Immunological hallmarks of stromal cells in the tumour microenvironment

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Abstract | A dynamic and mutualistic interaction between tumour cells and the surrounding stroma promotes the initiation, progression, metastasis and chemoresistance of solid tumours. Far less understood is the relationship between the stroma and tumour-infiltrating leukocytes; however, emerging evidence suggests that the stromal compartment can shape antitumour immunity and responsiveness to immunotherapy. Thus, there is growing interest in elucidating the immunomodulatory roles of the stroma that evolve within the tumour microenvironment. In this Review, we discuss the evidence that stromal determinants interact with leukocytes and influence antitumour immunity, with emphasis on the immunological attributes of stromal cells that may foster their protumorigenic function.

Extracellular matrix
(ECM). A dense network of various molecules that are secreted by cells into the extracellular space. This matrix provides physical support and structure for the cells and organs in addition to serving as a reserve for important signalling molecules such as chemokines.

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As cancer cells divide, form tumours and metastasize, they incite profound molecular, cellular and physical changes on the tissue in which they grow. The emergent tumour microenvironment (TME) comprises a complex milieu of non-malignant cells, including blood endothelial cells (BECs) and lymphatic endothelial cells (LECs), mesenchymal cells and immune cells, along with the extracellular matrix (ECM) and inflammatory mediators they secrete (BOX 1). Both healthy tissues and solid tumours have two distinct regions: the parenchyma (the tumour bed in the context of solid tumours) and a stromal region (part of the TME in tumours). However, the basal lamina, which normally separates these two regions in healthy tissues, is typically incomplete in solid tumours, and a poorly defined demarcation between the two areas is often observed. Thus, elements of the TME can interact closely with tumour cells and affect their growth in diverse ways; for example, factors derived from the TME can influence tumour cell survival, invasiveness and metastatic dissemination, as well as access and responsiveness to therapeutics[1–3] (BOX 2). The dynamic and mutualistic relationship between cancer cells and their microenvironment is formed early in malignant growth and evolves throughout the life history of a tumour.

Although the composition of the TME varies across cancer types, some features seem to be hallmarks of most, if not all, solid tumours. For instance, most tumours are abnormally vascularized by disorganized and leaky vessels. In addition, tumours are infiltrated by various innate and adaptive immune cells that can perform both protumour and antitumour functions. Finally, the TME contains several non-haematopoietic stromal cell types, which can include BECs and LECs, as well as cells of mesenchymal origin including mesenchymal stem cells (MSCs) and their differentiated progeny, cancer-associated fibroblasts (CAFs) and pericytes (FIG. 1). Other rarer stromal cell populations, including neurons, fibrocytes, adipocytes and follicular dendritic cells, have also been observed in some tumours.

It is now widely accepted that the immune system can recognize and respond to tumour cells either naturally or following therapeutic intervention. It has been proposed that a series of distinct steps, termed the cancer-immunity cycle[4], must occur for productive antitumour immunity to develop. Accordingly, antigens released from tumours are either passively transported in the lymph or captured and delivered by dendritic cells (DCs) to regional lymph nodes via afferent lymphatic vessels. In tumour-draining lymph nodes, DCs present tumour-derived peptides on MHC molecules and activate antigen-specific $CD4^+$ and $CD8^+$ T cells. Newly activated effector T cells then exit the lymph node and circulate throughout the body via the bloodstream. Chemokine gradients and adhesion molecules direct circulating T cells to extravasate through blood vessels and migrate into the tumour bed, where they scan for target cells displaying their cognate antigen and kill them. Cancer cell death can also lead to the release of additional tumour antigens that in turn are transported to the draining lymph node to initiate another revolution of the cancer-immunity cycle. Under ideal circumstances, the cancer-immunity cycle leads to the eradication of malignant cells by cytotoxic immune cells, establishes tumour-specific immunological memory and prevents further tumour progression.

Box 1 | **Stromal cell types in the tumour microenvironment**

Blood and lymphatic endothelial cells

Neoplastic progression is generally accompanied by a sustained increase in tissue vascularization. This process is fostered by increased levels of soluble factors such as vascular endothelial growth factor A (VEGFA), which promotes blood endothelial cell (BEC) proliferation and angiogenesis, and VEGFC and VEGFD, which induce proliferation and activation of lymphatic endothelial cells (LECs) and lymphangiogenesis[117]. Interestingly, the use of anti-angiogenic drugs in clinical practice has revealed a correlation between decreased vascularization and reduced immunosuppression in the tumour microenvironment (TME). In patients with renal cell carcinoma, for instance, administration of the pan VEGF inhibitor sunitinib (Sutent, Pfizer) results in decreased numbers of intratumoural regulatory T cells (T_{Reg} cells) and myeloid-derived suppressor cells (MDSCs)[118,119]. A growing body of evidence now exists to suggest that endothelial cells are crucial regulators of the host's immune response to cancer.

