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Lymphovascular and neural regulation of metastasis: Shared tumour signalling pathways and novel therapeutic approaches



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The progression of cancer is supported by a wide variety of non-neoplastic cell types which make up the tumour stroma, including immune cells, endothelial cells, cancer-associated fibroblasts and nerve fibres. These host cells contribute molecular signals that enhance primary tumour growth and provide physical avenues for metastatic dissemination. This article provides an overview of the role of blood vessels, lymphatic vessels and nerve fibres in the tumour microenvironment and highlights the interconnected molecular signalling pathways that control their development and activation in cancer. Further, this article highlights the known pharmacological agents which target these pathways and discusses the potential therapeutic uses of drugs that target angiogenesis, lymphangiogenesis and stress-response pathways in the different stages of cancer care.

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The tumour microenvironment influences cancer progression

The initiation and progression of cancer occurs in a microenvironment that consists of a broad range of non-neoplastic cells that make up the tumour stroma [1,2]. The stroma comprises the extracellular matrix, endothelial cells and pericytes, fibroblasts, mesenchymal stem cells, neural cells and immune cells including natural killer cells, macrophages and dendritic cells. The stroma connects to the broader circulatory system of blood and lymphatic vessels and has receptors that make stromal cells responsive to signals from the peripheral autonomic nervous system (Fig. 1). In the peripheral tissues and within the tumour stroma, blood and lymphatic vessels exchange fluid and cells via specialised small capillaries while nerve fibres exchange molecular information via specialised synapses with other cell types. Together, this collection of cells and matrix is the source of many important molecular signals that help to either promote or retard cancer growth and dissemination [3,4].

Prior to the initiation of cancer, normal human tissue is a highly ordered series of specialised cell types that function for a common purpose. Endothelial cells and nerve fibres share receptors for guidance cues that direct their growth and maturation [5]. During embryonic development, activation of these receptors results in common patterns of migratory behaviour by nerve fibres and blood vessels to support limb and muscle development [5]. In the adult, this results in co-localisation of nerve fibres, blood vessels and lymphatic vessels in neurovascular bundles. These three structures, while related in their migratory passage into tissue, carry out diverse functions relating to tissue homeostasis. Blood vessels are part of the high-pressure cardiovascular system that carries cells, protein-rich fluid and macromolecules to tissues. Arterial blood vessels connect to capillary beds where nutrients and waste products are exchanged from cells, and blood is then returned to the heart via veins. The function of the blood vasculature is complemented by lymphatic vessels that return cells and fluid from the interstitium to the blood circulation [6]. Lymphatics start as small blind-ended vessels in the periphery, which are known as initial lymphatics. The initial lymphatic vessels within tissue drain into pre-collecting and collecting lymphatic vessels that empty into regional lymph nodes and finally transit extracellular fluid back into circulation via the thoracic duct. Peripheral tissues are innervated by efferent fibres from the sympathetic branch of the autonomic nervous system. Autonomic neurons play a critical role in maintaining physiological homeostasis. They receive information integrated in the central nervous system (CNS) and communicate this via vertebral ganglia to control functions that are largely involuntary including heart rate, blood pressure, temperature and digestion.

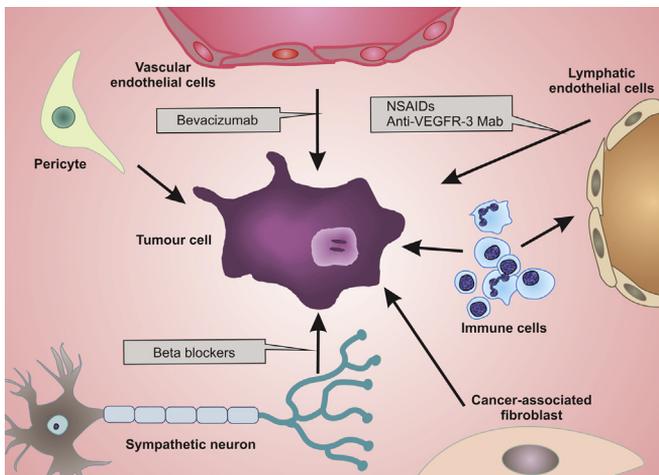


Fig. 1. Interactions between tumour cells and their microenvironment may be targeted by novel anticancer strategies. The stroma associated with a growing tumour consists of immune cell infiltrates, cancer-associated fibroblasts, sympathetic nerve fibres and endothelial cells and pericytes from blood and lymphatic vessels. Information flow in the form of soluble molecular signals and direct cell–cell contact occurs between the malignant tumour cells and the stromal cells. The boxes show examples of drug classes that may be used to manipulate these interactions *in vivo*.

