http://www.expertreviews.org/

expert reviews
in molecular medicine

Tumour–host interactions: implications for developing anti-cancer therapies

Bedrick B. Gadea and Johanna A. Joyce*

Cancers are a complex set of proliferative diseases that arise in most cases through multi-step pathways involving an accumulation of genetic and epigenetic changes. These steps include inactivation of tumour suppressor genes and activation of oncogenes. However, in addition to genetic mutations in the tumour cells themselves, the local host environment can act as a critical modulator of cancer progression, having either tumour-suppressive or tumour-promoting effects depending on the stage and site of cancer development. Because stromal cells can have these opposing functions during cancer development and progression, a recurring theme throughout this review will be that of balance: maintaining the normal functions of these co-opted cells, yet selectively inhibiting their pro-tumourigenic functions. To achieve this equilibrium, we need to understand the molecular mechanisms by which normal cells become modified by cancer cells before we can hope to target these functions selectively. Here, we will discuss recent efforts to address these key challenges and offer perspectives on the translation of discoveries made in model systems to the clinic.

The tumour microenvironment contains a multitude of different cell types and extracellular elements as depicted in Fig. 1. These include the tumour blood vasculature, comprising endothelial cells (ECs) and pericytes/smooth muscle cells, the lymphatic vasculature, stromal fibroblasts and immune cells (adaptive and innate), in addition to constituents of the basement membrane and extracellular matrix (ECM). The cells of the host environment can be co-opted or converted by the cancerous cells to produce an array of growth factors, matrix-degrading enzymes and pro-angiogenic molecules that enhance tumour cell proliferation, invasion and metastasis, as well as cytokines that suppress immunity against the tumour. Thus, understanding the mechanisms by which tumours recruit and modify normal stromal cells to participate in disease development and progression should identify important cellular and molecular targets for cancer prevention and treatment. Strategies that target many components of the tumour microenvironment have recently been reviewed (Refs 1, 2, 3, 4) and we will specifically focus on three key cell types – the blood

Cancer Biology and Genetics Program, Memorial Sloan Kettering Cancer Center, New York, NY 10021, USA.

*Corresponding author: Johanna A. Joyce, Cancer Biology and Genetics Program, Memorial Sloan Kettering Cancer Center, Box 372, New York, NY 10021, USA. Tel: +1 646 888 2048; Fax: +1 646 422 0231; E-mail: joycej@mskcc.org

Accession information: DOI: 10.1017/S1462399406000172; Vol. 8; Issue 30; December 2006
© 2006 Cambridge University Press

https://doi.org/10.1017/S1462399406000172 Published online by Cambridge University Press

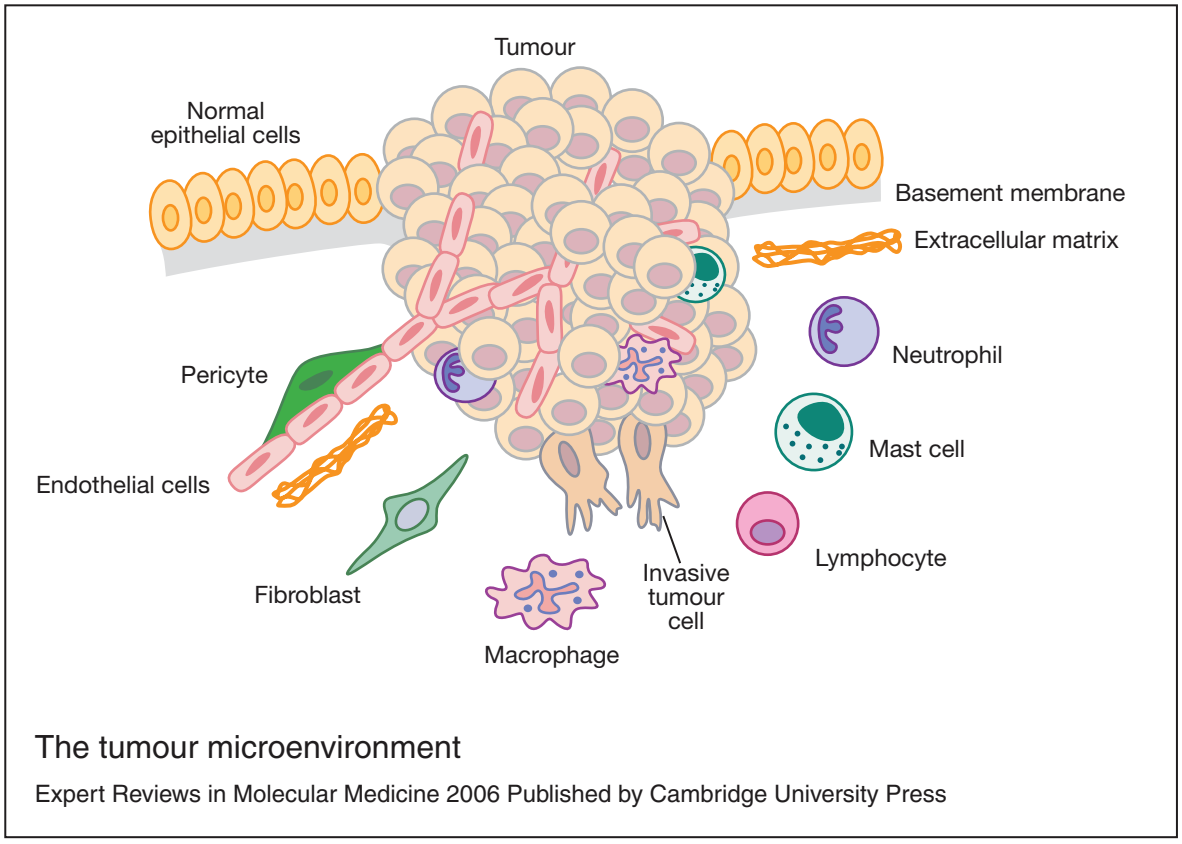


Figure 1. The tumour microenvironment. The tumour microenvironment comprises many different cell types in addition to the cancerous cells. These include vascular or lymphatic endothelial cells, supporting pericytes, fibroblasts, and both innate and adaptive infiltrating immune cells. In addition, there are non-cellular components of the tumour microenvironment, including the extracellular matrix and basement membranes. Adapted from Ref. 1 (© 2005) with permission from Elsevier.

vasculature, stromal fibroblasts and immune cells – highlighting therapeutic modalities that interfere with, or block, their pro-tumourigenic functions.

Cells of the tumour vasculature

The establishment of an independent blood vasculature is essential for tumour growth and can occur through two major pathways: angiogenesis, which is the formation of new vessels from pre-existing ones; and vasculogenesis, which is the recruitment of circulating endothelial progenitor cells to the nascent blood vasculature (Ref. 2). Because the tumour vasculature facilitates tumour growth and metastasis, great effort has been made to target molecules that are essential for the formation and maintenance of tumour blood vessels. Indeed, inhibiting tumour angiogenesis is the prototypical example of successful therapeutic targeting of the tumour microenvironment.

Targeting the vascular endothelial growth factor pathway in tumour angiogenesis

One of the major angiogenic pathways currently being targeted in cancer treatment is that of the vascular endothelial growth factor (VEGF) ligand and its receptors. VEGF-A binds to the tyrosine kinase receptors VEGFR1 and VEGFR2, but its effects on EC proliferation, angiogenesis and vascular permeability are mainly mediated through VEGFR2. Bevacizumab (Avastin®), which is a humanised monoclonal anti-VEGF antibody, was the first anti-angiogenic treatment approved for cancer treatment by the US Food and Drug Administration (FDA) (Table 1). In Phase III clinical trials for metastatic colorectal cancer, bevacizumab increased the median survival of patients by 4.7 months when

Accession information: DOI: 10.1017/S1462399406000172; Vol. 8; Issue 30; December 2006
© 2006 Cambridge University Press

https://doi.org/10.1017/S1462399406000172 Published online by Cambridge University Press

Table 1. Drugs that target cells of the tumour microenvironment[a]

Name	Cellular target	Mechanism of action	Status
Bevacizumab (Avastin®)	ECs	Humanised monoclonal antibody against VEGF-A	FDA approved
Neovastat (AE941)	ECs	Natural anti-angiogenic compound isolated from shark cartilage that inhibits VEGF signalling, MMP activity and specifically induces EC apoptosis	Phase III
Vatalanib (PTK787)	ECs	Small-molecule RTKI, inhibits VEGFR2, but also VEGFR1, VEGFR3 and PDGFRβ	Phase II/III
Interferon α	ECs	Inhibits angiogenesis in part by downregulating bFGF expression	Phase II/III
Combretastatin A4	ECs	Specifically interferes with endothelial microtubule assembly, resulting in rapid vascular dysfunction in tumours	Phase II
LY317615	ECs	Inhibitor of protein kinase Cβ	Phase II
Atrasentan	ECs	Small-molecule selective inhibitor of endothelin A receptor	Phase II
ZD6474	ECs	Small-molecule RTKI, inhibits VEGFR2 and EGFR and, to a lesser extent, VEGFR1 and VEGFR3	Phase I/II
VEGF-Trap	ECs	Soluble VEGFR: composite fusion protein of VEGFR1 and VEGFR2 with Fc fragment of IgG	Phase I/II
2-ME	ECs	2-ME is a small molecule inhibitor that blocks angiogenesis in part by downregulation of HIF-1α	Phase I
Thalidomide	ECs, other cell types	Glutamic acid derivative that inhibits angiogenesis, in part by inhibition of NF-κB, TNF-α, IL-6 and VEGF; also increases apoptosis and stimulates the immune response	Phase III
CC-5013	ECs, other cell types	Synthetic analogue of thalidomide, with more potent inhibition and less toxic side-effects	Phase II/III
Imatinib (Gleevec®)	Pericytes, stromal fibroblasts	RTKI; in addition to inhibiting Bcr-Abl kinase, also inhibits PDGFR and c-Kit kinases	FDA approved
Sunitinib (Sutent®, SU11248)	Pericytes, ECs	Small-molecule RTKI; inhibits VEGFR family members (VEGFR1, -2, -3), PDGFRβ and CSF-1R	FDA approved

(continued on next page)

Accession information: DOI: 10.1017/S1462399406000172; Vol. 8; Issue 30; December 2006
© 2006 Cambridge University Press

https://doi.org/10.1017/S1462399406000172 Published online by Cambridge University Press

Table 1. Drugs that target cells of the tumour microenvironment[a] *(continued)*

Name	Cellular target	Mechanism of action	Status
Sibrotuzumab	Carcinoma-associated fibroblasts	Humanised monoclonal antibody against fibroblast activation protein	Phase I
PI-88	ICs, ECs	Heparan sulphate mimetic; inhibits heparanase activity and function of heparin-binding growth factors	Phase I/II
Etanercept (Enbrel)	ICs, other cells	Recombinant human soluble p75 TNF receptor	Phase II
Zoledronic acid (Zometa®)	Macrophages, other cells	Bisphosphonate compound that inhibits osteoclast activity and suppresses MMP-9 synthesis in macrophages	FDA approved
Trabectedin (Yondelis)	Macrophages, dividing cells	Binds to the minor groove of DNA to effect gene expression and cell-cycle progression; reduces macrophage viability and expression of pro-inflammatory cytokines from macrophages and tumour cells	Phase II/III
INCB3284 and MK0812	ICs	CCR2 antagonists	Phase II for inflammatory disorders
Celecoxib (Celebrex®)	Macrophages, fibroblasts, tumour cells	Cox-2 inhibitors	Phase I/II
SU6668	Pericytes	RTKI that can target VEGFR-2, FGFR-1 and PDGFR-β	Phase I
Sorafenib (Nexavar®, BAY 43-9006)	ECs, pericytes, tumour cells	Serine/threonine and RTKI against Raf, VEGFR2 and VEGFR1	FDA approved

[a]List of agents that target one or more cells in the tumour microenvironment and that are approved by the FDA or are currently in clinical trials for cancer or inflammation-related disorders.

Abbreviations: 2-ME, 2-methoxyestradiol; bFGF, basic fibroblast growth factor; CCR2, CC chemokine receptor 2; CSF-1R, colony-stimulating factor 1 receptor; EC, endothelial cell; EGFR, epidermal growth factor receptor; FDA, US Food and Drug Administration; FGFR, fibroblast growth factor receptor; HIF-1, hypoxia-inducible factor; IC, inflammatory cell; IL-6: interleukin 6; MMP, matrix metalloproteinase; NF-κB, nuclear factor of κB; PDGFR, platelet-derived growth factor receptor; RTK, receptor tyrosine kinase; RTKI, receptor tyrosine kinase inhibitor; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

combined with standard chemotherapy (Ref. 5; reviewed by Ref. 3). In randomised Phase III trials that await publication, bevacizumab also increased the overall and/or progression-free survival of patients with lung cancer or metastatic breast cancer when combined with chemotherapy (http://www.cancer.gov/cancertopics/factsheet/AvastinFactSheet; reviewed by Ref. 6). However, a recent Phase III trial was ended prematurely because bevacizumab did

Accession information: DOI: 10.1017/S1462399406000172; Vol. 8; Issue 30; December 2006
© 2006 Cambridge University Press

https://doi.org/10.1017/S1462399406000172 Published online by Cambridge University Press

not provide survival benefits over chemotherapy in patients with pancreatic cancer. Although the combination of bevacizumab with chemotherapy has clear survival benefits in some cancers, patients under this regimen still develop end-stage disease, indicating that the dosage and/or timing of the combined treatment might need further optimisation to achieve maximal therapeutic efficacy.

Future studies could also be designed to determine whether other targeted therapeutics have synergistic effects when administered with bevacizumab, including targets on tumour cells such as the growth-stimulating epidermal growth factor receptor (EGFR), using approved inhibitors including erlotinib (Tarceva®) and gefitinib (Iressa®). Such a trial was recently presented in which patients with advanced renal cell carcinoma were treated with a three-drug combination using bevacizumab, erlotinib and imatinib (Gleevec®); these patients, however, experienced moderate-to-severe toxicity without increased efficacy (http://www.asco.org/portal/site/ASCO/menuitem.34d60f5624ba07fd506fe310ee37a01d/?vgnextoid=76f8201eb61a7010VgnVCM100000ed730ad1RCRD&vmview=abst_detail_view&confID=40&abstractID=34355). A challenge for the future will be to identify combinations of drugs and dosing schedules that have potent additive or synergistic therapeutic efficacy without the almost inevitable toxic side-effects.

Another approach to target VEGF is to block the kinase activity of its receptors. Small-molecule receptor tyrosine kinase inhibitors (RTKIs) that can bind and inhibit VEGF receptors (among other RTKs), such as sunitinib (SU11248), sorafenib (BAY 43-9006) and AG-013736, are currently being tested in clinical trials against several cancer types (reviewed by Ref. 3). Indeed, the results of the clinical trials performed to date indicate that RTKIs such as sunitinib and sorafenib, which inhibit other RTKs in addition to VEGFR2, including platelet-derived growth factor receptor (PDGFR)-β and c-Kit, have significant effects on patient survival as a monotherapy, compared with anti-VEGF (bevacizumab) treatment alone (Ref. 6). This suggests that broad-spectrum inhibitors that target VEGF receptors, as well as other receptors, on stromal cells and cancer cells might prove most efficacious, especially if there is minimal toxicity to normal cells.

Normalisation of the tumour vasculature

Bevacizumab only has significant survival benefits when administered in combination with chemotherapy, not when used alone. Thus, it has been proposed that anti-VEGF therapies enhance the sensitivity of tumours to cytotoxic drugs through normalisation of the tumour vasculature. Tumour vessels are extremely leaky and tortuous, which impedes uniform blood flow, oxygenation and drug delivery in tumours. Furthermore, the deficiency of oxygen (hypoxia) in certain areas of the tumour reduces the efficacy of radiation therapy and is responsible for the upregulation of hypoxia-inducible genes that promote malignant properties of tumour cells, including increased angiogenesis and metastatic potential (Ref. 7).

In contrast to tumour vessels, normal vessels have reduced permeability and more uniform blood flow that efficiently delivers oxygen. Since VEGF typically increases vessel permeability, anti-VEGF therapy is expected to reduce vessel leakage by normalising the tumour vasculature. Clinical evidence from human patients with rectal cancer supports the hypothesis that bevacizumab, and perhaps other anti-VEGF therapies, normalises the tumour vasculature and enhances drug delivery and oxygen distribution (Ref. 8). A transient increase in oxygenation may promote tumour cell proliferation, which would render tumours more sensitive to chemotherapeutic drugs. The prediction is that normalisation of the tumour vasculature would result in a temporary increase in drug and oxygen uptake, before the tumour vessels (and thus the delivery system) are ultimately destroyed. In this regard, establishing the correct treatment 'window' for each inhibitor, or for drugs in combination, will be essential to achieve optimal therapeutic results.

