved to be a slow and difficult process involving many mail and telephone requests to data custodian organisations across jurisdictions. It was clear that many custodians did their best and were successful in providing the requested data expeditiously, but uncertainties and delays arose with others due to privacy concerns or because of difficult and variable approval processes for data release, some of which were cumbersome with multijurisdictional approval points. Also, in-house resources were often limited for data extraction. Generally, there was a lack of policy on charging and other aspects that generated uncertainty. As a consequence, data extraction took around two years overall, and at the end of the project, some key data were still not available and had to be excluded from the Atlas. Data held by Commonwealth and other interstate authorities were generally more difficult to retrieve for this South Australian project than data held locally in South Australia.

Ideally, the research group would like to have commissioned construction of a de-identified linked data set that incorporated cancer registry data, hospital inpatient co-morbidity and treatment data, public and private radiotherapy data, Medicare Benefits Schedule/ Pharmaceutical Benefits Scheme data, cancer screening and HPV vaccination registry data, and data from population-based cohorts on relevant environmental and behavioural exposures.[3] Australia is well-practised in applying privacy-protecting data linkage methodologies to do this type of work, having gained extensive experience with the WA Data Linkage System and more recently with

*Figure 2: Case survival at five years among Aboriginal and non-Aboriginal cancer patients by primary cancer site: South Australia, 1977-2007[6] * Case survival at one year (data not available for case survival at five years)*



similar linkage systems in Queensland, NSW and SA/NT.[3] Examples of many successful projects using registries, administrative data and data linkage were presented at a Menzies Foundation Data Linkage Workshop.[3] However, these projects generally did not cover all cancers and broad aspects as risk factors, incidence, mortality and case survival. The data linkage option was not a realistic proposition for the Atlas due to the need to gain approvals from an insurmountably large number of research ethics committees and data-custodian organisations, given the time constraints. Barriers to data linkage have been described in a recent report of the Menzies Foundation Data Linkage Workshop and they were known to us at the start of this project.[3] All these barriers appear to be resolvable, but a significant national commitment would be needed across all jurisdictions to gain agreement and implement more manageable data governance arrangements."

Timely and routine access to multiple population-level data sources, such as those listed which include national as well as South Australian sources, would provide valuable evidence to Cancer Councils and other cancer-control agencies, and would facilitate better targeting of cancer control. Identification of inequalities across the cancer spectrum, from risk factor exposure through to mortality, and data showing how these patterns were changing over time in light of health promotion activities, population screening, changes to health care services delivery and policy changes, would assist Cancer Councils and other agencies to ensure that their resources were best utilised for cancer control.

Conclusion

The Atlas has provided an overview of differences in cancer risk, cancer rates and cancer outcomes across the South Australia population and shown the benefits of using combined data sources for this purpose. The Atlas was successful in bringing together data for residents of rural and remote areas, those living in areas of high socioeconomic disadvantage, and Aboriginal and Torres Strait Islander populations.

Importantly, the Atlas has provided much needed evidence on inequalities in risk and cancer outcomes for these populations. The information available was much broader than generally provided in routine reports from single data sources. The Atlas offers a breadth of data that enable more informed decision making and identification of targets for intervention (e.g. for health promotion, screening, treatment and health professional liaison). The data point to areas needing further research to identify high-risk groups for future programs. Baseline data have also been produced for assessing the effectiveness of such programs. The data can inform advocacy strategies at a local, regional and state level.

The Atlas demonstrates the value of using combination data sources to better understand cancer risk-factor prevalence, cancer rates and outcomes across the population. Data of much greater power could be assembled however, through linkage of data along the cancer trajectory for planning and evaluating preventive, screening, diagnostic, treatment and end-of-life care, and community support services. Opportunities to use existing Australian data to better inform the work of Cancer Councils and other cancer-control agencies clearly exist, but data governance arrangements need to be greatly simplified if there are to be timely data outputs. In particular, better access to nationally held data is required.

In the meantime, based on the project experience, the authors make the following recommendations in relation to South Australia.

Recommendations

Rural and remote communities and areas of most social disadvantage

Promote and advance the prevention, early detection and treatment of cancer in rural and remote areas:

- Increase promotion of sun protection and early diagnosis of lip and skin cancers
- Emphasise the importance of checking for lip and skin cancers in routine health checks
- Target rural communities in health promotion and cancer prevention programs, particularly focusing on tobacco and alcohol consumption, and excess sun exposure (due to the high proportion of cancers attributable to these risk factors), followed by diet, obesity and lack of physical activity. Readers are referred to the *National Cancer Prevention Policy* produced by Cancer Council Australia for effective strategies in these areas[12]
- Promote strategies for increasing access for rural and remote patients to cancer screening and specialist treatment services, including radiotherapy and medical oncology services.

Aboriginal and Torres Strait Islander populations

Work with Aboriginal partners in developing and proposing targeted initiatives to prevent cancer, and treat and provide support for cancer patients of Aboriginal and Torres Strait Islander background:

- Provide vaccination services for HPV and hepatitis B infection
- Undertake health promotion activities to combat high-risk health behaviours such as smoking and excess alcohol consumption, and to reduce the risk of diabetes and other co-morbidities
- Increase access to culturally acceptable screening and treatment services, particularly for rural and remote populations
- Address the broader social determinants of poor health in this sector of the population.

Data access

Improve ongoing data access:

- Ensure that key demographic and cancer data are routinely and systematically collected on cancer registration forms and by clinical cancer registries to aid monitoring for high risk groups (e.g. Aboriginal and Torres Strait Islander people and culturally and linguistically diverse groups); include cancer stage as a routine data item in cancer registries
- Make routine data supply to Cancer Council SA and other cancer-control agencies an integral part of the work of health authorities with dedicated budget lines for data provision
- Introduce programs of routine data release so that Cancer Council SA and other external agencies can obtain the data they need on a timely and regular basis for their work
- Extract additional data for high-risk groups including low SES populations, rural and remote communities, culturally and linguistically diverse groups, Aboriginal and Torres Strait Islander people and the elderly, to inform service delivery
- Use data linkage to construct repositories of linked de-identified data that cover the whole cancer trajectory and which can be used to extract data to support cancer-control initiatives
- Advocate to Australian Health Ministers for a greatly simplified and harmonised data governance arrangement across government jurisdictions, such that data access can be achieved across widely dispersed data repositories.

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UNDERSTANDING HOW CONSUMERS WOULD LIKE TO ENGAGE IN THE RESEARCH DECISION-MAKING PROCESS

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Abstract

There is an increasing emphasis on community and consumer engagement in cancer research, from identifying priorities to reviewing grants from a consumer perspective. It is clear that there is great interest from the community and consumers to be more actively involved in research, and many organisations and research institutions have responded by convening consumer advisory panels, including consumers on boards and committees, and including consumers and the community in forums and research seminars. While the opportunities available for consumers to participate in research are welcome, current mechanisms to engage with consumers often appear to be tokenistic and bureaucratic. Bedside to Bench, a research, community engagement and health education organisation, conducted

an online, consumer engagement in research survey over four weeks. The aim of the survey was to determine when and how cancer patients and their families how they would like to be involved in research. The survey was developed following feedback from consumers at the Australian Pancreatic Genome Initiative's annual research symposium, that suggested current opportunities for consumers to engage in research were limited. Eighty two cancer patients and carers responded to the survey. The majority of respondents (82%) stated that they were interested in being involved in the decision-making process in relation to cancer research. The greatest area of interest was in having access to the results of research projects (23%) and providing feedback to researchers once the projects are developed (23%). Other areas of interest were the development of research projects with researchers (17%), identification of research priorities (17%), with the lowest area of interest being grant reviews (13%).The results of this study suggest that the majority of consumers want to be involved in research in some way, however, given the option, there is potentially only a subset of consumers interested in the review of research grants. What is clear is that, whatever the mechanisms for consumer engagement, strategies, policies and resources need to be available in order to support all stakeholders improve the practice of research involving consumers. The results of this study will be useful to guide future research and policy decisions in relation to consumer engagement in research.

There is an increasing emphasis on community and consumer engagement in cancer research, from identifying priorities to reviewing grants from a consumer perspective. It is clear that there is great interest from the community and consumers to be more actively involved in research, and many organisations and research institutions have responded by convening consumer advisory panels, including consumers on boards and committees, and including consumers and the community in forums and research seminars.[1-6]

Bedside to Bench is a health education, community engagement and research not-for-profit that works with the community, researchers, health practitioners and policy makers to facilitate a meaningful and productive relationship between each stakeholder group through workshops, facilitated meetings and education. Our model of engagement creates an environment where consumers are engaged in research, as part of the research team, with the aim of ensuring that research addresses the needs of consumers and has a clear pipeline for application.

In May 2013, the Australian Pancreatic Genome Initiative (APGI, www.pancreaticcancer.net.au) held its annual research symposium. The symposium provided consumers and researchers with an update on the APGI's national study investigating the underlying genetic changes in pancreatic cancer, by studying the DNA from tissue and blood samples from pancreatic cancer patients across Australia. Of the 101 symposium attendants, 49 were consumers and community members, demonstrating a high level of consumer interest in the work of the APGI. This is a particularly high number of consumers given it is a poor prognosis cancer, which traditionally struggles to maintain high levels of consumer engagement or interest in research because of the nature of disease. The symposium included three presentations from APGI members in relation to pancreatic cancer research, and two non-pancreatic cancer specific presentations focusing on existing mechanisms for consumer engagement in research. During the two consumer engagement presentations, Cancer Council NSW, Cancer Australia and National Health and Medical Research Council consumer and community engagement mechanisms were described. Following the presentation, there was an open discussion, where many of the consumers suggested that the current mechanisms appeared to be tokenistic and bureaucratic.

It was from this ad-hoc feedback that an online survey was designed and conducted over a four week period, to ask cancer patients and their families how they would like to be involved in research, with the aim of informing policy decisions to ensure that future engagement is meaningful.

Methodology

An online survey was developed by the authors, following the collection of feedback from consumers at the Australian Pancreatic Genome Initiative's annual research symposium that suggested that the current opportunities for consumers to engage in research were limited. The survey was delivered through SurveyMonkey (Survey Monkey, Palo Alto, CA). The survey was solely advertised on the Bedside to Bench Facebook page. People who had experienced cancer as a patient or carer were invited to complete the survey. In an attempt to reduce bias, the advertisement on Facebook was intended to attract anyone who had been affected by cancer as a patient or carer, as opposed to experienced consumer representatives.

The survey was designed to collect minimal, non-identifiable, demographic information from participants and included a series of questions in relation to whether they were interested in participating in cancer research, whether they had previously been involved in the consumer review of grants, at what stage they would like to be involved in research, level of interest in the consumer review of grants, and barriers to participating in the consumer review of grants.

A content analysis was conducted by the authors using a conventional content analysis approach, in which categories were directly derived from the participant's open-ended responses, based on common themes.[7] The responses from participants who did not complete certain questions were excluded in the data analysis to ensure accuracy. Each week, the researchers met to discuss categories and resolve any discrepancies. The proportion of respondents in each category was analysed and the frequency and percentage that each category represented was calculated.

Results

Eight categories were identified by the researchers and had the following definitions:

Time burden: Responses that indicated participants had limited time available for grant reviews and/or had other commitments.

Lack of qualification and research experience: Expression of concerns that short-term training would not provide sufficient knowledge for participating in grant reviews and opinions that others were better qualified to make such important decisions.

Lack of interest: Expression of no interest in grant reviews.

Conflict of interest: Participants with previous/current contribution in cancer research expressed a concern that the participation might compromise their professional judgments.

Lack of current cancer experience: Opinion that past cancer experience might be irrelevant to the current grant review process.

Previous participation: Participants with previous grant reviewing experience did not want to be involved in the process again.

Health concerns: The health effects due to cancer treatment.

Travel burden: Expressed that travelling to the grant review venue was a deterrent.

Demographic information

Eighty two individuals responded to the survey, of which 31% were cancer patients, 62% were family members/carers of cancer patients and 7% were both a carer and a patient. Just over half (54%) of respondents were from NSW, 9% were from Victoria and Queensland, and the remaining respondents were from South Australia, Western Australia, Tasmania and overseas. The mean age for patients was 55 years and for carers 44 years; respondent ages were categorised into the following age groups, 35% 40-45 years, 32% 25-20, 18% 55-69, 4% under 25 years and 11% over 70 years of age.

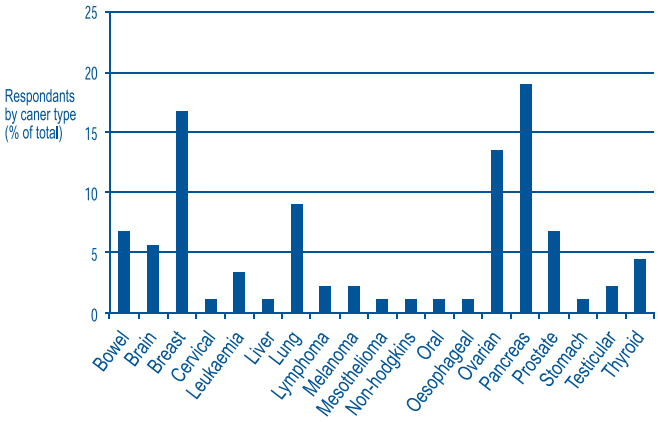
Cancer type and date of diagnosis

The majority of respondents had experienced pancreatic cancer (19%), followed by breast (17%), ovarian (14%), bowel (7%), prostate (7%) and brain (6%) cancers. The remaining types of cancer represented are available in figure 1. Participants were asked to identify the date of cancer diagnosis, either as a patient or a carer. A third (33%) of respondents stated a date of diagnosis between 2011-2013, 23% between 2006-2010, 11% between 2000-2005 and 15% before 2000. The remaining participants (18%) did not provide a response.

Questions in relation to consumer participation in research

Participants were able to select multiple responses to these questions. The majority of respondents (82%) stated they were interested in being involved in the decision-making process in relation to cancer research. The greatest area of interest was in having access to the results of research projects (23%) and providing feedback to researchers once the projects are developed (23%). Other areas of interest were the development of research projects with researchers (17%), identification of research priorities (17%), with the lowest area of interest being grant reviews (13%) (figure 2).

Figure 1: Cancer types represented in this survey.



Only a small proportion of respondents (18%) stated that they were not interested in participating in cancer research decision-making in general, with deterrents including lack of scientific knowledge and research experience, conflict of interest and time burden (figure 3).

Figure 2: Stages of research and decision-making process consumers expressed interest participating in.