Ordered tissue architecture is disrupted during cancer development which affects the structure and function of local and distant blood and lymphatic vessels to accelerate cancer progression [6,7]. This article describes the role of lymphatic and blood vessels in metastasis and characterises physiological factors that regulate their function, including inflammation and neural signals from the sympathetic nervous system (SNS). We describe therapeutic approaches that target molecular mediators of angiogenesis, lymphangiogenesis and neural signalling and discuss how these pro-metastatic molecules might be targeted in new anticancer drug combinations or novel clinical settings.

Angiogenesis and lymphangiogenesis

New blood and lymphatic vessels can form in and around tumours via the processes of angiogenesis and lymphangiogenesis, respectively [7]. During angiogenesis, endothelial cells that line pre-existing blood vessels are released from the extracellular matrix to form tip cells which lead a stalk of proliferating endothelial cells to re-form as a new vessel [8]. Angiogenesis and lymphangiogenesis play critical roles in epithelial tumour progression by facilitating key steps in metastasis including tumour cell escape from the primary tumour and metastatic colonisation of distant organs [9]. Metastasis is a complex multistep process that requires establishment of angiogenesis and evasion of the host immune defences for successful colonisation of distant organs [10]. The process begins with local invasion of the surrounding parenchyma by cancer cells followed by intravasation into nearby blood or lymphatic vessels. Both angiogenesis and lymphangiogenesis contribute to metastasis by providing new blood capillaries and initial lymphatics that tumour cells can use as entry points for dissemination [11,12]. Tumour cells may traffic through these vessels to distant lymph nodes or distant organs where metastasis requires extravasation into the surrounding parenchyma. Successful colonisation depends on the development of a micrometastasis and finally the formation of a macrometastasis with localised angiogenesis providing new tumour-associated vasculature [9].

In recent times, while much attention has been directed towards the formation of new vessels in a growing tumour, recent studies have also highlighted that molecular remodelling and re-differentiation of pre-existing vessels, without the formation of new vessels, contributes to metastasis [11,13]. In response to angiogenic and lymphangiogenic stimuli, blood vessel capillaries and initial lymphatic vessels undergo sprouting angiogenesis and lymphangiogenesis, while larger blood and lymphatic vessels (e.g., arteries or collecting lymphatics) undergo dilation and remodelling of the supporting smooth muscle cells. These changes in vascular structures promote increased lymph flow, dissemination of tumour cells and metastasis to regional lymph nodes [6,12].

Neural signalling

In response to stressful conditions, SNS nerve fibres convey information from the CNS to the periphery to mediate the flight-or-flight response to threat [14]. Increasing evidence indicates that these neural signals also remodel the tumour microenvironment in ways that promote cancer progression. SNS fibres innervate organs that support growth of primary and metastatic secondary tumours, including lung, liver, lymphoid tissues and bone marrow. Chronic stress induces the release of the catecholaminergic neurotransmitter noradrenaline from these nerve fibres and invokes the systemic release of adrenaline from the adrenal medulla. Stress-neurotransmitters bind beta-adrenergic receptors on target cells and induce a signalling cascade through cyclic adenosine monophosphate (cAMP) synthesis and protein kinase A phosphorylation to modulate cellular transcriptional programmes. In addition to regulating neurotransmitter release, chronic stress also remodels neural architecture to increase the density of nerve fibres in peripheral organs, which may serve to amplify adrenergic signalling in stressed individuals [15]. Beta-adrenergic receptors are present on both tumour cells and stromal cells in the tumour microenvironment [16–20], suggesting that tumour dynamics may be responsive to adrenergic stress-response signalling.

In vivo studies have provided persuasive evidence that beta-adrenergic signalling promotes cancer progression. Beta-adrenergic activation by chronic physiological stress or by pharmacological beta-agonists increased spontaneous metastasis from primary mammary tumours to clinically relevant target organs including lymph node, lung and bone [20–23]. Adrenergic stress signalling had minimal

effect on primary tumour growth rates in models of breast cancer [20,30], but it had increased metastatic growth in mouse models of ovarian cancer and prostate cancer [24–27] and increased lymphatic colonisation by tumour cells in a model of acute lymphoblastic leukaemia [28,29]. Of potential clinical importance, beta-blockers prevented metastasis suggesting a novel avenue for therapeutic intervention [20,21,24,27,31].

The effect of chronic stress on cancer progression in patients has been more challenging to define. Epidemiological studies and a meta-analysis of 131 prospective studies linked chronic stress to progression of established breast cancers [32–38], and a psychosocial intervention that sought to buffer stress showed improved cancer-related outcomes [39,40]. However, few consistent relationships have been found between stress and the initial incidence of breast cancer [41–45]. Consistent with a key role for beta-adrenergic signalling in the pro-tumour effects of stress, recent epidemiological studies show that beta-blocker use at the time of diagnosis is associated with improved outcomes in multiple tumour types (reviewed later, see Table 1). These studies highlight the emerging importance of stress-response pathways in influencing cancer progression and they suggest that effective disease management may include consideration of the whole patient, including their experience of chronic stress and other psychosocial factors.