The proof of this concept was elegantly demonstrated using VEGFR2 blockade followed by radiation therapy in an orthotopic glioma model. The effects of radiation synergised with the anti-angiogenic, normalisation therapy, but only in a very narrow treatment window (Ref. 9). Moreover, in addition to being expressed on ECs, VEGF receptors are also expressed by some human tumour cells, including colon cancer cells (Refs 10, 11). It is therefore possible that anti-VEGF therapies will target both tumour cells and ECs.

Accession information: DOI: 10.1017/S1462399406000172; Vol. 8; Issue 30; December 2006
© 2006 Cambridge University Press

https://doi.org/10.1017/S1462399406000172 Published online by Cambridge University Press

Targeting pericyte coverage of the tumour vasculature

An additional strategy to perturb the tumour vasculature is either to prevent the recruitment or to disrupt the coverage of perivascular cells on tumour blood vessels. Perivascular cells, which include pericytes and smooth muscle cells, make close contacts with the abluminal surface of ECs, where they provide structural support and regulate EC survival, proliferation and vascular permeability. ECs and pericytes communicate through multiple paracrine signals that may be targeted therapeutically, including the following interactions: PDGF-B–PDGFRβ, VEGF-A–VEGFR2, transforming growth factor β1 (TGF-β1)–TGF-βRs and Angiopoietin–Tie2.

The PDGF-B signalling pathway is essential for the recruitment of pericytes to newly formed blood vessels; indeed, the inhibition of PDGFRβ in pericytes, through the use of neutralising antibodies, reduced pericyte coverage and compromised EC viability in mice engineered to develop pancreatic islet tumours (Ref. 12). In these mice, alternatively targeting PDGFRβ using the tyrosine kinase inhibitor SU6668 also reduced pericyte coverage of tumour vessels and regressed late-stage tumours (Ref. 13). Furthermore, disrupting pericyte coverage makes tumours more sensitive to anti-VEGF therapy (Refs 13, 14). These publications suggest targeting both pericytes and ECs is an attractive therapeutic approach for achieving complete vascular disruption, which would block the flow of nutrients to the tumour and lead to tumour regression.

However, reducing pericyte coverage might also have adverse effects and possibly decrease vessel normalisation and drug delivery. For example, it was recently shown that genetic disruption of PDGF-B function in mouse pancreatic islet tumours reduced pericyte coverage and in fact enhanced metastasis to distant organs (Ref. 15). Limited pericyte coverage might increase tumour vessel permeability, which could then provide an escape route for invasive cancer cells from the primary tumour to distant sites. On the basis of these results, it will now be very important to examine carefully the consequences of PDGFRβ inhibition in various animal models of cancer and determine whether there is an increase in metastatic spread following pericyte disruption, especially as RTKIs that can target PDGFRβ (such as SU6668, imatinib, sunitinib) are currently at different phases of clinical trials. Taken together, it is not clear which strategy will ultimately prove most effective as a cancer therapy: either enhancing pericyte coverage and improving vessel normalisation or blocking pericyte recruitment and disrupting/regressing tumour vessels. Analyses in pre-clinical models are clearly needed to distinguish between these possibilities before proceeding to the clinic with more specific PDGFR inhibitors.

Anti-angiogenic therapy and drug resistance

One of the biggest challenges to cancer therapy is acquired drug resistance. This resistance might also be a problem in patients undergoing anti-VEGF therapy because most patients ultimately progress to end-stage disease. In addition to VEGF, tumour angiogenesis can be driven by factors such as fibroblast growth factor 1 (FGF-1), FGF-2, TGF-β, interleukin 8 (IL-8), ephrins, placental growth factor (PlGF) and others. In late-stage tumours, it is possible that any of these angiogenic factors could bypass the requirement of VEGF for neo-angiogenesis.

Using a mouse pancreatic islet cancer model, it was shown that anti-VEGF therapy initially decreased angiogenesis and tumour burden, but this reduction was then followed by a period of increased angiogenesis and tumour growth (Ref. 16). This resistance was found to be a result of the elevated expression of angiogenic factors such as FGFs and ephrins, suggesting that targeting multiple angiogenic pathways concurrently or in sequence might be necessary to sustain tumour regression in the long term. As an example, RTKIs that simultaneously target multiple tyrosine kinases involved in neovascularisation might have a reduced risk of acquired resistance.

In a separate study, downregulation of hypoxia-inducible factor 1α (HIF-1α) in a colorectal cancer cell line was shown to enhance the expression of another pro-angiogenic factor, the chemokine IL-8, both in culture and when these cells were transplanted subcutaneously into mice (Ref. 17). The growth of these transplanted tumour cells in vivo was inhibited in the presence of neutralising anti-IL-8 antibodies and reduced even further when VEGF was co-inhibited. HIF-1α is a transcription

Accession information: DOI: 10.1017/S1462399406000172; Vol. 8; Issue 30; December 2006
© 2006 Cambridge University Press

https://doi.org/10.1017/S1462399406000172 Published online by Cambridge University Press

factor that induces the expression of pro-angiogenic and pro-invasive genes in response to low oxygen concentrations. HIF-1α is actually downregulated by both microtubule-stabilising drugs (including taxanes and epothilones) and microtubule-destabilising compounds (e.g. 2-methoxyestradiol), which are anti-proliferative agents (Ref. 18). As 2-methoxyestradiol is currently being evaluated in clinical trials against solid tumours (Refs 19, 20), it will now be important to test whether such treatment indeed results in an increase in the levels of other angiogenic molecules, particularly IL-8. If this compensatory mechanism also occurs in patients undergoing anti-HIF-1α therapy, then targeting HIF-1α will probably be most effective in combination with other anti-angiogenic drugs. In summary, the above results indicate that therapies that target multiple, independent angiogenic pathways might have additive or even synergistic effects on tumour regression. The challenge, however, will be to develop cocktails of drugs that target multiple angiogenic pathways and at the same time determine what dosage, combination, timing and sequence is optimal, with minimal toxicity to normal tissues.

One distinct advantage associated with therapies targeting the microenvironment was the assumption that these non-cancerous cells are genetically stable – in contrast to the genetically unstable cancer cells – and thus should not accumulate adaptive mutations, leading to drug resistance. However, this view might not be entirely correct, as genetic changes have recently been reported in tumour-associated ECs. Cytogenetic abnormalities have been detected in ECs isolated from xenografts of human melanomas and liposarcomas (Ref. 21), and in many ECs associated with B-cell lymphomas in patients (Ref. 22). Whether these genetic alterations make ECs less sensitive to anti-angiogenic therapies remains to be tested. It is also not clear whether this EC-specific genetic instability is a common event in multiple types of human cancer.

The contribution of bone-marrow-derived cells to angiogenesis and tumour development

It is now recognised that bone-marrow-derived cells (BMDCs) participate in angiogenesis, but it is still controversial whether their role is direct, by incorporating into the tumour vasculature, or indirect, by providing angiogenic factors as accessory cells (Fig. 2). Depending on the tumour model used and/or the tumour stage analysed, bone-marrow-derived endothelial progenitor cells (EPCs) can either make up 50–90% of the tumour endothelium or represent only a minor population of ECs (Refs 23, 24, 25, 26, 27). In the human tumours that have been investigated to date, it is estimated that EPCs constitute fewer than 5% of the total EC population (Refs 28, 29, 30). Recruitment of EPCs from the bone marrow to tumour sites is dependent on VEGFR1 and matrix metalloproteinase 9 (MMP-9), and it remains to be determined whether blocking EPC recruitment, using VEGFR or MMP-9 inhibitors for example, will be a viable anti-angiogenic therapy (Fig. 2). However, it might be possible to measure the amount of EPCs or circulating ECs in the peripheral blood and to use this quantification as a read-out for determining the dosage and efficacy of anti-angiogenic therapies (Ref. 31).

Some recent studies suggest that the majority of BMDCs that home to the tumour vasculature might be progenitors of perivascular cells that supply the tumour vessels with pro-angiogenic factors (Refs 24, 26). Bone-marrow-derived c-Kit$^+$Sca-1$^+$VEGFR1$^+$PDGFRβ$^+$ perivascular progenitor cells are mobilised to tumour vessels where they differentiate into mature pericytes (Ref. 12). As discussed above, targeting pericytes, or pericyte progenitors, could lead to efficient vessel regression but it remains to be determined whether targeting this cell type alone is a viable therapeutic option.

BMDCs of haematopoeitic origin can also have profound effects on tumour development. There is accumulating evidence that immature myeloid cells are highly plastic and, once recruited to developing tumours, can differentiate into other cell types. Immature myeloid progenitor cells expressing monocyte and granulocyte markers CD11b and Gr1 were previously shown to differentiate into endothelial-type cells in vitro when exposed to angiogenic factors (Ref. 32). These cells also incorporated into the tumour endothelium when injected subcutaneously along with colon or lung cancer cells. In another study, CD11b$^+$ cells were found closely associated with newly formed blood vessels induced by VEGF expression in the heart or liver, but did not incorporate into the endothelium nor express endothelial or perivascular markers (Ref. 33).

Accession information: DOI: 10.1017/S1462399406000172; Vol. 8; Issue 30; December 2006
© 2006 Cambridge University Press
https://doi.org/10.1017/S1462399406000172 Published online by Cambridge University Press

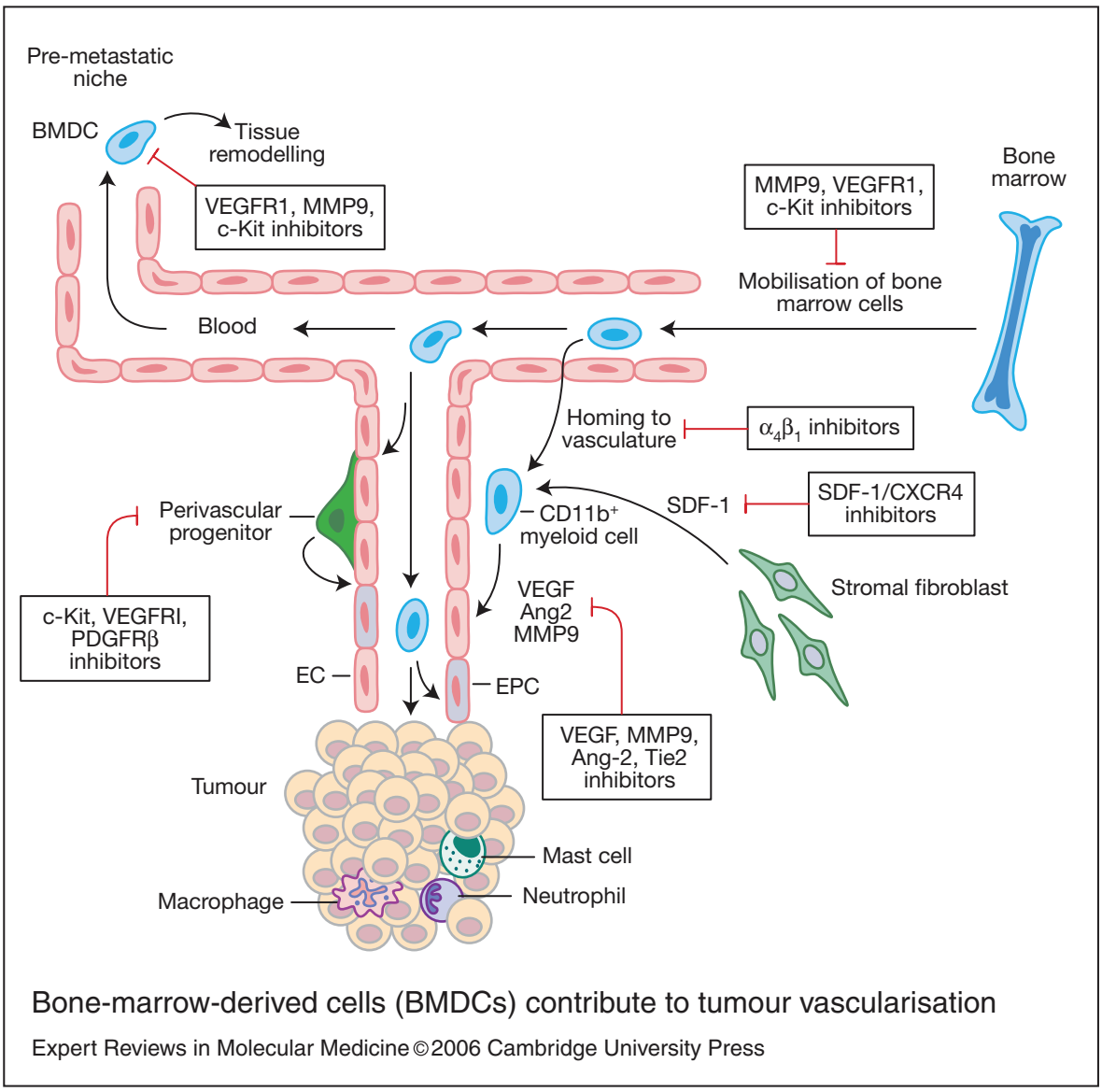


Figure 2. Bone-marrow-derived cells (BMDCs) contribute to tumour vascularisation. BMDCs mobilise from the bone marrow to sites of tumour growth and pre-metastatic niches in a manner dependent on matrix metalloproteinase 9 (MMP-9), vascular endothelial growth factor receptor 1 (VEGFR1) and c-Kit. Homing of BMDCs to the tumour vasculature depends on integrin $\alpha_1\beta_4$, which may be targeted with blocking antibodies. Depending on the tumour stage or site, BMDCs may either incorporate into the tumour endothelium as endothelial progenitor cells (EPCs) or differentiate into perivascular cells or myeloid progenitors. The latter haematopoietic cell is maintained near vascular sites by stroma-derived factor-1 (SDF-1), which is expressed by stromal fibroblasts. Myeloid cells, such as macrophages, mast cells and granulocytes, can also infiltrate within the tumour to supply pro-tumourigenic signals. Other abbreviations: Ang-2, Angiopoeitin-2; EC, endothelial cell.

These cells were retained near neovascular sites by secretion of stroma-derived factor 1α (SDF-1α) from stromal fibroblasts and promoted the growth of resident vessels through the release of angiogenic factors including VEGF, Angiopoietin 2 (Ang2) and MMP-9. Taken together, it appears that BMDCs receive signals from the tumour microenvironment to differentiate into various cell types that aid in the vascularisation required for tumour growth.

Accession information: DOI: 10.1017/S1462399406000172; Vol. 8; Issue 30; December 2006
© 2006 Cambridge University Press

https://doi.org/10.1017/S1462399406000172 Published online by Cambridge University Press

Determining whether BMDCs have similar roles in human cancers warrants future investigation. If so, mobilisation of BMDCs to areas of tumour neovascularisation could be blocked using agents that reduce MMP-9, VEGFR1 or c-Kit activity, and might also be perturbed by targeting SDF-1 and/or integrin $\alpha_4\beta_1$ (Ref. 34) (see Fig. 2).

A study recently identified a fraction of $CD11b^+$ myeloid cells expressing Tie2 (termed 'Tie2-expressing monocytes' or TEMs) as being crucial for development of the tumour vasculature in spontaneous and orthotopic tumours (Ref. 35). Tie2 is a tyrosine kinase receptor previously thought to have a restricted expression in ECs, where it regulates vessel maturation and stabilisation. Pericytes are known to secrete Ang1, which activates Tie2 receptors on the surface of ECs and promotes vessel stability. Thus, blocking the Ang1–Tie2 interaction could provide an additional strategy to disrupt tumour vessels.

Another angiopoietin family member, Ang2, antagonises many actions of Ang1, but also appears to function in tumour vessel formation. Pre-clinical trials in tumour-bearing mice using blocking antibodies or peptides that prevent the interaction between Ang2 and Tie2 delayed tumour development and reduced EC proliferation, suggesting that humanised anti-Ang2 antibodies or specific Tie2 inhibitors could also be used to treat tumours in which Ang2 is being actively released (Ref. 36). Moreover, recent experiments suggest an added benefit of targeting certain Angiopoietins, as Ang2 has been shown to enhance the response of ECs to inflammatory stimuli, whereas Ang1 suppresses it (Ref. 37). It is still not known whether targeting Ang1 or Ang2 provides a potent anti-angiogenic strategy or whether the use of RTKIs that block the activity of Tie2 (and possibly other RTKs) will be a more promising therapy.

