OPINION

Tumour-educated macrophages promote tumour progression and metastasis

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Evidence from clinical and experimental studies indicates that macrophages promote solid-tumour progression and metastasis. Macrophages are educated by the tumour microenvironment, so that they adopt a trophic role that facilitates angiogenesis, matrix breakdown and tumour-cell motility — all of which are elements of the metastatic process. During an inflammatory response, macrophages also produce many compounds — ranging from mutagenic oxygen and nitrogen radicals to angiogenic factors — that can contribute to cancer initiation and promotion. Macrophages therefore represent an important drug target for cancer prevention and cure.

Solid tumours comprise not only malignant cells, but also many other non-malignant cell types. This produces a unique microenvironment that can modify the neoplastic properties of the tumour cells. However, there has been an almost exclusive experimental focus on the malignant cells that make up tumours. This is probably because of the success of studies that defined the requirement for several mutational events in epithelial cells for the formation of malignant tumours; the isolation of transforming oncogenes, the epithelial-restricted expression of which causes cancers in animals; and the fact that these experimental results are intellectually compatible with epidemiological studies that indicate that multiple events are required for malignancy. Nevertheless, recent studies, particularly of oncogene-driven tumours in transgenic mice, have shown that the outcome of primary oncogenic events in epithelial cells can be significantly modified by the nature of the surrounding non-malignant cells[1–5]. So, it is becoming apparent that the microenvironment has an important role in allowing the tumour to express its full neoplastic phenotype and that non-malignant cells can be used as therapeutic targets.

The tumour microenvironment contains many resident cell types, such as adipocytes and fibroblasts, but it is also populated by migratory haematopoietic cells, most notably macrophages, neutrophils and mast cells. These haematopoietic cells have pivotal roles in the progression and metastasis of tumours[2,6–9], and this review will focus on one such class — the tumour-associated macrophages (TAMs). I will argue that the tumour microenvironment, in a similar way to that seen in normal development (BOX 1), educates macrophages to perform supportive roles that promote tumour progression and metastasis. In addition, because of their fundamental role in mediating the inflammatory response, and the growing appreciation that chronic inflammation is a significant cause of cancer (BOX 2), I suggest that macrophages can initiate and promote tumorigenesis.

TAMs: markers of poor prognosis

The observation of leukocytes in tumours dates back to the middle of the nineteenth century[6]. Until recently, however, they were usually overlooked[10]. Nevertheless, it is now appreciated that most solid tumours are abundantly populated with TAMs and that these cells can alter clinical outcomes[11]. In normal tissues, pathogenic challenge or wounding results in the local expression of a wide variety of growth factors — colony-stimulating factor 1 (CSF-1; also known as macrophage CSF), granulocyte–macrophage CSF (GM-CSF), macrophage-stimulating protein (MSP) and transforming growth factor-β1 (TGF-β1) — and chemokines (chemotactic cytokines), which include CCL2, CCL7, CCL8 (monocyte chemoattractant protein family-1-3), CCL3 (macrophage inflammatory protein-1α (MIP-1α)), CCL4 (MIP-1β) and macrophage migration inhibitory factor (MIF). These factors, together with the products of tissue breakdown, recruit circulating monocytes and stimulate them to differentiate into macrophages. Macrophages, in turn, mediate immune responses, kill pathogens, stimulate angiogenesis and effect tissue repair[12,13].

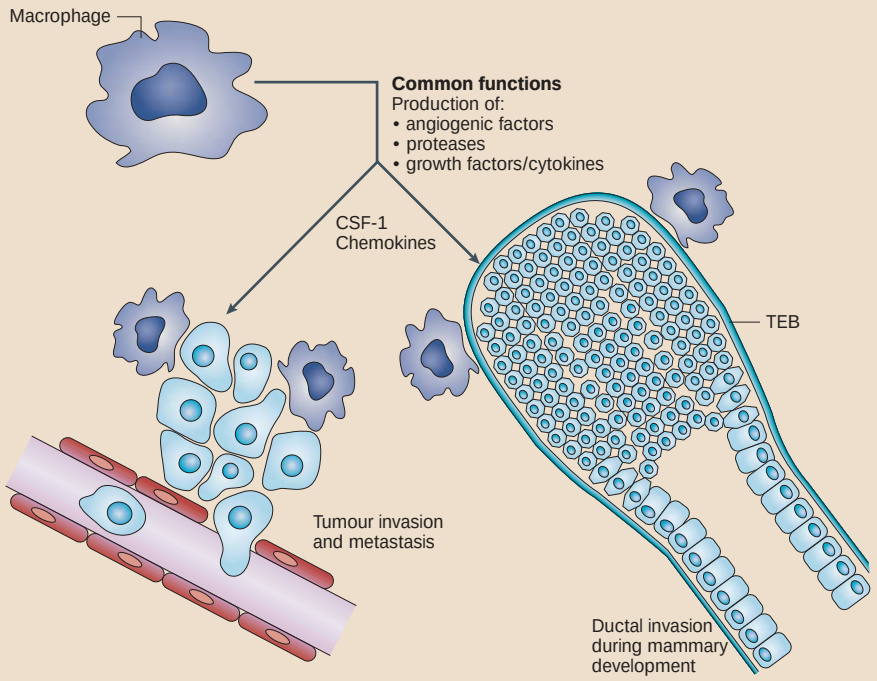
Macrophages — TAMs — are also recruited to tumours by a similar range of growth factors and chemokines, which are often produced by the tumour cells themselves[8,9,14]. Clinical studies have, on balance, shown a correlation between an abundance of TAMs and poor prognosis[14]. These data are particularly strong for breast, prostate, ovarian and cervical cancers; the data for stomach and lung cancers are contradictory[14], and in a small study in colorectal cancer, their presence was associated with good prognosis[15]. However, taking all reports into account — regardless of method and sample number — more than 80% show a significant correlation between TAM density and poor prognosis, whereas less than 10% associate TAM density with a good prognosis[14]. So, increased TAM density is usually associated with advanced tumour progression and metastasis.

It is also striking that, in the tumour types in which high TAM density correlates with poor prognosis, there is also a substantial body of clinical literature that shows that overexpression of macrophage growth factors or chemokines correlates with poor prognosis. CCL2 is widely expressed in tumours, including ovarian, cervical, bladder and breast tumours, and gliomas — this last being the original source of its purification — and high levels of CCL2 correlate with poor prognosis in breast, cervical and bladder cancer[8,16,17]. CSF-1 — the main growth factor that is responsible for the survival, proliferation, differentiation and chemotaxis of mononuclear phagocytes, such as macrophages — is widely overexpressed in tumours of the reproductive system, including ovarian, uterine, breast and prostate tumours[7,18-21]. In each case, overexpression of CSF-1 correlates with poor prognosis[7,20]. In breast cancer, CSF-1 expression correlated with high grade and poor prognosis and was also associated with a dense leukocytic infiltrate in 90% of the tumours that were analysed[21]. These clinical data therefore provide significant support for the theory that, in most types of solid tumour, TAMs are involved in tumour progression through their recruitment to these sites by chemokines and CSF-1.