Molecular mediators of cancer progression

Molecular crosstalk between tumour cells and stromal cells induces the synthesis of pro-inflammatory mediators, growth factors and chemokines to create a microenvironment that is permissive for angiogenesis, lymphangiogenesis and tumour progression [46]. Inflammatory mediators play a pivotal role in growth and remodelling of blood and lymphatic vessels in and around the tumour, which influences the local immune response and tumour cell dissemination. Specifically, inflammatory cells including M2-oriented macrophages and neutrophils are recruited to the tumour and release molecules including prostaglandins (PGs), cytokines, chemokines and proteases which regulate angiogenesis and lymphangiogenesis and modulate cancer cell adhesion, migration and invasion to promote metastasis [47]. Neural signalling provides a higher level of physiological control to regulate inflammation and tumour cell dissemination [20,31].

Angiogenesis

Oncogenic signalling, hypoxia and inflammation in the tumour microenvironment induce secretion of angiogenic factors by tumour cells or tumour stroma [4,48]. The best-validated tumour angiogenic factor is glycoprotein vascular endothelial growth factor (VEGF)-A (also known as VEGF or vascular permeability factor) [49], which is induced by hypoxia and induces sprouting and proliferation of blood vascular endothelial cells via VEGF receptor-2 (VEGFR-2), a tyrosine kinase on the surface of these cells [50]. The activation of VEGFR-2 signals for other facets of angiogenesis such as remodelling of extracellular matrix by matrix metalloproteinases [51] and integrins [52], endothelial cell guidance through semaphorins, ephrins, slits and their cognate receptors [48], through Delta-like ligand 4/Notch signalling [53] and vessel formation and recruitment of pericytes (involving platelet-derived growth factors (PDGFs) [54]) (see Ref. [48] for an overview). Alternative tumour angiogenic factors have been characterized in animal models and are expressed in human cancers, including fibroblast growth factors (FGFs) [55], placenta growth factor (PlGF) [56,57], angiopoietins [58], VEGF-C [59–61] and VEGF-D [62–64]. The importance of these alternative angiogenic proteins in prevalent human cancers remains to be established in clinical trials with agents specifically targeting these molecules. This complex signalling generates a tumour vasculature which is tortuous and leaky but nevertheless able to facilitate tumour growth and spread.

Lymphangiogenesis

Tumour cells and stromal cells secrete VEGF-C and VEGF-D, which drive lymphangiogenesis or lymphatic remodelling in the tumour microenvironment. These growth factors can be proteolytically activated [65–69] leading to high-affinity binding of VEGFR-3 to promote lymphangiogenesis involving

Table 1
Cancer-related outcomes associated with beta-blocker use.

Cancer type	Key outcomes associated with beta-blocker use	Subjects	Cohort	Year	Reference
Breast	Reduced incidence of cancer-related events Reduced distant metastasis Reduced cancer specific mortality	800	European Institute of Oncology, Milan, Italy	1997–2008	Botteri et al., 2013
Breast	No association with cancer-related outcomes	18,733	Danish Breast Cancer Cooperative Group Registry	1996–2003	Sorensen et al., 2013
Breast	No association with cancer-related outcomes	4,661	Nurses' Health Study, USA	1985–2005	Holmes et al., 2013
Breast	No association with cancer-related outcomes	2,763	Cancer Surveillance System, SEER program, NCI, USA	2000–2008	Li et al., 2013
Breast	Increased 3 year relapse-free survival Reduced Lymphovascular Invasion (LVI)	1,413	Breast Cancer Management System, MD Anderson Cancer Center, TX, USA	1995–2007	Melhem-Bertrandt et al., 2011
Breast	No association with cancer-related outcomes	2,269	LACE Study, Kaiser Permanente Northern California subset, USA	2000–2002	Ganz et al., 2011
Breast	Propranolol use was associated with: Lower tumour grade at diagnosis Lower stage, reduced metastasis Reduced 5 year cancer specific mortality Atenolol use was not associated with cancer-related outcomes	5,333	General Medical Services National Cancer Registry, Ireland	2001–2006	Barron et al., 2011
Breast	Increased time to distant metastasis Reduced recurrence and metastasis Reduced cancer specific mortality	466	Nottingham City Hospital, UK	1987–1994	Powe et al., 2010
Breast	No association with cancer risk Progression was not investigated	49,950	Danish Cancer Registry	1989–2002	Fryzek et al., 2006
Breast	No association with cancer risk Progression was not investigated	17,861	General Practice Research Database, London, UK	1992–1997	Meier et al., 2000
Lung (NSCLC)	Increased metastasis-free survival Increased disease-free survival Increased overall survival	722	MD Anderson Cancer Center, TX, USA	1998–2010	Wang et al., 2012
Lung	No association with cancer mortality	8,039	Department of Health Hypertension Care Computing Project, UK	1971–7986	Fletcher et al., 1993