BMDCs and metastasis

In addition to stimulating neovascularisation, BMDCs may have an unexpected role in establishing sites of future tumour metastasis. Indeed, bone-marrow-derived haematopoietic progenitor cells expressing VEGFR1 were shown to be mobilised by tumour-derived soluble factors to specific pre-metastatic sites, where they modify the tissue environment to attract and support the colonisation of circulating tumour cells and endothelial progenitors (Ref. 38). Depletion of $VEGFR1^+$ cells from the bone marrow, or inhibition of VEGFR1 signalling using specific antibodies, prevented metastasis and formation of the pre-metastatic environment. Thus, agents that block VEGFR1 signalling (such as the RTKI sorafenib) or other pathways implicated in the mobilisation of bone marrow progenitor cells to pre-metastatic sites, such as MMP-9 or c-Kit, might also represent effective anti-metastatic therapies. Some of the anti-metastatic therapies would be limited, however, to preventative strategies in patients that have not yet developed metastatic disease. The exact factors released from tumour cells that mobilise BMDCs to particular pre-metastatic niches have yet to be identified and could provide additional targets to prevent tumour dissemination or could represent prognostic factors for disease progression.

Cancer and inflammation

The immune system activates chemical pathways that eliminate foreign pathogens and elicit long-term immunity. During the acute activation of these pathways, innate immune cells enter and exit the affected area, eliminate the invading agent, repair the damaged tissue, and initiate adaptive immunity against the pathogen. The resolution of acute inflammation then restores normal tissue homeostasis to the afflicted area. However, when acute inflammation is not resolved, chronic inflammation ensues and results in excessive tissue remodelling, genetic alterations as a result of oxidative stress and loss of normal tissue architecture. These changes are associated with an increased risk of pathological disorders such as arthritis and cancer, indicating that, to maintain normal tissue homeostasis, a fine balance must be reached between the initiation and complete execution of an inflammatory response.

Insights from clinical studies

Epidemiological studies have long revealed a significant connection between chronic inflammation and increased susceptibility to various cancers. Inflammatory stimuli associated with cancer predisposition include chronic pancreatitis, *Helicobacter pylori* infection, chemical irritants and viral infections (Refs 39, 40). In fact, infectious agents or pro-inflammatory conditions are believed to be the

Accession information: DOI: 10.1017/S1462399406000172; Vol. 8; Issue 30; December 2006
© 2006 Cambridge University Press

https://doi.org/10.1017/S1462399406000172 Published online by Cambridge University Press

causative force underlying approximately 15% of all human cancers, by inducing the changes associated with chronic inflammation mentioned above. Notable examples of cancers arising from chronic inflammatory conditions include ultraviolet (UV)-induced melanoma, chronic pancreatitis-associated pancreatic adenocarcinoma and Crohn's disease-associated colorectal cancer (Ref. 40). These observations suggest that either reducing chronic inflammation itself or blocking the action of pro-inflammatory molecules could lower the risk of developing certain types of cancer.

Clinical studies demonstrate that long-term use of non-steroidal anti-inflammatory drugs (NSAIDs; e.g. aspirin), or cyclo-oxygenase 2 (Cox-2) inhibitors reduce the risk of colon, breast and gastric cancer (Ref. 41). Cox-2 is an enzyme involved in the conversion of arachidonic acid into prostaglandins, which bind to cell-surface receptors to promote inflammation and tumour angiogenesis, invasion and metastasis (reviewed by Ref. 42). Elevated expression of Cox-2 correlates with poor patient prognosis in many human tumours including oesophageal adenocarcinomas (Ref. 43) and carcinomas of the breast (Ref. 44), ovary (Ref. 45) and colon (Ref. 46). Mice lacking Cox-2 have reduced tumour growth and angiogenesis when injected with Lewis lung carcinoma cells (Ref. 47) and form smaller and fewer polyps in a mouse model of human familial adenomatous polyposis (Ref. 48). These and other studies provide rational support for the use of Cox-2 inhibitors in cancer patients. However, there is considerable controversy surrounding the association of long-term usage of high doses of Cox-2 inhibitors with elevated risk of cardiovascular events (Ref. 49). Furthermore, NSAIDs have many targets, which may also lead to toxic effects. These epidemiological studies indicate that selectively inhibiting alternative molecules that mediate the pro-tumourigenic effects of chronic inflammation might be more efficacious and less toxic, as outlined below.

In many tumours, the presence of adaptive immune cells, particularly $CD4^+$ and $CD8^+$ T cells, is generally associated with a good clinical prognosis. However, there are a few examples in animal models where B and T cells were shown to have stimulating effects on tumour progression depending on the tissue (Ref. 50; reviewed by Ref. 51); these results require further experimental validation in other organs to determine whether this effect is a tissue-specific phenomenon. Many tumours develop ways to evade immune surveillance by suppressing the function of T cells and by enhancing the activities of subtypes of immune cells that promote tumour development. In cancers associated with chronic inflammation, for example, the presence of innate immune cells belonging to the myeloid lineage, such as macrophages, mast cells (MCs) and neutrophils, invariably correlates with a poor prognosis for patients (Refs 41, 52).

Innate immune cells modified by the tumour environment provide tumours with factors that promote tumourigenic processes, such as tumour growth, survival, vascularisation and tissue remodelling, and suppress the anti-tumour activities of adaptive immune cells (Refs 40, 52). Innate immune cells can also promote genomic instability and mutagenesis of tumour cells by producing reactive oxygen and nitrogen species (Refs 53, 54). Thus, it is possible that targeting molecules provided by tumour-infiltrating innate immune cells might provide beneficial outcomes in the treatment of various cancers (as discussed below).

General pro-inflammatory molecules

Infiltrating innate immune cells produce numerous pro-inflammatory molecules that influence cancer development; these include the transcriptional regulator nuclear factor κB (NF-κB), tumour necrosis factor α (TNF-α), various interleukins (IL-1, IL-6, IL-8), certain chemokines and their receptors, and matrix-degrading enzymes. The role of some of these molecules in tumour progression has been evaluated in animal models and in patients.

NF-κB

Using an inflammation-induced model of hepatocellular carcinoma, it was found that NF-κB signalling in both epithelial and inflammatory cells promotes tumour growth, the release of pro-inflammatory cytokines and progression to a malignant state (Ref. 55). NF-κB promotes tumourigenesis through the transcriptional regulation of genes that inhibit apoptosis, promote cell-cycle progression and inflammatory responses, and enhance angiogenesis, invasion and metastasis (reviewed by Ref. 56). Sustained activation of NF-κB is

Accession information: DOI: 10.1017/S1462399406000172; Vol. 8; Issue 30; December 2006
© 2006 Cambridge University Press

https://doi.org/10.1017/S1462399406000172 Published online by Cambridge University Press

observed in many human cancers, where it might contribute to the acquired resistance of tumours to various therapies as a result of suppressing apoptosis, which is the major mechanism by which chemotherapeutic drugs kill tumour cells. Direct inhibitors of NF-κB, such as those that can block its interaction with DNA, may be difficult to generate for therapeutic delivery and thus it might be more effective to target the kinase activity of IKKβ, which is a kinase that indirectly activates NF-κB. Deletion of IKKβ in myeloid cells reduced tumour incidence in inflammation-induced colon cancers, indicating that IKKβ inhibitors could be useful in cancer therapy to combat the action of infiltrating immune cells (Ref. 57). Many such inhibitors have been generated by multiple pharmaceutical companies and have been shown to significantly attenuate NF-κB signalling in vivo and delay or prevent the progression of several inflammatory disorders in animal models (reviewed by Ref. 58). Surprisingly, these agents have not yet been used in humans and thus their efficacy and toxicity need to be critically evaluated in pre-clinical cancer models.

The use of IKKβ inhibitors will need to be assessed in multiple organ-specific tumour models, since some tissues may respond differently to anti-NF-κB therapy. For example, blocking NF-κB signalling in the skin actually promoted squamous cell carcinoma progression in mice (Refs 59, 60) and hepatocyte-specific deletion of IKKβ enhanced chemical-induced hepatocarcinoma development (Ref. 61). In the latter model, IKKβ deletion in haematopoietic-derived cells decreased tumour incidence, showing that immune cells use the NF-κB pathway to stimulate tumourigenesis. A possible explanation for such results may be that NF-κB promotes the expression of pro-tumourigenic inflammatory cytokines in immune cells, while increasing the expression of apoptotic genes in hepatocytes that are improperly dividing. Thus, unless these functions can be separately inhibited, current strategies to target NF-κB or IKKβ may be premature for clinical use.

TNF-α

TNF-α is a major cytokine that mediates inflammatory reactions and acts by binding to its receptors TNFR1 or TNFR2 on the surface of cells. Although high expression of TNF-α can be anti-angiogenic, its chronic production is associated with the tissue remodelling necessary for tumour growth and spread. TNF-α production is also associated with poor prognosis in lymphomas (Ref. 62) and ovarian carcinomas (Ref. 63). TNF-α expression during malignancy has been detected in both epithelial tumour cells and/or infiltrating immune cells (reviewed by Ref. 64). In breast carcinomas, for example, tumour-infiltrating macrophages are the major source of TNF-α expression (Ref. 65). TNF-α production may promote tumourigenesis by stimulating the production of inflammatory cytokines, pro-angiogenic cytokines and chemokines, and matrix-degrading enzymes.

Targeting TNF-α could attenuate the inflammatory responses that are associated with cancer progression. This is corroborated by studies in mice, in which deletion of TNF-α or its receptors (TNFR1 and TNFR2) significantly inhibited the progression of chemical-induced skin carcinogenesis (Refs 66, 67). Studies in mouse models of inflammation-induced liver and colon cancer showed that stromal production of TNF-α activated NF-κB, suggesting that inhibitors of NF-κB might alleviate some TNF-α-induced tumourigenic effects (Refs 55, 57). TNF-α inhibitors, such as the soluble TNF receptor etanercept (Enbrel®), the chimaeric IgG1 monoclonal antibody against TNF infliximab (Remicade®) and pegylated humanised anti-TNF fragment CDP870 are currently being used in the clinic for the treatment of inflammatory-related diseases. Etanercept is approved by the FDA for the treatment of rheumatoid arthritis and was shown to decrease cytokine production [IL-6 and CC chemokine ligand 2 (CCL2)] in Phase II clinical trials for metastatic breast cancer and Phase I trials for ovarian cancer (Refs 68, 69). In the former trial, no significant survival advantages were reported, whereas the latter study reported an overall 33% stable disease response at the doses administered. Further trials are required to determine whether higher and/or more frequent doses can yield more favourable outcomes and if there are advantages to combining anti-TNF agents with other conventional therapies.

Matrix-degrading enzymes

Inflammatory cells also produce proteases that degrade basement membrane and extracellular

Accession information: DOI: 10.1017/S1462399406000172; Vol. 8; Issue 30; December 2006
© 2006 Cambridge University Press

https://doi.org/10.1017/S1462399406000172 Published online by Cambridge University Press

matrix components; these activities are an essential prerequisite for their normal migration throughout the body. Matrix-degrading enzymes can also be released from inflammatory cells within the tumour microenvironment to stimulate the formation of the tumour vasculature, and enable tumour invasion and metastasis. Some matrix-degrading enzymes that are upregulated in tumour-infiltrating inflammatory cells include urokinase-type plasminogen activator (uPA) (Ref. 70), MMPs (Refs 71, 72), cathepsins (Refs 73, 74) and heparanase (Ref. 75); pharmacological or genetic ablation of these proteases has been shown to perturb tumour progression in different animal models.

MMPs

Numerous MMPs have been implicated in tumourigenic processes such as ECM turnover, tumour invasion and metastasis, and angiogenesis. Many proteases in the MMP family can be released from stromal cells, including fibroblasts and immune cells, and accumulate in the extracellular environment where they influence normal or cancer cell behaviour. MMP-3, for example, is produced by stromal fibroblasts and causes cellular transformation of adjacent normal mammary epithelial cells into mesenchymal-like tumours both in mice and tissue culture (Refs 76, 77). MMP-7 is produced primarily from cancer cells and was recently shown to promote osteolysis during bone metastasis of prostate cancer cells by activating osteoclasts at the tumour–bone interface (Ref. 78). Furthermore, in a mouse model of intestinal adenoma, MMP-7 deficiency significantly reduced tumour size (Ref. 79). MMP-9 promotes tumourigenesis by releasing angiogenic growth factors (e.g. VEGF) sequestered within the ECM and is involved in the generalised matrix remodelling important for angiogenesis and metastasis. MMP-9 is also implicated in the mobilisation of bone-marrow-derived progenitor cells into the tumour (Fig. 2). Specific inhibitors of MMP-9 would be expected to diminish the capability of tumours to undergo angiogenesis and metastatic spread, as well as having the added advantage of reducing the contribution of BMDCs to the tumour vasculature as discussed above. MMP-9-deficient mice have a reduced ability to develop malignant tumours in the skin (Ref. 71), cervix (Ref. 72) and pancreas (Ref. 80). In these tumours, MMP9 is predominantly expressed by innate immune cells such as macrophages and MCs, with little or no expression observed in tumour cells.

Although MMPs have important roles in various cancer models, broad-spectrum MMP inhibitors have had disappointing outcomes in clinical trials, perhaps owing to adverse effects related to targeting multiple MMP family members, such as those known to have anti-tumour functions including MMP-8 and MMP-12 (reviewed by Ref. 81). Therefore, if more selective MMP inhibitors are developed in the future, it will be very important to test them thoroughly in pre-clinical models for adverse effects. For example, caution should be exercised when administering specific MMP-9 inhibitors since one study reported that although skin tumours that arose in MMP-9-deficient mice were reduced in size compared with their wild-type counterparts, they were less differentiated and more aggressive (Ref. 71), suggesting that MMP-9 inhibitors could potentially select for more malignant tumours over time. It would be interesting to determine whether the aggressive tumours that arise in MMP-9-deficient mice upregulate additional MMPs and/or other protease family members as a compensatory mechanism to enable tumour progression.

Cathepsins

Recently, cysteine cathepsins have emerged as possible anti-cancer targets from functional studies in mice. In pancreatic islet, cervical and lung cancer mouse models, it was found that multiple cathepsin family members were upregulated during tumour progression (Refs 73, 82). Interestingly, the expression of four cathepsin family members (cathepsins B, C, H and S) was abundant in infiltrating innate immune cells in pancreatic tumours (Ref. 73). Collective inhibition of cathepsins using a broad-spectrum inhibitor reduced pancreatic islet cell cancer progression and even regressed well-established tumours. This result was encouraging, since it suggested cathepsin proteases have important functions in late-stage disease. Subsequent genetic experiments have identified cathepsins B, L and S as the key tumour-promoting cathepsins in pancreatic cancer (Ref. 74), supporting the future development of inhibitors that are selective for

Accession information: DOI: 10.1017/S1462399406000172; Vol. 8; Issue 30; December 2006
© 2006 Cambridge University Press

https://doi.org/10.1017/S1462399406000172 Published online by Cambridge University Press

these cathepsins, which can then be tested in pre-clinical and clinical trials.

In human cancers, high cathepsin B and/or L expression and activity is frequently associated with poor patient prognosis, increased tumour grade, and enhanced invasion and metastasis in melanomas, colorectal carcinomas, gliomas, prostate carcinomas and breast carcinomas among others (reviewed by Ref. 83). Increased cathepsin B expression has been observed at the invading tumour front of many human cancers, consistent with a role in invasion and metastasis through destruction of basement membrane/ECM proteins. In addition to being expressed in tumour cells, cathepsin B expression is high in tumour-associated stromal fibroblasts in melanomas and breast and colorectal carcinomas; its expression is also high in macrophages that have infiltrated colorectal carcinomas (Ref. 83). Targeting cathepsin B and/or other cathepsin family members in human cancers represents an exciting new therapeutic strategy that warrants further clinical development.

uPA/uPAR

The uPA serine protease and its receptor, uPAR, are implicated in tumour cell invasion and metastasis owing to the ability of uPA to activate plasminogen, an enzyme that stimulates degradation of ECM proteins such as fibrin, fibronectin, laminin and collagen IV. Cathepsin B has previously been shown to activate extracellular uPA under certain culture conditions, but whether cathepsin B activates uPA during tumour invasion remains unknown (Ref. 84). Interestingly, uPAR expression also appears to induce the release of tumour cells from a dormant state, which might represent a mechanism by which dormant cancer cells that escaped surgery of the primary tumour could induce tumour recurrence (Ref. 85). High levels of uPAR expression correlate with increased tumour grade and decreased survival in cancers of the breast, lung, colon and gastrointestinal tract, which is indicative of the potential use of the uPA/uPAR system as a prognostic indicator and/or drug target (reviewed by Ref. 85).