Box 1 | Macrophages have important developmental roles

The immunological and repair functions of macrophages are well documented. It is known that they are among the first cells to arrive at sites of wounding and/or infection, where they perform several functions. They produce cytokines and chemokines to orchestrate the recruitment and actions of other immune cells, and produce growth factors, angiogenic factors and proteases to promote tissue repair. They also kill pathogens through the production of reactive oxygen and nitrogen radicals and present foreign antigens to cytotoxic T cells. What is less well appreciated is their important role in tissue morphogenesis during development. Nevertheless, analysis of mice that are deficient for macrophages and other mononuclear phagocytic cells (such as osteoclasts) because of a null mutation in the gene that encodes colony-stimulating factor 1 (Csf-1) shows that these cells have a significant role in the morphogenesis of many tissues[83,84]. Developmental defects include osteopetrosis, dermal hypoplasia, aberrant development of the sex-steroid-hormone feedback response in the brain, delayed and aberrant pancreatic morphogenesis and impaired branching morphogenesis of the mammary gland. In this last case[85], as shown in the figure, the ends of the developing ducts form a unique multi-laminate structure called the terminal end bud (TEB), which grows out through the mammary fat pad and gives rise to the basal arborized ductal tree. As these TEBs form, they recruit macrophages; the absence of these in the *Csf-1*-null mutant mouse results in delayed ductal development and a poorly branched, atrophic ductal tree. Similar experiments, in which macrophages are ablated by other means, show important roles in eye development[86]. Together, these experiments show that cells of the mononuclear phagocytic lineage have important developmental roles through their remodelling and trophic functions.

It seems likely that tumours co-opt the normal developmental roles of macrophages to promote their own development and invasion through the surrounding stroma[87]. In contrast to normal epithelia, however, tumour cells — owing to intrinsic transforming mutations — have lost positional identity, and so they continue to send out 'help me' signals that result in invasion into the vasculature.



Cytokines alter TAM function

The conventional wisdom about TAM function is that they are recruited to reject the tumour, which has been recognized as foreign because tumours express unique antigens. However, there is a growing body of evidence that the tumour microenvironment is immunosuppressive[22,23], perhaps as a result of selection for such an environment — a process recently termed 'immunoediting'[24]. So, even when *bona fide* tumour antigens — such as MUC1 — are expressed, there seems to be an attenuated immune response to these antigens[25]. Recent data indicate that TGF-β1 has an important role in suppressing these local responses and that inhibiting this molecule can result in tumour rejection[26]. It is noteworthy that TAMs can both produce TGF-β1 and process latent TGF-βs to produce their active forms[27]. In addition, the local cytokine milieu in the tumour tends to block the immunological functions of these newly recruited mononuclear phagocytes — such as antigen presentation and cytotoxicity — towards tumours, and diverts them towards specialized TAMs that are immunosuppressed and trophic[28] (FIG. 1). A principal component of this cytokine mixture is CSF-1, which locally blocks the maturation of dendritic cells so that they are unable to present antigens and promotes the development of immunosuppressed trophic TAMs. In support of this hypothesis, studies have shown that renal-carcinoma cell-line production of interleukin-6 (IL-6) and CSF-1 inhibits dendritic-cell maturation. This effect can be reversed by cytokines such as IL-4 and IL-13, which divert the immune response to one that favours cytotoxic T cells[28–31] (FIG. 1).

The cytokine profile of the tumour microenvironment is therefore extremely important to the phenotype of the local mononuclear phagocytes. This is probably why, under certain circumstances, a high density of TAMs correlates with good prognosis. One can hypothesize that, in these cases, the environment pushes the TAMs

Box 2 | Cancer as an inflammatory disease

The concept of inflammation as a cause of cancer dates back to the work of Virchow in the 1850s and to the work of Fibiger and Yamagiwa in the early twentieth century, which showed that chronic irritation could trigger cancer[6]. Although this topic has been largely overlooked in the past, a growing body of evidence has recently indicated that this inflammatory process is a contributor, if not a cause, of a wide variety of neoplasms[2]. First, it is now recognized that at least 15% of tumours worldwide have a direct infectious origin[88]. Generally, the pathogens that are responsible establish chronic infections that cause a persistent inflammatory response. Perhaps the best documented are the roles of *Helicobacter pylori* in stomach cancer, herpes viruses in cervical cancer and schistosomes in bladder cancers[89–91]. Continuous irritants, such as asbestos, silica and cigarette smoke, also increase the probability of developing bronchial cancer[92]. Colonic inflammation, such as that found in ulcerative colitis or Crohn's disease, predisposes sufferers to colorectal cancer[93]. Indeed, even the prototypical oncogenic retrovirus, Rous sarcoma virus, requires an inflammatory response to induce tumours at secondary sites in chickens[94]. Furthermore, the classical studies of skin carcinogens showed that a second, non-carcinogenic compound, such as a phorbol ester, could increase the effects of low doses of a primary carcinogen. These promoters set up an acute inflammatory response and cause alterations in cytokine signalling pathways that are required for the carcinoma to form[75].

Inflammatory responses recruit many immune cells, among which macrophages are key players[95]. Macrophages produce angiogenic factors, proteases and growth factors, which result in an environment that stimulates epithelial-cell migration, survival and proliferation. They are also key signalling cells that help to organize the responses of other cells — most notably mast cells and neutrophils. Both of these cell types have, along with tumour-associated macrophages (TAMs), been shown to have causal roles in tumour progression[1,2,96,97]. The signals responsible for this are thought to be immune cytokines, including tumour necrosis factor-α (TNF-α), interleukin-1 (IL-1), IL-6 and a plethora of chemokines, such as IL-8. Not only do these cytokines and chemokines recruit immune cells to specific sites that stimulate tumour progression, but it has also recently been shown that their receptors are often found on tumour cells themselves, where they can increase tumour growth and migration. For example, GRO (growth-related oncogene, which is probably the same as CXCL1) — an IL-8-related chemokine — stimulates melanoma migration and proliferation[98]. Such data indicate that chemokines might make a permissive environment for tumour-cell growth and migration. Consistent with this are recent studies that show that the expression of specific chemokines defines the site of tumour metastasis[99]. Indeed, it seems more and more likely that tumour cells might subvert the pathways that leukocytes use to migrate to specific sites.

Altogether, the data show that inflammation creates a microenvironment that causes neoplastic transformation and potentiates the progression of cancers. Such a realization should alter therapeutic strategies, both for prophylaxis and for treatment of acute disease.

either to be immunologically neutral, or to differentiate to become active participants in the immune response against tumours through their presentation of tumour antigens to T cells or by direct tumour-cell killing. In determining this balance, another macrophage colony-stimulating factor — GM-CSF — together with interferon-γ (IFN-γ), are likely to be important, as these direct macrophages towards more cytotoxic and antigen-presenting phenotypes[32]. Interestingly, even the type of CSF-1 that is expressed seems to determine the nature of the immune response. CSF-1 is made both as a cell-surface glycoprotein and as a secreted proteoglycan[33]. Glioma cells that express the cell-surface form of CSF-1 were rejected when transplanted intracranially into mice, and 80% of the mice survived. By contrast, 100% of mice that were implanted with the same cells died if they had been transfected with a complementary DNA encoding the soluble form of CSF-1. Other studies indicate that this difference is because macrophages 'lock' onto the cell-surface form of CSF-1 and kill the tumour cells — either directly, or indirectly through antigen presentation to T cells[34–36]. In humans, the soluble form predominates in those tumours that overexpress CSF-1[18,19]. So, one hypothesis that explains the relative lack of immune response in tumours is that they produce soluble CSF-1 and cytokines, which suppress dendritic-cell maturation and recruit TAMs, the immune and killing functions of which are suppressed and the trophic and remodelling functions of which are enhanced (FIG. 1).It will be important to know how other macrophage chemokines, such as CCL2 and CCL3 — which are produced in high concentrations by tumours — affect local immune responses, and whether manipulation of microenvironments containing these cytokines through therapies using GM-CSF, IFN-γ and IL-12 will be effective means of promoting the immune rejection of tumours. These issues are discussed in depth in another article in the same issue of this journal[32], which indicates that such approaches might be used effectively to cause immunological rejection of tumours.