(continued on next page)

Table 1 (continued)

Cancer type	Key outcomes associated with beta-blocker use	Subjects	Cohort	Year	Reference
Ovarian	No association with cancer mortality	6,626	Danish Cancer Registry	1999–2010	Johannesdottir et al., 2013
Ovarian	No association with progression-free survival	381	AGO-OVAR clinical trial subset	1997–2002	Heitz et al., 2013
Ovarian	Increased progression-free survival Increased overall survival	248	Gynecologic Oncology Service, Cedars-Sinai Medical Center, CA, USA	1999–2006	Diaz et al., 2012
Melanoma	No association with cancer mortality	709	Eindhoven Cancer Registry, The Netherlands	1998–2010	Livingstone et al., 2013
Melanoma	Reduced disease progression Increased disease-free survival	121	Department of Dermatology, University of Florence, Italy	1993–2009	De Giorgi et al., 2011
Melanoma	Trend toward reduced mortality	4,179	Danish Cancer Registry	1943–2006	Lemeshow et al., 2011
Prostate	Reduced Prostate Specific Antigen levels at diagnosis Reduced metastasis Reduced risk of cancer specific mortality	3,561	Cancer Registry of Norway	2004–2009	Grytli et al., 2013 (Euro Onco)
Prostate	No association with cancer specific risk or mortality in whole cohort Decreased cancer specific mortality in patients who received androgen deprivation therapy	6,515	Oslo II Study 2000 – Cancer Registry of Norway	1972–2000	Grytli et al., 2013 (The Prostate)
Colorectal	No association with cancer risk Long-term (6+ year) use was associated with higher stage	3,470	DACHS study, Odenwald, Germany	2003–2007	Jansen et al., 2012
Multiple	Reduced overall risk of cancer Progression was not investigated	1,340 diabetes patients	Diabetes Clinic, University of Florence, Italy	1998–2007	Monami et al., 2013
Multiple	No association with cancer risk	12,877	Kaiser Permanente Medical Care Program, CA, USA	1994–2008	Friedman et al., 2011
Multiple	Reduced survival from pancreatic or prostate cancer	3,462	DIN-LINK Database, UK	1997–2006	Shah et al., 2011

initial lymphatic vessels and of VEGFR-2 which promotes the circumferential expansion of initial lymphatics [7,70–74]. VEGF-C and VEGF-D mediated lymphangiogenesis that promoted metastasis to lymph nodes [61,62,75] and distant organs [76,77] in animal models, and their expression in human cancers correlated with metastasis and poor outcome (for reviews see Refs. [78,79]). Furthermore, VEGF-C promoted lymphangiogenesis in distant lymph nodes, even before the onset of metastasis, which may facilitate metastasis beyond sentinel lymph nodes [80]. In addition to their effects on the growth of new blood vessels, VEGF-C and VEGF-D promoted dilation of tumour-draining collecting lymphatics, via VEGFR-2 and VEGFR-3 signalling and PG-dependent mechanisms, which facilitated lymph node metastasis [13]. Hence, VEGF-C and VEGF-D modulate the growth and structure of different types of lymphatic vessels to promote metastasis, which led to the notion of targeting VEGF-C/VEGF-D/VEGFR-3 signalling in cancer [6,81–83].

Other secreted proteins have been reported to promote lymphangiogenesis including VEGF-A, FGF-2, angiopoietin-2, insulin-like growth factor-1 and -2, PDGF-B, hepatocyte growth factor and erythropoietin [84,85]. There is emerging evidence that some of these proteins play roles in tumour lymphangiogenesis [86,87], but their effects on lymphogenous metastasis require further characterisation.

Inflammation and lymphangiogenesis

PGs play a key role in creating an inflammatory environment that supports angiogenesis and lymphangiogenesis in tumours. They are bioactive lipids that are produced from arachidonic acid by the actions of cyclooxygenases (COXs) in a wide variety of human tissue and they have a central role in inflammation and cancer progression [88,89]. Two COX isoforms are responsible for PG biosynthesis: COX-1 is constitutively expressed in most tissues and is important in maintaining basal PG levels, whereas COX-2 is induced in tissues during inflammation and cancer. Consequently, COX-2 is up-regulated by inflammatory mediators in many types of cancer including breast, colon, lung, pancreas and head and neck [90–98]. PG synthesis is balanced by 15-hydroxyprostaglandin dehydrogenase (PGDH), which degrades PGs [99]. PGDH expression was shown to be abrogated in animal models of colon cancer suggesting that PGDH is a tumour suppressor [100–102]. The pro-tumourigenic effects of COX-2 are believed to be largely attributed to its role in producing PGE2 [103,104]. PGE2 exerts its effects via G-protein-coupled prostaglandin E (EP)1–4 receptors [105]. Tumour studies conducted in EP receptor knockout animals or using pharmacological inhibition revealed that these receptors contribute to tumour angiogenesis and lymphangiogenesis [106–110]. PGs are released by tumour cells and stromal cells, and they play a central role in the tumour inflammatory response by down-regulating anti-neoplastic cytokines tumour necrosis factor-alpha (TNF- α) and interferon-gamma (IFN γ) whilst up-regulating immunosuppressive cytokines including interleukin (IL)-10, IL-4 and IL-6 [111,112]. PGE2 promotes tumour immunosuppression by inhibiting maturation of cytotoxic T cells [113] and increasing the migration of dendritic cells to lymph nodes [114]. Naïve T cells fail to respond to tumour-derived antigens presented by dendritic cells or antigen presenting cells, which enhances tumour cell evasion of host immune defences and permits metastatic dissemination through blood or lymphatic vasculature.