Heparanase

Heparanase, another matrix-degrading enzyme, degrades heparan sulphate proteoglycans, resulting in the release of heparin-bound growth factors and chemokines, rendering them bioavailable and thus promoting tumourigenesis (Ref. 86). Heparanase is predominantly expressed by tumour-infiltrating immune cells and its inhibition using a sulphated oligosaccharide mimetic, PI-88, significantly perturbed tumour progression in pancreatic islet cancers (Ref. 75) and in a rat mammary adenocarcinoma model (Ref. 87). High levels of heparanase correlate with poor prognostic survival in patients with colon, pancreatic, oesophageal, head and neck, cervical, liver and gastric cancers (Refs 86, 88, 89, 90, 91, 92, 93). PI-88 is currently being evaluated in Phase II clinical trials for the treatment of melanoma, multiple myeloma, lung cancer and hepatocellular carcinoma. The results from these trials are currently awaited.

Common downstream targets of matrix-degrading proteases?

It is clear that matrix-degrading proteases are essential for tumour progression, but it is not known whether they all act in independent pathways or have some common downstream targets. Such information will be important to obtain, as inhibition of one type of matrix-degrading enzyme could potentially lead to compensatory upregulation and activation of others. For example, a recent report provided evidence that genetic deletion of cathepsin B in a mouse model of mammary gland tumourigenesis induced by the expression of Polyoma middle T antigen (PyMT) led to an increase in the expression and activity of the related protease cathepsin X (Ref. 94). If such is the case in human cancers, then the simultaneous administration of combinations of protease inhibitors may be more efficacious and might be expected to circumvent any acquired resistance. The challenge lies in being able to target only those proteases that have pro-tumourigenic functions and not those that have suppressive roles. Developing allosteric inhibitors to these enzymes may yield ways to prevent non-specific binding to the active sites of off-target proteases.

Innate immune cells and their molecules

Tumour-associated macrophages (TAMs)

Circulating monocytes are recruited to sites of wounding, infection, or tumour growth by high concentrations of certain chemoattractive molecules including VEGF, CCL2 and colony-stimulating factor 1 (CSF-1). Following infiltration

Accession information: DOI: 10.1017/S1462399406000172; Vol. 8; Issue 30; December 2006
© 2006 Cambridge University Press

https://doi.org/10.1017/S1462399406000172 Published online by Cambridge University Press

of these tissues, monocytes differentiate into macrophages that exist in one of two polarised states known as type 1 (M1) macrophages or type 2 (M2) macrophages (Fig. 3). Different stimuli can influence macrophage activation and differentiation. M1 macrophages are activated by cytokines such as interferon γ (IFN-γ) or microbial agents such as lipopolysaccharide. Once activated, they produce factors [IL-12, TNF-α and inducible nitric oxide synthetase (iNOS)] that help execute type 1 reactions, which include killing microorganisms or tumour cells, reducing angiogenesis and presenting foreign antigens to T cells (Refs 95, 96). Thus, M1 macrophages have tumour-suppressive activities. Some tumour-derived cytokines and factors released during tissue breakdown can cause macrophages to take on an M2 macrophage phenotype. In contrast to M1 macrophages, M2 macrophages perform type 2 reactions including scavenging for debris, suppressing adaptive immunity and promoting proliferation, angiogenesis and tissue remodelling in normal tissues. M2 macrophages are also believed to contribute to tumour progression by performing the above functions through the release of growth factors, pro-angiogenic molecules and matrix-degrading enzymes.

Although macrophages can take on tumour-promoting or -suppressive functions, TAMs are

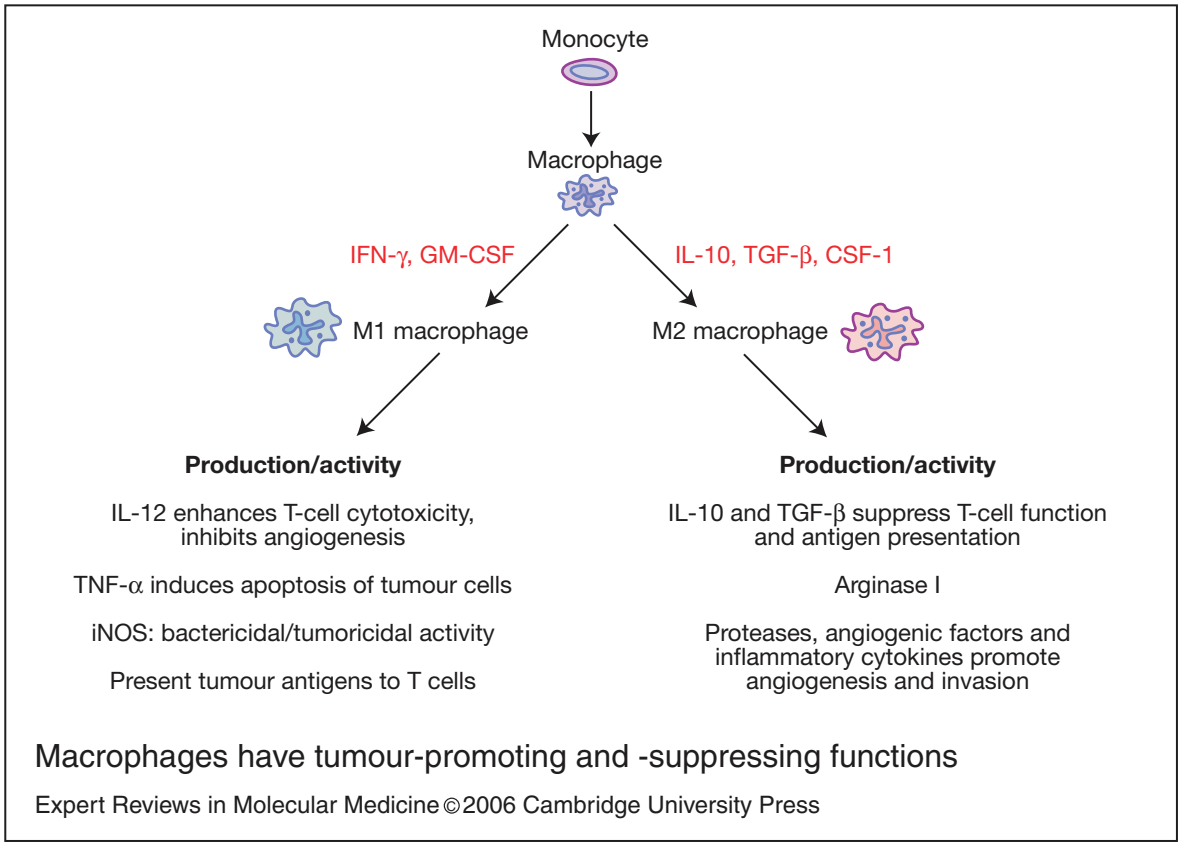


Figure 3. Macrophages have tumour-promoting and -suppressing functions. Monocytes differentiate into tissue macrophages. Depending on the cytokine makeup of the tissue, macrophages can exist as one of two different states: M1 type or M2 type. M1 macrophages actively present tumour antigens to T cells to elicit an immune response against tumours. These macrophages also produce IL-12, TNF-α and iNOS, which can have cytotoxic effects towards tumour cells. In tissues containing high levels of immune-suppressive molecules such as IL-10 and TGF-β, macrophages take on a pro-tumour function, supplying molecules that aid tumour progression and tumour escape from the immune system. Abbreviations: CSF-1, colony-stimulating factor 1; GM-CSF, granulocyte–macrophage colony-stimulating factor; IFN-γ, interferon γ; IL, interleukin; iNOS, inducible nitric oxide synthase; TGF-α, transforming growth factor α; TNF-α, tumour-necrosis factor α.

Accession information: DOI: 10.1017/S1462399406000172; Vol. 8; Issue 30; December 2006
© 2006 Cambridge University Press

https://doi.org/10.1017/S1462399406000172 Published online by Cambridge University Press

generally indicators of poor prognosis in patients with breast, bladder and cervical cancer (reviewed by Ref. 97). There are contradicting clinical studies, however, in patients with non-small-cell lung carcinoma (NSCLC) or pancreatic adenocarcinoma, whereby macrophages have been either linked to poor (Refs 98, 99, 100) or more favourable (Refs 101, 102, 103) prognosis. Although many variables such as sample size, tumour stage and methods used to obtain the data could account for these opposing conclusions, differences in clinical outcome might also depend on the local cytokine balance present in a particular tumour environment that stimulates macrophages to either attenuate or promote disease progression. Macrophage function could also depend on the particular organ where the tumour arises, the tumour stage and its size. A comparison in parallel of different tumour types at distinct stages of development might help answer some of the above debate about the exact correlation between TAMs and disease prognosis.

When M1-type macrophages infiltrate developing tumours, they contribute to tumour rejection by releasing cytotoxic factors (nitrogen species, reactive oxygen species), presenting tumour antigens to T cells, inducing the maturation of dendritic cells (DCs) and secreting molecules such as IL-12 and TNF-α that stimulate T-cell cytotoxicity and tumour cell apoptosis, respectively. The cytokines granulocyte–macrophage colony-stimulating factor (GM-CSF) and IFN-γ enhance the ability of macrophages to present antigens to T cells and to be cytotoxic towards tumour cells (reviewed by Ref. 96). Certain macrophages also express MMP-12, which can suppress the growth of lung metastases in mice (Ref. 104). In many tumours, however, the presence of immunosuppressive cytokines (e.g. IL-10, TGF-β and CSF-1) prevents tumour rejection by blocking anti-tumour activities of both macrophages and DCs (Ref. 52).

Furthermore, TAMs can express high levels of IL-10 and TGF-β, which can reduce adaptive immunity by suppressing T-cell activation and proliferation, indicating that TAMs directly contribute to the immunosuppressive tumour environment (Ref. 95). Thus, the cytokines present in the tumour microenvironment greatly affect the polarisation state of macrophages, such that locally altering the cytokine concentration or composition may be a viable therapeutic option. Indeed, local treatment of tumours in mice with a bacterial stimulus and anti-IL-10 receptor antibodies quickly induced an M1 macrophage phenotypic conversion from the M2 macrophage state, which resulted in reduced growth of the implanted tumours by stimulating anti-tumour adaptive and innate immunity functions (Ref. 105). Thus, cytokine administration directly into the tumour (Ref. 106) or strategies that restore the cytokine balance to improve tumour recognition and destruction by M1 macrophages might represent effective therapies.

Pro-tumourigenic functions of TAMs

In most tumour types, TAMs appear to predominantly promote tumour progression. This is supported by genetic experiments showing that mice deficient in CSF-1 failed to recruit TAMs to PyMT-induced mammary tumours, resulting in a reduced incidence of advanced malignant tumours and metastasis to the lung (Ref. 107). These results are consistent with clinical studies showing that high levels of macrophage chemoattractants CSF-1 and CCL2 correlate with poor prognosis and high TAM infiltration in numerous cancer types (Refs 108, 109). Blocking the signalling of CSF-1, CCL2, or their receptors (CSF-1R and CCR2, respectively) might reduce TAM infiltration and thus suppress late-stage tumours.

One possible way to inhibit CSF-1R is through the use of the general RTKI sunitinib, which blocks CSF-1R in tissue culture cells (Ref. 110) and possibly in human tumours. CCR2 antagonists are currently being developed and evaluated by numerous pharmaceutical companies, but their efficacy in animal models or human patients has not been disclosed (Ref. 111). Incyte in partnership with Pfizer has developed an oral CCR2 antagonist (INCB3284) that is currently in Phase II clinical trials for the treatment of rheumatoid arthritis and type 2 diabetes (http://www.incyte.com/drugs_product_pipeline.html). Merck is also evaluating its CCR2 antagonist MK0812 in Phase II clinical trials for relapsing multiple sclerosis (http://www.clinicaltrials.gov/ct/show/NCT00239655). Future studies should determine whether these agents or their derivatives will be efficacious towards different inflammation-induced diseases and cancer.

Accession information: DOI: 10.1017/S1462399406000172; Vol. 8; Issue 30; December 2006
© 2006 Cambridge University Press

https://doi.org/10.1017/S1462399406000172 Published online by Cambridge University Press

Selectively targeting the pro-tumourigenic functions of TAMs

TAMs can promote tumour neovascularisation by producing pro-angiogenic factors such as VEGF, FGF-2, IL-8 and Cox-2 (Refs 112, 113). Anti-VEGF therapy may efficiently reduce TAM numbers, as VEGF can act as a macrophage attractant in tumour tissue, or block the contribution of VEGF-expressing TAMs to tumour vessel formation in cancers where TAMs are the major producers of VEGF. Furthermore, TAMs might participate in the tumour revascularisation induced by tumour cell necrosis and hypoxia that can occur following some conventional cytotoxic therapies, as they are found in hypoxic and necrotic areas of tumours (Ref. 112). Thus, strategies to reduce TAM numbers, or deplete hypoxia-associated macrophages, following chemotherapy could reduce the recurrence of tumours after conventional treatments.

Under the influence of the tumour microenvironment, TAMs produce numerous growth factors [e.g. EGF, hepatocyte growth factor (HGF), PDGF and TGF-β] that aid tumour growth, and matrix-degrading enzymes that potentially enhance invasion and metastasis. For example, TAMs are major producers of EGF in human breast carcinomas (Ref. 114). In PyMT oncogene-induced mammary tumours in mice, EGF secreted from TAMs promoted carcinoma cell migration and invasion, which was hypothesised to recruit aggressive, chemotherapy-resistant tumour cells to blood vessels where they can exit the primary tumour and metastasise to distant organs (Ref. 115). Blocking these signals using RTKIs that target the EGFRs expressed by tumour cells may prevent TAM-induced invasion and metastasis of $EGFR^{+}$ cancer cells in human patients. EGFR inhibitors such as gefitinib and erlotinib, or EGFR-blocking antibodies such as cetuximab, are being evaluated in clinical trials; cetuximab is approved by the FDA for the treatment of colorectal cancer. TAMs can be found in areas of basement membrane degradation during tumour invasion, where they release proteases such as MMP-9, MMP-7, cathepsins and uPA (Ref. 52). Thus, several protease inhibitors are likely to target macrophages.

Some compounds known to have anti-tumourigenic properties have also recently been shown to alter TAM function. Treatment with the amino-bisphosphonate zoledronic acid (ZA), which reduces skeletal problems associated with bone metastasis in human patients, diminished tumour angiogenesis and growth in a mouse cervical cancer model, probably through the suppression of MMP-9 expression by TAMs (Ref. 72). ZA was not toxic to TAMs, nor did it directly inhibit MMP-9 activity, but rather blocked MMP-9 accumulation. The mechanism as to how ZA reduced MMP-9 accumulation is not known, nor whether it is specific to TAMs or a general suppressor of MMP-9 expression in other cell types, such as ECs and tumour cells, or TAMs in other tumour sites. Also, whether ZA blocks the expression of other MMPs is not known. If ZA is a specific MMP-9 suppressor, this agent might well represent a means to block MMP-9 accumulation without targeting other MMPs that are know to have anti-tumour functions. Trabectedin (Yondelis™), an alkaloid originally extracted from the tunicate *Ecteinascidia turbinata*, has potent anti-tumour activity in vivo and is currently in Phase III clinical trials for patients with advanced relapsed ovarian cancer in combination with the chemotherapeutic drug Doxil, and in Phase II trials as a monotherapy for patients with advanced prostate cancer. The drug binds to the minor groove of DNA and causes structural changes that are believed to block cell-cycle progression of dividing cells (Ref. 116). In addition to targeting cancer cells and being anti-proliferative, it was recently shown to reduce monocyte numbers in patients with advanced ovarian cancer, prevent monocyte differentiation into macrophages in vitro, and reduce expression of tumour-promoting cytokines IL-6 and CCL2 in both macrophages and tumour cells (Ref. 117). The latter effect shows that it has anti-inflammatory properties. In tissue culture cells, trabectedin interfered with the activation of the transcription factor NF-Y, which has been implicated in monocyte differentiation to macrophages (Ref. 118). Studies in animal cancer models using this drug should shed some light on whether its mode of action in vivo is indeed related to its ability to reduce TAM numbers and their production of pro-tumourigenic molecules.