TAMs potentiate tumour progression

The clinical evidence described above indicates that, in most tumour types in which it has been studied, an abundance of TAMs that are matured in the right cytokine environment has a negative impact on patient survival. Several recent experiments with animal models support this view by showing that TAMs potentiate tumour progression and metastasis. Lin *et al.*[1] used mice carrying a null mutation in the gene that encodes Csf-1 to prevent macrophages accumulating in mammary tumours that were induced by the mammary-epithelial-restricted expression of the polyoma middle-T oncoprotein (known as PyMT mice). In the macrophage-deficient mice, the incidence and initial rates of growth of primary tumours were not different from those seen in normal mice, but the rate of tumour progression was slowed and their metastatic ability was almost completely abrogated when compared with mice that contained normal numbers of macrophages. Increasing the abundance of TAMs in the null mutant mice by expressing Csf-1 in a restricted fashion in tumours, using transgenic technology, accelerated the rate of tumour progression and restored the rate of metastasis to wild-type levels. Interestingly, overexpression of Csf-1 in wild-type mice also accelerated tumour progression and increased their metastatic rate. These data indicate that the clinical correlation between overexpression of CSF-1 and poor prognosis might be due to the ability of CSF-1 to recruit and modulate the behaviour of TAMs.

Consistent with these genetic experiments, when immunocompromised mice that had been xenografted with either a human malignant embryonic tumour or human colon-cancer cells were treated with antisense oligonucleotides directed against mouse *Csf-1*, the growth of the embryonic tumour was completely suppressed, and the growth rate of the colon cancer was halved, with an increased survival rate in these mice. As the human cells did not express the CSF-1 receptor, and the antisense oligonucleotides did not inhibit human CSF-1 expression, these data argue for an effect on the host, and not on the tumour.

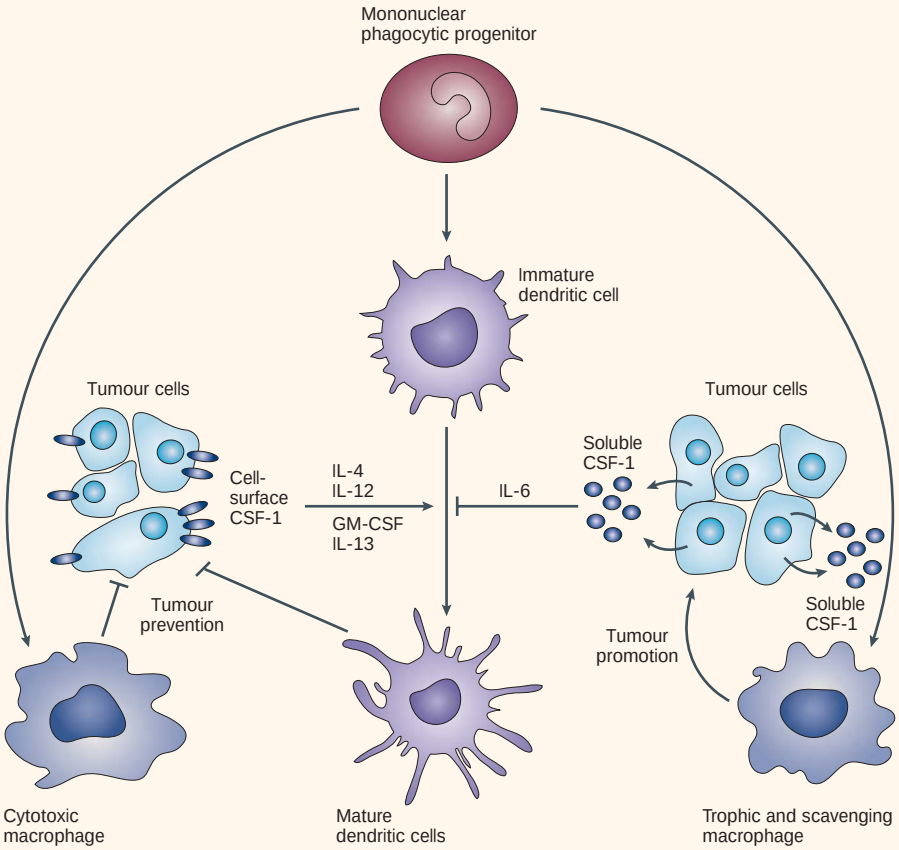


Figure 1 | **Pro- and anti-tumorigenic properties of macrophages depend on the cytokine microenvironment in the tumour.** Tumours are populated by macrophages and dendritic cells that are derived from mononuclear phagocytic progenitor cells. In many tumours, a high concentration of soluble colony-stimulating factor-1 (CSF-1) educates macrophages to be trophic to tumours and, together with interleukin-6 (IL-6), inhibits the maturation of dendritic cells. This creates a microenvironment that potentiates progression to metastatic tumours. By contrast, CSF-1 presented in a transmembrane form on the tumour surface activates macrophages to kill tumour cells. This — together with high concentrations of IL-4, IL-12, IL-13 and GM-CSF — causes dendritic cells to mature, allowing the presentation of tumour antigens to cytotoxic T cells, with the consequent rejection of the tumour.

This was confirmed by the reduction seen in *Csf-1* messenger RNA levels and serum Csf-1 concentrations, which correlated with a reduction in the number of TAMs[37].

In another set of experiments in mice, primary-tumour-stimulated macrophages were shown to increase the metastatic ability of tail-vein-injected tumour cells. In these experiments, the ability of the injected cells to colonize and grow in the host lungs only occurred in mice that also carried a separate primary tumour[38]. This study provided evidence that macrophages — modified by their exposure to the primary tumour — promoted the seeding and vascularization of the injected tumour cells through the induction of matrix metalloproteinase 9 (Mmp-9) and vascular endothelial growth factor (Vegf).

Taken together, these recent experiments support a role for TAMs in tumour progression and metastasis and provide experimental support for the clinical observations that increased TAM density promotes tumour malignancy.

Functions of TAMs

Macrophages are therefore multifunctional cells, the phenotypes of which are modified by the local environment, and have important roles in the morphogenesis of tissues. They take on non-immunological functions, which provide trophic support to tissues (BOX 1). I suggest that a similar 'education' process within a tumour results in macrophages that, on balance, promote the progression of the tumour to a more malignant state. In addition, in the context of an inflammatory response, macrophage functions can result in the initiation or promotion of tumorigenesis. These diverse functions are summarized in FIG. 2, and are discussed below.

Macrophages promote angiogenesis. It is widely recognized that tumours require angiogenesis to grow beyond a certain size. This process involves a wide range of soluble mediators that are both stimulatory and inhibitory, including basic fibroblast growth factor (bFGF), VEGF, the angiopoietins (ANG1 and ANG2), IL-1, IL-8, tumour necrosis factor-α (TNF-α), thymidine phosphorylase (TP; also known as vascular-derived endothelial growth factor), the matrix metalloproteinases MMP-9 and MMP-2, and nitric oxide (NO)[39,40]. These molecules, which are expressed in a coordinated spatial and temporal fashion, result in the proliferation and migration of endothelial cells, matrix remodelling and the eventual formation of stabilized vessels[41]. Macrophages are perfectly designed to promote these processes, as their monocytic precursors can migrate into sites where they differentiate into macrophages, and these wandering cells can synthesize the required angiogenic molecules on demand in specific locations[42].