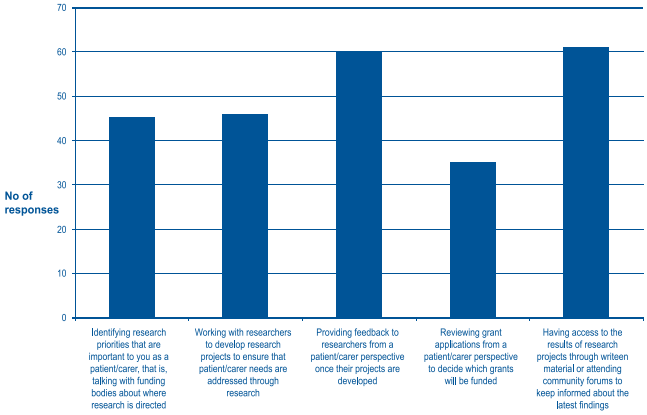
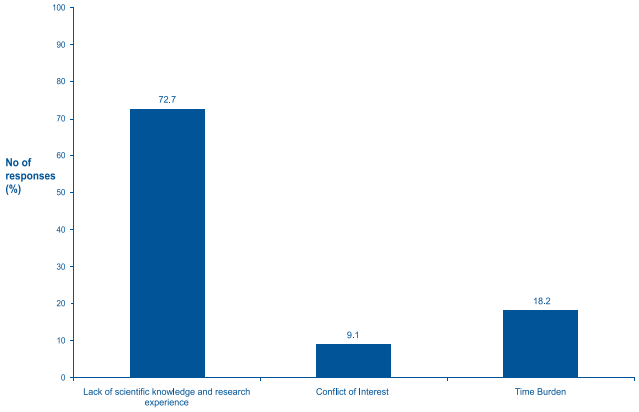


Figure 3: Reasons consumers state for not wanting to participate in research.

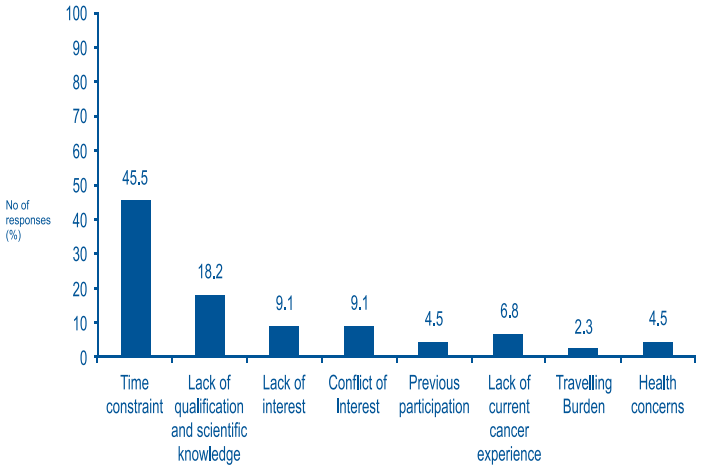


Questions specifically related to the consumer review of grants

Respondents were asked specifically about their interest in participating in the consumer review of grants, and if yes, how much time they were prepared to commit to the review. Of the respondents who stated that they would be interested in reviewing grants (43%, n=35), the majority stated that they were prepared to travel (71%). In relation to time commitment, 10 respondents were prepared to commit at least six days to the process, four would commit three-five days, 11 would commit two days and 10 would commit one day. Of the 35 respondents who stated that they were interested in the review of grants, 14 had previously attended a training or seminar in relation to community engagement in research.

A higher number of participants (52%, n=43) stated that they were not interested in the consumer review of grants. The reasons respondents did not want to participate were time burden (46%), others better qualified to make decisions about funding (18%), lack of interest (9%), conflict of interest (9%), lack of current cancer experience (7%), previous participation (5%), health concerns (5%), and travelling burden (2%) (figure 4).

Figure 4: Reasons consumers state for not wanting to participate in the consumer review of grants.



Discussion

Consumer and community engagement seeks to involve participants to play an active role in the decision making process of research.[8] The results of this survey suggest that the greatest area of interest for consumers participating in research is at the beginning of the research process, providing feedback to researchers once research projects are developed, and at the end of the process, hearing the results of research projects. The area of least interest in relation to involvement was in priority setting and making decisions about research funding. This is in contrast to existing literature, where consumer involvement in the decision-making process has been observed as evolving over time to address several concerns found in traditional research, particularly concerns in relation to the relevance of research to patient needs and cancer care services, and the need to increase community engagement in health related decisions.[9-11]

Within our survey results, there were inconsistencies in relation to the responses to the question about participating in the consumer review of grants. The inconsistency arose when the question of grant review was posed as a separate question, rather than an option in a series of questions. One explanation for this may be that when information on the amount of time attendance at workshops and overall commitment needed was provided, participation in the review of grants was less appealing. While there is no way in this review to accurately determine the reason for this, we can infer that in the absence of options, consumers may be more willing to participate in the consumer review of grants, however given the option, the preference would be to engage in research in other ways.

There have been a number of studies involving consumers in all stages of research, from study design, proposal review, data collection and analysis and result dissemination.[12-15] The joint partnership allowed a direct influence on all aspects of the studies and was found mutually beneficial. Many studies have also found that consumer involvement in the research agenda provided different perspectives and insight into major concerns of cancer patients and the experience in dealing with the disease,[1,2,4,5,16] therefore enhanced the relevance, appropriateness and practicability of research questions and protocols to the community and potentially improved participation rates.[3,13,15,17] It is also acknowledged that additional resources are required, including support for researchers who wish to welcome consumers as co-researchers.[3] The importance of an organisational framework,[6] sufficient funding and resources to support consumer involvement,[10,12] and a change in attitudes from traditional research to partnership have been recognised,[10] and it is recommended that research and institutional policy adapt to reflect this need.

Consumer collaboration in disseminating research findings to the community has been suggested to increase the credibility and accessibility of the findings.[2,3,6] For the community, scientific skill and knowledge enhancement increased consumers' confidence and provided them a degree of control in the research.[6,9,10,17] There are established benefits to consumer engagement in all aspects of research, however our survey suggests that there is a preference for participation at specific points in time, leading to a misalignment between consumer preference in how they wish to be engaged in research, and the overall benefits that consumer engagement offers to research outcomes.

There were some concerns from consumers in our study in relation to whether they were suitably qualified to make decisions on research funding. Andejeski et al found that both consumers and researchers sitting on scientific merit and grant review panels were concerned about the lack of scientific background of consumers.[10] Personal bias due to a consumer's personal perspective and experience was also added to the concerns of researchers, and could lead to power imbalance between consumers and researchers.[11] While these problems could be avoided by

ensuring plain language was used whenever possible, making the language more accessible by researchers,[13] and providing training to consumers,[3,6,13,15] it does not address the key issue identified in this study which questions whether the consumer preference is being involved in the review of grants, or other activities.

This study was limited, as only a small sample population was included, it was open for a short period of time (four weeks), and the number of people who saw the survey is unknown, so it is not possible to provide an estimate of response rate. There is also limited existing research, making it difficult to discuss findings in the context of consumer engagement in research. In addition, the generalisability of consumers' interests across a larger population was questionable, due to an absence of participants coming from minority ethnic backgrounds and with rare cancer types.[4-6,15,16] This survey did not account for ethnic backgrounds, however rare cancer types were well represented. This was largely opportunistic and a result of the recent work of Bedside to Bench across a number of low prevalence and poor prognosis diseases. Nevertheless, the issue of underrepresentation should be considered in any study design involving consumers in order to maximise inclusion while avoiding tokenism.[6]

Although tension, distrust and conflicts have been observed in the interaction between consumers and researchers,[12,13] this interaction also serves as a platform for negotiating the problems. Through communication and agreement on both parties' roles and obligations, and having flexibility to renegotiate those roles and obligations, successful collaboration could be fostered.[11] This is where the concept of inviting consumers to be involved as part of the research team demonstrates an opportunity for meaningful engagement. Previous models of consumer engagement in Australia have focused on building networks of consumers for researchers to draw upon. While there are benefits to this model in relation to resourcing, it does not facilitate the development of a working relationship between researchers and consumers, and encourages the tokenistic interaction that has been a criticism of consumer engagement in research from many stakeholders, as researchers need only 'access' a consumer once a year for the purpose of grant applications.

Conclusion

Consumer and community engagement in research has been gradually implemented. A number of benefits, limitations and challenges have been identified to both consumers and the community, and researchers. The results of this study suggest that consumers want to be involved in research in some way, however given the option, there is potentially only a subset of consumers interested in the review of research grants. What is clear is that there is a place for consumers in all facets of research. Whatever the mechanisms adopted for consumer engagement, strategies, policies and resources need to be available in order to support all stakeholders, which in turn will improve the overall practice of involving consumers in research decision-making.

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REPORTS

SUPPORT FOR RESEARCH FUNDING 2014

The state and territory Cancer Councils, which comprise the member bodies of Cancer Council Australia, are the main sponsors of cancer research and related activities in Australia. Grants are made following a competitive, peer-reviewed assessment of funds derived from donations and bequests.

In 2014, the value of these grants is more than $64 million.

Please note: for research grants spanning more than one year, only funds to be dispersed in 2014 have been included.

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Priority-driven Collaborative Cancer Research Scheme through Cancer Australia*

Grimison University of Sydney	Accelerating First-Line Chemotherapy to Improve Cure Rates for Advanced Germ Cell Tumours: An Australian-Led, International Randomised Trial	$111,000
Friedlander Prince of Wales Hospital	An international multi-stage randomised phase III trial of dose-fractionated chemotherapy compared to standard three-weekly chemotherapy for women with newly diagnosed epithelial ovarian cancer	$84,000
Nowak University of Western Australia	Phase III trial of Concurrent and Adjuvant Temozolomide chemotherapy in non-1p/19q non deleted anaplastic glioma. The CATNON Intergroup Trial.	$64,000
Severi Cancer Council Victoria	Risk and Prognostic Factors for Glioma in Australia	$ 121,000
Hayes Queensland University of Technology	ECHO trial: Exercise during chemotherapy for ovarian cancer	$ 98,000
Total research funded		**$478,000**

IARC Fellowships

Muller International Agency for Research on Cancer	IARC Research Fellowship	$55,033
El-Zaemey International Agency for Research on Cancer	IARC Research Fellowship	$51,305
Total research funded		**$106,338**

ARC Linkage Grant

Australian Research Council (ARC)	Public and ethical responses to mandated alcohol warning labels about increased long-term risk of cancer	$ 20,000
Total research funded		**$20,000**
TOTAL RESEARCH FUNDED		**$604,338**

*The funds for these research grants are pooled from state and territory Cancer Councils.

CANCER COUNCIL ACT

Research Grants

Dr Anneke Blackburn	Understanding and targeting metabolic regulators of cancer cell proliferation and death	$60,000
Total research funded		**$60,000**
TOTAL RESEARCH FUNDED		**$60,000**

CANCER COUNCIL NSW

Externally funded research programs - RSU

		Cancer Council charitable funding amount 2014	Other Funding Amount For 2014	Total
New Research Grants				
Project Grants				
Prof Christopher Liddle University of Sydney	RG 14-02 - Novel approaches to target cancer stem cells in liver cancer	$120,442		$120,442
Prof Jacqui Matthews University of Sydney	RG 14-03 - Developing inhibitors of the LMO4 oncoprotein	$119,037		$119,037
Dr Jeremy Henson Children's Medical Research Institute	RG 14-04 - Development of the C-Circle biomarker as a cancer diagnostic	$116,149		$116,149
Prof John Rasko Centenary Institute	RG 14-05 - Consequences of CTCF haploinsufficiency in endometrial carcinoma	$120,000		$120,000
Dr Lionel Hebbard University of Sydney	RG 14-05 - Metabolic drivers of liver cancer progression	$120,000		$120,000
Prof Peter Croucher Garvan Institute of Medical Research	RG 14-07 - Defining the critical role of osteoclasts in multiple myeloma cell growth and activation in bone	$120,000		$120,000
Dr Paul Timpson Garvan Institute of Medical Research	RG 14-08 - Optimising ECM-targeted therapy in cancer using live intravital FRET biosensor imaging	$119,037		$119,037
Prof John Rasko Centenary Institute	RG 14-09 - Consequences of CTCF mutation in acute lymphoblastic leukaemia	$119,899		$119,899
Prof David Thwaites University of Sydney	RG 14-11 - Do treatment delivery uncertainties limit the effectiveness of advanced technology radiotherapy treatments?	$119,259		$119,259
Prof Michael Rogers Garvan Institute of Medical Research	RG 14-12 - A new use for old drugs: Anti-tumour effects of bisphosphonates via tumour-promoting myeloid cells	$120,000		$120,000
Dr Megan Chircop Children's Medical Research Institute	RG 14-13 - Defining the cellular determinants that drive dynamin inhibitor induced cell death and in vivo efficacy against glioblastoma	$120,000		$120,000
Dr Scott Byrne University of Sydney	RG 14-14 - Skin cancer prevention and treatment by targeting sunlight-activated regulatory B cells	$120,000		$120,000
Prof Michael Henderson University of Sydney	RG 14-15 - ANZMTG 03.12 Melanoma Margins Trial - A Phase III, Multi-centre Randomised Controlled Trial Investigating 1cm v 2cm Wide Excision Margins for Primary Cutaneous Melanoma (MelMarT)	$120,000		$120,000
Dr Hilda Pickett Children's Medical Research Institute	RG 14-16 - Altered teleomeric chromatin and its role in Alternative Lengthening of Telomeres	$106,149		$106,149
Dr Glen Reid Asbestos Diseases Research Institute	RG 14-17 - MicroRNA replacement: A novel therapeutic approach for malignant mesothelioma	$117,380		$117,380
Prof John Mattick Garvan Institute of Medical Research	RG 14-18 - Modular RNA structures guiding epigenetic differentiation	$117,719		$117,719
TOTAL RESEARCH FUNDED (New projects)		$1,895,071		$1,895,071

Continuing research grants

Program Grants

Prof Roger Reddel Children's Medical Research Institute	PG 11-08 - Alternative Lengthening of Telomeres: from basic biology to drug discovery	$450,000	$450,000
Prof Murray Norris University of NSW	PG 11-06 - Toward cure of childhood ALL: improved diagnostics, therapeutics and prevention strategies	$450,000	$450,000
Prof Christopher Ormandy Garvan Institute of Med Res	PG 11-07 - Personalising breast cancer management by discovering the transcriptional basis for tumour phenotype	$450,000	$450,000
Prof Philip Hogg University of NSW	PG 11-03 - Metabolism inhibitors for the treatment of brain and pancreatic cancer	$450,000	$450,000
A/Prof Susan Henshall/ Horvath Garvan Institute of Med Res	Building capacity in Pharmacogenomics across NSW: PRIMe (Pharmacogenomic Research for Individualised Medicine)	$299,724	$299,724

Special

Prof Andrew Biankin Garvan Institute of Med Res	International Cancer Genome Consortium (ICGC)	$250,000	$250,000

STREP

Prof Andrew Biankin Garvan Institute of Med Res	SRP 11-01 - Genotype Guided Cancer Therapy (Genomic Theranostics)	$300,000	$300,000
Prof Sanson-Fisher University of Newcastle	CSR 11-02 - New 3C	$200,000	$200,000