The link between the VEGF family members and inflammatory PG signalling pathways was first observed in animal models of colon cancer, where an association between COX-2, VEGF-A expression and angiogenesis was demonstrated [115,116]. PG signalling by stromal cells including fibroblasts and endothelial cells was also shown to contribute to angiogenesis [108,117,118]. Clinical studies have demonstrated a correlation between the level of COX-2 expression and the extent of angiogenesis in breast, endometrial and gastric cancers [119–121]. Likewise, clinical and histopathological studies have revealed a correlation between COX-2 expression, lymphatic vessel density and lymph node metastasis in several human cancer types [122–124]. The PG pathway can influence lymphatic vessel density and therefore lymph node metastasis by regulating the levels of VEGF-C and VEGF-D produced within both the tumour and the tumour stroma [125–127]. PGE2 mediates this effect by acting via different EP receptors expressed on the surface of various tumour or stromal cells. EP3 activation caused an increase in VEGF-C and VEGF-D secretion by cultured macrophages, whereas EP4 activation elevated VEGF-C secretion by tumour-associated macrophages and VEGF-D secretion by cancer-associated fibroblasts leading to enhanced lymphangiogenesis within the primary tumour [127]. In addition, local secretion

of PG by endothelial cells can affect the morphology of the lymphatics by dilation and can facilitate the dissemination of cancer cells to draining lymph nodes [13]. These findings suggest that inhibition of PG synthesis by pharmacological or therapeutic targeting of the VEGF-C/VEGF-D signalling pathway might slow cancer progression.

Neural regulation of angiogenesis and metastasis

Neural signalling through beta-adrenergic pathways was recently identified as a physiological regulator of angiogenesis and metastasis [20,24]. Activation of beta-adrenergic receptors through physiological or pharmacological stimuli induces a pro-metastatic gene expression signature in the tumour microenvironment, remodels primary tumour architecture and increases the frequency of tumour cell escape [20,24–26,28]. Physiological activation of stress pathways using repeated restraint in mouse models of breast and ovarian cancer resulted in genetic switching with transcriptional up-regulation of angiogenesis genes including *Vegf-a* and increased blood vessel density [19,20,24,128]. Regulation of inflammation plays a central role in the beta-adrenergic regulation of angiogenesis. The effect of stress on angiogenesis was dependent on recruitment of macrophages to the primary tumour [20], suggesting an intimate relationship between neural signalling, tumour-associated inflammation and angiogenesis. Consistent with this, several lines of evidence suggest that macrophages are particularly sensitive to adrenergic signalling [20,129,130]. Noradrenaline increased matrix metalloproteinase 9 (MMP9) production by cultured macrophages [130]. Similarly, significant life stress prior to diagnosis with ovarian cancer was associated with increased MMP9 production by CD68+ cells in primary tumours [130], which has been associated with aggressive tumour progression likely due to altered basement membrane integrity.

The adverse effects of stress on angiogenesis may be reversed by alternate neurotransmitter signalling, although the clinical application of this is unclear. Dopamine was found to act on tumour cells and pericytes to reverse stress-induced angiogenesis and increase pericyte coverage to stabilise blood vessels [131]. The effect of stress-response pathways on lymphangiogenesis has not been evaluated. However, beta-adrenergic regulation of both PG synthesis and normal lymphatic development and lymphatic flow [132,133] suggests that neural signalling may regulate tumour-associated lymphangiogenesis.

In addition to modulating the tumour microenvironment, neural signalling may impact metastasis by directly modulating invasive, inflammatory and anti-apoptotic behaviour by tumour cells. Tumour cells express beta-adrenergic receptors, and receptor levels have been associated with adverse outcomes [16,17]. Beta-adrenergic signalling increased the expression of inflammatory cytokines including IL-6 and IL-8 by tumour cells [19,134] and impelled the invasion of cancer cells in culture assays [135–137]. Beta-adrenergic activation promoted ovarian cancer cell resistance to anoikis through focal adhesion kinase-mediated pathways [138] and protected cells against chemotherapy induced apoptosis [26,139].