Macrophages are important, although not the only, producers of VEGF, which is a key component of the process of angiogenesis in tumours[43,44]. Studies by Harris and co-workers in human breast cancer showed that TAMs cluster in 'hot spots' in avascular areas[45]. These hot spots correlate with a high level of angiogenesis, and also with decreased relapse-free and overall survival[8]. It was suggested that hypoxia — or the cytokines that are produced in response to this condition — is one of the local attractants for macrophages, and that hypoxia itself upregulates the transcription factor hypoxia-inducible factor-2α (HIF-2α) in macrophages. HIF-2α, in turn, induces VEGF expression[8,43] (FIG. 2). VEGF is also upregulated by CSF-1 in macrophages[46], and the *CSF-1* antisense experiments described above showed a reduction in VEGF expression and an inhibition of angiogenesis[37]. VEGF is also a chemoattractant for macrophages[44,47] and, as such, this might result in a positive-feedback loop, providing rapid vascularization to tumours.

TAMs also produce many other pro-angiogenic cytokines[8,40]. They are key producers of TNF-α[48], the expression of which increases in these cells as tumours become invasive carcinomas[48], and which upregulates TP in breast cancer cell lines[49]. Correlative studies indicate that it also does so in breast tumours, and that TP expression is significantly correlated with angiogenesis and poor prognosis in these tumours[50]. TNF-α also induces MMP-9 expression, and this in turn can release bioactive VEGF from its extracellular-matrix (ECM)-bound latent form[6]. TAMs

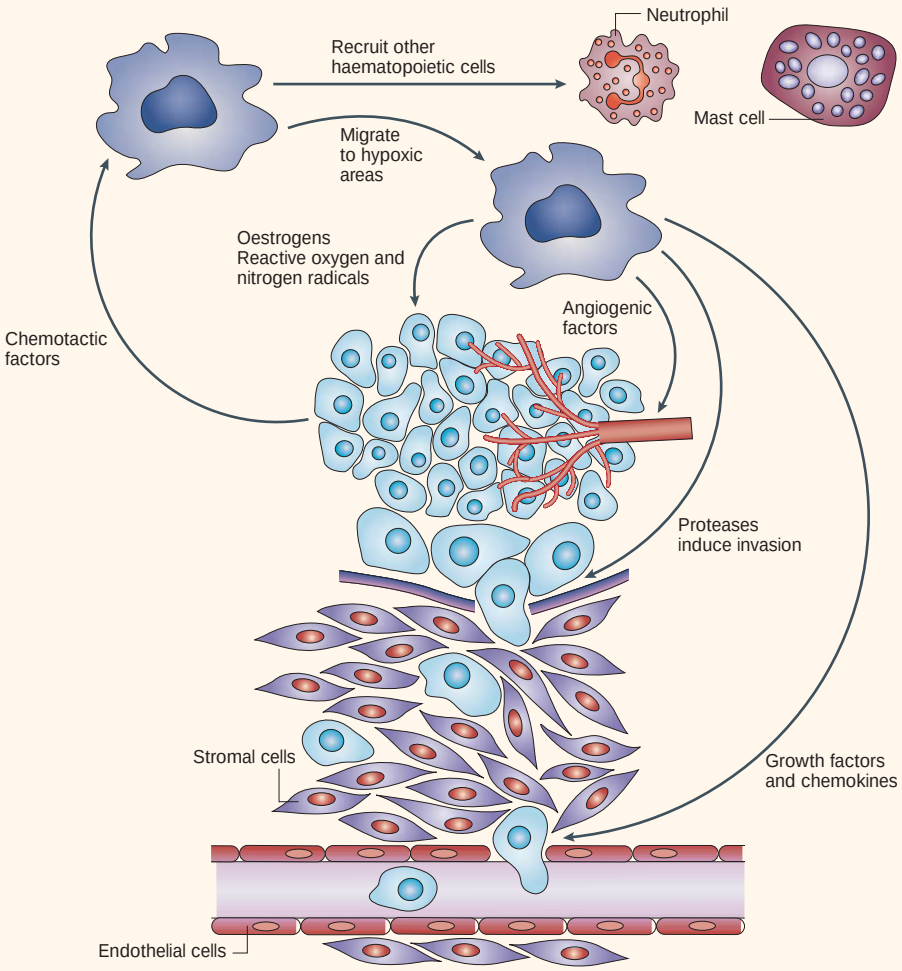


Figure 2 | **Pro-tumorigenic functions of tumour-associated macrophages.** Macrophages are recruited to tumours by chemotactic factors and provide many trophic functions that promote tumour progression and metastasis. These tumour-associated macrophages (TAMs) migrate to hypoxic areas within the tumour, where they stimulate angiogenesis by expressing factors such as vascular endothelial growth factor (VEGF), angiopoietin 1 (ANG1) and ANG2, and recruit other haematopoietic cells — mast cells and neutrophils — that can perform similar tasks. TAMs also promote tumour invasion by producing proteases — such as urokinase-type plasminogen activator (uPA), matrix metalloproteinase 9 (MMP-9) and cathepsins — that break down the basement membrane and remodel the stromal matrix. MMP-9 also contributes to angiogenesis. Various growth factors and chemokines — epidermal growth factor (EGF), transforming growth factor-β (TGF-β), interleukin-8 (IL-8) and tumour necrosis factor-α (TNF-α) — contribute to the migration of tumour cells towards vessels and provide proliferative and anti-apoptotic signals to these cells. Macrophages that are attracted to sites of inflammation or tissue breakdown can also initiate or promote tumorigenesis through their synthesis of oestrogens and the generation of mutagens as a by-product of their production of reactive oxygen and nitrogen-oxide radicals.

also synthesize other proteins that influence angiogenesis — for example, urokinase-type plasminogen activator (uPA)[51], which is activated by CSF-1 receptor signalling in macrophages[52] and by TGF-β1 (REF. 53). Expression of uPA and its inhibitor, plasminogen-activator inhibitor type 1 (PAI-1), has clinical and prognostic value[54]. uPA and its receptor are upregulated in TAMs in breast cancer[51]. The complex of uPA and its receptor is fully active, and might contribute to the ECM breakdown that is required for vascular invasion to occur. Consistent with this, the expression of the uPA receptor in TAMs has been clinically correlated with high microvessel density and poor prognosis[54,55]. In addition, a significant correlation exists between PAI-1 expression, vessel remodelling, and node status and tumour grade[56–58]. These data indicate that the uPA system is important in establishing the vascular network in tumours and that TAMs have an important role in its expression and regulation.

TAMs also release other molecules that can influence angiogenesis. They produce IL-1, which — through cyclooxygenase 2 (COX 2) — upregulates HIF-1α, resulting in an increase in the transcription of *VEGF*[59]. VEGF production is also increased by IL-1β in co-cultures of tumour cells and macrophages. IL-1 is synthesized by macrophages and, in these experiments, IL-1β was required for tumour-cell invasiveness and angiogenesis[60]. TAMs also release NO through the induction of the enzyme inducible NO synthase (iNOS)[61]. Expression of iNOS has been correlated with tumour grade[62], and its ablation delays tumour progression in mouse models of breast cancer[63]. Increased NO is likely to result in vasodilation and increased vascular flow. Interestingly, endothelin 2 — another vasoactive molecule involved in inflammation and angiogenesis that is expressed in tumours — is a chemoattractant for macrophages, which indicates that it might recruit TAMs to hypoxic areas[64].