Special

Sax Institute	45 and Up	$300,000	$300,000

Project Grants

A/Prof Tracy Bryan University of Sydney	RG 12-01 - Involvement of helicase DHX36 in human telomere maintenance	$97,508	$97,508
Dr Scott Cohen University of Sydney	RG 12-02 - Structure and Inhibition of the Human Telomerase Enzyme complex	$120,000	$120,000
Dr Sue Firth University of Sydney	RG 12-03 - IGFBP-3 enhances autophagy to promote breast cancer cell survival during stress	$120,000	$120,000
Dr Beric Henderson University of Sydney	RG 12-04 - Novel regulation of beta-catenin intracellular transport and its role in cell polarity and migration	$120,000	$120,000
Dr Megan Hitchins University of NSW	RG 12-05 - The mechanistic basis for prediction of response to alkylating chemotherapy in high grade glioma patients by molecular markers of MGMT activity	$98,725	$98,725
A/Prof Geraldine O'Neill University of Sydney	RG 12-06 - A Sting in the Tail: Focal Adhesion Targeting and Mechanotransduction	$108,724	$108,724
Dr Nicole Verrills University of Newcastle	RG 12-07 - RG 12-07 Activating a tumour suppressor for leukaemia therapy	$120,000	$120,000
Dr Stuart Tangye Garvan Institute of Med Res	RG 12-08 - Mechanisms underlying impaired anti-EBV and anti-tumour immune responses in the absence of SAP	$118,570	$118,570

Prof Minoti Apte University of NSW	RG 13-01 - Targeting the stroma in pancreatic cancer - a novel therapeutic approach focussing on the hepatocyte growth factor/c-MET pathway	$120,000	$120,000
Dr Linda Bendall University of Sydney	RG 13-02 - Sphingosine Kinases as Potential Therapeutic Targets for Acute Lymphoblastic Leukemia	$120,000	$120,000
Prof Samuel Breit University of NSW	RG 13-03 - The role of the TGF-b superfamily cytokine MIC-1/GDF15 in cancer growth and spread	$119,551	$119,551
A/Prof Xu Dong Zhang University of Newcastle	RG 13-04 - Targeting PP2A to improve the therapeutic efficacy of mutant BRAF inhibitors in melanoma	$119,750	$119,750
Prof Christine Clarke University of Sydney	RG 13-05 - Determinants of genomic binding of the progesterone receptor in endocrine target cells	$120,000	$120,000
Dr Nickolas Haass University of Sydney	RG 13-06 - Effect of three-dimensional tumour organisation on the sensitivity of individual melanoma cells to endoplasmic reticulum stress	$119,891	$119,891
Dr Megan Hitchins University of NSW	RG 13-07 - Genetic determination of hereditary MLH1 epimutation as a cause for familial cancer	$99,891	$99,891
A/Prof Lisa Horvath Garvan Institute	RG 13-08 - Novel strategies to overcome Docetaxel resistance in castration-resistant prostate cancer (CRPC)	$120,000	$120,000
Dr Tao Liu University of NSW	RG 13-09 - The critical role of the long intergenic noncoding RNA MALAT1 in neuroblastoma	$118,551	$118,551
A/Prof Deborah Marsh University of Sydney	RG 13-10 - Monoubiquitinated histone H2B – marking key pathways in ovarian cancer	$114,891	$114,891
Prof Markus Seibel University of Sydney	RG 13-12 - Novel Cytoplasmic Functions of the Vitamin D Receptor in Bone Metastases	$119,891	$119,891
Dr Elena Shklovskaya University of Sydney	RG 13-13 - Role of dendritic cell subsets in regulating CD4 T cell memory responses in inflammation and cancer	$119,061	$119,061
A/Prof Janette Vardy University of Sydney	RG 13-14 - Evaluation of a Web-based Cognitive Rehabilitation Programme in Cancer Survivors with Self-reported Cognitive Impairment	$89,252	$89,252
A/Prof Xu Dong Zhang University of Newcastle	RG 13-15 - Functional consequences of epigenetic repression of PIB5PA in melanoma	$119,750	$119,750
Dr Kerrie McDonald University of NSW	RG 13-16 - The biological basis of success or failure to the anti-VEGF agent, bevacizumab in patients with recurrent glioblastoma	$117,422	$117,422
Prof Edna Hardeman University of NSW	RG 13-17 - The role of epigenetic modifications in longterm memory of irradiation in cancer survivors	$120,000	$120,000
STREP			
A/Prof Gail Garvey Menzies School of Health Research	SRP 13-01 - Strategic Research Partnership to improve cancer control for Indigenous Australians (STREP Ca-CINDA)	$395,170	$395,170
Dr Gillian Mitchel Peter MacCallum Cancer Centre	SRP 13-02 - The Inherited Cancer Connect (Icon) Partnership	$391,952	$391,952
Prof Andrew Grulich Kirby Institute UNSW	SRP 13-11 - Preventing morbidity and mortality from anal cancer	$398,629	$398,629
CA PdCCRS			
Dr Kerrie McDonald University of NSW	RG 12-09 PdCCRS Mechanisms underpinning how brain cancer cells respond to drugs	$160,000	$160,000

A/Prof Gianluca Severi CCVIC	RG 12-10a (PdCCRS) - Risk and Prognostic Factors for Glioma in Australia	$40,000		$40,000
Dr Lorraine O'Reilly Walter and Eliza Hall Institute of Med. Res	RGPd 13-01 - Understanding the role of NF-KB in the progression of gastric adenocarcinomas and assessment of new therapies.	$200,000		$200,000
Total research funded (Continuing projects)		**$7,496,903**		**$7,496,903**
TOTAL EXTERNALLY FUNDED RESEARCH PROGRAMS - RSU (including new and continuing research grants)		**$9,391,974**		

Internally Funded Research Programs

Research programs

Cancer Research Division - (internal + external but excluding NHMRC funding)		$2,580,804		$2,580,804
Total research funded		**$2,580,804**		**$2,580,804**

Externally Funded Research Programs - Cancer Programs Division

New Research Grants

NHMRC Partnership Grant

Professor Robert Sanson-Fisher University of Newcastle	Who decides and at what cost? Comparing patient, surrogate, and oncologist perspectives on end of life care	$43,833	$84,037	$127,870

Cancer Institute Evidence to Practice Grant

Dr Dean Dudley, Jackie McIver, Vanessa Rock Charles Sturt University	Evaluation of a SunSmart primary school policy support intervention	$22,000	$33,000	$55,000
Scott Walsberger Cancer Council NSW	Tackling tobacco among Aboriginal families with dependent children: integrating smoking care within family services		$44,250	$44,250
TOTAL RESEARCH FUNDED		**$65,833**	**$161,287**	**$227,120**

Continuing Research Grants

Australian Research Council Linkage Program

Professor Robert Sanson-Fisher, Anita Tang, Kathy Chapman, Elizabeth Humphries University of Newcastle	Improving cancer treatment systems: a randomised controlled trial of a consumer action model for cancer patients receiving chemotherapy	$56,610	$83,515	$140,125
Associate Professor Debbie Horsfall, Susan Goldie University of Western Sydney	Caring at End of Life: Understanding the nature and effect of informal community care networks for people dying at home	$22,000	$46,091	$68,091
Dr Catalina Lawsin, Annie Miller University of Sydney	Rekindle sexuality after cancer: development and testing of a novel web-based psychoeducational resource for both survivors and their partners	$35,000	$107,521	$142,521

NHMRC Partnership Grant

Associate Professor Billie Bonevski, Scott Walsberger University of Newcastle	Cost effectiveness of a systems change intervention for smoking cesation in drug and alcohol treatment centres.	$43,154	$256,163.12	$299,317.12
Associate Professor Christine Paul, Lorna O'Brien University of Newcastle	An RCT of online versus telephones based information and support: Can electronic platforms deliver effective care for lung cancer patients?	$63,000	$103,750	$166,750

NSW Office of Preventive Health

Clare Hughes Cancer Council NSW	Eat It To Beat It Evaluation		$74,250	$74,250

Department of Health and Ageing Grant

Clare Hughes Cancer Council NSW	Extend Cancer Council NSW's Eat It To Beat It program to create a strategy for delivering the program to disadvantaged families who are already accessing social welfare organisations.		$99,000	$99,000
Total research funded		**$219,764**	**$770,290**	**$990,054**
TOTAL EXTERNALLY FUNDED RESEARCH PROGRAMS - CANCER PROGRAMS DIVISION (including new and continuing research grants)		**$285,597**	**$931,577**	**$1,217,174**

Internally funded research programs - Cancer Programs Division

New Research Grants

Lyndal Wellard, Clare Hughes, Cancer Council NSW	Exploratory research to understand how to engage and support GPs to address alcohol and cancer risk	$29,250		$29,250
Kelly Williams, Anita Tang, Scott Walsberger, Rae Fry, Eleonora Feletto, Cancer Council NSW	Qualitative study of former tobacco retailers	$6,000		$6,000
TOTAL RESEARCH FUNDED		**$35,250**		**$35,250**

CANCER COUNCIL QLD

Cancer Council QLD

EXTERNALLY FUNDED RESEARCH PROGRAMS
New Research Grants

		Cancer Council charitable funding amount 2014	Other funding amount for 2014	Total
Prof Andrew Boyd QIMR Berghofer Medical Research Institute	Characterisation of the function and therapeutic potential of EphA2 and EphA3 in prostate cancer	$100,000		$100,000
Dr Glenn Boyle QIMR Berghofer Medical Research Institute	Investigating phenotype plasticity in melanoma progression and drug resistance	$100,000		$100,000
Prof Judith Clements Queensland University of Technology	KLK4 is a key regulator of the reactive stromal microenvironment in prostate cancer	$100,000		$100,000
Dr Nicole Cloonan QIMR Berghofer Medical Research Institute	MicroRNAs and isomiRs as chemosensitizers in Double-stranded Break Repair defective cancer	$100,000		100000
Dr Bryan Day QIMR Berghofer Medical Research Institute	Understanding the function of salinomycin as a DNA damaging agent and its relevance as a potential therapeutic agent for the treatment of malignant brain tumours	$100,000		100000
Assoc Prof Greig de Zubicaray University of Queensland	A prospective study of language function following surgical resection of left hemisphere primary brain tumours	$100,000		100000
Dr Thomas Haselhorst Griffith University	Development of a novel glycotherapy for the treatment of B cell derived non-Hodgkin lymphoma	$100,000		100000
Dr Graham Leggatt University of Queensland	Memory CD8 T cell subsets in non-melanoma skin cancer	$100,000		$100,000

Dr Kelli MacDonald QIMR Berghofer Medical Research Institute	Investigations of the cellular and molecular mediators of chronic graft versus host disease	$100,000	$100,000
Assoc Prof Jennifer Martin University of Queensland	Targeting existing therapies with innovative technology platforms to improve survival in brain cancer	$100,000	$100,000
Prof George Muscat University of Queensland	Protein Arginine Methyltransferase 6 dependent signalling in breast cancer	$100,000	$100,000
Prof Colleen Nelson Queensland University of Technology	Characterising insulin signalling in androgen-deprived prostate cancer cells; rationalising current metabolic therapies for adjuvant use in advanced prostate cancer	$100,000	$100,000
Dr Allison Pettit Mater Research Institute, University of Queensland	The role of macrophages in facilitating hematopoietic stem cell engraftment and reconstitution	$100,000	$100,000
Assoc Prof Sally-Ann Poulsen Griffith University	Development of bimodel MRI/PET imaging agents for imaging of hypoxia: The best of both worlds	$100,000	$100,000
Dr Tarl Prow University of Queensland	Automated image analysis development for early non-melanoma skin cancer detection	$81,000	$81,000
Dr Derek Richard Queensland University of Technology	BanF1: A critical regulator of the ageing process and genome stability Koa Iris Greer Research Grant	$100,000	$100,000
Dr Aaron Smith University of Queensland	Investigating the role of the NR4A nuclear receptor family in melanocyte function and tumourigenesis	$100,000	$100,000
Prof Stephen Taylor University of Queensland	Complement C3a receptor, a novel therapeutic target for melanoma	$100,000	$100,000
Assoc Prof Jolieke Van der Pols, University of Queensland	Risk factors for sessile serrated adenoma	$100,000	$81,000
Dr Graeme Walker QIMR Berghofer Medical Research Institute	Characterization of a novel naevus modifier gene on murine chromosome 8	$100,000	$100,000
Dr James Wells University of Queensland	Chemokine involvement in the differential response of Actinic Keratosis and Squamous Cell Carcinoma to Imiquimod therapy	$100,000	$100,000
Dr Ingrid Winkler Mater Research Institute, University of Queensland	A new approach to tackling chemotherapy-induced mucositis	$100,000	$100,000

Special Project Grant

Dr Bena Cartmill University of Queensland	Does a computerised swallowing, nutrition, and distress screening tool capture those patients and carers who need face-to-face intervention during (chemo) radiotherapy for head and neck cancer?	$25,000	$25,000

Senior Research Fellowship

Assoc Prof Sandra Hayes Queensland University of Technology	Exercise is medicine: a non-pharmacological approach to cancer care	$130,020	$130,020

PhD Scholarship

Arabella Young QIMR Berghofer Medical Research Institute	Targeted therapy and immunotherapy in breast cancer	$30,000	$30,000

Dr Matthew Roberts University of Queensland	Improving the early detection of prostate cancer: a non-invasive, systems biology approach	$30,000		$30,000

Cancer Clinical Trial Data Manager Grants

Cairns Hospital
Gold Coast Hospital
Icon Cancer Care - HOCA Research Centre
Mater Health Services - Medical Oncology & Palliative Care
Nambour Hospital
Oncology Research Australia
Premion
Prince Charles Hospital
Princess Alexandra Hospital – Surgery, Haematology & Medical Oncology, Radiation Oncology
Radiation Oncology Services - Mater Centre
Royal Brisbane and Women's Hospital – Gynaecological Cancer, Medical Oncology, Radiation Oncology,
Brisbane Colorectal Group
Royal Children's Hospital
Townsville Hospital
Wesley Research Institute

Total	$682,132	$667,868	$1,350,000

Travel Grants

By application	$85,000		$85,000

Travelling Fellowships

By application or invitation	$85,000		$85,000
TOTAL RESEARCH FUNDED (new research grants)	**$3,248,152**	**$667,868**	**$3,897,020**

Continuing Research Project Grants

Dr Mark Appleyard Royal Brisbane & Women's Hospital	The effects of butyrylated high amylose maize starch on polyposis in familial adenomatous polyposis volunteers	$75,000	$75,000
Assoc Prof Helen Blanchard Griffith University	Design of specific chemical probes to target and inhibit galectin-3	$100,000	$100,00
Prof Melissa Brown University of Queensland	The role of BRCA non-coding mutations in breast cancer susceptibility	$99,891	$99,891
Prof Judith Clements Queensland University of Technology	Kallikrein proteases have key roles in tumour cell aggregation in ascites and chemoresistance in epithelial ovarian cancer	$100,000	$100,000
Dr Jermaine Coward Mater Research Institute University of Queensland	Targeting inflammatory pathways in epithelial ovarian cancer	$99,891	$99,891
Dr Mathias Francios University of Queensland	Inhibitors of SOX18 transcription factor: from developmental biology to pre-clinical trial of novel anti-metastatic compounds	$100,000	$99,891
Prof Maher Gandhi Princess Alexandra Hospital	Monocytic myeloid derived suppressor cells and antiCD20-antibody dependent cellular cytotoxicity in diffuse large B-cell lymphoma	$99,981	$99,981
Prof Thomas Gonda University of Queensland	Targeting Myb transcriptional elongation in cancer	$99,891	$99,981
Dr Benjamin Hogan University of Queensland	A novel mechanism regulating lymphatic vascular precursor cell migration	$99,891	$99,981