Drugs that target angiogenesis and lymphangiogenesis

Anti-angiogenics and anti-lymphangiogenics

Various targeted anti-angiogenic agents have been approved by the US Food and Drug Administration (FDA) for clinical use in oncology, most of which target VEGF-A/VEGFR-2 signalling with varying degrees of specificity [140] (Fig. 1). The first agent to be approved was bevacizumab (also known as Avastin), a humanised neutralising anti-VEGF-A monoclonal antibody (mab) used to treat multiple cancers, typically in combination with cytotoxic chemotherapy [141]. The observed lack of efficacy of bevacizumab as a single agent led to the suggestion that it may be more effectively utilised as a chemosensitisation agent by enhancing entry of chemotherapeutics into tumours, consequent to the vascular normalisation it induced [142].

Other anti-angiogenic agents in oncology include aflibercept ('VEGF Trap'), a soluble receptor which binds VEGF-A, VEGF-B and PlGF, that was recently approved for treatment of metastatic colorectal cancer [143]. Various small-molecule protein kinase inhibitors (PKIs) which target VEGFR-2 and other

kinases have been approved, including pazopanib (Votrient), vandetanib (Zactima), sunitinib (Sutent) and sorafenib (Nexavar) [140]. PKIs typically target multiple kinases, which can enhance efficacy but lead to side effects. The resulting dose-limiting toxicities have restricted PKI use in oncology due to difficulties in combining PKIs with cytotoxic chemotherapeutics. A challenge associated with anti-angiogenic agents is the emergence of drug resistance during treatment; hence, considerable effort is being made to define mechanisms of resistance that may lead to improved treatment strategies [140,144].

The concept of targeting tumour lymphangiogenesis to restrict metastasis arose more recently than that of anti-angiogenesis; hence, the development of anti-lymphangiogenic drugs is less advanced. Nevertheless, a range of inhibitors targeting VEGF-C/VEGF-D/VEGFR-3 signalling have been developed for blocking tumour lymphangiogenesis. These include neutralising monoclonal antibodies against VEGF-C [77], VEGF-D [145], VEGFR-3 [146], neuropilin-2 (a co-receptor for VEGF-C and VEGF-D) [147] and soluble versions of VEGFR-3 that sequester VEGF-C and VEGF-D [148]. Notably, anti-VEGF-C and anti-VEGFR-3 monoclonal antibodies recently progressed to phase I clinical trials. Other potential anti-lymphangiogenic therapeutics include antibodies and small molecules targeting angiopoietin/Tie2 signalling [149]. Further, small-molecule PKIs targeting VEGFR-2 and VEGFR-3 or Tie2 have been developed [150–154]. The exact clinical settings in which these drugs would be used in oncology will require careful consideration of tumour type and timing of treatment relative to surgery and other therapeutic interventions [81].

Non-steroidal anti-inflammatory drugs (NSAIDs)

NSAIDs are a diverse group of drugs that have been traditionally used to treat inflammatory disease. These drugs alter PG levels by regulating the activity of COX enzymes and they are used during surgery as analgesics and for pain management post-surgery. NSAIDs include selective inhibitors against COX-1 (e.g., ketoprofen) or COX-2 (e.g., celecoxib, etodolac and rofecoxib) or non-selective drugs that inhibit both COX enzymes (e.g., aspirin, naproxen and ibuprofen) [155]. While some experimental evidence indicated that NSAIDs had anticancer effects, recent clinical trial data have shown a significant link between NSAID intake and restricted cancer development [115,156–158]. The data further suggested that this effect was at the level of preventing metastasis [159].

By inhibiting PG synthesis, NSAIDs have profound effects on key characteristics of tumourigenesis, including cell proliferation, migration, apoptosis and angiogenesis [155]. Epidemiologic studies found that long-term use of NSAIDs was associated with reduced risk of breast, colon, lung and prostate cancer and recurrence [160–162]. Consistent with this, clinical trials revealed that NSAIDs restricted tumour progression in breast- and prostate cancer patients [159,161,163–166]. In order to define the cellular and molecular mechanisms by which NSAIDs restrict metastasis, the impact was assessed on both lymphangiogenic growth factor expression and structure of the lymphatic vasculature in pre-clinical models of metastatic disease. The *in vitro* treatment of breast and oesophageal cancer cell lines with nimesulide, diclofenac, rofecoxib or SC-5600 induced a down-regulation of VEGF-C expression [167,168]. In a model of gastric cancer, treatment with a COX-2 inhibitor, etodolac, reduced lymphangiogenesis, which decreased metastasis to sentinel lymph nodes. NSAIDs regulate lymphangiogenesis by reducing the macrophage secretion of VEGF-C to modulate lymphatic vessel density and by modulating the morphology of lymphatic collector vessels that facilitated metastasis [13,126,169,170]. While most clinical trials involving NSAIDs have emphasised tumour growth, mortality and metastasis, future clinical trials evaluating the efficacy of NSAIDs on tumour metastasis should also focus on clinical evaluations of tumour lymphatics.