In conclusion, migratory TAMs are equipped to enter areas of the tumour where vascularization is needed. Here, these cells synthesize angiogenic regulators, which results in the formation of new vessels that allows further tumour growth and access of tumour cells to the vasculature for escape into the circulation. At these sites, a complex mixture of factors — ranging from hypoxia to cytokines — controls the expression of these regulators. This is consistent with the hypothesis that macrophages are educated to perform specialized tasks at specific sites.

Macrophages produce growth factors and proteases that enhance tumour progression. It is apparent from the discussion above that macrophages are important producers of proteases, which range from uPA to a variety of matrix metalloproteinases — especially MMP-7 and MMP-9. In our detailed study of the progression of PyMT-induced tumours[7,65], we noted that at the time of malignant transition, leukocytic infiltrates were present that coincided with areas of basement-membrane breakdown and tumour-cell egress (FIG. 3). It is not clear whether these invading leukocytes cause the initial breakage of the basement membrane or if this is a result of the activities of the tumour cells themselves. Regardless of the initiating mechanism, approximately 50% of the leukocytes are TAMs, which produce proteases that degrade the basement membrane, so creating a portal through which tumour cells enter the stroma. This is a key step in tumour metastasis.

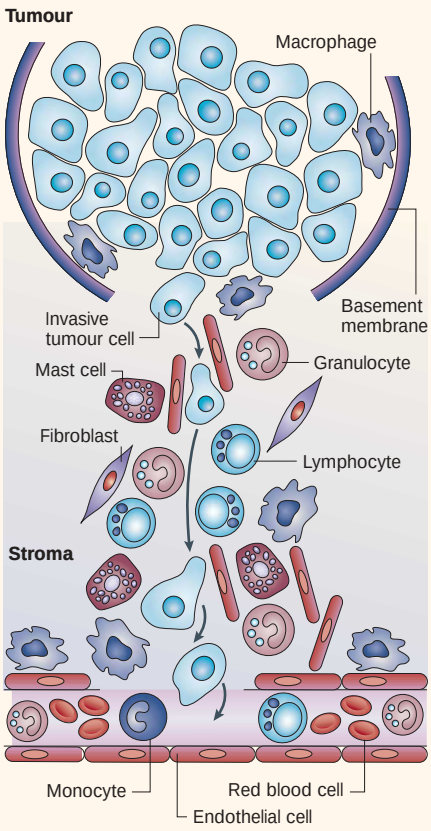


Figure 3 | **The leukocytic infiltration site as a portal for the exit of tumour cells.** Many tumours, as they become invasive, also have leukocytic infiltration sites that are abundantly populated by macrophages. So, a hypothesis to explain the role of macrophages in tumour invasion and metastasis is that these cells, through their proteolytic activity, break down the basement membrane around pre-invasive tumours, thereby enhancing the ability of tumour cells to escape into the surrounding stroma. Macrophages also stimulate angiogenesis at these sites of tumour egress and send 'come hither' signals to cells, causing them to move out of the tumour mass towards blood vessels. So, tumour cells flow out from the ductally constrained tumour mass into the surrounding stroma, thereby gaining access to the vasculature, with the consequent ability to colonize distant sites.

TAMs also produce a wide variety of growth factors that can stimulate the growth and motility of tumour cells[8,66]. These include fibroblast growth factor (FGF), hepatocyte growth factor (HGF), epidermal-growth-factor receptor (EGFR)-family ligands, platelet-derived growth factor (PDGF) and transforming growth factor-βs (TGF-βs). Ligands of the EGFR family seem to be very important in cancer, particularly in cancers of the breast and lung, and the receptors that belong to this family form a complex group that are able to both heterodimerize and homodimerize. Some, such as ERBB3, do not have an active kinase domain and need to heterodimerize to transduce a signal. ERBB2, for which a ligand has yet to be identified, is overexpressed in approximately 20% of breast cancers and is an effective target for therapy[67]. Similarly, a high level of ERBB1 (also known as EGFR) expression in breast cancer is correlated with poor survival and has important diagnostic value[68]. TAMs have been reported to be the most significant source of EGF in tumours[69] and are associated with EGFR expression[44] and poor prognosis. EGF can promote tumour-cell proliferation and is also a potent chemoattractant of breast cancer cells in culture[70]. We have recently shown that tumour cells respond to macrophage-produced EGF ligands *in vivo* by chemotaxis and invasion. These macrophages are often associated with vessels, which indicates that they provide chemotactic signals that recruit tumour cells to blood vessels and enhance their egress into the vasculature (J. Wycoff *et al.*, unpublished observations). The absence of these macrophage signals might be part of the reason why mice that lack macrophages show a low rate of metastasis[1].

A unifying concept for TAM action. In our studies of PyMT tumours that were discussed earlier, focal sites of leukocytic infiltration were found at the point of transition of tumours[71]. Such sites are also commonly found in human breast cancers[71]. So, a scenario can be proposed in which intrinsic mutations that accumulate in the tumour cells cause them to send out chemoattractive signals that are similar to those produced in the developing mammary gland (BOX 1), indicating that they want to break out through the basement membrane and into the stroma. These signals recruit macrophages and other haematopoietic cells, which interact to cause focal breakdown of the basement membrane (FIG. 3). Tumour cells consequently exit into the stroma and TAM-induced angiogenesis occurs at the location of this escape. These vessels become sites of macrophage alignment. TAMs then provide chemotactic signals to the tumour cells to promote their migration towards vessels and cause their intravasation. Growth factors that are secreted by TAMs might also promote the viability of tumour cells by overcoming the apoptotic signals that are induced by their detachment from the basement membrane. The same growth factors might also promote tumour-cell proliferation locally, and so further select for tumour cells that contain mutations that enhance apoptosis resistance and increase motility. The consequence of all these processes is a more malignant and invasive phenotype.

Macrophages can initiate tumorigenesis

The presence of TAMs in a tumour provides an environment that enhances the survival, proliferation and migration of epithelial cells that have accumulated primary oncogenic mutations. However, macrophages might have an even more sinister role by playing a significant part in establishing the primary oncogenic events in epithelial cells (FIG. 2). There is a growing body of evidence to indicate that inflammation — as a result of chronic infection, continuous exposure to irritants or genetic makeup — is a causative event in many cancers (BOX 2). Macrophages comprise a key component of the inflammatory response and function as key regulators of the activities of many of the other cell types that are involved in inflammation. At these inflammatory sites, macrophages produce high levels of reactive oxygen and nitrogen species and these — through the formation of peroxynitite — can react with DNA, resulting in mutagenic events in epithelial and surrounding cells[72,73]. This continuous generation of mutagenic compounds in response to persistent infection is thought to be the mechanism by which *Helicobacter pylori* causes stomach cancer (BOX 2). Similarly, some cytokines that are produced by TAMs and other immune cells, such as TNF-α and MIF, might also contribute to the generation of chromosomal abnormalities. MIF, for example, suppresses *TP53* transcription in tumour cells, which results in the lack of a DNA-damage-repair response and, consequently, the accumulation of mutations[74]. TNF-α treatment of carcinogen-treated fibroblasts renders them capable of tumour formation in nude mice[65]. Mechanistically, this may be through the induction of iNOS in the tumour cells, which results in NO production. Studies of mice that lack Tnf-α showed that this cytokine is also required for 7,12-dimethylbenz(*a*)-anthracene (DMBA)-induced, 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA)-promoted skin carcinogenesis, and this is related to the reduced inflammation that is seen in the absence of Tnf-α, which decreases the incidence of *de novo* carcinogenesis[75].