Clinical considerations for anti-TAM therapies

When developing agents that target TAMs, it is important to take into account that macrophages elicit both anti-tumour and pro-tumour responses. Therefore, completely depleting

Accession information: DOI: 10.1017/S1462399406000172; Vol. 8; Issue 30; December 2006
© 2006 Cambridge University Press

https://doi.org/10.1017/S1462399406000172 Published online by Cambridge University Press

macrophages for extended periods may not be entirely beneficial. For example, clodronate-liposome-mediated depletion of macrophages from the liver and peritoneal cavity of rats before the injection of colon cancer cell lines resulted in differentiated tumours that had a reduced immune infiltrate and tumour vasculature but increased tumour burden and decreased overall survival (Ref. 119).

These results suggest that anti-tumour activities of macrophages are crucial and that simply trying to block macrophage recruitment may have unwanted side-effects. Agents that target molecules with known tumour-promoting functions that are highly enriched in TAMs such as VEGF, TNF-α, EGF, MMPs or cathepsins should provide a more selective approach (Fig. 4). Finding additional molecules provided by these cells could yield further therapeutic targets. For example, isolating TAMs from different tumour types at different stages and in distinct tumour areas such as in the tumour stroma, hypoxic areas, invading fronts, or near vessels, and comparing their expression profile to macrophages from non-tumour tissue might yield differentially expressed drug candidates. Furthermore, the validation of such targets will need to be conducted in animal models of cancer known to have a significant macrophage infiltrate. Clinical trials would probably require pre-screening patients to quantify TAM numbers, ideally using non-invasive imaging techniques.

Mast cells

MCs have been implicated in allergic responses, inflammation, adaptive and innate immunity, and tumour invasion and angiogenesis (reviewed by Ref. 120). MCs participate in these biological processes by releasing stored and newly synthesised cytokines, proteases and proteoglycans from cytoplasmic granules. MC precursors are first produced in the bone marrow as pluripotent cells that circulate in the blood and lymphatic vasculature. These precursor cells then mature in vascularised peripheral tissues through the influence of specific cytokines. One important maturation and survival cytokine is stem cell factor (SCF), which is produced by stromal cells and binds to the tyrosine kinase receptor c-Kit present on MCs (reviewed by Ref. 120). Stimuli ranging from allergens to bacterial cell wall components activate receptors on MCs that lead to signalling cascades, culminating in the immediate exocytosis of cytoplasmic granules, generation of lipid-derived substances, and synthesis of cytokines and chemokines (Ref. 121). These activated MCs also recruit additional leukocytes, such as macrophages and neutrophils, which help elicit the inflammatory response.

Mice containing mutations in the gene encoding c-Kit (Kit^W mutations) are almost completely deficient in tissue MCs, which has made them useful in determining the role of MCs in tumour progression. Mice deficient in tissue MCs had reduced tumour growth, angiogenesis and lung metastasis when subcutaneously injected with melanoma cells; these effects were corrected by restoring MC levels (Ref. 122). In a spontaneous mouse model of squamous cell carcinoma, MCs were also shown to be essential for the early stages of neoplastic progression, including keratinocyte hyperproliferation and angiogenesis (Ref. 123). Some clinical evidence corroborates these findings: the presence of MCs in tumours is generally associated with poor patient outcome and enhanced tumour angiogenesis, growth and invasion in malignant melanoma, breast carcinoma, and colorectal and pulmonary adenocarcinoma (Refs 124, 125, 126, 127, 128, 129, 130, 131). In these tumours, MCs associate closely with small capillaries located at the periphery of the tumour and their presence near vessels is believed to directly enhance tumour angiogenesis through the production of pro-angiogenic proteins (VEGF, FGF-2, TGF-β, IL-8 and TNF-α), proteases known to elicit angiogenic responses (MMP-9, tryptase and chymase), and histamine and heparin (Ref. 132). Interestingly, in malignant melanoma, basal cell carcinoma and laryngeal squamous cell carcinoma, MCs are the major source of VEGF, supporting their direct contribution to angiogenesis in these tumour types (Refs 133, 134, 135).

Therapeutic targeting of mast cells

Targeting MC-derived molecules might help treat tumours containing a significant MC infiltrate. Of the proteases released from MC granules, tryptase and chymase are highly MC specific and both have been implicated in tumour progression. Tryptase stimulates EC

Accession information: DOI: 10.1017/S1462399406000172; Vol. 8; Issue 30; December 2006
© 2006 Cambridge University Press

https://doi.org/10.1017/S1462399406000172 Published online by Cambridge University Press

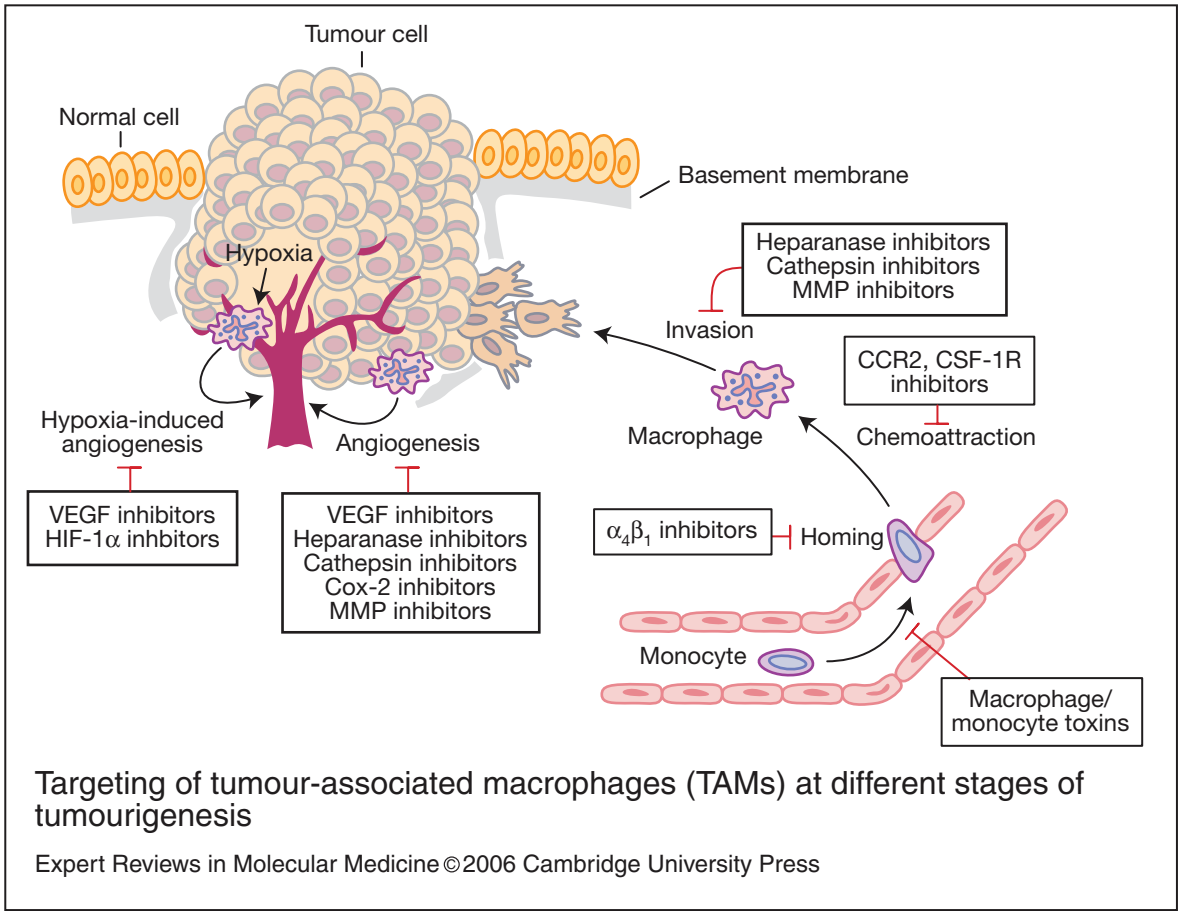


Figure 4. Targeting of tumour-associated macrophages (TAMs) at different stages of tumourigenesis. Monocytes/macrophages are recruited to tumour sites by tumour-derived chemoattractants CCL2 and CSF-1, which can be targeted using inhibitors of their receptors CCR2 and CSF-1R, respectively. Homing of these cells to tumour areas requires integrin $\alpha_1\beta_4$. Blocking the contribution of TAMs to neovessel formation within the tumour and at hypoxic sites might be accomplished using inhibitors of VEGF, heparanase, cathepsins, Cox-2 and MMPs, which are enriched in TAMs. Signals between cancer cells and TAMs that aid in tumour cell migration and metastasis might also be blocked using combinations of heparanase, cathepsin, MMP and EGFR inhibitors. Abbreviations: CCL2, CC chemokine ligand 2; CCR2, CC chemokine receptor 2; CSF-1R, colony-stimulating factor 1 receptor; EGFR, epidermal growth factor receptor; MMP, matrix metalloproteinase; VEGF, vascular endothelial growth factor.

growth and organisation into capillary-like structures in cell culture (Ref. 136) and induces the proliferation of fibroblasts and their deposition of type I collagen (Refs 137, 138). This stimulation of stromal fibroblasts might be responsible for the matrix remodelling that precedes neovascularisation in tumours containing a high MC infiltrate. Specific tryptase inhibitors have been generated and tested in animal models of asthma and inflammatory diseases (reviewed by Ref. 139). In patients with mild cases of asthma or ulcerative colitis, the peptidic tryptase inhibitor APC-366 showed encouraging clinical results (Ref. 140; reviewed by Ref. 139). APC-366 has since been discontinued, but other companies have now begun developing more potent tryptase inhibitors as antagonists of asthma and inflammatory diseases. Future studies should be directed to test whether these compounds reduce tumour progression in cancers where MC infiltration is high.

The other major MC protease, chymase, can elicit angiogenic effects by proteolytically

Accession information: DOI: 10.1017/S1462399406000172; Vol. 8; Issue 30; December 2006
© 2006 Cambridge University Press

https://doi.org/10.1017/S1462399406000172 Published online by Cambridge University Press

converting Angiotensin I into pro-angiogenic Angiotensin II (reviewed by Ref. 141) and/or by directly activating MMP-9 (Refs 123, 142). Angiotensin II is produced by stromal cells and activates G-protein-coupled receptors present in many tumour cells to stimulate their proliferation and invasion (reviewed by Ref. 143). Angiotensin II also stimulates angiogenesis by increasing VEGF levels secreted by tumours. Blocking chymase activity would thus be expected to reduce Angiotensin II- and MMP-9-mediated angiogenesis. Several peptide and small-molecule inhibitors that block chymase activity have been generated but have not been tested in humans (Ref. 144). In animal models for vascular diseases, chymase inhibitors have therapeutic efficacy. One inhibitor, BCEAB, blocks bFGF-mediated angiogenesis and reduces VEGF levels in a hamster sponge granuloma model (Ref. 145). The above results are encouraging and suggest that tryptase and chymase inhibitors might indeed be anti-angiogenic and should be tested in pre-clinical cancer models.

These studies collectively indicate that MCs contribute to physiological and pathological angiogenesis. However, compounds such as tranilast that block MC degranulation have not had positive results in clinical trials against cardiovascular injury, indicating that targeting specific molecules released by MCs in different diseases may be more efficient (reviewed by Ref. 144). In tumours where MC infiltration is high, inhibitors of specific MC mediators of angiogenesis, such as VEGF, tryptase and/or chymase, might yield more favourable therapeutic outcomes when compared with blocking MC degranulation. Furthermore, as c-Kit appears to be crucial for MC survival and maturation in various tissues, c-Kit inhibitors could provide a useful treatment for tumours where MCs are implicated. RTKIs such as imatinib, sorafenib and sunitinib have been shown to inhibit the tyrosine kinase activity of c-Kit in vivo, making them attractive suppressors of MC function in various tumour types. Yet-to-be-identified tumour-derived factors that recruit MCs may be additional targets. For these therapies to be most efficient, prior knowledge of the patients would be needed, such as levels of MCs, tryptase and chymase present in the malignant lesions. Because MC depletion delays early tumourigenic events in animal models, it will also be important to test whether inhibitors of tryptase or chymase are effective as preventative agents and/or target late-stage disease as well.

Neutrophils

Neutrophils are granular, phagocytic cells produced in the bone marrow and released into the circulation, where they are usually short lived. During the early stages of inflammation, neutrophil production in the bone marrow is enhanced and neutrophil survival is increased at sites of inflammation. Neutrophils represent the first line of defence against numerous foreign pathogens and are usually the first immune cells to infiltrate inflamed tissues and possibly tumours (Ref. 146).

Opposing functions of neutrophils in cancer

The exact role of neutrophils in cancer is not entirely clear, as clinical studies in different cancer types have cited contradictory associations between neutrophil infiltration and patient prognosis. High neutrophil infiltration was associated with a better prognostic outcome in patients with advanced gastric carcinoma (Ref. 147). This favourable prognosis might reflect the ability of infiltrating neutrophils to stimulate tumour toxicity depending on the cytokine makeup of the tumour. For example, tumours engineered to release cytokines, such as various interleukins (IL-1, -2, -3, -4, -7, -10 and -12), IFN-α, IFN-β, granulocyte colony-stimulating factor (G-CSF) or TNF-α, stimulated neutrophil-mediated tumour cytotoxicity and the establishment of immunity against the tumour in mice (reviewed by Ref. 148). As tumours often reduce expression of anti-tumour cytokines, approaches that restore cytokine production in a localised manner could potentially enhance these tumour-suppressive functions of neutrophils.

Clinical evidence has also shown that neutrophil infiltration can be associated with poor prognosis in patients with pulmonary adenocarcinoma of the bronchioalveolar (BAC) subtype (Ref. 149). In these patients, neutrophils produced high levels of HGF, which activates growth and survival signals in tumour cells by binding to its receptor c-Met (Ref. 150). Furthermore, bronchioalveolar lavage fluid supernatants from patients with BAC enhanced neutrophil survival and HGF production, indicating that soluble factors present in the

Accession information: DOI: 10.1017/S1462399406000172; Vol. 8; Issue 30; December 2006
© 2006 Cambridge University Press

https://doi.org/10.1017/S1462399406000172 Published online by Cambridge University Press

tumour microenvironment contribute to the tumour-promoting effects of neutrophils (Refs 150, 151). As HGF expression is associated with a poor prognosis in many cancers, antagonising HGF function presents a possible therapeutic option for these patients. Antagonists of HGF/c-Met could be in the form of monoclonal antibodies to HGF to prevent it from activating c-Met on tumour cells (Ref. 152), monoclonal antibodies to c-Met that block its interaction with HGF, or specific small-molecule inhibitors of c-Met that interfere with its tyrosine kinase activity (reviewed by Ref. 153).

RTKIs of c-Met are in development for the treatment of numerous cancer types. The most advanced compound in the clinic is XL880, which is orally active and also inhibits VEGFR2, as well as other RTKs (PDGFR, c-Kit, FLT-3 and Tie2). Phase I trials have not established a maximum tolerated dose for the compound, but a patient with hereditary papillary renal cell carcinoma, a tumour driven by c-Met overexpression, experienced a 15% reduction in tumour volume after failing multiple other therapies (http://library.corporate-ir.net/library/12/120/120923/items/174688/XL880.pdf). Future, larger studies should establish the efficacy of c-Met treatment in cancers where neutrophils provide high levels of HGF.

The importance of neutrophil infiltration for tumour progression has been validated in some animal models. Depletion of neutrophils from mice using RB6-8C5, which depletes $Gr1^+$ mature granulocytes from the peripheral blood, did not support growth of an aggressive skin tumour cell line that had lost cytotoxic T-cell recognition (Ref. 154). Neutrophils enhanced metastasis to the lung of mammary adenocarcinoma cells when both are co-injected intravenously into rats (Ref. 155). Exactly how neutrophils aid in the metastatic process is not known, but in vitro data suggest that tumour cells might use neutrophils to cross EC barriers (Ref. 156). Furthermore, neutrophils produce cytokines and proteases that could stimulate tumour progression. For example, human breast cancer cells stimulated peripheral blood neutrophils to secrete high levels of the cytokine Oncostatin M in culture (Ref. 157). Oncostatin M production by neutrophils caused tumour cells to become more invasive in vitro and produce higher levels of VEGF. Neutrophils also secrete proteases such as elastase, cathepsin G and proteinase-3, which in culture can induce invasion of tumour cells by activating MMP-2 (Ref. 158). Whether these mechanisms operate in vivo is not known and needs to be investigated. Similarly, with regard to the situation with TAMs, there also appears to be a fine balance between tumour-cytotoxic and tumour-promoting roles of neutrophils. Understanding what determines this balance will be paramount to creating effective therapies against the particular cancers where these inflammatory cells aid disease progression.