Beta-blockers

Beta-adrenergic regulation of cancer progression suggests that inhibiting adrenergic stress-response pathways with beta-blockers might protect against metastasis and cancer progression. Sir James W. Black developed the beta-blocker propranolol in 1964 [171] and in 1975, he set up the first clinical trial of beta-blockers for heart failure. Beta-blockade revolutionised the medical treatment of heart disease including hypertension, arrhythmias and angina and is used perioperatively to maintain

physiological homeostasis. Beta-blockers bind beta-adrenergic receptors and prevent their activation by endogenous catecholaminergic neurotransmitters. These compounds can therefore regulate the effects of SNS signalling on heart and smooth muscle contractility. Beta-blockers, due to their ability to block stress-neurotransmitter signalling at the cellular level, may also be a novel anticancer therapeutic strategy for targeted blockade of the stress-responsive pathways.

The perioperative period may be particularly sensitive to stress biology, and it offers opportunity for a novel application of NSAIDs and beta-blockers. Surgical stress increased retention of tumour cells in the lung in rodent models of breast cancer [23,31] and increased tumour growth in ovarian cancer models [172]. Induction of inflammation with PGE2 increased tumour cell retention and promoted an immunosuppressive environment by decreasing natural killer cell activity [31]. Both COX inhibitors and beta-blockers reduced tumour cell retention, but the effect was additive when the drugs were combined [31]. Combined propranolol and etodolac treatment also increased survival rates in mice following tumour excision [173]. NSAIDs and beta-blockers are already used in the perioperative setting to limit inflammation and regulate heart rate and blood pressure. These findings suggest that perioperative use of NSAIDs in combination with beta-blockers may provide opportunities to improve immune competence and reduce the risk of tumour metastasis.

In addition to the preclinical studies described earlier indicating that beta-blockade slows progression of multiple tumour types, epidemiological studies suggest that beta-blockers may protect patients against the progression of cancer. Many of these large retrospective cohort studies found that beta-blocker use for co-morbid hypertension was associated with improved cancer-related outcomes, including increased metastasis-free survival and negative lymphovascular status [174,175]. (see Table 1). By contrast, use of other anti-hypertensive medications including angiotensin-converting enzyme inhibitors or calcium-channel blockers was not associated with beneficial effects [176–180]. Metoprolol and atenolol were the most frequently prescribed beta-blockers in these studies, followed by non-selective propranolol. Drug exposure varied, with some studies looking at associations of beta-blocker use prior to diagnosis while others investigated beta-blockade concurrent with neoadjuvant chemotherapy.

It is unclear whether all beta-blockers protect equally against cancer progression. Preclinical studies suggest that signalling through beta2-adrenergic receptors promotes cancer progression, but few studies have investigated the effect of signalling through beta1- or beta3-adrenergic receptors [24]. Antagonists that show mild selectivity towards beta1-adrenergic receptors including metoprolol and atenolol (2- to 5-fold selectivity for beta1 over beta2-adrenergic receptors) are now more frequently prescribed than the older generation of nonspecific beta-blockers. It will be critical to define receptors that mediate beta-adrenergic effects on cancer prior to randomised clinical trials. Similarly, not all tumour types may be equally responsive to beta-blockade. Beta-blocker use was associated with favourable outcomes in women with triple-negative breast cancer [174,175] but not in those with ER+ cancer. Beta-blocker use was associated with reduced prostate cancer specific mortality in men who received androgen-deprivation therapy but not in those with androgen-refractive tumours [181,182]. Randomised trials of target populations will be required to validate these observations; however, they suggest that adrenergic signalling may interact with other signalling pathways in tumours to affect progression.

Future directions

The bidirectional relationship between tumour cells and their microenvironment provides novel opportunities for interventions to slow down or stop cancer progression (Fig. 2). Tumour-related angiogenesis and lymphangiogenesis have critical roles in tumour cell dissemination, and they are tightly regulated by inflammatory and neural signals in the immediate microenvironment. This provides opportunities to slow down or prevent metastasis by complementing existing anti-angiogenic therapies with interventions that modulate immune cell recruitment or function or with drugs that modulate peripheral neural signalling.