TAMs are abundant in breast cancers[8]. Recent studies indicate that these cells might both respond to and produce oestrogens[76,77]. TAMs in the breast cancer bed express

aromatase, which converts androgens to oestrogens[76]. Interestingly, in a cohort of women at risk of breast cancer who had been given intraductal lavages to access epithelial-cell morphology, it was found that more than 50% of the retrieved cells were macrophages[78]. These cells might synthesize oestrogen, which could explain the fivefold higher concentration of oestrogens in the ductal fluid than in serum. As oestrogen exposure is the primary risk factor for breast cancer[79], macrophages in both the pre-malignant and malignant breast could have an important role in increasing exposure to high concentrations of this steroid hormone. This, coupled with their mutagenic properties, which are described above, could significantly enhance the risk of breast cancer.

Therapeutic opportunities

The data indicate that continuous inflammation as a result of persistent infection or other irritants has a causal role in tumour progression. Is it possible that cancer is often a pathology of chronic infectious diseases (BOX 2)? If so, therapies that are directed at reducing inflammation or inhibiting the function of inflammatory cytokines could reduce cancer risk. Consistent with this notion is the ability of non-steroidal anti-inflammatory drugs (NSAIDs) to significantly lower colon-cancer risk and, perhaps, to prevent lung, oesophageal and stomach cancers[80,81]. These drugs target cyclooxygenase enzymes, which are involved in the metabolism of prostaglandins. Prostaglandins have an important role in inflammatory responses and are produced abundantly by macrophages. It will be important to determine whether macrophages are the site of action of these NSAIDs, as COX2 can also be expressed in tumour cells.

I have argued in this article that the microenvironment educates macrophages to take on specific phenotypes that can increase the risk of cancer and, once cancers are formed, promote their progression. Although there is a considerable amount of data on the phenotype of 'activated' macrophages that are involved in immune responses, there are relatively few data about the macrophages that are found at different sites in the body. However, recent advances that allow macrophages to be marked *in vivo*[82] will allow their isolation, in a relatively unperturbed state, from specific sites. The analysis of these cells using DNA or protein microarrays will then define their phenotypes. Although most of the expressed genes will be common to all macrophages, these experiments will, I suspect, show cohorts that are unique to particular macrophage environments. TAMs will therefore be shown to have a unique set of expressed genes. The intracellular regulators of these different pathways might therefore be potential therapeutic targets. The experiments in mouse models that are described above — in which tumour progression and metastasis are reduced by the ablation of *CSF-1*, either genetically[1] or by the use of antisense *CSF-1* oligonucleotides[37] — support this idea. In the future, new methods to reduce the continuous over-exuberant production of macrophage chemoattractants, combined with anti-inflammatory drugs, might result in a significant abatement of the aggressive phenotypes of epithelial neoplasias.

Summary

The data described above indicate that macrophages affect many processes, ranging from tumour initiation to the acceleration of tumour progression and metastasis. The abilities of these cells to move to specific sites, increase matrix remodelling and induce angiogenesis are also essential during normal development and in normal physiological processes, such as wound healing and inflammation. This indicates that tumours recapitulate these developmental signals to co-opt the normal developmental roles of macrophages (BOX 1). However, unlike normal epithelial structures, neoplastic cells have lost their positional identity due to intrinsic mutations and, so, they continue to invade and enter the vascular and lymphatic systems. Furthermore, these transformed epithelial cells send out continuous and unrestrained signals that constantly recruit macrophages and other haematopoietic cells to perform these morphogenic roles. This results in a state that is described by the phrase "tumours are wounds that never heal"[6]. But this could perhaps be better stated as "tissues that never cease to develop". This continuous 'on' signal indicates that strategies that are directed to dampen the recruitment and tumour-associated functions of macrophages could find an important place within the therapeutic arsenal against tumours.

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1. Lin, E. Y., Nguyen, A. V., Russell, R. G. & Pollard, J. W. Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. *J. Exp. Med.* **193**, 727–740 (2001).
2. Coussens, L. M. & Werb, Z. Inflammation and cancer. *Nature* **420**, 860–867 (2002).
3. Coussens, L. M., Tinkle, C. L., Hanahan, D. & Werb, Z. MMP-9 supplied by bone marrow-derived cells contributes to skin carcinogenesis. *Cell* **103**, 481–490 (2000).
4. Iyengar, P. *et al.* Adipocyte-secreted factors synergistically promote mammary tumorigenesis through induction of anti-apoptotic transcriptional programs and proto-oncogene stabilization. *Oncogene* **22**, 6408–6423 (2003).
5. Krtolica, A. *et al.* Senescent fibroblasts promote epithelial cell growth and tumorigenesis: a link between cancer and aging. *Proc. Natl Acad. Sci. USA* **98**, 12072–12077 (2001).
6. Balkwill, F. & Mantovani, A. Inflammation and cancer: back to Virchow? *Lancet* **357**, 539–545 (2001).
7. Lin, E. Y., Gouon-Evans, V., Nguyen, A. V. & Pollard, J. W. The macrophage growth factor, CSF-1, in mammary gland development and cancer. *J. Mammary Gland Biol. Neoplasia* **7**, 147–162 (2002).
8. Leek, R. D. & Harris, A. L. Tumor-associated macrophages in breast cancer. *J. Mammary Gland Biol. Neoplasia* **7**, 177–189 (2002).
9. Brigati, C., Noonan, D. M., Albini, A. & Benelli, R. Tumors and inflammatory infiltrates: friends or foes? *Clin. Exp. Metastasis* **19**, 247–258 (2002).
10. Normann, S. J. Macrophage infiltration and tumor progression. *Cancer Metastasis Rev.* **4**, 277–291 (1985).
11. Mantovani, A. Tumor-associated macrophages in neoplastic progression: a paradigm for the *in vivo* function of chemokines. *Lab. Invest.* **71**, 5–16 (1994).
12. Lingen, M. W. Role of leukocytes and endothelial cells in the development of angiogenesis in inflammation and wound healing. *Arch. Pathol. Lab. Med.* **125**, 67–71 (2001).
13. Nathan, C. Points of control in inflammation. *Nature* **420**, 846–852 (2002).
14. Bingle, L., Brown, N. J. & Lewis, C. E. The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies. *J. Pathol.* **196**, 254–265 (2002).
15. Nakayama, Y. *et al.* Relationships between tumor-associated macrophages and clinicopathological factors in patients with colorectal cancer. *Anticancer Res.* **22**, 4291–4296 (2002).
16. Ueno, T. *et al.* Significance of macrophage chemoattractant protein-1 in macrophage recruitment, angiogenesis, and survival in human breast cancer. *Clin. Cancer Res.* **6**, 3282–3289 (2000).
17. Saji, H. *et al.* Significant correlation of monocyte chemoattractant protein-1 expression with neovascularization and progression of breast carcinoma. *Cancer* **92**, 1085–1091 (2001).
18. Smith, H. O. *et al.* The role of colony-stimulating factor 1 and its receptor in the etiopathogenesis of endometrial adenocarcinoma. *Clin. Cancer Res.* **1**, 313–325 (1995).
19. Kacinski, B. M. CSF-1 and its receptor in breast carcinomas and neoplasms of the female reproductive tract. *Mol. Reprod. Dev.* **46**, 71–74 (1997).
20. Kacinski, B. M. CSF-1 and its receptor in ovarian, endometrial and breast cancer. *Ann. Med.* **27**, 79–85 (1995).
21. Scholl, S. M. *et al.* 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 (1994).
22. Elgert, K., Alleva, D. & Mullins, D. Tumor-induced immune dysfunction: the macrophage connection. *J. Leukoc. Biol.* **64**, 275–290 (1998).
23. Ohm, J. E. & Carbone, D. P. VEGF as a mediator of tumor-associated immunodeficiency. *Immunol. Res.* **23**, 263–272 (2001).
24. Dunn, G. P. *et al.* Cancer immunoediting: from immunosurveillance to tumor escape. *Nature Immunol.* **3**, 991–998 (2002).
25. Taylor-Papadimitriou, J. *et al.* MUC1 and the immunobiology of cancer. *J. Mammary Gland Biol. Neoplasia* **7**, 209–221 (2002).
26. Gorelik, L. & Flavell, R. A. Immune-mediated eradication of tumors through the blockade of transforming growth factor-β signaling in T cells. *Nature Med.* **7**, 1118–1122 (2001).
27. Chong, H., Vodovotz, Y., Cox, G. W. & Barcellos-Hoff, M. H. Immunocytochemical localization of latent transforming growth factor-β activation by stimulated macrophages. *J. Cell Physiol.* **178**, 275–283 (1999).