Platelets

Platelets are small anucleated cells arising from megakaryocytes in the bone marrow that take part in normal processes such as homeostasis and thrombosis, but have also been implicated in pathological angiogenesis and tumour metastasis. Platelets adhere to sites of vessel injury, where they interact with ECs to release various molecules stored in intracellular granules. Proteins released from platelet granules include coagulation factors, both pro-angiogenic (VEGF, bFGF, PDGF, TGF, Ang1, MMPs) and anti-angiogenic proteins (platelet factor-4, thrombospondin, TGF-β1, plasminogen activator inhibitor type I) and the lipid lysophosphatidic acid (LPA). In several models of pathological angiogenesis, depletion of platelets using anti-platelet antibodies reduced neovessel formation and increased vessel leakage, supporting an important role for platelets in angiogenesis (Ref. 159). However, the mechanisms that direct platelets to release pro- versus anti-angiogenic proteins are not known, nor whether this discrimination is depend on the stage or cancer type. Platelets have also been implicated in mediating metastatic spread of cancer cells through several mechanisms: protecting clusters of circulating cancer cells from immune cell recognition in the circulation, extravasation of such clusters by adhering to ECs, and/or providing mitogenic and angiogenic signals to improve survival conditions at metastatic sites (Ref. 160). LPA, for example, acts as a tumour cell mitogen and an indirect promoter of osteolysis during bone metastasis (Ref. 161). The above results are in line with clinical findings showing that many cancer patients have abnormally high levels of platelets (Ref. 162). Thus, compounds that perturb interactions between platelets and

Accession information: DOI: 10.1017/S1462399406000172; Vol. 8; Issue 30; December 2006
© 2006 Cambridge University Press

https://doi.org/10.1017/S1462399406000172 Published online by Cambridge University Press

cancer cells yet allow platelets to retain their normal functions should provide therapeutic options for cancer patients.

Carcinoma-associated fibroblasts (CAFs)

Inflammatory cells are not the only host cell type that tumour cells modify during cancer development. It has been appreciated for some time that in response to neoplastic conversion in the adjacent epithelium, fibroblasts undergo changes that promote tumour progression. These alterations include the proliferation and differentiation of stromal fibroblasts into myofibroblasts, also termed CAFs, which express markers typical of smooth muscle differentiation such as vimentin and α-smooth muscle actin. These cells enhance malignant epithelial cell transformation and contribute to tumour progression by secreting multiple factors and by altering the ECM through the secretion of collagens, fibronectin and matrix-degrading proteases (reviewed by Ref. 163).

Co-culture experiments and in vivo studies indicate that stromal fibroblasts can have tumour-suppressive or -promoting effects on adjacent epithelium. These bipolar effects depend on the differentiation state of fibroblasts and on the tumour stage. TGF-β signalling in normal fibroblasts, for example, has tumour-suppressive functions. Using a genetic approach, it was recently demonstrated that deletion of TGF-β receptor II in stromal fibroblasts caused mice to develop epithelial neoplastic lesions in the forestomach and prostate, indicating that TGF-β signalling in normal fibroblasts helps suppress the proliferation and oncogenic potential of adjacent epithelial cells (Ref. 164). In the absence of TGF-β signalling, fibroblasts secrete HGF, which binds to its receptor c-Met on epithelial cells to promote cell proliferation and survival. As mentioned above, c-Met inhibitors are being developed and may be effective at reducing the contribution of fibroblasts to cancer progression. In addition, normal fibroblasts can prevent transformation of epithelial cells in culture (Ref. 165), suggesting that fibroblasts normally have intrinsic tumour-suppressive properties.

Therapeutic strategies to inhibit CAFs selectively

Although fibroblasts can have intrinsic tumour-suppressive properties, fibroblasts co-opted by the tumour microenvironment promote tumourigenesis. These CAFs express growth factors [HGF, insulin-like growth factor (IGF), EGF, bFGF], matrix-degrading proteases (MMPs and uPA) and pro-angiogenic molecules (VEGF, PlGF, Cox-2) that aid in tumour progression. Interestingly, VEGF expression occurs primarily in CAFs in multiple subcutaneous tumours and in PyMT-induced spontaneous mammary tumours in mice, indicating that CAFs can directly stimulate angiogenesis (Ref. 166). In addition to expressing high levels of VEGF, fibroblasts isolated from human breast tumours, but not from normal tissue, recruit endothelial progenitor cells into the tumour vasculature and enhance tumour angiogenesis and growth through the production of the chemokine SDF-1/CXCL12 in subcutaneously growing tumours (Ref. 167). SDF-1 is a ligand for the chemokine receptor CXCR4 and is a major contributor to chronic inflammation through the recruitment and retention of leukocytes in inflamed tissues (reviewed by Ref. 168). Interestingly, blocking CXCR4 has anti-tumour effects, as the bicyclam antagonist AMD3100 inhibited growth of intracranial glioblastoma and medulloblastoma xenografts (Ref. 169). AMD3100 is currently being evaluated in clinical trials as it might also interfere with the retention of BMDCs near the tumour vasculature (Ref. 170).

CAFs also highly express other molecules, including the serine protease fibroblast-activating protein (FAP) (Ref. 171) and the cysteine protease cathepsin K (Ref. 172). FAP is predominantly expressed in activated fibroblasts during wound healing and tumourigenesis and some efforts have been made to target its activity in human cancers using the humanised neutralising antibody sibrotuzumab (Refs 173, 174). However, in Phase II clinical trials, sibrotuzumab gave no significant results in patients with metastatic colorectal cancer, where fibroblasts play important functions (Ref. 174). In addition to being expressed by CAFs, cathepsin K is also produced by osteoclasts in the bone, where it causes the bone resorption associated with diseases such as osteoporosis and bone metastasis. GlaxoSmithKline has produced a small-molecule inhibitor of cathepsin K called relacatib, which has progressed to Phase II clinical trials for the treatment of bone metastasis, and Novartis is currently conducting Phase II clinical trials with

Accession information: DOI: 10.1017/S1462399406000172; Vol. 8; Issue 30; December 2006
© 2006 Cambridge University Press
https://doi.org/10.1017/S1462399406000172 Published online by Cambridge University Press

its cathepsin K inhibitor AAE-581 in patients with osteoporosis. Preclinical trials using cathepsin K inhibitors should provide answers as to whether cathepsin K is an attractive novel drug target in the many cancer types with pronounced stromal fibroblast responses.

Genetic changes in tumour and stromal cells

Point mutations in key regulatory genes, epigenetic changes, abnormal chromosome numbers and chromosomal rearrangements are frequently observed in tumour cells. However, recent studies report that these changes also occur in stromal fibroblasts. Epigenetic DNA methylation changes were detected both in human breast carcinoma cells and in the associated stromal fibroblasts (Ref. 175). Point mutations in the tumour suppressor genes *PTEN* and *p53* have been found in fibroblasts of invasive breast carcinomas (Ref. 176). These results suggest that genetic and epigenetic changes in the tumour stroma alter epithelial–stroma interactions to support tumour progression. Indeed, mixing human breast carcinoma cells (MCF7 cells) with fibroblasts deficient in p53 results in rapid and aggressive tumour growth when both of these cell types are co-injected into mice (Ref. 177). Tumours also grow much more rapidly in p53-deficient mice when injected with human mammary adenocarcinoma cells compared with wild-type or heterozygous animals. Furthermore, a recent study used a spontaneous prostate cancer model induced by an N-terminal fragment of SV40 large T antigen (which results in Rb inactivation) to show that epithelial cells lacking cell-cycle control induced a selective pressure on the fibroblasts to lose p53 expression, which caused them to expand and contribute to tumour progression (Ref. 178). Unexpectedly, loss of p53 in stromal fibroblasts was shown to occur before p53 was lost in epithelial tumour cells, suggesting that fibroblasts generated signals that promoted the loss of key regulatory genes in the epithelium.

Whether other cell-regulatory genes are also lost in addition to p53 and whether fibroblasts in other tissues undergo similar changes remains to be determined. Identifying the paracrine signals that promote these genetic changes in both epithelial and stromal cells should provide the means to block tumour progression in some tumour types. Taken together with the limited clinical studies discussed above showing that ECs acquire genetic abnormalities in tumours, it is possible that fibroblasts are also exposed to similar selective pressures. Thus, the tumour-associated stroma not only undergoes transient changes in gene expression, it may in fact acquire permanent genetic changes that promote cancer progression. Whether other stromal cells, such as inflammatory cells, also experience such genetic alterations remains to be investigated. Finally, it will be essential to determine whether these effects are widely identified in cancer patients.

Clinical implications

During the evolution of a tumour, malignant cells acquire properties that allow them to evade immune recognition and co-opt normal cells of the vasculature, bone marrow, immune system and stroma to support cancer progression. Thus, strategies that block the actions of these normal resident and/or infiltrating cells are beginning to be exploited in the clinic. Anti-VEGF therapy targeting the tumour vasculature is clearly the most advanced and successful of such approaches to date. Blocking VEGF and its receptors is expected not only to have anti-angiogenic effects, but also to inhibit BMDC recruitment in tumour vasculogenesis and metastasis. Furthermore, this therapy can enhance tumour vessel normalisation, which improves delivery of chemotherapeutics. Although targeting the VEGF signalling cascade would appear to be a promising anti-cancer therapy, the fact that multiple angiogenic pathways exist suggests that combinatorial therapy will ultimately have the greatest efficacy and lowest risk of acquired drug resistance. However, optimising the dosing and timing regimen will be one of the hurdles in attempting to develop therapeutically efficacious yet non-toxic drug combinations.

Taming the inflammatory response that precedes and/or accompanies cancer progression is also an attractive therapeutic approach for treating cancer patients. Pro-inflammatory molecules produced by infiltrating immune cells that are indicative of poor prognosis, such as various cytokines (CCL2, CSF-1), interleukins (IL-10), TNF-α, Cox-2, IKKβ and NF-κB, are important targets that are beginning to yield potential

Accession information: DOI: 10.1017/S1462399406000172; Vol. 8; Issue 30; December 2006
© 2006 Cambridge University Press

https://doi.org/10.1017/S1462399406000172 Published online by Cambridge University Press

treatments. These targets, however, may represent cytostatic therapies that will need to be used in conjunction with conventional chemotherapeutic drugs or as preventative treatments for patients with chronic inflammatory diseases at high risk for cancer development. Blocking the actions of innate immune cells, especially tumour-infiltrating macrophages and mast cells, should be considered as a viable therapeutic option in many tumours, as they are potent inducers of pro-angiogenic and pro-invasive cellular programs. However, as macrophages and neutrophils can also participate in tumour rejection, the challenge will be to develop therapies that interfere with the functions of the tumour-promoting population, yet not perturb the anti-tumour fraction of cells. For these therapies to be successful, we would need to develop non-invasive pre-screening methods to measure the degree and type of immune infiltrate in patients.

Unlike tumour cells, it had long been assumed that cells of the tumour microenvironment were genetically stable and that drugs that target these cells would have a reduced capacity to develop acquired drug resistance through mutational selection. However, there are now several examples of genetic changes in ECs and fibroblasts in the tumour microenvironment, raising the possibility that therapies targeted against components of the infiltrating 'normal' cells might also acquire drug resistance through genetic instability. Future studies should address whether ECs from tumours treated with anti-VEGF therapy, for example, acquire drug resistance over time.

Conclusions

In conclusion, co-opted cells in the tumour microenvironment have emerged as important therapeutic targets in recent years, with anti-stromal therapies now being added to the armament of anti-cancer weapons, as exemplified by the FDA approval of bevacizumab for an increasing number of clinical applications. The immediate challenges for the future, as raised throughout this review, will be first to distinguish the tumour-promoting functions from the tumour-suppressive functions of co-opted normal cells and selectively target those molecular differences. The second hurdle will be in patient selection, specifically being able to identify and target those patients with a pronounced TAM infiltrate for example, and treat them accordingly. The third challenge will be in the appropriate administration of anti-stromal therapies, with drugs targeting either the cancer cells (cytotoxic or cytostatic) or additional stromal targets, and the optimisation of therapeutic efficiency while minimising side-effects from these drug combinations.

Although these challenges and others exist, there are also many reasons for optimism, as demonstrated in part by the variety of prospective targets and inhibitors discussed in this review. We can already envisage how treatment options could change in the future to accommodate therapeutic targeting of the tumour microenvironment. For example, we would suggest a multi-step treatment in which the stromal microenvironment is first 'normalised' by anti-angiogenic inhibitors, which should then enhance the cytotoxic effects of subsequent chemotherapy or radiation therapy to shrink or even eradicate the tumour. Finally, a long-term 'maintenance' treatment might include low-dose chemotherapy or additional anti-stromal inhibitors to keep any remaining cancer cells under control, and thus potentially prevent metastatic spread or the development of pre-metastatic niches. Using sophisticated pre-clinical animal models in which the tumour microenvironment is faithfully recapitulated, we are now in a position to test these combinatorial approaches using the multitude of inhibitors discussed here, as well as others that are currently in development, and hopefully to translate this knowledge to the clinic in the near future.

Acknowledgements and funding

We thank members of the Joyce lab for helpful discussions. Research in the author's lab is supported in part by American Cancer Society Grant #PF-06-064-01-MGO (B.B.G.), the Rita Allen Foundation (J.A.J.), the V Foundation (J.A.J.) and the Society of MSKCC (J.A.J.). We acknowledge the anonymous referees' time and efforts in reviewing the manuscript and appreciate their remarks.

References

1 Joyce, J.A. (2005) Therapeutic targeting of the tumor microenvironment. Cancer Cell 7, 513-520
2 Carmeliet, P. and Jain, R.K. (2000) Angiogenesis in cancer and other diseases. Nature 407, 249-257

Accession information: DOI: 10.1017/S1462399406000172; Vol. 8; Issue 30; December 2006
© 2006 Cambridge University Press

https://doi.org/10.1017/S1462399406000172 Published online by Cambridge University Press

3 Ferrara, N. and Kerbel, R.S. (2005) Angiogenesis as a therapeutic target. Nature 438, 967-974
4 Achen, M.G., McColl, B.K. and Stacker, S.A. (2005) Focus on lymphangiogenesis in tumor metastasis. Cancer Cell 7, 121-127
5 Hurwitz, H. et al. (2004) Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 350, 2335-2342
6 Jain, R.K. et al. (2006) Lessons from phase III clinical trials on anti-VEGF therapy for cancer. Nat Clin Pract Oncol 3, 24-40
7 Pennacchietti, S. et al. (2003) Hypoxia promotes invasive growth by transcriptional activation of the met protooncogene. Cancer Cell 3, 347-361
8 Willett, C.G. et al. (2004) Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. Nat Med 10, 145-147
9 Winkler, F. et al. (2004) Kinetics of vascular normalization by VEGFR2 blockade governs brain tumor response to radiation: role of oxygenation, angiopoietin-1, and matrix metalloproteinases. Cancer Cell 6, 553-563
10 Ryden, L. et al. (2003) Tumor specific VEGF-A and VEGFR2/KDR protein are co-expressed in breast cancer. Breast Cancer Res Treat 82, 147-154
11 Fan, F. et al. (2005) Expression and function of vascular endothelial growth factor receptor-1 on human colorectal cancer cells. Oncogene 24, 2647-2653
12 Song, S. et al. (2005) PDGFRbeta+ perivascular progenitor cells in tumours regulate pericyte differentiation and vascular survival. Nat Cell Biol 7, 870-879
13 Bergers, G. et al. (2003) Benefits of targeting both pericytes and endothelial cells in the tumor vasculature with kinase inhibitors. J Clin Invest 111, 1287-1295
14 Benjamin, L.E. et al. (1999) Selective ablation of immature blood vessels in established human tumors follows vascular endothelial growth factor withdrawal. J Clin Invest 103, 159-165
15 Xian, X. et al. (2006) Pericytes limit tumor cell metastasis. J Clin Invest 116, 642-651
16 Casanovas, O. et al. (2005) Drug resistance by evasion of antiangiogenic targeting of VEGF signaling in late-stage pancreatic islet tumors. Cancer Cell 8, 299-309
17 Mizukami, Y. et al. (2005) Induction of interleukin-8 preserves the angiogenic response in HIF-1alpha-deficient colon cancer cells. Nat Med 11, 992-997
18 Escuin, D., Kline, E.R. and Giannakakou, P. (2005) Both microtubule-stabilizing and microtubule-destabilizing drugs inhibit hypoxia-inducible factor-1alpha accumulation and activity by disrupting microtubule function. Cancer Res 65, 9021-9028
19 Sweeney, C. et al. (2005) A phase II multicenter, randomized, double-blind, safety trial assessing the pharmacokinetics, pharmacodynamics, and efficacy of oral 2-methoxyestradiol capsules in hormone-refractory prostate cancer. Clin Cancer Res 11, 6625-6633
20 Dahut, W.L. et al. (2006) Phase I clinical trial of oral 2-methoxyestradiol, an antiangiogenic and apoptotic agent, in patients with solid tumors. Cancer Biol Ther 5, 22-27
21 Hida, K. et al. (2004) Tumor-associated endothelial cells with cytogenetic abnormalities. Cancer Res 64, 8249-8255
22 Streubel, B. et al. (2004) Lymphoma-specific genetic aberrations in microvascular endothelial cells in B-cell lymphomas. N Engl J Med 351, 250-259
23 Lyden, D. et al. (2001) Impaired recruitment of bone-marrow-derived endothelial and hematopoietic precursor cells blocks tumor angiogenesis and growth. Nat Med 7, 1194-1201
24 De Palma, M. et al. (2003) Targeting exogenous genes to tumor angiogenesis by transplantation of genetically modified hematopoietic stem cells. Nat Med 9, 789-795
25 Li, H., Gerald, W.L. and Benezra, R. (2004) Utilization of bone marrow-derived endothelial cell precursors in spontaneous prostate tumors varies with tumor grade. Cancer Res 64, 6137-6143
26 Rajantie, I. et al. (2004) Adult bone marrow-derived cells recruited during angiogenesis comprise precursors for periendothelial vascular mural cells. Blood 104, 2084-2086
27 Spring, H. et al. (2005) Chemokines direct endothelial progenitors into tumor neovessels. Proc Natl Acad Sci U S A 102, 18111-18116
28 Hilbe, W. et al. (2004) CD133 positive endothelial progenitor cells contribute to the tumour vasculature in non-small cell lung cancer. J Clin Pathol 57, 965-969
29 Yu, Y. et al. (2004) Endothelial progenitor cells in infantile hemangioma. Blood 103, 1373-1375