Dr John Hooper Mater Research Institute University of Queensland	A novel Src regulated protease activated signalling pathway in hematogenous metastasis	$100,000		$100,000
Dr Susan Jordan QIMR Berghofer Medical Research Institute	Patterns of care in renal cell carcinoma	$94,127		$94,127
Prof Kum Kum Khanna QIMR Berghofer Medical Research Institute	Role of FBXO31-mediated protein degradation in mitotic progression	$97,551		$97,551
Prof Martin Lavin QIMR Berghofer Medical Research Institute	Role of ATM-dependent Mre11 Phosphorylation in the DNA damage response	$100,000		$100,000
Dr Graham Leggatt University of Queensland	Immunotherapy of non-melanoma skin cancer and their precancerous lesions during lymphopenia Gavin Chase Research Grant	$100,000		$100,000
Dr Nigel McMillan Griffith University	Nanoparticle mucosal delivery systems for siRNA-based cancer therapies	$93,000		$93,000
Dr Andreas Möller QIMR Berghofer Medical Research Institute	Regulation of the pre-metastatic niche by hypoxia Ralph Carroll Research Grant	$100,000		$100,000
Prof Gregory Monteith University of Queensland	Identification and characterisation of calcium signalling modifying proteins as drug targets for basal breast cancer	$99,891		$99,891
Prof Jiri Neuzil Griffith University	How to efficiently treat resistant breast cancer	$100,000		$100,000
Dr Pamela Pollock Queensland University of Technology	Mechanisms of resistance to FGFR inhibition in endometrial cancer	$100,000		$100,000
Assoc Prof Stephen Rose Australian e-Health Research Centre, CSIRO	Understanding radiation insensitivity and temozolomide resistance in Glioblastoma Multiforme	$99,920		$99,920
Dr Fiona Simpson University of Queensland	The role of epidermal growth factor receptor trafficking in tumor progression and patient therapy resistance	$97,705		$97,705
Dr Graeme Walke QIMR Berghofer Medical Research Institute	In vivo functional dissection of the respective roles of the CDKN2A and MTAP loci in naevus susceptibility	$99,891		$99,891
Dr Stephen Wood Griffith University	Dissecting Usp9x's tumour suppression function in pancreatic ductal adenocarcinoma Daphne May Brown Research Grant	$100,000		$100,000

Senior Research Fellowship

Prof Nicholas Saunders University of Queensland	Translating basic science into better cancer treatments	$149,920		$149,920
Dr Kelli MacDonald QIMR Berghofer Medical Research Institute	Requirements for class II antigen presentation to generate curative anti-leukaemic responses and immunocompetence after transplantation	$141,961		$141,961

John McCaffrey Senior Clinical Research Felllowship

Prof Maher Gandhi Princess Alexandra Hospital	Lymphoma: translating research into better outcomes.	$80,000	$75,000	$155,000

Chair of Cancer Prevention Research

Prof Michael Kimlin Queensland University of Technology	Joint Cancer Council Queensland /QUT Professor of Cancer Prevention Research	$100,000		$100,000

PhD Scholarship

Mr Nicholas Ashton Queensland University of Technology	Characterisation of the role and regulation of human single-stranded DNA binding protein (hSSB1) post-translational modifications Marylyn Mayo Scholar	$30,000		$30,000
Ms Mary Mihuta Grifith University	Joint CCQ/Griffith University PhD Scholarship: A modern-day approach: assessing the effectiveness of web-based cognitive rehabilitation in cancer survivors	$15,000	$15,000	$30,000
Mr Mark Bettington QIMR Berghofer Medical Research Institute	The histological, immunohistochemical and molecular genetic features of serrated colorectal polyps	$30,000		$30,000
Dr Donald McLeod QIMR Berghofer Medical Research Institute	The role of abnormal thyroid function and autoimmunity in thyroid cancer John Earnshaw Scholar	$30,000		$30,000
Ms Bryony Thompson QIMR Berghofer Medical Research Institute	Validation of bioinformatic predictions and their application to a model for mismatch repair gene variant classification	$15,000		$15,000
Total research funded (continuing research grants)		**$2,848,511**	**$90,000**	**$2,863,582**
TOTAL EXTERNALLY FUNDED RESEARCH PROGRAMS (including new and continuing research grants)		**$6,096,663**	**$757,868**	**$6,760,602**

Internally Funded Research Programs

Viertel Centre for Research in Cancer Control	$3,073,750	$1,000,000	$4,073,750
Epidemiology - Breast Cancer Outcomes Study - Geographical inequalities in colorectal cancer survival	$368,201	$190,891	$559,092
Psycho-oncology - Prostate Cancer and supportive care - Mindfulness intervention for advanced prostate cancer - Proscan for Life - CancerCope	$98,425	$582,155	$680,580
Australian Paediatric Cancer Registry	$99,288		$99,288
Queensland Cancer Registry	$446,545	$910,499	$1,357,044
TOTAL INTERNALLY FUNDED RESEARCH (including new and continuing research grants)	**$4,086,209**	**$2,683,545**	**$6,769,754**

CANCER COUNCIL SOUTH AUSTRALIA

EXTERNALLY FUNDED RESEARCH PROGRAMS

New Research Grants		Cancer Council charitable funding amount 2014	Other funding amount for 2014	Total
BEAT CANCER PROJECT - A joint initiative of Cancer Council SA, South Australian Health and Medical Research Institute, SA Health and University of Adelaide, University of South Australia and Flinders University				
Project Grants				
A/Professor Stuart Pitson	Characterising a highly oncogenic variant of sphingosine kinase 1	$49,519	$49,519	$99,037
Professor Eric Yeoh	Colonic and anal sphincteric dysmotility after radiotherapy for prostate cancer	$49,841	$49,841	$99,681
Dr Caroline Miller	Assessing community knowledge, beliefs and attitudes about sugar-sweetened beverages, including responses to potential regulatory measures aimed at curbing obesity	$50,000	$50,000	$100,000
Hospital Packages				
Professor Guy Maddern	Individualised Risk Assessment and Therapeutic Intervention for Colorectal Cancer in South Australia	$187,500	$562,500	$750,000
Professor David Watson	Flinders Centre for Gastrointestinal Cancer Prevention	$187,500	$562,500	$750,000
Professor Tim Hughes	Advancing T-cell therapy for leukaemia and glioblastoma	$187,500	$562,500	$750,000
Travel Grants	Awarded to 10 recipients	$12,500	$12,500	$25,000
PhD Scholarships	Under review	$15,000	$15,000	$30,000
One Year Infrastructure Grants	To be awarded	$336,250	$1,008,750	$1,345,000
Partnership Grant				
Professor Alex Brown	Cancer Data and Aboriginal Disparities Project	$125,000	$375,000	$500,000
Total research funded		**$1,200,609**	**$3,248,109**	**$4,448,718**
Continuing research grants				
Peter Nelson Leukaemia Research Fellowship				
Dr Hayley Ramshaw		$100,000		$100,000
CCSA Foundation Chair in Cancer Prevention (Behavioural Science)				
Professor Carlene Wilson		$250,000		$250,000
Cancer Genome Facility				
Centre for Cancer Biology SA Pathology - Professor Angel Lopez		$105,000		$105,000
Clinical Cancer Registry				
Clinical Cancer Registry		$132,500	$177,500	$310,000

Research Chair				
Professor Charles Mullighan		$125,000	$375,000	$500,000
Professor David Roder		$125,000	$375,000	$500,000
Professor Ross McKinnon		$125,000	$375,000	$500,000
Principal Research Fellow				
Dr Daniel Worthley	Identifying and targeting the important supportive cells in cancer	$105,000	$315,000	$420,000
Professor Shudong Wang	New therapeutics for cancer treatment	$105,000	$315,000	$420,000
Dr Caroline Miller	Packaging and labeling of tobacco products, food and alcohol	$105,000	$315,000	$420,000
Data Managers Program				
A/Professor Karapetis	Data management support	$8,500	$8,500	$17,000
A/Professor Dusan Kotasek	Data management support	$7,500	$7,500	$15,000
A/Professor Kim Moretti	Prostate cancer data management support	$12,000	$12,000	$24,000
Professor Graeme Suthers	Familial cancer data management support	$22,500	$22,500	$45,000
Dr Heather Tapp	Paediatric data management support	$12,500	$12,500	$25,000
Microarray				
Professor Greg Goodall		$12,000	$12,000	$24,000
Infrastructure Funding				
Mr Andrew Stanley	SANT DataLink	$145,600	$436,800	$582,400
Total research funded		**$1,498,100**	**$2,759,300**	**$4,257,400**
INTERNALLY FUNDED RESEARCH PROGRAMS				
Research Program				
Behavioural Research and Evaluation Unit		$1,099,101	$154,260	$1,253,361
Total research funded		**$1,099,101**	**$154,260**	**$1,253,361**
TOTAL RESEARCH FUNDED (including internally and externally funded research projects)		**$3,797,810**	**$6,161,669**	**$9,959,479**

CANCER COUNCIL TASMANIA

Research Grants

CCTAS NHMRC Grant Associate Professor Jo Dickinson	Familial hematological malignancies: understanding the role of inherited causative factors	$20,000
CCTAS NHMRC Grant Dr Johnson Liu	Targeting drug transporters in colorectal cancer	$10,000

Cancer Council Tasmania Fellowship

Dr Mai Frandsen	Supporting expectant mothers to be smoke free	$95,000

Other

Royal Hobart Hospital	Data Management Clinical Trials	$32,500
Launceston General Hospital	Data Management Clinical Trials	$37,500

Jeanne Foster Scholarship

Caroline Knipe		$1,000
Kym Nutting		$1,000
Susan Wright		$3,000

Cancer Council Tasmania Honours

Alexandra Woodworth		$10,000

Continuing Research Grants

Cancer Council Tasmania Elite Research Scholarship Jessica Phillips	Regulation of Integrins by RUNX transcription factors in cancer	$7,500
NHMRC Associate Professor Jo Dickinson	Genetic aetiology of familial haemotological malignancies	$17,500
NHMRC Associate Professor Jo Dickinson	Combining deep sequencing and linkage approaches to identify rare variants contributing to familial prostate cancers	$17,500
TOTAL RESEARCH FUNDED		**$252,500**

CANCER COUNCIL VICTORIA

EXTERNALLY FUNDED RESEARCH PROGRAMS

		Cancer Council charitable funding amount 2014	Other funding amount for 2014	TOTAL
New Research Grants				
Colebatch Fellowship				
Dr Sherene Loi Peter MacCallum Cancer Centre	Advancing personalised medicine for breast cancer patients	$300,000		$300,000
Mesothelioma Grant				
Dr Thomas John Ludwig Institute for Cancer Research	Melbourne Mesothelioma Research Collaborative	$139,100	$100,000	$239,100
Grant-in-Aid				
Dr Jeffrey Babon Walter and Eliza Hall Institute of Medical Research	The role of SOCS1 in inflammatory disease and cancer	$99,705		$99,705
Dr Walter (Doug) Fairlie Walter and Eliza Hall Institute of Medical Research	Mechanisms of Bcl-2 pro-survival protein regulation in normal physiology, tumourigenesis and drug responsiveness	$100,000		$100,000

Dr Stephan Glaser Walter and Eliza Hall Institute of Medical Research	Investigating the requirement of pro-survival Bcl-2 family proteins in leukaemia	$100,000		$100,000
A/Prof Kieran Harvey Peter MacCallum Cancer Centre	Upstream signalling in the Hippo tumour suppressor pathway	$100,000		$100,000
Dr Duangporn Jamsai Monash University	RNA binding motif 5 is a lung tumour suppressor	$99,418		$99,418
A/Prof Brendan Jenkins Monash Institute of Medical Research	Elucidation of the molecular basis by which the innate immune receptor TLR2 promotes gastric tumourigenesis	$99,649		$99,649
A/Prof Michael Kershaw Peter MacCallum Cancer Centre	Genetic redirection of immunity against cancer	$100,000		$100,000
Dr Daniel Park University of MelbourneUniversity of Melbourne	Mouse phenotype-driven breast cancer risk gene discovery	$98,691		$98,691
A/Prof Richard Pearson University of Melbourne	Targeting PI3K/AKT-dependent cellular senescence to treat cancer	$100,000		$100,000
Dr Leonie Quinn University of Melbourne	Investigating roles for the FUBP family of Myc-regulators and their novel transcriptional targets in coordinating animal growth in vivo	$100,000		$100,000
Dr Oliver Sieber Walter and Eliza Hall Institute of Medical Research	Identification of novel colon cancer genes predictive for prognosis and 5-fluorouracil therapy benefit	$100,000		$100,000
Postdoctoral Fellowship				
Dr Katrina Falkenberg Peter MacCallum Cancer Centre	Identification and characterisation of novel mechanisms of resistance to the histone deacetylae inhibitor, vorinostat	$71,149		$71,149
Dr Francine Ke Walter and Eliza Hall Institute of Medical Research	Investigating the role of the pro-apoptotic BCL-2 family member BOK in intestinal cancer	$71,149		$71,149
Two fellowships to be appointed mid-year		$71,149		$71,149
Vacation Studentships				
17 summer Vacation Studentships were awarded		$30,420		$30,420
TOTAL RESEARCH FUNDED (new research grants)		**$1,780,430**	**$100,000**	**$1,880,430**
Continuing Research Grants				
Carden Fellowship				
Prof Don Metcalf Walter and Eliza Hall Institute of Medical Research	Regulatory control of normal and leukaemic cells	$320,000		$320,000
Dunlop Fellowship				
A/Prof Clare Scott Walter and Eliza Hall Institute of Medical Research	Improvement of ovarian cancer models to support preclinical development of new therapies for ovarian cancer	$300,856		$300,856
Grant-in-Aid				
Dr Michael Buchert Walter and Eliza Hall Institute of Medical Research	Molecular elucidation of PI-3K/mTor pathway as a therapeutic target in inflammation-associated (gastrointestinal) cancers	$97,184		$97,184