28. Menetrier-Caux, C. *et al.* Inhibition of the differentiation of dendritic cells from CD34$^+$ progenitors by tumor cells: role of interleukin-6 and macrophage colony-stimulating factor. *Blood* **92**, 4778–4791 (1998).
29. Menetrier-Caux, C. *et al.* IL-4 prevents the blockade of dendritic cell differentiation induced by tumor cells. *Cancer Res.* **61**, 3096–3104 (2001).
30. Boggio, K. *et al.* Ability of systemic interleukin-12 to hamper progressive stages of mammary carcinogenesis in *HER2/neu* transgenic mice. *Cancer Res.* **60**, 359–364 (2000).
31. Nanni, P. *et al.* Prevention of *HER-2/neu* transgenic mammary carcinoma by tamoxifen plus interleukin 12. *Int. J. Cancer* **105**, 384–389 (2003).
32. Dranoff, G. Cytokines in cancer pathogenesis and cancer therapy. *Nature Rev. Cancer* **4**, 11–22 (2004).
33. Price, L. K. H., Choi, H. U., Rosenberg, L. & Stanley, E. R. The predominant form of secreted colony stimulating factor-1 is a proteoglycan. *J. Biol. Chem.* **267**, 2190–2199 (1992).
34. Jadus, M. R. *et al.* Macrophages kill T9 glioma tumor cells bearing the membrane isoform of macrophage colony stimulating factor through a phagocytosis-dependent pathway. *J. Immunol.* **160**, 361–368 (1998).
35. Jadus, M. R. *et al.* Macrophages can recognize and kill tumor cells bearing the membrane isoform of macrophage colony-stimulating factor. *Blood* **87**, 5232–5241 (1996).
36. Williams, C. C. *et al.* Membrane macrophage colony-stimulating factor on MADB106 breast cancer cells does not activate cytotoxic macrophages but immunizes rats against breast cancer. *Mol. Ther.* **3**, 216–224 (2001).
37. Aharinejad, S. *et al.* Colony-stimulating factor-1 antisense treatment suppresses growth of human tumor xenografts in mice. *Cancer Res.* **62**, 5317–5324 (2002).
38. Hiratsuka, S. *et al.* MMP9 induction by vascular endothelial growth factor receptor-1 is involved in lung-specific metastasis. *Cancer Cell* **2**, 289–300 (2002).
39. Carmeliet, P. & Jain, R. K. Angiogenesis in cancer and other diseases. *Nature* **407**, 249–257 (2000).
40. Boudreau, N. & Myers, C. Breast cancer-induced angiogenesis: multiple mechanisms and the role of the microenvironment. *Breast Cancer Res.* **5**, 140–146 (2003).
41. Hanahan, D. & Folkman, J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* **86**, 353–364 (1996).
42. Bando, H. & Toi, M. Tumor angiogenesis, macrophages, and cytokines. *Adv. Exp. Med. Biol.* **476**, 267–284 (2000).
43. Lewis, J. S. *et al.* Expression of vascular endothelial growth factor by macrophages is up- regulated in poorly vascularized areas of breast carcinomas. *J. Pathol.* **192**, 150–158 (2000).
44. Leek, R. D. *et al.* Macrophage infiltration is associated with VEGF and EGFR expression in breast cancer. *J. Pathol.* **190**, 430–436 (2000).
45. Leek, R. D. *et al.* Association of macrophage infiltration with angiogenesis and prognosis in invasive breast carcinoma. *Cancer Res.* **56**, 4625–4629 (1996).
46. Eubank, T. D. *et al.* M-CSF induces vascular endothelial growth factor production and angiogenic activity from human monocytes. *J. Immunol.* **171**, 2637–2643 (2003).
47. Barleon, B. *et al.* Migration of human monocytes in response to vascular endothelial growth factor (VEGF) is mediated via the VEGF receptor flt-1. *Blood* **87**, 3336–3343 (1996).
48. Miles, D. W. *et al.* Expression of tumour necrosis factor (TNFα) and its receptors in benign and malignant breast tissue. *Int. J. Cancer* **56**, 777–782 (1994).
49. Eda, H. *et al.* Cytokines induce thymidine phosphorylase expression in tumor cells and make them more susceptible to 5'-deoxy-5-fluorouridine. *Cancer Chemother. Pharmacol.* **32**, 333–338 (1993).
50. Leek, R. D. *et al.* Association of tumour necrosis factor α and its receptors with thymidine phosphorylase expression in invasive breast carcinoma. *Br. J. Cancer* **77**, 2246–2251 (1998).
51. Hildenbrand, R., Dilger, I., Horlin, A. & Stutte, H. J. Urokinase and macrophages in tumour angiogenesis. *Br. J. Cancer* **72**, 818–823 (1995).
52. Stacey, K. J. *et al.* Regulation of urokinase-type plasminogen activator gene transcription by macrophage colony-stimulating factor. *Mol. Cell. Biol.* **15**, 3430–3441 (1995).
53. Hildenbrand, R. *et al.* Transforming growth factor-β stimulates urokinase expression in tumor-associated macrophages of the breast. *Lab. Invest.* **78**, 59–71 (1998).
54. Foekens, J. A. *et al.* The urokinase system of plasminogen activation and prognosis in 2780 breast cancer patients. *Cancer Res.* **60**, 636–643. (2000).
55. Hildenbrand, R. *et al.* Urokinase receptor localization in breast cancer and benign lesions assessed by *in situ* hybridization and immunohistochemistry. *Histochem. Cell. Biol.* **110**, 27–32 (1998).
56. Fox, S. B. *et al.* Plasminogen activator inhibitor-1 as a measure of vascular remodelling in breast cancer. *J. Pathol.* **195**, 236–243 (2001).
57. Knoop, A. *et al.* Prognostic significance of urokinase-type plasminogen activator and plasminogen activator inhibitor-1 in primary breast cancer. *Br. J. Cancer* **77**, 932–940 (1998).
58. Hildenbrand, R. *et al.* 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 (1999).
59. Jung, Y. J. *et al.* IL-1β mediated up-regulation of HIF-1α via an NFκB/COX-2 pathway identifies HIF–1 as a critical link between inflammation and oncogenesis. *FASEB J.* **17**, 2115–2117 (2003).
60. Voronov, E. *et al.* IL-1 is required for tumor invasiveness and angiogenesis. *Proc. Natl Acad. Sci. USA* **100**, 2645–2650 (2003).
61. MacMicking, J., Xie, Q. W. & Nathan, C. Nitric oxide and macrophage function. *Annu. Rev. Immunol.* **15**, 323–350 (1997).
62. Wink, D. A. *et al.* The multifaceted roles of nitric oxide in cancer. *Carcinogenesis* **19**, 711–721 (1998).
63. Ellies, L. G. *et al.* Mammary tumor latency is increased in mice lacking the inducible nitric oxide synthase. *Int. J. Cancer* **106**, 1–7 (2003).