Accession information: DOI: 10.1017/S1462399406000172; Vol. 8; Issue 30; December 2006
© 2006 Cambridge University Press

https://doi.org/10.1017/S1462399406000172 Published online by Cambridge University Press

30 Peters, B.A. et al. (2005) Contribution of bone marrow-derived endothelial cells to human tumor vasculature. Nat Med 11, 261-262
31 Shaked, Y. et al. (2005) Genetic heterogeneity of the vasculogenic phenotype parallels angiogenesis; Implications for cellular surrogate marker analysis of antiangiogenesis. Cancer Cell 7, 101-111
32 Yang, L. et al. (2004) Expansion of myeloid immune suppressor Gr+CD11b+ cells in tumor-bearing host directly promotes tumor angiogenesis. Cancer Cell 6, 409-421
33 Grunewald, M. et al. (2006) VEGF-Induced Adult Neovascularization: Recruitment, Retention, and Role of Accessory Cells. Cell 124, 175-189
34 Jin, H. et al. (2006) A homing mechanism for bone marrow-derived progenitor cell recruitment to the neovasculature. J Clin Invest 116, 652-662
35 De Palma, M. et al. (2005) Tie2 identifies a hematopoietic lineage of proangiogenic monocytes required for tumor vessel formation and a mesenchymal population of pericyte progenitors. Cancer Cell 8, 211-226
36 Oliner, J. et al. (2004) Suppression of angiogenesis and tumor growth by selective inhibition of angiopoietin-2. Cancer Cell 6, 507-516
37 Fiedler, U. et al. (2006) Angiopoietin-2 sensitizes endothelial cells to TNF-alpha and has a crucial role in the induction of inflammation. Nat Med 12, 235-239
38 Kaplan, R.N. et al. (2005) VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. Nature 438, 820-827
39 Balkwill, F., Charles, K.A. and Mantovani, A. (2005) Smoldering and polarized inflammation in the initiation and promotion of malignant disease. Cancer Cell 7, 211-217
40 Coussens, L.M. and Werb, Z. (2002) Inflammation and cancer. Nature 420, 860-867
41 Vakkila, J. and Lotze, M.T. (2004) Inflammation and necrosis promote tumour growth. Nat Rev Immunol 4, 641-648
42 Ristimaki, A. (2004) Cyclooxygenase 2: from inflammation to carcinogenesis. Novartis Found Symp 256, 215-221; discussion 221–216, 259–269
43 Buskens, C.J. et al. (2002) Prognostic significance of elevated cyclooxygenase 2 expression in patients with adenocarcinoma of the esophagus. Gastroenterology 122, 1800-1807
44 Ristimaki, A. et al. (2002) Prognostic significance of elevated cyclooxygenase-2 expression in breast cancer. Cancer Res 62, 632-635
45 Erkinheimo, T.L. et al. (2004) Elevated cyclooxygenase-2 expression is associated with altered expression of p53 and SMAD4, amplification of HER-2/neu, and poor outcome in serous ovarian carcinoma. Clin Cancer Res 10, 538-545
46 Sheehan, K.M. et al. (1999) The relationship between cyclooxygenase-2 expression and colorectal cancer. Jama 282, 1254-1257
47 Williams, C.S. et al. (2000) Host cyclooxygenase-2 modulates carcinoma growth. J Clin Invest 105, 1589-1594
48 Oshima, M. et al. (1996) Suppression of intestinal polyposis in Apc delta716 knockout mice by inhibition of cyclooxygenase 2 (COX-2). Cell 87, 803-809
49 Bresalier, R.S. et al. (2005) Cardiovascular events associated with rofecoxib in a colorectal adenoma chemoprevention trial. N Engl J Med 352, 1092-1102
50 de Visser, K.E., Korets, L.V. and Coussens, L.M. (2005) De novo carcinogenesis promoted by chronic inflammation is B lymphocyte dependent. Cancer Cell 7, 411-423
51 de Visser, K.E., Eichten, A. and Coussens, L.M. (2006) Paradoxical roles of the immune system during cancer development. Nat Rev Cancer 6, 24-37
52 Pollard, J.W. (2004) Tumour-educated macrophages promote tumour progression and metastasis. Nat Rev Cancer 4, 71-78
53 Maeda, H. and Akaike, T. (1998) Nitric oxide and oxygen radicals in infection, inflammation, and cancer. Biochemistry (Mosc) 63, 854-865
54 Hussain, S.P., Hofseth, L.J. and Harris, C.C. (2003) Radical causes of cancer. Nat Rev Cancer 3, 276-285
55 Pikarsky, E. et al. (2004) NF-kappaB functions as a tumour promoter in inflammation-associated cancer. Nature 431, 461-466
56 Karin, M. (2006) Nuclear factor-kappaB in cancer development and progression. Nature 441, 431-436
57 Greten, F.R. et al. (2004) IKKbeta links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. Cell 118, 285-296
58 Karin, M., Yamamoto, Y. and Wang, Q.M. (2004) The IKK NF-kappa B system: a treasure trove for drug development. Nat Rev Drug Discov 3, 17-26
59 van Hogerlinden, M. et al. (1999) Squamous cell carcinomas and increased apoptosis in skin with

Accession information: DOI: 10.1017/S1462399406000172; Vol. 8; Issue 30; December 2006
© 2006 Cambridge University Press

https://doi.org/10.1017/S1462399406000172 Published online by Cambridge University Press

inhibited Rel/nuclear factor-kappaB signaling. Cancer Res 59, 3299-3303
60 Dajee, M. et al. (2003) NF-kappaB blockade and oncogenic Ras trigger invasive human epidermal neoplasia. Nature 421, 639-643
61 Maeda, S. et al. (2005) IKKbeta couples hepatocyte death to cytokine-driven compensatory proliferation that promotes chemical hepatocarcinogenesis. Cell 121, 977-990
62 Warzocha, K. et al. (1997) Tumor necrosis factor ligand-receptor system can predict treatment outcome in lymphoma patients. J Clin Oncol 15, 499-508
63 Naylor, M.S. et al. (1993) Tumor necrosis factor and its receptors in human ovarian cancer. Potential role in disease progression. J Clin Invest 91, 2194-2206
64 Baldwin, A.S. (2001) Control of oncogenesis and cancer therapy resistance by the transcription factor NF-kappaB. J Clin Invest 107, 241-246
65 Leek, R.D. et al. (1998) Association of tumour necrosis factor alpha and its receptors with thymidine phosphorylase expression in invasive breast carcinoma. Br J Cancer 77, 2246-2251
66 Moore, R.J. et al. (1999) Mice deficient in tumor necrosis factor-alpha are resistant to skin carcinogenesis. Nat Med 5, 828-831
67 Arnott, C.H. et al. (2004) Expression of both TNF-alpha receptor subtypes is essential for optimal skin tumour development. Oncogene 23, 1902-1910
68 Madhusudan, S. et al. (2004) A phase II study of etanercept (Enbrel), a tumor necrosis factor alpha inhibitor in patients with metastatic breast cancer. Clin Cancer Res 10, 6528-6534
69 Madhusudan, S. et al. (2005) Study of etanercept, a tumor necrosis factor-alpha inhibitor, in recurrent ovarian cancer. J Clin Oncol 23, 5950-5959
70 Hildenbrand, R. et al. (1999) Urokinase plasminogen activator receptor (CD87) expression of tumor-associated macrophages in ductal carcinoma in situ, breast cancer, and resident macrophages of normal breast tissue. J Leukoc Biol 66, 40-49
71 Coussens, L.M. et al. (2000) MMP-9 supplied by bone marrow-derived cells contributes to skin carcinogenesis. Cell 103, 481-490
72 Giraudo, E., Inoue, M. and Hanahan, D. (2004) An amino-bisphosphonate targets MMP-9-expressing macrophages and angiogenesis to impair cervical carcinogenesis. J Clin Invest 114, 623-633
73 Joyce, J.A. et al. (2004) Cathepsin cysteine proteases are effectors of invasive growth and angiogenesis during multistage tumorigenesis. Cancer Cell 5, 443-453
74 Gocheva, V. et al. (2006) Distinct roles for cysteine cathepsin genes in multistage tumorigenesis. Genes Dev 20, 543-556
75 Joyce, J.A. et al. (2005) A functional heparan sulfate mimetic implicates both heparanase and heparan sulfate in tumor angiogenesis and invasion in a mouse model of multistage cancer. Oncogene 24, 4037-4051
76 Sternlicht, M.D. et al. (1999) The stromal proteinase MMP3/stromelysin-1 promotes mammary carcinogenesis. Cell 98, 137-146
77 Radisky, D.C. et al. (2005) Rac1b and reactive oxygen species mediate MMP-3-induced EMT and genomic instability. Nature 436, 123-127
78 Lynch, C.C. et al. (2005) MMP-7 promotes prostate cancer-induced osteolysis via the solubilization of RANKL. Cancer Cell 7, 485-496
79 Wilson, C.L. et al. (1997) Intestinal tumorigenesis is suppressed in mice lacking the metalloproteinase matrilysin. Proc Natl Acad Sci U S A 94, 1402-1407
80 Bergers, G. et al. (2000) Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. Nat Cell Biol 2, 737-744
81 Overall, C.M. and Kleifeld, O. (2006) Tumour microenvironment - opinion: validating matrix metalloproteinases as drug targets and anti-targets for cancer therapy. Nat Rev Cancer 6, 227-239
82 Grimm, J. et al. (2005) Use of gene expression profiling to direct in vivo molecular imaging of lung cancer. Proc Natl Acad Sci U S A 102, 14404-14409
83 Jedeszko, C. and Sloane, B.F. (2004) Cysteine cathepsins in human cancer. Biol Chem 385, 1017-1027
84 Guo, M. et al. (2002) Phorbol ester activation of a proteolytic cascade capable of activating latent transforming growth factor-betaL a process initiated by the exocytosis of cathepsin B. J Biol Chem 277, 14829-14837
85 Laufs, S., Schumacher, J. and Allgayer, H. (2006) Urokinase-receptor (u-PAR): an essential player in multiple games of cancer: a review on its role in tumor progression, invasion, metastasis, proliferation/dormancy, clinical

Accession information: DOI: 10.1017/S1462399406000172; Vol. 8; Issue 30; December 2006
© 2006 Cambridge University Press

https://doi.org/10.1017/S1462399406000172 Published online by Cambridge University Press

outcome and minimal residual disease. Cell Cycle 5, 1760-1771
86 Simizu, S., Ishida, K. and Osada, H. (2004) Heparanase as a molecular target of cancer chemotherapy. Cancer Sci 95, 553-558
87 Parish, C.R. et al. (1999) Identification of sulfated oligosaccharide-based inhibitors of tumor growth and metastasis using novel in vitro assays for angiogenesis and heparanase activity. Cancer Res 59, 3433-3441
88 Nobuhisa, T. et al. (2005) Heparanase expression correlates with malignant potential in human colon cancer. J Cancer Res Clin Oncol 131, 229-237
89 Quiros, R.M. et al. (2006) Elevated serum heparanase-1 levels in patients with pancreatic carcinoma are associated with poor survival. Cancer 106, 532-540
90 Okawa, T. et al. (2005) Heparanase is involved in angiogenesis in esophageal cancer through induction of cyclooxygenase-2. Clin Cancer Res 11, 7995-8005
91 Beckhove, P. et al. (2005) Heparanase expression at the invasion front of human head and neck cancers and correlation with poor prognosis. Clin Cancer Res 11, 2899-2906
92 Shinyo, Y. et al. (2003) Heparanase expression is an independent prognostic factor in patients with invasive cervical cancer. Ann Oncol 14, 1505-1510
93 Takaoka, M. et al. (2003) Heparanase expression correlates with invasion and poor prognosis in gastric cancers. Lab Invest 83, 613-622
94 Vasiljeva, O. et al. (2006) Tumor cell-derived and macrophage-derived cathepsin B promotes progression and lung metastasis of mammary cancer. Cancer Res 66, 5242-5250
95 Mantovani, A. et al. (2002) Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Trends Immunol 23, 549-555
96 Dranoff, G. (2004) Cytokines in cancer pathogenesis and cancer therapy. Nat Rev Cancer 4, 11-22
97 Bingle, L., Brown, N.J. and Lewis, C.E. (2002) The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies. J Pathol 196, 254-265
98 Koukourakis, M.I. et al. (1998) Different patterns of stromal and cancer cell thymidine phosphorylase reactivity in non-small-cell lung cancer: impact on tumour neoangiogenesis and survival. Br J Cancer 77, 1696-1703
99 Lissbrant, I.F. et al. (2000) Tumor associated macrophages in human prostate cancer: relation to clinicopathological variables and survival. Int J Oncol 17, 445-451
100 Chen, J.J. et al. (2003) Up-regulation of tumor interleukin-8 expression by infiltrating macrophages: its correlation with tumor angiogenesis and patient survival in non-small cell lung cancer. Clin Cancer Res 9, 729-737
101 Kerr, K.M. et al. (1998) Partial regression in primary carcinoma of the lung: does it occur? Histopathology 33, 55-63
102 Shimura, S. et al. (2000) Reduced infiltration of tumor-associated macrophages in human prostate cancer: association with cancer progression. Cancer Res 60, 5857-5861
103 Welsh, T.J. et al. (2005) Macrophage and mast-cell invasion of tumor cell islets confers a marked survival advantage in non-small-cell lung cancer. J Clin Oncol 23, 8959-8967
104 Houghton, A.M. et al. (2006) Macrophage elastase (matrix metalloproteinase-12) suppresses growth of lung metastases. Cancer Res 66, 6149-6155
105 Guiducci, C. et al. (2005) Redirecting in vivo elicited tumor infiltrating macrophages and dendritic cells towards tumor rejection. Cancer Res 65, 3437-3446
106 Forni, G. et al. (1988) Helper strategy in tumor immunology: expansion of helper lymphocytes and utilization of helper lymphokines for experimental and clinical immunotherapy. Cancer Metastasis Rev 7, 289-309
107 Lin, E.Y. et al. (2001) Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. J Exp Med 193, 727-740
108 Scholl, S.M. et al. (1994) Anti-colony-stimulating factor-1 antibody staining in primary breast adenocarcinomas correlates with marked inflammatory cell infiltrates and prognosis. J Natl Cancer Inst 86, 120-126
109 Lin, E.Y. and Pollard, J.W. (2004) Role of infiltrated leucocytes in tumour growth and spread. Br J Cancer 90, 2053-2058
110 Murray, L.J. et al. (2003) SU11248 inhibits tumor growth and CSF-1R-dependent osteolysis in an experimental breast cancer bone metastasis model. Clin Exp Metastasis 20, 757-766
111 Dawson, J. et al. (2003) Targeting monocyte chemoattractant protein-1 signalling in disease. Expert Opin Ther Targets 7, 35-48
112 Lewis, C. and Murdoch, C. (2005) Macrophage responses to hypoxia: implications for tumor