Prof Ian Campbell Peter MacCallum Cancer Centre	Identification of novel genes predisposing to familial colorectal cancer by full exome sequencing	$100,000	$100,000
Dr Suzanne Cutts La Trobe University	Tumour-targeted nanoparticles as sensitisers for cancer chemotherapy	$95,902	$95,902
Dr Christine Hawkins La Trobe University	Are direct apoptosis inducers less mutagenic than chemotherapy drugs?	$100,000	$100,000
Dr Nicole Haynes The University of Melbourne	Characterisation of the immunological factors that influence the local and abscopal anti-tumour effects of radiotherapy in preclinical models of solid and metastatic cancer	$99,797	$99,797
Prof John Hopper The University of Melbourne	Mammographic density of young women and their relatives	$99,822	$99,822
Dr Patrick Humbert Peter MacCallum Cancer Centre	The role of cell polarity regulators in mammary gland development and breast cancer	$100,000	$100,000
Prof Ricky Johnstone Peter MacCallum Cancer Centre	Defining the apoptotic and therapeutic activities of histone deacetylase inhibitors	$98,723	$98,723
Dr Kathryn Kinross The University of Melbourne	Mechanisms of resistance to P13K pathway inhibitors in ovarian cancer	$100,000	$100,000
Prof Graham Lieschke Australian Regenerative Medicine Institute	The role of ZBTB11, a novel transcriptional regulator in liver development and the pathogenesis of hepatocellular carcinoma	$100,000	$100,000
Dr Pavel Lobachevsky Peter MacCallum Cancer Centre	Radioprotection by combination of DNA binding antioxidants and aminothiol radical scavengers	$100,000	$100,000
Dr Matthew McCormack Walter and Eliza Hall Institute of Medical Research	Identifying commonality amongst T cell oncogenes	$100,000	$100,000
A/Prof Helena Richardson The University of Melbourne	Regulation of signalling pathways and protein trafficking by Lgl/aPKC	$100,000	$100,000
Prof Andrew Roberts Walter and Eliza Hall Institute of Medical Research	Targeting pro-survival Bcl-2 family proteins for cancer therapy: exploring and defining new applications	$99,736	$99,736
Prof Jamie Rossjohn Monash University	A structural and functional investigator into tumour recognition by NKT cells	$99,891	$99,891
Prof Andrew Scott Ludwig Institute for Cancer Research	siRNA therapies for colorectal cancer	$100,000	$100,000
Prof Arthur Shulkes Austin Health	Targeting proGRP as a therapeutic strategy for gastrointestinal cancers	$98,551	$98,551
Prof Andreas Strasser Walter and Eliza Hall Institute of Medical Research	The role of necroptosis in tumour suppression and the response of malignant tumour cells to anti-cancer therapy	$99,730	$99,730
Prof David Vaux Walter and Eliza Hall Institute of Medical Research	Regulation and function of RIP kinase 1 and RIP kinase 3	$100,000	$100,000
Dr Carl Walkley St Vincent's Institute of Medical Research	Novel approaches to understanding osteosarcoma	$100,000	$100,000

Postdoctoral Fellowship

Dr Sarah To Prince Henry's Institute of Medical Research	Epigenetic regulation of oestrogen biosynthesis in breast cancer	$34,946	$34,946

Dr Michael White Walter and Eliza Hall Institute of Medical Research	Molecular regulation of stem cells and leukaemia	$34,946		$34,946

Postgraduate Scholarship

Mr Dustin Flanagan University of Melbourne	The role of frizzled 7 in colorectal cancer	$32,942		$32,942
Miss Kai Syin Lee Monash University	Evaluating novel targeted therapies for prevention and treatment of squamous cell carcinoma of the skin and head and neck	$32,942		$32,942
Ms Samantha Boyle Peter MacCallum Cancer Centre	Understanding melanoma progression and therapy resistance using in vivo modelling	$19,217		$19,217
Dr Annette Lim University of Melbourne	Defining the molecular landscape of oral tongue squamous cell carcinomas and its impact on patient outcome	$28,924		$28,924
Miss Sarah Sawyer Peter MacCallum Cancer Centre	Translational studies of the genomic variation associated with breast cancer in clinic-based breast and ovarian cancer families	$4,748		$4,748
Ms Soo Hyun Kim University of Melbourne	Breast Cancer Metastasis to Brain: Mechanisms and New Therapies	$28,493		$28,493
Miss Hendrika Duivenvoorden La Trobe University	The role of myoepithelial proteins in blocking breast cancer invasion	$28,493		$28,493
Miss Emma Nolan Walter and Eliza Hall Institute of Medical Research	Identification of Novel Breast Cancer Genes using a Transposon-Based Mutagenesis Screen in Mice	$28,493		$28,493
Miss Antonia Policheni Walter and Eliza Hall Institute of Medical Research	Discovery of cancer genes in lymphomas	$28,493		$28,493
Ms Fiona Chang Monash University	Telomere maintenance mechanism (TMM) in human cancers	$16,513		$16,513
Ms Gemma Ryan Monash University	Designing dendrimer-based lymphatic drug vectors as improved treatent for metastatic cancer	$16,513		$16,513
Support for medical and scientific activities		$116,000		$116,000
Total research funded (continuing research grants)		**$2,945,855**		**$2,945,855**
TOTAL EXTERNALLY FUNDED (including new and continuing research grants)		**$4,726,285**	**$100,000**	**$4,826,285**

INTERNALLY FUNDED RESEARCH PROGRAMS

Research Program	Cancer Council charitable funding amount 2014	Other funding amount for 2014	Total
Cancer Epidemiology Centre	$3,303,000	$3,311,000	$6,614,000
Centre for Behavioural Research in Cancer	$1,409,000	$3,231,000	$4,640,000
Nigel Gray Fellowship Group	$233,000	$642,000	$875,000
Victorian Cancer Biobank		$2,030,000	$2,030,000
Victorian Cancer Registry	$2,629,000	$1,281,000	$3,910,000
TOTAL INTERNALLY FUNDED RESEARCH FUNDED	**$7,574,000**	**$10,495,000**	**$18,069,000**
TOTAL RESEARCH FUNDED (including internally and externally funded research projects)	**$12,300,285**	**$10,595,000**	**$22,895,285**

CANCER COUNCIL WESTERN AUSTRALIA

New Research Grants		Cancer Council charitable funding amount 2014	Other funding amount for 2014	Total
Capacity Building and Collaboration Grant				
W/Prof Eric Moses University of Western Australia and Curtin University	Integrating personalised genomics into risk-stratification models of population screening for cancer	$400,000		$400,000
Research Project Grants				
Dr Bernard Callus University of Western Australia	Establishing a causal relationship between the deletion of the CDKN2A gene in liver progenitor cells and liver cancer thereby providing a genetic mechanism for hepatocarcinogenesis	$100,000		$100,000
Prof Mariapia Degli-Esposti Lions Eye Institute	Establishing a causal relationship between the deletion of the CDKN2A gene in liver progenitor cells and liver cancer thereby providing a genetic mechanism for hepatocarcinogenesis	$100,000		$100,000
W/Prof Wendy Erber University of Western Australia	Megakaryocyte pathology in myeloproliferative neoplasms	$100,000		$100,000
A/Prof Roslyn Francis University of Western Australia	Determining prognosis and treatment response: novel imaging modalities for glioblastoma	$99,985		$99,985
Asst/Prof Juliana Hamzah Harry Perkins Institute of Medical Research	Targeting extracellular matrix in solid tumours	$99,649		$99,649
A/Prof Evan Ingley Harry Perkins Institute of Medical Research	Role of Lyn tyrosine kinase in blood development and disease	$100,000		$100,000
Prof Peter Leedman Harry Perkins Institute for Medical Research	Investigating the mechanisms for treatment resistance in head and neck cancer	$100,000		$100,000
A/Prof Robert McLaughlin University of Western Australia	Intra-operative assessment of lymph node metastasis in breast cancer with optical imaging	$99,937		$99,937
Clin/Prof Bill Musk University of Western Australia	From asbestos miners to DIY home renovators: understanding the consequences of changing patterns of asbestos exposure	$58,000		$58,000
Prof Anna Nowak University of Western Australia	Outcomes and predictors of efficacy of palliative radiotherapy in patients with malignant pleural mesothelioma	$99,679		$99,679
Early Career Investigator Grants				
Dr Claire Harma Sir Charles Gairdner Hospital	Morphology and molecular profiling of interval colorectal cancers	$21,000		$21,000
Dr Suzanne Mashtoub University of Western Australia	Emu oil and protection from inflammation-associated colorectal cancer	$25,000		$25,000
Asst/Prof Katie Meehan University of Western Australia	Sensitive blood-based monitoring of breast cancer	$25,000		$25,000

Dr Elke Seppanen Telethon Institute for Child Health Research	Adjuvant therapy in a preclinical melanoma brain tumour model	$25,000	$25,000

Research Fellowships

A/Prof Pilar Blancafort University of Western Australia	Epigenetic tailoring of the cancer genome: novel targeted strategies for the treatment of aggressive breast cancer	$20,000	$20,000
Prof Daniel Galvao Edith Cowan University	Improving health outcomes after cancer through exercise: a survivorship program	$80,000	$80,000

Postdoctoral Fellowship

Dr Angela Ives University of Western Australia	Upper gastro-intestinal surgery as treatment for cancer: what influences its use and outcomes?	$75,000	$75,000

PhD Top Up Scholarship

Ms Joanne Gardner Curtin University	Does aging impact on anti-cancer immune responses?	$12,000	$12,000

Honours Scholarships

Ms Courtney George Telethon Insitute for Child Health Research"	The role of DACH1 and the mir-200 family in medulloblastoma pathogenesis	$2,000	$2,000
Ms Tenielle George Curtin University	Recycling of melanoma cell adhesion in melanoma metastasis	$7,500	$7,500
Ms Sarah Henn Curtin University	Assessing the therapeutic and immunological consequences of anti-vascular agents in lung cancer and malignant mesothelioma	$5,500	$5,500
Mr Brett Patterson Telethon Insitute for Child Health Research	Examining the efficacy of a PI3K inhibitor (BKM120) on pineoblastoma and medulloblastoma cell lines using in vitro and in vivo models	$7,500	$7,500
Ms Olivia Ruhen University of Western Australia	Sensitive blood-based monitoring of breast cancer	$7,500	$7,500

Vacation Scholarships

Mohammad Rizwan bin Hussain Ahamed University of Western Australia	Synthesis of fluorescent multifunctional calix[4]arene nanobaskets to study their cellular uptake in ovarian cancer cell lines	$3,000	$3,000
Mr Jeremy Duczynski University of Western Australia	Better cancer detection using gold nanoparticles as optical contrast agents	$3,000	$3,000
Ms Sarah Howarth Harry Perkins Institute of Medical Research	Investigating the novel funtional role of the metalloproteinase ADAM19 in human prostate cancer	$3,000	$3,000
Mr Yu Jie Kan Curtin University	Design of peptide-cisplatin conjugates as novel molecular cancer therapeutics to target breast and ovarian tumours	$3,000	$3,000
Ms Hayley Peel Curtin University	CT virtual colonoscopy screening for colorectal polyps: a phantom study for optimal low-dose scanning protocols in the early detection of colorectal cancer	$3,000	$3,000
Ms Natalie Seed Harry Perkins Institute of Medical Research	Does passive smoking cause cancers of the kidney and bladder? A systematic literature review and meta-analysis	$3,000	$3,000

Ms Rebecca Weselman University of Western Australia	Translational genomics for stratification of mantle cell lymphoma	$3,000		$3,000
Ms Serena Wong Siew Yee University of Western Australia	Synthesis and testing of the anticancer properties of the novel vitamin D derivative 20,25-dihydroxyvitamin D3	$3,000		$3,000
James Crofts Hope Foundation Vacation Scholarships				
Mr Brett Patterson Telethon Insitute for Child Health Research	Determining the efficacy of a PI3K inhibitor, BKM-120, in combination with conventional chemotherapy for treatment of childhood brain cancer.	$3,000		$3,000
Ms Olivia Ruhen University of Western Australia	The genetics of glioblastoma: towards translation into routine diagnostic laboratory practice	$3,000		$3,000
John Nott Cancer Fellowship Travel Support Fund				
Irmgard Irminger-Finger University of Geneva, Geneva Switzerland	Together with leading cancer researchers in Peth Dr Irminger-Finger will set up and promote a translational cancer research program focusing on genes that supress tumours	$9,000		$9,000
Travel Grants		$15,000		$15,000
Awards				
Dr Terry Boyle Harry Perkins Institute of Medical Research	Cancer Council WA Early Career Cancer Researcher of the Year	$10,000		$10,000
Prof Ruth Ganss Harry Perkins Institute of Medical Research	Cancer Council WA Cancer Resarcher of the Year	$20,000		$20,000
W/Prof D'Arcy Holman University of Western Australia	Cancer Council WA Cancer Research Career Achievement Award	$20,000		$20,000
TOTAL RESEARCH FUNDED		**$1,774,250**		**$1,774,250**
Continuing research grants				
Research Project Grants				
W/Prof Cameron Platell University of Western Australia	Predicting response to neo-adjuvant chemo-radiotherapy in patients with resectable rectal cancer	$100,912		$100,912
STREP				
A/Prof Gail Garvey Menzies School of Health Research	To improve cancer control for Indigenous Australians	$100,000	$395,170	$495,170
NHMRC Partnership Grant				
Prof Lin Fritschi Curtin University	The Extended Australian Workplace Exposures Study (AWES2)	$20,000	$208,166	$228,166
Research Fellowships				
Prof Lin Fritschi Curtin University	Occupational cancer epidemiology	$20,000		$20,000
A/Prof Archa Fox Harry Perkins Institute of Medical Research	Novel gene regulation targets for cancer therapy	$100,000		$100,000
Prof Steven Mutsaers Lung Institute of Western Australia	Small non-coding RNAs in malignant mesothelioma	$80,000		$80,000

Clinical Research Fellowship			
A/Prof Andrew Redfern Royal Perth Hospital	Clinical Research Fellowship in Cancer at Royal Perth Hospital	$100,000	$100,000
Postdoctoral Fellowships			
Dr Prue Cormie Edith Cowan University	Exercise as medicine for the management of cancer	$75,000	$75,000
Dr Anna Johansson Harry Perkins Institute for Medical Research	Targeting of LIGHT to tumour vessels for anti-cancer combination therapy	$75,000	$75,000
Asst/Prof Claire Johnson University of Western Australia	A program of research to optimise quality of care for people with cancer and their families: A peer review framework to promote best practice in multi-disciplinary cancer teams in Australia	$75,000	$75,000
Cancer Pathology Postdoctoral Fellowship			
University of Western Australia	Translational Pathology Research in Cancer	$75,000	$75,000
PhD Top Up Scholarship			
Mr Samuel Taylor University of Western Australia	Tyrosine kinase inhibitors for treating c-Cbl and Flt3-driven leukaemias	$12,000	$12,000
Lions Cancer Institute PhD Top Up Scholarshp			
Mr Philip Hardy University of Western Australia	Identifying chromosomal and molecular aberrations that correlate to various stress events in human and mouse liver models	$12,000	$12,000
Chairs			
Curtin University	Chair in Behavioural Research and Cancer Control	$160,000	$160,000
W/Prof Michael Millward University of Western Australia	Chair in Clinical Cancer Research	$341,295	$341,295
Bone Tumour Registry		$5,000	$5,000
Total research funded		**$1,351,207**	**$1,954,543**
TOTAL RESEARCH FUNDED (including new and continuing research grants)		**$3,125,457**	**$3,728,793**

AUSTRALIAN BEHAVIOURAL RESEARCH IN CANCER

Behavioural Research and Evaluation Unit (BREU) Cancer Council South Australia

Does sun protection policy influence comprehensiveness of sun protection practices in primary schools?