64. Grimshaw, M. J., Wilson, J. L. & Balkwill, F. R. Endothelin-2 is a macrophage chemoattractant: implications for macrophage distribution in tumors. *Eur. J. Immunol.* **32**, 2393–2400 (2002).
65. Arnott, C. H. *et al.* Tumour necrosis factor-α mediates tumour promotion via a PKCα- and AP-1-dependent pathway. *Oncogene* **21**, 4728–4738 (2002).
66. Ogmundsdottir, H. M., Petursdottir, I. & Gudmundsdottir, I. Interactions between the immune system and breast cancer. *Acta Oncol.* **34**, 647–650 (1995).
67. Menard, S., Tagliabue, E., Campiglio, M. & Pupa, S. M. Role of HER2 gene overexpression in breast carcinoma. *J. Cell Physiol.* **182**, 150–162 (2000).
68. Nicholson, S. *et al.* Epidermal growth factor receptor (EGFR); results of a 6 year follow-up study in operable breast cancer with emphasis on the node negative subgroup. *Br. J. Cancer* **63**, 146–150 (1991).
69. O'Sullivan, C., Lewis, C. E., Harris, A. L. & McGee, J. O. Secretion of epidermal growth factor by macrophges associated with breast carcinoma. *Lancet* **342**, 872–873 (1993).
70. Wyckoff, J. B., Segall, J. E. & Condeelis, J. S. The collection of the motile population of cells from a living tumor. *Cancer Res.* **60**, 5401–5404 (2000).
71. Lin, E. Y. *et al.* Progression to malignancy in the polyoma middle T oncoprotein mouse breast cancer model provides a reliable model for human diseases. *Am. J. Pathol.* **163**, 2113–2126 (2003).
72. Maeda, H. & Akaike, T. Nitric oxide and oxygen radicals in infection, inflammation, and cancer. *Biochemistry* (Mosc.) **63**, 854–865 (1998).
73. Fulton, A. M., Loveless, S. E. & Heppner, G. H. Mutagenic activity of tumor-associated macrophages in *Salmonella typhimurium* strains TA98 and TA100. *Cancer Res.* **44**, 4308–4311 (1984).
74. Hudson, J. D. *et al.* A proinflammatory cytokine inhibits p53 tumor suppressor activity. *J. Exp. Med.* **190**, 1375–1382 (1999).
75. Moore, R. J. *et al.* Mice deficient in tumor necrosis factor-α are resistant to skin carcinogenesis. *Nature Med.* **5**, 828–831 (1999).
76. Mor, G. *et al.* Macrophages, estrogen and the microenvironment of breast cancer. *J. Steroid Biochem. Mol. Biol.* **67**, 403–411 (1998).
77. Mor, G. *et al.* Interaction of the estrogen receptors with the Fas ligand promoter in human monocytes. *J. Immunol.* **170**, 114–122 (2003).
78. King, B. L. *et al.* Immunocytochemical analysis of breast cells obtained by ductal lavage. *Cancer* **96**, 244–249 (2002).
79. Travis, R. C. & Key, T. J. Oestrogen exposure and breast cancer risk. *Breast Cancer Res.* **5**, 239–247 (2003).
80. Ricchi, P., Zarrilli, R., Di Palma, A. & Acquaviva, A. M. Nonsteroidal anti-inflammatory drugs in colorectal cancer: from prevention to therapy. *Br. J. Cancer* **88**, 803–807 (2003).
81. Ryan, B. M., Russel, M. G., Langholz, E. & Stockbrugger, R. W. Aminosalicylates and colorectal cancer in IBD: a not-so bitter pill to swallow. *Am. J. Gastroenterol.* **98**, 1682–1687 (2003).
82. Sasmono, R. T. *et al.* A macrophage colony-stimulating factor receptor-green fluorescent protein transgene is expressed throughout the mononuclear phagocyte system of the mouse. *Blood* **101**, 1155–1163 (2003).
83. Pollard, J. W. & Stanley, E. R. Pleiotropic roles for CSF-1 in development defined by the mouse mutation osteopetrotic (*op*). *Adv. Dev. Biochem.* **4**, 153–193 (1996).
84. Pollard, J. W. Role of colony-stimulating factor-1 in reproduction and development. *Mol. Reprod. Dev.* **46**, 54–61 (1997).
85. Gouon-Evans, V., Rothenberg, M. E. & Pollard, J. W. Postnatal mammary gland development requires macrophages and eosinophils. *Development* **127**, 2269–2282 (2000).
86. Lang, R. A. & Bishop, J. M. Macrophages are required for cell death and tissue remodeling in the developing mouse eye. *Cell* **74**, 453–462 (1993).
87. Gouon-Evans, V., Lin, E. Y. & Pollard, J. W. Requirement of macrophages and eosinophils and their cytokines/chemokines for mammary gland development. *Breast Cancer Res.* **4**, 155–164 (2002).
88. Kuper, H., Adami, H. O. & Trichopoulos, D. Infections as a major preventable cause of human cancer. *J. Intern. Med.* **248**, 171–183 (2000).
89. Rosin, M. P., Anwar, W. A. & Ward, A. J. Inflammation, chromosomal instability, and cancer: the schistosomiasis model. *Cancer Res.* **54** (Suppl.), S1929–S1933 (1994).
90. Mostafa, M. H., Sheweita, S. A. & O'Connor, P. J. Relationship between schistosomiasis and bladder cancer. *Clin. Microbiol. Rev.* **12**, 97–111 (1999).
91. Castellsague, X., Bosch, F. X. & Munoz, N. Environmental co-factors in HPV carcinogenesis. *Virus Res.* **89**, 191–199 (2002).
92. Shacter, E. & Weitzman, S. A. Chronic inflammation and cancer. *Oncology* (Huntingt.) **16**, 217–226, 229; discussion 230–232 (2002).
93. Kornfeld, D., Ekbom, A. & Ihre, T. Is there an excess risk for colorectal cancer in patients with ulcerative colitis and concomitant primary sclerosing cholangitis? A population based study. *Gut* **41**, 522–525 (1997).
94. Sieweke, M. H., Stoker, A. W. & Bissell, M. J. Evaluation of the cocarcinogenic effect of wounding in Rous sarcoma virus tumorigenesis. *Cancer Res.* **49**, 6419–6424 (1989).
95. Libby, P. Inflammation in atherosclerosis. *Nature* **420**, 868–674 (2002).
96. Di Carlo, E. *et al.* The intriguing role of polymorphonuclear neutrophils in antitumor reactions. *Blood* **97**, 339–345 (2001).
97. Coussens, L. M. *et al.* Inflammatory mast cells up-regulate angiogenesis during squamous epithelial carcinogenesis. *Genes Dev.* **13**, 1382–1397 (1999).
98. Haghnegahdar, H. *et al.* The tumorigenic and angiogenic effects of MGSA/GRO proteins in melanoma. *J. Leukoc. Biol.* **67**, 53–62 (2000).
99. Muller, A. *et al.* Involvement of chemokine receptors in breast cancer metastasis. *Nature* **410**, 50–56 (2001).

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Competing interests statement

The author declares that he has no competing financial interests.

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