Accession information: DOI: 10.1017/S1462399406000172; Vol. 8; Issue 30; December 2006
© 2006 Cambridge University Press

https://doi.org/10.1017/S1462399406000172 Published online by Cambridge University Press

progression and anti-cancer therapies. Am J Pathol 167, 627-635
113 Nakao, S. et al. (2005) Infiltration of COX-2-expressing macrophages is a prerequisite for IL-1beta-induced neovascularization and tumor growth. J Clin Invest 115, 2979-2991
114 O'Sullivan, C. et al. (1993) Secretion of epidermal growth factor by macrophages associated with breast carcinoma. Lancet 342, 148-149
115 Wyckoff, J. et al. (2004) A paracrine loop between tumor cells and macrophages is required for tumor cell migration in mammary tumors. Cancer Res 64, 7022-7029
116 Erba, E. et al. (2001) Ecteinascidin-743 (ET-743), a natural marine compound, with a unique mechanism of action. Eur J Cancer 37, 97-105
117 Allavena, P. et al. (2005) Anti-inflammatory properties of the novel antitumor agent yondelis (trabectedin): inhibition of macrophage differentiation and cytokine production. Cancer Res 65, 2964-2971
118 Minuzzo, M. et al. (2000) Interference of transcriptional activation by the antineoplastic drug ecteinascidin-743. Proc Natl Acad Sci U S A 97, 6780-6784
119 Oosterling, S.J. et al. (2005) Macrophages direct tumour histology and clinical outcome in a colon cancer model. J Pathol 207, 147-155
120 Mekori, Y.A. and Metcalfe, D.D. (2000) Mast cells in innate immunity. Immunol Rev 173, 131-140
121 Metcalfe, D.D., Baram, D. and Mekori, Y.A. (1997) Mast cells. Physiol Rev 77, 1033-1079
122 Starkey, J.R., Crowle, P.K. and Taubenberger, S. (1988) Mast-cell-deficient W/Wv mice exhibit a decreased rate of tumor angiogenesis. Int J Cancer 42, 48-52
123 Coussens, L.M. et al. (1999) Inflammatory mast cells up-regulate angiogenesis during squamous epithelial carcinogenesis. Genes Dev 13, 1382-1397
124 Hartveit, F. et al. (1984) Mast cell changes and tumour dissemination in human breast carcinoma. Invasion Metastasis 4, 146-155
125 Lachter, J. et al. (1995) Mast cells in colorectal neoplasias and premalignant disorders. Dis Colon Rectum 38, 290-293
126 Kankkunen, J.P., Harvima, I.T. and Naukkarinen, A. (1997) Quantitative analysis of tryptase and chymase containing mast cells in benign and malignant breast lesions. Int J Cancer 72, 385-388
127 Takanami, I., Takeuchi, K. and Naruke, M. (2000) Mast cell density is associated with angiogenesis and poor prognosis in pulmonary adenocarcinoma. Cancer 88, 2686-2692
128 Imada, A. et al. (2000) Mast cells correlate with angiogenesis and poor outcome in stage I lung adenocarcinoma. Eur Respir J 15, 1087-1093
129 Ribatti, D. et al. (2001) The role of mast cells in tumour angiogenesis. Br J Haematol 115, 514-521
130 Ribatti, D. et al. (2003) Tumor vascularity and tryptase-positive mast cells correlate with a poor prognosis in melanoma. Eur J Clin Invest 33, 420-425
131 Ibaraki, T. et al. (2005) The relationship of tryptase- and chymase-positive mast cells to angiogenesis in stage I non-small cell lung cancer. Eur J Cardiothorac Surg 28, 617-621
132 Ribatti, D. et al. (2004) Mast cell contribution to angiogenesis related to tumour progression. Clin Exp Allergy 34, 1660-1664
133 Sawatsubashi, M. et al. (2000) Association of vascular endothelial growth factor and mast cells with angiogenesis in laryngeal squamous cell carcinoma. Virchows Arch 436, 243-248
134 Toth-Jakatics, R. et al. (2000) Cutaneous malignant melanoma: correlation between neovascularization and peritumor accumulation of mast cells overexpressing vascular endothelial growth factor. Hum Pathol 31, 955-960
135 Aoki, M. et al. (2003) Mast cells in basal cell carcinoma express VEGF, IL-8 and RANTES. Int Arch Allergy Immunol 130, 216-223
136 Blair, R.J. et al. (1997) Human mast cells stimulate vascular tube formation. Tryptase is a novel, potent angiogenic factor. J Clin Invest 99, 2691-2700
137 Gruber, B.L. et al. (1997) Human mast cells activate fibroblasts: tryptase is a fibrogenic factor stimulating collagen messenger ribonucleic acid synthesis and fibroblast chemotaxis. J Immunol 158, 2310-2317
138 Cairns, J.A. and Walls, A.F. (1997) Mast cell tryptase stimulates the synthesis of type I collagen in human lung fibroblasts. J Clin Invest 99, 1313-1321
139 Cairns, J.A. (2005) Inhibitors of mast cell tryptase beta as therapeutics for the treatment of asthma and inflammatory disorders. Pulm Pharmacol Ther 18, 55-66
140 Tremaine, W.J. et al. (2002) Treatment of mildly to moderately active ulcerative colitis with a tryptase inhibitor (APC 2059): an open-label pilot study. Aliment Pharmacol Ther 16, 407-413

Accession information: DOI: 10.1017/S1462399406000172; Vol. 8; Issue 30; December 2006
© 2006 Cambridge University Press

https://doi.org/10.1017/S1462399406000172 Published online by Cambridge University Press

141 Takai, S. et al. (2004) Chymase as a novel target for the prevention of vascular diseases. Trends Pharmacol Sci 25, 518-522
142 Fang, K.C. et al. (1997) Dog mast cell alpha-chymase activates progelatinase B by cleaving the Phe88-Gln89 and Phe91-Glu92 bonds of the catalytic domain. J Biol Chem 272, 25628-25635
143 Ino, K. et al. (2006) Manipulating the angiotensin system–new approaches to the treatment of solid tumours. Expert Opin Biol Ther 6, 243-255
144 Doggrell, S.A. and Wanstall, J.C. (2004) Vascular chymase: pathophysiological role and therapeutic potential of inhibition. Cardiovasc Res 61, 653-662
145 Muramatsu, M. et al. (2002) Suppression of basic fibroblast growth factor-induced angiogenesis by a specific chymase inhibitor, BCEAB, through the chymase-angiotensin-dependent pathway in hamster sponge granulomas. Br J Pharmacol 137, 554-560
146 Stoppacciaro, A. et al. (1993) Regression of an established tumor genetically modified to release granulocyte colony-stimulating factor requires granulocyte-T cell cooperation and T cell-produced interferon gamma. J Exp Med 178, 151-161
147 Caruso, R.A. et al. (2002) Prognostic value of intratumoral neutrophils in advanced gastric carcinoma in a high-risk area in northern Italy. Mod Pathol 15, 831-837
148 di Carlo, E. et al. (2001) Neutrophils in anti-cancer immunological strategies: old players in new games. J Hematother Stem Cell Res 10, 739-748
149 Bellocq, A. et al. (1998) Neutrophil alveolitis in bronchioloalveolar carcinoma: induction by tumor-derived interleukin-8 and relation to clinical outcome. Am J Pathol 152, 83-92
150 Wislez, M. et al. (2003) Hepatocyte growth factor production by neutrophils infiltrating bronchioloalveolar subtype pulmonary adenocarcinoma: role in tumor progression and death. Cancer Res 63, 1405-1412
151 Wislez, M. et al. (2001) Tumor-derived granulocyte-macrophage colony-stimulating factor and granulocyte colony-stimulating factor prolong the survival of neutrophils infiltrating bronchoalveolar subtype pulmonary adenocarcinoma. Am J Pathol 159, 1423-1433
152 Cao, B. et al. (2001) Neutralizing monoclonal antibodies to hepatocyte growth factor/scatter factor (HGF/SF) display antitumor activity in animal models. Proc Natl Acad Sci U S A 98, 7443-7448
153 Christensen, J.G., Burrows, J. and Salgia, R. (2005) c-Met as a target for human cancer and characterization of inhibitors for therapeutic intervention. Cancer Lett 225, 1-26
154 Pekarek, L.A. et al. (1995) Inhibition of tumor growth by elimination of granulocytes. J Exp Med 181, 435-440
155 Welch, D.R. et al. (1989) Tumor-elicited polymorphonuclear cells, in contrast to 'normal' circulating polymorphonuclear cells, stimulate invasive and metastatic potentials of rat mammary adenocarcinoma cells. Proc Natl Acad Sci U S A 86, 5859-5863
156 Wu, Q.D. et al. (2001) Human neutrophils facilitate tumor cell transendothelial migration. Am J Physiol Cell Physiol 280, C814-822
157 Queen, M.M. et al. (2005) Breast cancer cells stimulate neutrophils to produce oncostatin M: potential implications for tumor progression. Cancer Res 65, 8896-8904
158 Shamamian, P. et al. (2000) Neutrophil-derived serine proteinases enhance membrane type-1 matrix metalloproteinase-dependent tumor cell invasion. Surgery 127, 142-147
159 Kisucka, J. et al. (2006) Platelets and platelet adhesion support angiogenesis while preventing excessive hemorrhage. Proc Natl Acad Sci U S A 103, 855-860
160 Gupta, G.P. and Massague, J. (2004) Platelets and metastasis revisited: a novel fatty link. J Clin Invest 114, 1691-1693
161 Boucharaba, A. et al. (2004) Platelet-derived lysophosphatidic acid supports the progression of osteolytic bone metastases in breast cancer. J Clin Invest 114, 1714-1725
162 Nash, G.F. et al. (2002) Platelets and cancer. Lancet Oncol 3, 425-430
163 Bhowmick, N.A., Neilson, E.G. and Moses, H.L. (2004) Stromal fibroblasts in cancer initiation and progression. Nature 432, 332-337
164 Bhowmick, N.A. et al. (2004) TGF-beta signaling in fibroblasts modulates the oncogenic potential of adjacent epithelia. Science 303, 848-851
165 Olumi, A.F. et al. (1999) Carcinoma-associated fibroblasts direct tumor progression of initiated human prostatic epithelium. Cancer Res 59, 5002-5011
166 Fukumura, D. et al. (1998) Tumor induction of VEGF promoter activity in stromal cells. Cell 94, 715-725
167 Orimo, A. et al. (2005) Stromal fibroblasts present in invasive human breast carcinomas promote

Accession information: DOI: 10.1017/S1462399406000172; Vol. 8; Issue 30; December 2006
© 2006 Cambridge University Press

https://doi.org/10.1017/S1462399406000172 Published online by Cambridge University Press

tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell 121, 335-348
168 Douglas, M.R. et al. (2002) Why does inflammation persist: a dominant role for the stromal microenvironment? Expert Rev Mol Med 4, 25, 1-18
169 Rubin, J.B. et al. (2003) A small-molecule antagonist of CXCR4 inhibits intracranial growth of primary brain tumors. Proc Natl Acad Sci U S A 100, 13513-13518
170 Devine, S.M. et al. (2004) Rapid mobilization of CD34+ cells following administration of the CXCR4 antagonist AMD3100 to patients with multiple myeloma and non-Hodgkin's lymphoma. J Clin Oncol 22, 1095-1102
171 Park, J.E. et al. (1999) Fibroblast activation protein, a dual specificity serine protease expressed in reactive human tumor stromal fibroblasts. J Biol Chem 274, 36505-36512
172 Allinen, M. et al. (2004) Molecular characterization of the tumor microenvironment in breast cancer. Cancer Cell 6, 17-32
173 Scott, A.M. et al. (2003) A Phase I dose-escalation study of sibrotuzumab in patients with advanced or metastatic fibroblast activation protein-positive cancer. Clin Cancer Res 9, 1639-1647
174 Hofheinz, R.D. et al. (2003) Stromal antigen targeting by a humanised monoclonal antibody: an early phase II trial of sibrotuzumab in patients with metastatic colorectal cancer. Onkologie 26, 44-48
175 Hu, M. et al. (2005) Distinct epigenetic changes in the stromal cells of breast cancers. Nat Genet 37, 899-905
176 Kurose, K. et al. (2002) Frequent somatic mutations in PTEN and TP53 are mutually exclusive in the stroma of breast carcinomas. Nat Genet 32, 355-357
177 Kiaris, H. et al. (2005) Evidence for nonautonomous effect of p53 tumor suppressor in carcinogenesis. Cancer Res 65, 1627-1630
178 Hill, R. et al. (2005) Selective evolution of stromal mesenchyme with p53 loss in response to epithelial tumorigenesis. Cell 123, 1001-1011

Accession information: DOI: 10.1017/S1462399406000172; Vol. 8; Issue 30; December 2006
© 2006 Cambridge University Press

https://doi.org/10.1017/S1462399406000172 Published online by Cambridge University Press

Further reading, resources and contacts

The following website has the transcript of a lecture given by L. Matrisian, Vanderbilt University, as part of the American Association for Cancer Research (AACR) Scientist-Survivor Workshop in which she discusses 'The tumour microenvironment: how do the normal cells surrounding a tumour affect a cancer's growth?':

http://www.aacr.org/home/survivors--advocates/on-cancer-research--educational-series-on-science-and-advocacy/scientists-on-science/scientists-on-science-the-tumor-microenvironment.aspx

This website at the US National Cancer Institute (NCI) gives a general overview of the tumour microenvironment and discusses the importance of research initiatives in this field of cancer biology:

http://www.cancer.gov/think-tanks-cancer-biology/page3

The following textbook provides an excellent and comprehensive overview of cancer biology, with several chapters that are of specific relevance to the tumour microenvironment:

Weinberg, R.A. (2007) The Biology of Cancer. Garland Science, Taylor & Francis Group, LLC

Chapter 11: Multi-step tumorigenesis

Chapter 13: Dialogue replaces monologue: heterotypic interactions and the biology of angiogesis

Chapter 15: Crowd control: tumor immunology and immunotherapy

Cancer Cell published a series of Focus articles on the Tumor Microenvironment in 2005:

Bissell, M.J. and LaBarge, M.A. (2005) Context, tissue plasticity, and cancer: are tumor stem cells also regulated by the microenvironment? Cancer Cell 7, 17-23

Achen, M.G., McColl, B.K. and Stacker, S.A. (2005) Focus on lymphangiogenesis in tumor metastasis. Cancer Cell 7, 121-127

Balkwill, F., Charles, K.A. and Mantovani, A. (2005) Smoldering and polarized inflammation in the initiation and promotion of malignant disease. Cancer Cell 7, 211-217

Joyce, J.A. (2005) Therapeutic targeting of the tumor microenvironment. Cancer Cell 7, 513-520

Nature compiled an extensive Insight series on Angiogenesis in December 2005 (Vol. 438, No. 7070). The following reviews are relevant for tumour angiogenesis and lymphangiogenesis:

Carmeliet, P. (2005) Angiogenesis in life, disease and medicine. Nature 438, 932-936

Alitalo, K., Tammela, T. and Petrova, T.V. (2005) Lymphangiogenesis in development and human disease. Nature 438, 946-953

Ferrara, N. and Kerbel, R.S. (2005) Angiogenesis as a therapeutic target. Nature 438, 967-974

Nature Reviews Cancer are publishing a series of articles throughout 2006 on the Tumour Microenvironment; these can all be found at the following website:

http://www.nature.com/nrc/series/tumourmicro/index.html

Features associated with this article

Figures

Figure 1. The tumour microenvironment.
Figure 2. Bone-marrow-derived cells (BMDCs) contribute to tumour vascularisation.
Figure 3. Macrophages have tumour-promoting and -suppressing functions.
Figure 4. Targeting of tumour-associated macrophages (TAMs) at different stages of tumourigenesis.

Table

Table 1. Drugs that target cells of the tumour microenvironment.

Accession information: DOI: 10.1017/S1462399406000172; Vol. 8; Issue 30; December 2006
© 2006 Cambridge University Press

https://doi.org/10.1017/S1462399406000172 Published online by Cambridge University Press

Citation details for this article

Bedrick B. Gadea and Johanna A. Joyce (2006) Tumour–host interactions: implications for developing anti-cancer therapies. Expert Rev. Mol. Med. Vol. 8, Issue 30, December 2006, DOI: 10.1017/S1462399406000172

Accession information: DOI: 10.1017/S1462399406000172; Vol. 8; Issue 30; December 2006
© 2006 Cambridge University Press

https://doi.org/10.1017/S1462399406000172 Published online by Cambridge University Press