Research has shown that the SunSmart program has contributed to a long-term increase in daily sun protective practices for students participating in the program. Primary schools with SunSmart membership have more comprehensive policies and higher compliance with sun protection practice recommendations than schools without SunSmart membership. However, in 2005, 80% of schools had a written sun protection policy, whereas 52% of schools had SunSmart status, suggesting that there may be a gap between having a written policy and meeting best practice recommendations for sun protection.

A study was conducted at Cancer Council SA based on the results of the most recent National Primary Schools Sun Protection Survey (2011/12) to examine the relationship between having a written sun protection policy (and if so, how comprehensive this policy is) and the comprehensiveness of school sun protection practices. The influence of SunSmart status and school demographics upon this relationship was also investigated. The survey was distributed to 1573 randomly selected schools nationally that catered to primary school-aged students. It was completed by 857 schools, giving a response rate of 57%. The survey included items surrounding the existence of a written sun protection policy at the school and the types of items included in the policy, as well as the school's sun protection practices for key sun protection areas (hat-wearing, uniform, sunglasses, use of sunscreen, shade availability and consideration of UV levels for outdoor activities).

The study found that schools with a written sun protection policy had more comprehensive sun protection practices in place than schools without a policy. This relationship was stronger for remotely located schools and for schools catering to both primary and secondary students. Furthermore, the comprehensiveness of written policy was associated with comprehensiveness of sun protection practices. Membership in Cancer Council's National SunSmart Schools Program was found to indirectly affect comprehensiveness of sun protection practices i.e. members tended to have comprehensive written policies in place, which is strongly associated with having comprehensive practices. The findings further reinforce the importance of written sun protection policies for the implementation of comprehensive sun protective practices in primary schools and membership in the SunSmart program. The study was recently published in the journal *Health Education Research.*

Evaluation of the Give Up Smokes for Good campaign among Aboriginal students

Cancer Council SA is undertaking an evaluation of a school-based anti-tobacco social marketing campaign aimed at reducing smoking initiation and promoting smoking cessation among Aboriginal youth. The evaluation is being funded by Drug and Alcohol Services South Australia, who have developed the Give Up Smokes for Good social marketing campaign. This campaign is part of the Tackling Smoking program, a public health initiative that aims to reduce the smoking rate among Aboriginal communities in South Australia.

A partnership between Drug and Alcohol Services South Australia and the Department of Education and Child Development allowed the Give Up Smokes for Good social marketing campaign to reach Aboriginal students attending the South Australian Aboriginal Sports Training Academy. As a result of the partnership, approximately 300 Aboriginal students have been exposed to the Give Up Smokes for Good campaign materials and anti-tobacco educational materials, which appear throughout the students' workbooks.

The purpose of the evaluation is to assess whether there have been any changes in the smoking-related attitudes and behaviours of students as a result of being exposed to the campaign. The outcome of the campaign is being evaluated with a pre-post survey design. The first phase of data collection took place at the beginning of the school year. Students are currently being followed-up to take part in the post-survey.

Centre for Behavioural Research in Cancer (CBRC), Victoria

Evaluation of tobacco plain packaging

In December 2012, Australia became the first country to implement plain packaging of tobacco, accompanied by refreshed graphic health warnings covering 75% of the front-of-pack. Over the past two years, CBRC has been undertaking a raft of studies to evaluate aspects of the legislation. These include government-funded survey research to track responses of adult smokers and recent quitters over time, and to study early effects on adolescents, the results of which are scheduled to be reported in late 2014. CBRC has also undertaken a national panel study of tobacco retail outlets, funded by Quit Victoria, Cancer Council and other UK funders. Published papers from that dataset have so far demonstrated no change in the time taken to serve tobacco (Wakefield et al. Addiction 2013) and continued extremely low prevalence of willingness-to-sell illicit tobacco in small mixed business retail outlets (Scollo et al. Tobacco Control 2014). A third study, found a decline in the rate of cigarette pack display at outdoor café strips in Melbourne and Adelaide (which do not yet have outdoor smoking bans) with the introduction of plain packaging (Zacher et al. Addiction 2014). Overall, these early published studies suggest the plain packaging legislation is working as intended, with few of the adverse outcomes predicted by the tobacco industry. Many more papers in the pipeline from these datasets, along with

the studies of other researchers, will provide further key information about the progress of the policy.

Population-based evaluation of the WA 'LiveLighter' obesity campaign

The 'LiveLighter' campaign is targeted toward adults aged 25-64 and aims to increase awareness and understanding of the health consequences of overweight and to encourage the adoption of simple changes towards leading a healthier lifestyle. Phase I graphically depicts visceral fat of an overweight individual, while supporting ads demonstrate simple changes to increase physical activity and eat healthier. Phase II reminds viewers of this visceral imagery and focuses on the contribution of sugar-sweetened beverages to its development and ultimately disease. Phase I was launched in WA in June 2012 and Phase II in July 2013. The television campaign is complemented by cinema, radio, print and online advertising and a website (www.livelighter.com.au). The evaluation aimed primarily to measure proximal outcomes of campaign recall and perceived effectiveness. In addition, the population-level impact on awareness and attitudes related to diet and physical activity and more distal outcomes of contemplating and making behaviour changes. At Phase I, cross-sectional population surveys (25-49 years) were undertaken pre-campaign (May/Jun N=2013) and following the two media waves (Jul/Aug and Sept/Oct N=2010 each) in the intervention (WA) and comparison states (Victoria). Phase II employed a cohort design with a population survey of WA adults undertaken pre-campaign (May/Jun 2013 N=1504) and followed after each of the two media waves (Aug/Sept N=822 and Oct/Nov N=557). Findings have been provided to the National Heart Foundation (WA), undertaking the campaign in partnership with Cancer Council WA.

Newcastle Cancer Control Collaborative (New-3C), NSW

Cancer patients' preferences and priorities for health service initiatives

Consumer involvement in developing health policy and evaluating services is widely recognised as important for promoting patient-centred care, and is incorporated into National Health and Medical Research Council and Institute of Medicine guidelines. The Consumer Preferences Survey was developed to enable consumers to participate in quality evaluation activities. The electronic touch-screen survey asks participants to select up to 23 general service changes that could improve their experiences when: 1) making an appointment; 2) arriving at the clinic; 3) during an appointment; and 4) managing their condition at home. The interactive survey also allows participants to select up to 107 specific initiatives based on their previous responses. A pilot test of the survey was completed to examine patients' preferences for health service changes and acceptability of the survey. A total of 675 individuals with a chronic illness, including 400 oncology patients, completed the survey while attending an outpatient clinic. Preliminary results suggest oncology patients select an average of two general service changes. Examples of frequently identified initiatives include: scheduling convenient appointment times; improving parking; reducing clinic wait times; providing opportunities to discuss concerns with a health care provider; providing up-to-date and personalised information on condition and treatment progress; and information about how to handle a medical emergency. Participants reported the survey was easy to complete and comprehensive. Time to complete was 8.5 minutes and 85% of participants indicated they would be willing to complete a similar survey in the future. The Consumer Preferences Survey can be used to guide patient-centred changes within health services, and provides a list of patient prioritised targets. This offers an alternative and reliable method to introduce strategic initiatives to medical oncology outpatient services with the objective of improving patient-centred care. This tool will be used in an Australian Research Council funded project conducted by the Priority Research Centre for Health Behaviour at the University of Newcastle, and Cancer Council New South Wales.

Prevalence and predictors of anxiety and depression among haematological cancer patients

A cross-sectional study was conducted to explore the prevalence of, and factors associated with, anxiety and depression among patients with haematological cancer. Outpatients (n=304) with a confirmed diagnosis of haematological cancer were recruited from three haematology clinics across Australia. Participants completed a self-report survey which included questions about demographic characteristics, disease and treatment characteristics, and the Hospital Anxiety and Depression Scale. The prevalence of anxiety and depression among participants was 27% and 17% respectively. More specifically, 12% of haematological cancer patients reported experiencing both anxiety and depression, 15% reported anxiety only, and 5% reported depression only. Participants who had to relocate to receive treatment had almost three times the odds of reporting anxiety and/or depression compared to those who did not have to move. Former smokers also had significantly greater odds of reporting anxiety and/or depression. These findings suggest that the proportion of haematological cancer outpatients experiencing psychological distress may be much higher than in the general population. Additional psychological support may need to be provided to former smokers and to patients who have to relocate for treatment.

Cancer Council Queensland Viertel Centre for Research in Cancer Control (VCRCC)

Survivor study

Around 128,000 new cases of cancer will be diagnosed in Australia this year, with that number set to increase to 170,000 by 2025 (Baade, Meng, Sinclair, and Youl, 2012; Smith et al. 2007). At the same time, more people are surviving cancer today than ever before – around 66 per cent of people diagnosed with cancer in Australia survive for at least five years after their diagnosis (Australian Institute of Health and Welfare, 2012). The combination of increasing cancer incidence and longer survival means that more and more people are living with a diagnosis of

cancer. However, while more people are now surviving cancer, they are not necessarily surviving well. The need to understand the challenges faced by cancer survivors and how to address these is becoming more and more pressing. A significant proportion of cancer survivors experience ongoing difficulties including physical, psychological and practical problems related to the diagnosis and/or treatment of their cancer, and there remain unmet needs for supportive care. The Survivor Study is a community-based study, led by Cancer Council Queensland, assessing the needs and concerns of cancer survivors. Respondents are currently being recruited in order to investigate the emotional, physical and practical concerns of cancer survivors and whether they have received care for those concerns. Of particular interest is whether the concerns of cancer survivors and the receipt of supportive care differ based on geography. It is anticipated that the information provided will facilitate the development of improved support programs for this growing group of Australians.

CANCER COUNCIL AUSTRALIA

Cancer Council hosts World Cancer Congress

Cancer Council Australia is proud to be hosting the World Cancer Congress, which is coming to Australia for the first time in December.

To be staged at the Melbourne Convention and Exhibition Centre, the Congress will be held in conjunction with the Clinical Oncology Society of Australia's Annual Scientific Meeting.

With a theme of 'Joining forces - accelerating progress', the World Cancer Congress will bring together cancer control experts and leaders in global health to find solutions and actions to reduce the impact of cancer world-wide.

The theme for Clinical Oncology Society of Australia's 41st Annual Scientific Meeting is 'Cancer survivorship, supportive care and palliative care', with a disease focus on lung cancer and metastases.

The Clinical Oncology Society of Australia Annual Scientific Meeting will be held 2-4 Dec and the World Cancer Congress 4-6 Dec, with 4 Dec being a joint day.

From policy, fundraising and cancer control, through to treatment, prevention and palliative care, these events will appeal to a wide range of professionals working with cancer.

Discounts are available for those interested in attending both events.

New report shows cancer the No. 1 global killer

A global scientific report released to coincide with World Cancer Day (4 February) showed cancer was the biggest cause of mortality worldwide, responsible for 8.2 million deaths per year and rising.

The World Cancer Report also predicted that cancer incidence would increase by 75% over the next two decades, exceeding 20 million new cases a year in 2025.

Cancer Council Australia spokesperson, Terry Slevin, said reasons for the increase varied in different countries. "Australia has one of the world's highest cancer incidence rates, third in the world behind Denmark and France, largely because of our ageing population," Mr Slevin said.

"Australians are living longer than previous generations, thanks to improved infection and cardiovascular disease control. Unfortunately, cancer is a disease that is more likely to affect us later in life, so the longer Australians live, the more cancer cases we see".

"Extended life expectancy in the developing world is also increasing cancer rates globally. Unfortunately, developing countries are also adopting the worst of our western lifestyle, such as smoking, poor diet and inactivity, which is significantly contributing to global cancer prevalence.

"We need to act as a global community and do what we know works to reduce the cancer burden – promoting a healthy lifestyle, evidence-based screening programs, and access to life-saving medicine."

Screening mammography saves lives

Cancer Council Australia confirmed that the weight of scientific evidence shows mammographic screening for breast cancer is a lifesaving public health intervention irrespective of a Canadian study that questions mortality benefit.

Cancer Council Australia CEO, Professor Ian Olver, said the Canadian study, published in the *British Medical Journal* in February, was not relevant to the Australian context.

"The Canadian study found no mortality benefit for women aged 40 to 59 undergoing annual mammograms," Professor Olver said. "However, evidence shows mammography as a screening tool is most beneficial for women aged 50 and over, undertaken every two years.

"So you are comparing apples with oranges if you try to apply conclusions from the Canadian study to Australia, where mammography is appropriately targeted through the BreastScreen Australia program.

"The Canadian study also looked at screening over five years, while the most comprehensive study of BreastScreen reviewed mammography outcomes over 15 years and found an overall mortality benefit between 21 and 30 per cent, at existing screening participation levels.

"The message is simple: the weight of scientific evidence supports mammography as a population screening tool,

and many Australian women are alive today thanks to 20 years of BreastScreen."

Cancer Council Australia congratulates UK on tobacco plain packaging progress

Cancer Council Australia has congratulated the UK Government for taking another step closer to introducing plain packaging for tobacco.

Professor Olver said the UK Government's announcement that the case for plain packaging was 'compelling' and that it would soon table draft regulations was a victory for evidence over scaremongering.

He said public health groups worldwide would welcome the UK's response to the independent Chantler review of the evidence on plain packaging.

"Having had this experience in Australia, it was clear that the most vocal opposition came from those who want to sell cigarettes and a few uninformed ideologues," he said.

"The only credible, independent research on the effects of plain packaging in Australia shows it is doing what 20 years' worth of analysis suggested it would achieve – making smoking far less appealing to young people."

Professor Olver said tobacco companies had produced numerous reports claiming that plain packaging would not work.

"The louder the tobacco industry screamed, the more it showed that plain packaging was a threat to their attempts to lure and addict new smokers," he said.

"Today's newly addicted smokers are tomorrow's cancer patients."

Recommended changes to cervical screening good news for Australian women

Cancer Council has welcomed recommended changes to Australia's cervical screening program announced by Australia's Medical Services Advisory Committee.

Professor Olver said evidence showed a new HPV (human papillomavirus) test every five years, which is recommended to become the primary cervical screening tool, would be more effective than the Pap test and just as safe.

Professor Olver emphasised that the proposed changes were recommendations only and that women should continue to have Pap tests every two years for now. Pending decisions by government, it is likely the changes would not be implemented before 2016.

"The Pap test based screening program has been a great public health success story since its introduction in 1991 and is the main reason cervical cancer mortality rates in Australia are among the world's lowest," Professor Olver said.

"In its first 10 years, the Pap test based program reduced mortality by 50%, a figure that plateaued in the subsequent decade. The HPV test is predicted to further reduce mortality by 15%."

Professor Olver said the changes should also include improved targeting of the program to Indigenous women, who have not shared equitably in Australia's cervical cancer successes.

"Cancer Council has been formally involved in the renewal of the screening program, and the review team has undertaken a comprehensive analysis of the evidence."

Mixed messages on DNA test a risk to bowel cancer screening

Cancer Council is urging GPs to encourage asymptomatic patients 50 or over to screen for bowel cancer with a faecal occult blood test (FOBT), which remains the gold standard for population screening.