Targeting SNS signalling may provide a novel leverage point for new anticancer therapies. Translation of findings from recent preclinical and epidemiological studies of beta-blockers to the cancer clinic requires characterisation of the receptors and downstream signalling pathways that mediate

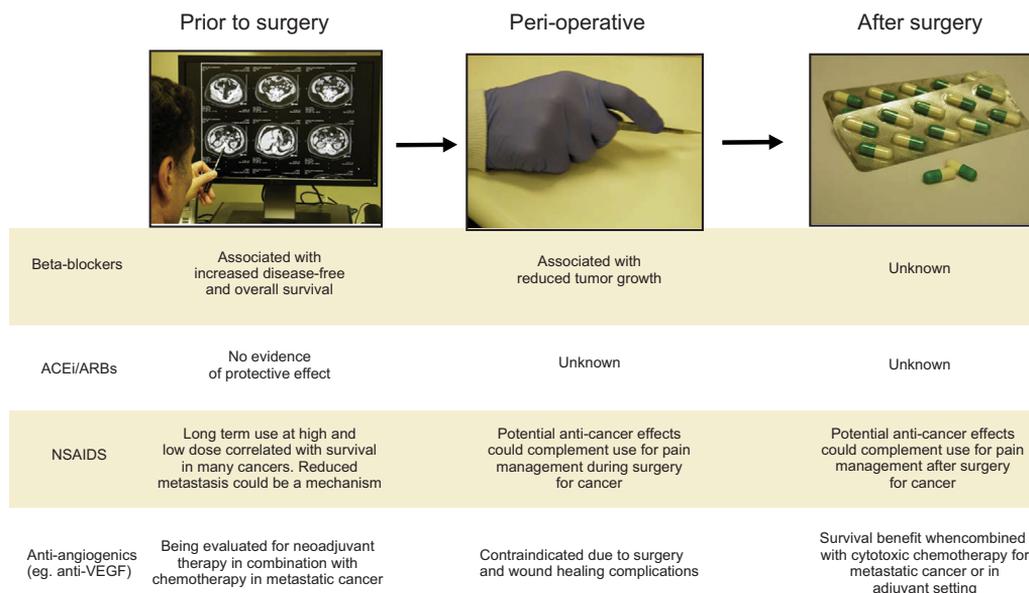


Fig. 2. Potential perioperative application of drugs that target vasculature and neural signalling. Summary of the potential applications of different major drugs classes that target blood vessels, lymphatic vessels and neural signalling for cancer patients prior to surgery, in the perioperative phase, and after surgery.

beta-adrenergic control of metastasis. The variable outcomes of clinical observational studies (see Table 1) indicate that not all patients or tumour types may be sensitive to beta-blockade and not all beta-blockers may provide protective effects against cancer progression. Prospective randomised clinical trials will be essential for unbiased assessment of beta-blockade in specific patient cohorts. Comprehensive and systematic profiling of beta-adrenergic signalling in tumour cells and in tumour stromal cells is needed to provide a mechanistic framework for rational selection and development of beta-blockers to treat cancer. These pharmaco-biological studies may additionally identify biomarkers of tumours that are sensitive to beta-adrenergic intervention to pinpoint cancer patients who will optimally benefit from beta-blockade.

The use of drug combinations that target multiple metastatic processes including angiogenesis or lymphangiogenesis, neural signalling and inflammation may be effective in slowing or preventing cancer progression. The results of trials of small-molecule inhibitors and monoclonal antibodies that target VEGF-dependent angiogenesis have shown some success and these drugs are now FDA-approved for a range of cancer indications [183]. Nonetheless, the heterogeneity of the response in patients and the development of resistance means that further studies are required to gain the most patient benefit from anti-angiogenic strategies. Patient profiling of molecular angiogenic drivers may allow a more selective use of anti-angiogenic drugs, and their combination with anti-inflammatory drugs or beta-blockers may provide additional therapeutic support. Beta-blockade may also enhance the efficacy of chemotherapy by increasing tumour cell apoptosis [26,184] and through modulation of immune cell recruitment to tumours [20], which has been linked to chemotherapy resistance [185]. In addition to maintaining homeostasis, NSAID and beta-blocker use in the perioperative setting may reduce the risk of tumour metastasis. In future, rational selection of these drugs and their application in specific patient populations may improve cancer outcomes.

Insights from vascular and neural biology have demonstrated the developmental links between the blood vasculature, lymphatic vasculature and the peripheral nervous system. These relationships extend to pathologies including cancer, where stress responses modify angiogenic and lymphangiogenic responses to facilitate cancer progression. Knowledge of these relationships opens the way for

evaluation of a number of well-accepted therapeutic agents to be used in preventative or perioperative strategies to combat cancer.

Research and clinical agenda

- Beta-blockers and NSAIDs may complement existing anti-angiogenesis strategies to treat cancer.
- Further research is required to define the receptors and signalling pathways that mediate these effects and windows of therapeutic opportunity.
- An unbiased evaluation of beta-blockers to treat cancer requires randomised clinical trials in defined patient populations.

Conflict of interest

Steven A. Stacker and Marc G. Achen are shareholders in Circadian Technologies Ltd., a company developing anticancer therapeutics.

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