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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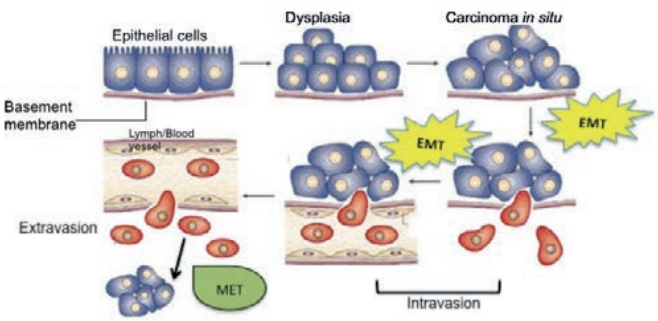
Abstract

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