The advice is aimed at addressing 'mixed messages' in the Australian media about a new blood test for bowel cancer, which is only a third as sensitive for advanced adenomas and stage one cancer as immunochemical FOBT.

Cancer Council Australia CEO, Professor Ian Olver, said the evidence clearly showed that FOBT was the most effective tool for bowel cancer screening.

"New biomarkers for major disease usually attract media coverage, but it's important to remain focused on the evidence," Professor Olver said. "As the developers of the DNA test have noted, it could have a role as an adjunct to FOBT."

Professor Olver stressed that the evidence, including major pilot programs in Australia, overwhelmingly supported FOBT as the best population screening tool.

Professor James St John, gastro-enterologist, researcher and longstanding member of Cancer Council's National Cancer Screening Committee, said the FOBT-based national program, when fully implemented, had the potential to prevent 70,000 bowel cancer deaths over the next four decades.

"Identifying cancer and precancerous conditions early is the key to effective screening," Professor St John said.

Immunochemical FOBT are around three times more sensitive for advanced adenomas and stage one bowel cancers than the blood-based DNA test. On a population basis, this can make an enormous difference to mortality and morbidity.

CLINICAL GUIDELINES NETWORK

Cancer Council Australia aims to produce concise, clinically relevant and up-to-date electronic clinical practice guidelines for health professionals and is the core activity of the Clinical Guidelines Network. Its custom designed, collaborative Cancer Guidelines Wiki platform (wiki.cancer.org.au) facilitates the guideline development and revision processes.

New guidelines in development

Clinical practice guidelines for the diagnosis and management of Barrett's oesophagus and mucosal neoplasia

Working party authors met to review and finalise the draft guidelines on the Cancer Guidelines Wiki for release for public consultation in May. Relevant organisations, experts and interested parties were consulted. The final guidelines should be established and launched as online guidelines later this year.

Clinical practice guidelines for PSA testing and management of test-detected prostate cancer

These guidelines are undergoing systematic literature review. Cancer Council Australia, together with the Prostate Cancer Foundation of Australia, are planning to release the draft guidelines for public consultation at the UICC World Congress in December 2014.

Guidelines under revision

Clinical practice guidelines for the prevention, diagnosis and management of lung cancer

The prevention and diagnosis section of the 2004 guidelines is planned for revision and will be updated as online guidelines on the Cancer Guidelines Wiki this year.

The Lung Cancer Screening Guidelines Working Party has developed topic groups, key clinical questions and search strategies.

Clinical practice guidelines for the management of melanoma

Planning for the revision of the 2008 melanoma guidelines began earlier this year with the guideline scope focusing on treatment of melanoma (including diagnosis and follow-up).

The literature review is to be informed by the German S3 Melanoma Guidelines, which Cancer Council Australia is looking to adapt for this revision.

Completed guidelines

Clinical practice guidelines for the management of adult onset sarcoma

These recently launched guidelines are designed to be used as a resource for the sarcoma community, both clinicians and consumers, and to help to assist in identifying priority research areas. Paediatric and gynaecological topics will be considered for inclusion in future iterations.

The working party will meet in November to review any changes made following literature updates since the development of the guidelines.

Clinical practice guidelines for the prevention, diagnosis and management of lung cancer

These guidelines focus on the revision of the treatment section of the 2004 guidelines, comprising management of non-small cell lung cancer and small cell lung cancer topics.

Clinical practice guidelines for the treatment and management of endometrial cancer

These guidelines focus on the management and treatment of apparent early stage low risk and high risk endometrial cancer.

Additional resources

Algorithms for colonoscopic surveillance intervals in adenoma follow-up; following curative resection of colorectal cancer, and for colorectal cancer screening (family history).

Algorithms based on the *Clinical practice guidelines for surveillance colonoscopy* and the *Clinical practice guidelines for the prevention, early detection and management of colorectal cancer* have been developed and reviewed.

The algorithms are now available on the Cancer Guidelines Wiki to accompany the clinical practice guidelines as a derivative resource for health professionals working in this specialty area.

- Algorithm for colonoscopic surveillance intervals – adenomas
- Algorithm for colonoscopic surveillance intervals – following surgery for colorectal cancer
- Algorithm for colorectal cancer screening – family history

An algorithm for colonoscopic surveillance intervals in inflammatory bowel disease is being planned.

For more information regarding clinical practice guidelines contact the Clinical Guidelines Network Manager on 02 8063 4100.

Clinical practice guidelines can also be accessed from Cancer Council Australia's website at cancer.org.au/clinicalguidelines.

CLINICAL ONCOLOGY SOCIETY OF AUSTRALIA, COSA

Annual Scientific Meeting (ASM)

The theme for COSA's 41st ASM will highlight cancer survivorship, supportive care and palliative care – all important areas of interest for COSA members and delegates. Our disease themes will focus on lung cancer and metastases, particularly oligometastases.

Building on the strength of previous meetings and to appeal to COSA's multidisciplinary membership, the Local Organising Committee led by our expert convenor, Mei Krishnasamy, is keen to ensure the inclusion of broad content in every session. Many international experts have confirmed their participation in the program.

- Director of the Cancer Survivorship Program at Memorial Sloan-Kettering Cancer Center, Mary McCabe, has kindly agreed to present on her expertise in standards of survivorship care and the ethics of access to care.
- Professor Harvey Pass from the NYU Langone Medical Center is a surgeon scientist whose work focuses on the early detection, surgical management, and adjuvant therapy of thoracic malignancies. Professor Pass will present on new techniques in the surgical management of lung cancer.
- Dr Sumitra Thongpreasert, a medical oncologist from Chiang Mai in Thailand, will discuss the challenges of treating lung cancer patients in the Asian region.
- Professor Jim Bishop AO will present an Australian perspective on comprehensive cancer centres during his Presidential Lecture on Thursday 4 December.

A full list of confirmed speakers and the draft program are available at www.cosa2014.org

As previously reported, the COSA ASM will be held in conjunction with the Union for International Cancer Control World Cancer Congress in the first week of December at the Melbourne Convention and Exhibition Centre. The COSA ASM will run Tuesday 2nd to Thursday 4th December, and World Cancer Congress 4th to 6th December, with Thursday 4th being a joint day. Discounts are available for delegates attending both events.

Collaboration in cancer control and research

COSA attended two important national meetings in March – the Cancer Drugs Alliance Forum and the Australia Clinical Trials Alliance.

The newly formed Cancer Drugs Alliance held a forum in Canberra on 26-27 March. Delegates at the forum included consumers, clinicians and industry, with COSA represented by its Executive Officer. This is the first time this breadth of stakeholders across our cancer community has come together. The forum reinforced the seriousness of the cancer challenge and the need for a more collaborative, proactive approach going forward. The key issues explored were: how we ensure the consumer voice truly gets heard; how we help inform evidentiary requirements; and how we shape a fit-for-purpose Australian system – one that is equitable, affordable and sustainable. The alliance has committed to tapping into the expertise and passion of those involved and will support this collaboration by forming working groups to explore the priorities identified:

- ensuring a meaningful and impactful public and consumer voice
- system improvement
- innovative access models
- reducing red tape.

A national summit of investigator-initiated clinical trials networks was hosted by the Australian Clinical Trials Alliance in Melbourne in March. Many COSA members and representatives of the COSA Affiliated Cancer Cooperative Trials Groups were in attendance. The meeting objectives included providing an opportunity to discuss innovative opportunities for increasing the impact of investigator-initiated clinical trials and the capacity of collaborative networks to answer important clinical questions and provide better evidence to support the delivery of high-quality health care. It is evident that cancer has a long history in running investigator initiated trials through the Cancer Cooperative Trials Groups, yet there are always opportunities to learn from other disease areas and share knowledge.

Marie Malica
Executive Officer, COSA

MEDICAL ONCOLOGY GROUP OF AUSTRALIA, MOGA

The Medical Oncology Group of Australia Incorporated (MOGA) is pleased to report on another successful quarter. Membership of the Association continues to grow with the number of trainees entering speciality training in medical oncology through the Royal Australasian College of Physicians growing annually, consultant membership running at an all-time high as new Fellows have joined the Association and increasing numbers of members participating in Association initiatives. Currently, there are 411 consultant and 200 trainee members of the Association.

Oncology drugs, treatments and advocacy

The MOGA Oncology Drugs Working Group, set up to pursue oncology drugs and treatment matters, as

well as meet quarterly with the Pharmaceutical Benefits Advisory Committee as a clinical advisory body, is now well into its third year of operations. The Association has continued to work closely with regulatory agencies on long standing matters such as amending indications to reflect clinical practice for off patent drugs and strategies to address national drug shortages. The Association has been working with the Therapeutics Goods Administration (TGA), the pharmaceutical industry through Medicines Australia and the Generic Medicines Industry of Australia, as well as other professional groups, for some months to develop an initiative to better communicate and manage the effects of drug shortages. One outcome of this work has been the development of a searchable website and email alert service by the TGA to provide information from the pharmaceutical industry about current and anticipated shortages. The website also provides information to minimise the impact on patients' continuing health care.

2014 Annual Scientific Meeting

Integrating Molecular and Immunologic Advances into Practice is the theme of the 2014 MOGA Annual Scientific Meeting (Sydney Hilton, 6-8 August; Best of ASCO Australia, 9 August). Professor Paul de Souza, Professor and Foundation Chair, Medical Oncology, School of Medicine University of Western Sydney and Director, Medical Oncology, Liverpool Hospital, is the Meeting Convenor. Along with the planning team, he has organised an innovative meeting program that explores many of the contemporary challenges and advances in medical oncology research, discovery and clinical practice. The meeting's focus on immunology, immunotherapy, biomarkers and genomics provides a timely opportunity for Australian medical oncology practitioners to review the role they play in the management of patients with cancer and how they guide drug development, as well as impact on targeted therapy. International guest speakers, Professor Alison Stopeck (US), Professor James Gulley (US) and Professor Klaus Pantel (Germany) will provide a range of perspectives on molecular and immunologic advances and related scientific and research trends. Sessions on specific tumour types will include a symposium on lung cancer with international speaker Professor Ramaswamy Govindan (US). The presidents of the major medical oncology professional organisations in Japan, Singapore and Korea will also be attending the meeting as part of the Association's international and regional collaborative and networking activities.

YOGA

At this year's ASM, MOGA will be launching the Young Oncologists Group of Australia (YOGA). Established by three young consultant members of the Association, Drs George Au-Yeung, Deme Karikios and Hui-li Wong, YOGA aims to provide young medical oncologists who have attained their fellowship within the last five years and are members of the Association with a networking framework and assistance to facilitate their transition from advanced trainees to consultants. The group has developed a special education program to be held at the MOGA ASM.

ACORD

The Association has received a record number of applications for the 10th Anniversary Australia and Asia Pacific Clinical Oncology Research Development Workshop (ACORD) to be held in September. ACORD has continued to grow as a major international oncology education program, with increased support from long-standing and collaborating partners: the American Association for Cancer Research, the American Society of Clinical Oncology, the European Society for Medical Oncology, Cancer Council Australia, Clinical Oncology Society of Australia, Cancer Australia, the US National Cancer Institute and the Cancer Council NSW.

To facilitate workshop applications from across the Asia Pacific region and spread clinical trials protocol development skills, late in 2013, Professor Martin Stockler, ACORD Convenor and, previous ACORD Faculty member, Dr Andrew Martin from the NHMRC Clinical Trials Centre, University of Sydney, presented Turning Good Ideas into Successful Studies…Getting Started in Clinical Research: Writing a Concept Outline to start the Clinical Trails Process Workshop. This series of six one day workshops aimed to help early career researchers in India and Pakistan turn their new ideas for cancer clinical research studies into persuasive one-page research concept outlines

Associate Professor Gary Richardson
Chairman, MOGA

FACULTY OF RADIATION ONCOLOGY, RANZCR

According to a report released on World Cancer Day, cancer is now the leading cause of death worldwide. As medical professionals providing cancer care, we have huge challenges ahead. The Faculty is committed to working closely with all stakeholders to advocate for increased and improved services. We also need to take any possible opportunity to influence decision making from hospital to government levels, to improve healthcare in Australia and New Zealand.

Change of Dean in Faculty of Radiation Oncology

In March, Professor Gill Duchesne decided to step down as Dean of the Faculty of Radiation Oncology, due to personal reasons. I am honoured to be appointed by the RANZCR Board to fill the casual vacancy until the end of 2014.

Prof Duchesne has made significant contributions to

the running of the Faculty and the College by serving on Council/Board since 2001. She has demonstrated dedication to and impeccable leadership in various Faculty initiatives – including the Tripartite Radiation Oncology Practice Standards, the Tripartite National Strategic Plan for Radiation Oncology and the recent Radiation Oncology Targeting Cancer Campaign. The Faculty and the College are very appreciative of Prof Duchesne's tireless work and significant contributions over the last 13 years at both Victorian and bi-national levels.

I am currently the Head of Radiation Oncology at Liverpool and Campbelltown Hospitals in south western Sydney. I have been on the Faculty Board/Council since 2011, and have been the Chair of the Economics and Workforce Committee since that time. I have had the opportunity to assume leading roles in the Faculty's Horizon Scanning and the annual Radiation Therapy Innovation Summit, as well as work related to radiation oncology funding and workforce issues.

I look forward to leading the Faculty and working closely with all stakeholders to advocate for optimal patient care that is safe, accessible, efficient, affordable and of the highest quality.

Radiation therapy innovation summit and stakeholder forum

The Faculty, on behalf of the Radiation Oncology Tripartite Committee, hosted an Innovation Summit, as well as a Tripartite Stakeholder Forum in Canberra on 4 February (World Cancer Day). Representatives from the federal and state governments in Australia, cancer peak bodies, consumer organisations and medical professions participated in discussions on contemporary radiation therapy, and implementation strategies for the Tripartite National Strategic Plan for Radiation Oncology (2012 – 2022).

A report on the Forum is available on the RANZCR website: www.ranzcr.edu.au/component/docman/doc_download/3038-2014-ro-tripartite-stakeholder-forum-report. Workforce and quality issues such as the national standards are a major priority for our sector.

The Faculty will continue to engage with governments and stakeholders in the broader cancer arena to advocate for radiation oncology as an essential pillar of cancer control.

Quality assurance for radiation therapy services

Delivery of safe and high quality radiation therapy services is of paramount importance to our patients. The Faculty, through the Quality Improvement Committee, is developing a number of guidelines and position papers on quality matters – including the use of imaging in radiation oncology, delivery of stereotactic body radiation therapy and volumetric delineation. These will become available on the College website over the coming months.

The Faculty's Horizon Scan Position Paper on Radiation Oncology Techniques and Technologies presents our position on the uptake of techniques used for safe delivery of high quality radiation therapy. This document was recently updated with the latest available evidence and is now available on the College website: www.ranzcr.edu.au/resources/consumers/764-radiotherapy-technologies.

Supporting research activities in radiation oncology

Research and academia as foundations of future radiation oncology practice has been identified as one of the key areas for development in the next decade. The Faculty is committed to providing world class specialty training and promoting research in radiation oncology.

A position statement on Clinical Academic Pathways in Medicine was released by the Australian Medical Association (AMA) last year, and is available at https://ama.com.au/position-statement/clinical-academic-pathways-medicine-2013.

The Faculty council strongly supports the position statement and agrees that there is an urgent need to encourage more junior doctors to choose a clinical academic career path. Federal and state governments, health departments, universities, medical colleges and research institutes must work together to develop a strategy to cultivate and retain a well-trained and skilled clinical academic workforce

The Faculty has developed a clinician-scientist pathway as part of its training program, which will enable trainees to undertake full-time research activities while maintaining the quality of clinical radiation oncology training. The model of the 'clinician-scientist' is becoming more attractive as a means of combining specialist training with a formal research higher degree, and there are a growing number of trainees seeking to engage with this model. Details about this program are available from the RANZCR website: www.ranzcr.edu.au/research/radiation-oncology/research-opportunities

In order to provide trainees with an environment which promotes academic development, the Faculty also recently introduced the Research Mentorship position, to assist those trainees embarking on research for the first time and to promote a culture of research in the profession

The Faculty looks forward to collaborating with all radiation oncology practices and other stakeholders to promote and foster a research culture in the radiation oncology sector, and to make Australia an international leader in radiation oncology research, to ultimately improve patient outcomes.

Dr Dion Forster
Dean, Faculty of Radiation Oncology,
RANZCR

CALENDAR OF MEETINGS

AUSTRALIA AND NEW ZEALAND

Date	Name of Meeting	Place	Secretariat
July			
13-15	Australian and New Zealand Urogenital and Prostate (ANZUP) Annual Scientific Meeting 2014	Melbourne, Victoria	YRD (Aust) Pty Ltd Website: www.anzup.org.au Email: anzup@yrd.com.au Phone: +61 7 3368 2422
16-19	2014 Australia and New Zealand Breast Cancer Trials Group (ANZBCTG) Annual Scientific Meeting	Wellington, New Zealand	ANZBCTG Business Department Website: www.bcia.org.au Email: asm@anzbctg.org Phone: +61 2 4925 5255
24-26	Cancer Nurses Society of Australia (CNSA) Winter Congress 2014	Melbourne, Victoria	Chillifox Events Website: www.chillifoxevents.com.au Email: cnsa@chillifoxevents.com.au Phone: +61 2 8005 1867
August			
1-3	Royal Australian and New Zealand College of Radiologists (RANZCR) New Zealand Branch Annual Scientific Meeting	Wellington, New Zealand	Outshine Website: www.ranzcr2014.co.nz Email: ranzcr@outshine.co.nz Phone: +64 7 823 2316
6-8	Medical Oncology Group of Australia (MOGA) Annual Scientific Meeting 2014	Sydney, New South Wales	Daniel Evans, ASM Project Manager Website: www.moga.org.au Email: projects2@moga.org.au Phone: +61 2 9256 9656
7-8	Australian Palliative Care Research Colloquium	Melbourne, Victoria	Try Booking.com Website: www.trybooking.com Email: centreforpallcare@svhm.org.au Phone: +61 3 9416 0000
20-22	16th Australasian Gastro-intestinal Trials Group (AGITG) Annual Scientific Meeting	Brisbane, Queensland	ASN Events Pty Ltd Website: www.agitg.asnevents.com.au Email: eg@asnevents.net.au Phone: +61 3 9329 6600
31-2 Sep	15th Asia-Pacific Prostate Cancer Conference 2014	Melbourne, Victoria	ICMS Pty Ltd Website: www.prostatecancercongress.org.au Email: pcwc2013@icms.com.au Phone: +61 1300 792 466
September			
2-5	Australian and New Zealand Society of Palliative Medicine (ANZSPM) Conference 2014	Gold Coast, Queensland	Australian and New Zealand Society of Palliative Medicine Website: www.etouches.com/ehome/65181 Email: anzspm@willorganise.com.au Phone: +61 2 4973 6573
5	The Second Victorian Psycho-Oncology Research Conference	Melbourne, Victoria	Victorian Comprehensive Cancer Centre Project Website: www.victoriancc.org.au Email: fiona.macken@unimelb.edu.au Phone: +61 3 9035 8170
14-19	Australia and Asia Pacific Clinical Oncology Research Development Workshop (ACORD)	Coolum, Queensland	Medical Oncology Group of Australia (MOGA) Website: www.moga.org.au Email: moga@moga.org.au Phone: +61 2 8247 6210

October			
9-11	Australasian Breast Congress	Surfers Paradise, Queensland	Australasian Breast Congress Website: www.asbd.org.au Email: info@asbd.org.au Phone: +61 7 3847 1946
16-18	5th Australian Lung Cancer Conference	Brisbane, Queensland	The Australian Lung Cancer Conference Website: www.alcc.net.au Email: info@alcc.net.au Phone: +61 7 3751 3600
16-18	BreastScreen Australia Conference 2014	Melbourne, Victoria	Think Business Events Website: bsaconference.com.au Email: bsa@thinkbusinessevents.com.au Phone: +61 3 9417 1350
24-25	7th Cooperative Trials Group for Neuro-Oncology (COGNO) Annual Scientific Meeting	Melbourne, Victoria	Cooperative Trials Group for Neuro-Oncology Website: www.cogno.org.au Email: cogno@cogno.org.au Phone: +61 2 9562 5000
26	Australasian Lung Cancer Trials Group (ALTG) Meeting	Sydney, New South Wales	Australasian Lung cancer Trials Group Website: www.altg.com.au Email: enquiries@altg.com.au Phone: +61 7 3251 3648
November			
8-11	15th Biennual Meeting of the International Gynaecological Cancer Society (IGCS)	Melbourne, Victoria	The Australian Lung Cancer Conference Website: www.alcc.net.au Email: info@alcc.net.au Phone: +61 7 3751 3600
11-14	Australasian Leukaemia & Lymphoma Group (ALLG) Annual Scientific Meeting 2014	Sydney, New South Wales	Think Business Events Website: bsaconference.com.au Email: bsa@thinkbusinessevents.com.au Phone: +61 3 9417 1350
16-19	Australian Health and Medical Research Congress	Melbourne, Victoria	Cooperative Trials Group for Neuro-Oncology Website: www.cogno.org.au Email: cogno@cogno.org.au Phone: +61 2 9562 5000
26-28	Sydney Cancer Conference 2014	Sydney, New South Wales	Australasian Lung cancer Trials Group Website: www.altg.com.au Email: enquiries@altg.com.au Phone: +61 7 3251 3648
December			
2-4	Clinical Oncology Society of Australia's (COSA's) 41st Annual Scientific Meeting	Melbourne, Victoria	ASN Events Pty Ltd Website: www.asnevents.net.au Email: eg@asnevents.net.au Phone: +61 3 5983 2400
4-6	Union for International Cancer Control (UICC) World Cancer Congress	Melbourne, Victoria	Union for International Cancer Control Website:

INTERNATIONAL

Date	Name of Meeting	Place	Secretariat
September			
7-11	18th International Conference on Cancer Nursing (ICCN)	Panama City, Panama	ISNCC Secretariat Website: www.isncc.org/ Email: info@isncc.org Phone: +1 604 630 5516
11-13	3rd World Congress on Controversies in Hematology (COHEM) ESMO	Istanbul, Turkey	ComtecMed Website: www.comtecmed.com/cohem/2014 Email: cohem@comtecmed.com Phone: +972 3 5666166
26-30	European Society for Medical Oncology (ESMO) 2014 Congress	Madrid, Spain	European Society for Medical Oncology Website: www.esmo.org Email: esmo@esmo.org Phone: +41 0 91 973 19 00
October			
6-10	9th International Conference of Anticancer Research	Sithonia, Greece	International Institute of Anticancer Research Website: www.iiar-anticancer.org/ Email: conference@iiar-anticancer.org Phone: +30 22950 53389
9-11	14th International Cancer Imaging Society Meeting & Annual Teaching Course	Heidelberg, Germany	ICIS Secretariat Website: www.icimagingsociety.org.uk/ Email: louise.mustoe@cancerimagingsociety.org.uk Phone: +44 0 207 036 8805
16-19	18th Senologic International Society (SIS) World Congress on Breast Healthcare	Orlando, United States	Kenes International Website: www2.kenes.com/sis/Pages/Home.aspx Email: sis2014@kenes.com Phone: +41 22 908 0488
20-24	16th World Congress of Psycho-Oncology and Psychosocial Academy	Lisbon, Portugal	International Psycho-Oncology Society Website: www.ipos2014.com Email: info@ipos-society.org Phone: +1 434.293.5350
23-25	International Society of Geriatric Oncology (SIOG) Annual Meeting 2014	Lisbon, Portugal	International Society of Geriatric Oncology Website: www.siog.org Email: siog2014@mci-group.com Phone: +41 22 552 3305
29-31	American Institute for Cancer Research (AICR) 2014 Annual Research Conference on Food, Nutrition, Physical Activity and Cancer	Washington, United States	The Pearson Group Website: www.aicr.org/ Email: research@aicr.org Phone: 540 373 4493
29-31	34th Congress of the European Society of Surgical Oncology (ESSO) in partnership with BASO	Liverpool, United Kingdom	European Cancer Organisation Website: www.ecco-org.eu/ESSO34 Email: ESSO34@ecco-org.eu Phone: +32 2 775 02 01
November			
6-7	2nd Breast Cancer in Young Women Conference (BCY2)	Tel Aviv, Israel	European School of Oncology Website: www.eso.net Email: efiore@eso.net
December			
9-13	37th Annual San Antonio Breast Cancer Symposium	San Antonio, United States	Rich Markow, Director Website: Email: sabcs@uthscsa.edu Phone: 210 450 1550

CANCER COUNCIL AUSTRALIA

Cancer Council Australia is the nation's peak independent cancer control organisation.

Its members are the leading state and territory Cancer Councils, working together to undertake and fund cancer research, prevent and control cancer and provide information and support for people affected by cancer.

MEMBERS

Cancer Council ACT
Cancer Council New South Wales
Cancer Council Northern Territory
Cancer Council Queensland
Cancer Council South Australia
Cancer Council Tasmania
Cancer Council Victoria
Cancer Council Western Australia

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Clinical Oncology Society of Australia

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CLINICAL ONCOLOGY SOCIETY OF AUSTRALIA

The Clinical Oncology Society of Australia (COSA) is a multidisciplinary society for health professionals working in cancer research or the treatment, rehabilitation or palliation of cancer patients.

It conducts an annual scientific meeting, seminars and educational activities related to current cancer issues. COSA is affiliated with Cancer Council Australia.

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Cancer Council Australia nominee
Professor I Olver AM

MEMBERSHIP

Further information about COSA and membership applications are available from:

www.cosa.org.au or cosa@cancer.org.au

Membership fees for 2014
Medical Members: $170
Non Medical Members: $110 (includes GST)

COSA Groups

Adolescent & Young Adult
Biobanking
Breast Cancer
Cancer Biology
Cancer Care Coordination
Cancer Pharmacists
Clinical Trials & Research Professionals
Complementary & Integrative Therapies
Developing Nations
Epidemiology
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Lung Cancer
Melanoma & Skin Cancer
Neuroendocrine Tumours
Neuro-Oncology
Nutrition
Paediatric Oncology
Palliative Care
Psycho-Oncology
Radiation Oncology
Regional & Rural Oncology
Social Work
Surgical Oncology
Survivorship
Urologic Oncology

Information for contributors

Cancer Forum provides an avenue for communication between all those involved in cancer control and seeks to promote contact across disciplinary barriers. To this end, articles need to be comprehensible to as wide a section of the readership as possible. Authors should provide sufficient introductory material to place their articles in context for those outside their field of specialisation. *Cancer Forum* is primarily a review journal, with each issue addressing a particular topic in its 'Forum'. The Forum topic and appointment of Guest Editor(s) are determined by the Editorial Board, which welcomes suggestions. Proffered papers containing primary research findings will be considered for publication in *Cancer Forum* in limited circumstances. Articles will be considered by the Editorial Board and then published subject to two peer-reviews. Generally speaking, authors are encouraged to submit their primary research findings to established cancer research or clinical oncology journals. The following information is provided for contributors invited to prepare manuscripts for *Cancer Forum*.

Format

Prospective authors are encouraged to examine recent editions of *Cancer Forum* for an indication of the style and layout of Forum papers (www.cancerforum.org.au). All manuscripts should be submitted by email to the Forum's Guest Editor(s) and Executive Editor (rosannah.snelson@cancer.org.au) as MS Word documents.
Length: 2000-2500 words.
Font: Arial - 20pt and bold for title, 12pt and bold for headings, 12pt and italics for subheadings and 10pt for text.
Following the title, include your full name, organisation and email address.
Include introductory headings and sub-headings that describe the content.
Number pages in the footer.

Abstract

All manuscripts must include an abstract of approximately 200 words, providing a summary of the key findings or statements. No references or abbreviations should be included in the abstract.

Abbreviations and acronyms

Abbreviations and acronyms should only be used where the term appears more than five times within the paper. They must be explained in full in the first instance, with the abbreviation in brackets.
The Editorial Board reserves the right to remove the heavy use of abbreviations and acronyms that may be confusing to the diversity of our readership.

Photographs, tables and graphs

Photographs and line drawings can be submitted via email, preferably in tiff or jpeg format. If images are not owned by the author, written permission to reproduce the images should be provided with the submission. A maximum of five illustrations and figures and three tables can be submitted with the manuscript. Inclusion of additional items is subject to approval by the Editorial Board. Unless otherwise specified by the authors or requested by the Editorial Board, all images, graphs and tables will be printed in black and white. All figures – including tables and graphs – will be reproduced to *Cancer Forum's* style. Figures containing data (eg. a line graph) must be submitted with corresponding data so our designers can accurately represent the information. Figures and images should be labelled sequentially, numbered and cited in the text in the correct order e.g. (table 3, figure 1). Tables should only be used to present essential data. Each must be on a separate page with a title or caption and be clearly labelled.

Referencing

Reference numbers within the text should be placed after punctuation and superscripted. The maximum number of references is 75. Only papers closely related to the subject under review should be quoted and exhaustive lists should be avoided. Only one publication can be listed for each number. Citation of more than one reference to make a point is not recommended. The Editorial Board prefers a focus on more recent references (in the last 10 years). The list of references at the end of the paper should be numbered consecutively in the order in which they are first mentioned and be consistent with the National Library of Medicine's International Committee of Medical Journal Editors' Uniform Requirements for Manuscripts Submitted to Biomedical Journals. i.e. Halpern SD, Ubel PA, Caplan AL. Solid-organ transplantation in HIV-infected patients. N Engl J Med. 2002 Jul 25;347(4):284-7.

A full guide is available at www.nlm.nih.gov/bsd/uniform_requirements.htmlA guide to abbreviation of journal names can be found at https://www.library.uq.edu.au/faqs/endnote/medical_2010.txt

The Editorial Board will make the final decision on inclusion of manuscripts and may request clarifications or additional information.

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