Mesenchymal stem cells

Mesenchymal stem cells (MSCs) are multipotent stromal cells that support the maintenance of healthy tissue and wound healing through their differentiation into various tissue-resident cell types, including adipocytes, chondrocytes, myocytes and osteoblasts. MSCs are also recruited to tumours, where they represent a source of fibroblasts and pericytes[120]. MSCs are immunoregulatory cells, and numerous studies suggest that they may contribute to dampening effective antitumour immunity. However, as many of the markers often used to identify MSCs are also expressed by fibroblasts and pericytes (TABLE 1), it is still difficult to confidently ascribe particular functional contributions to MSCs versus their more-differentiated progeny.

Cancer-associated fibroblasts

Fibroblasts represent the predominant non-haematopoietic stromal cell type in the TME. Usually identified by expression of proteins such as vimentin, α-smooth muscle actin (αSMA) and fibroblast activation protein (FAP) (TABLE 1), cancer-associated fibroblasts (CAFs) are characterized by an activated, highly contractile, myofibroblastic phenotype[32,120]. It is now becoming increasingly recognized, however, that CAFs are a heterogeneous cell population of multiple origins[121]. CAF accumulation in the TME is often correlated with poor prognosis in many tumours (see for example REFS 122,123). Indeed, CAFs have been shown to support cancer cell growth and metastatic dissemination in several ways (BOX 2). Additionally, there is now compelling evidence demonstrating that CAFs may hinder antitumour immune responses.

Pericytes

Pericytes differentiate from mesenchymal precursors and are recruited to tumours by platelet-derived growth factor-β (PDGFβ) gradients[124], where they reside on the abluminal side of blood vessels. Pericytes in both healthy and tumour tissues possess characteristic cellular markers including 3G5 ganglioside and chondroitin sulfate proteoglycan 4 (CSPG4; also known as NG2) (TABLE 1). Furthermore, αSMA is highly expressed by pericytes in the tumour, although it is often absent in quiescent cells in non-neoplastic sites[125]. In addition to their role in blood vessel formation and maturation, pericytes can actively modulate the magnitude of immune responses in several conditions, including cancer. Indeed, immunization of melanoma-bearing mice with a vaccine against the chondroitin sulfate proteoglycan high molecular weight melanoma-associated antigen (HMW-MAA), a molecule highly expressed by tumour pericytes, was previously shown to promote intratumoural infiltration of $CD8^+$ T cells and tumour regression[126], supporting the idea that targeting tumour pericytes can alleviate local immunosuppression. Indeed, other reports[14,68,59] suggest that pericytes may prevent lymphocyte extravasation and activation in malignant lesions.

However, the relationship between the TME and the immune system is far more complex, and tumour cells and various components of their microenvironment can impair protective T cell immunity in diverse ways at each step of this cycle. For example, tumour antigens are often derived from altered or overexpressed proteins, and thus presentation of such self-antigens in tumour-draining lymph nodes can favour the induction of peripheral tolerance. Even if T cells are effectively primed, their migration into the tumour bed can be hindered by the disorganized vasculature and chemotactic cues[5]. Furthermore, within tumours, T cells encounter many inhibitory cells and molecules that can impair their survival, activation, proliferation and effector functions (discussed in detail below).

Evidence for the suppressive role of inhibitory factors in the cancer-immunity cycle has been provided by recent clinical studies using neutralizing antibodies that block the inhibitory molecules cytotoxic T lymphocyte antigen 4 (CTLA4; also known as CD152), programmed cell death protein 1 (PD1; also known as CD279) and programmed cell death ligand 1 (PDL1; also known as B7-H1 and CD274). The expression of PDL1 is often upregulated on cancer cells, myeloid cells and endothelial cells, and this surface protein inhibits T cell activation and survival upon binding to its receptor PD1 on activated T cells. Therapies that use antibodies to disrupt the PDL1–PD1 interaction to unleash $CD8^+$ T cell-mediated killing of tumour cells have shown promising response rates in several human cancers[6–8]. However, many patients do not experience therapeutic benefit, possibly owing to a lack of pre-existing antitumour immunity or to the presence of other immunosuppressive mechanisms in the TME. Understanding the nature of the different obstacles that $CD8^+$ T cells face in the cancer-immunity cycle will help researchers to develop novel therapeutic approaches to overcome them. In the future, combining approaches to target multiple immunosuppressive signals may improve the therapeutic index for patients with cancer.

In this Review, we discuss our current understanding of the immunomodulatory properties of non-haematopoietic stromal cells in the TME. Much of what we know so far about stromal–immune interactions in the TME is derived from experimental mouse models of cancer. Although these models, as with any experimental disease model, have obvious limitations and caveats, they have also taught us crucial lessons on immune cell interactions with tumours and host responsiveness to immunotherapies, including checkpoint blockade, adoptive cell therapy, chimeric-antigen receptor (CAR) T cells and vaccination. Furthermore, with increasing access to human tissues and clinical trial data, the principles established in experimental models are now being validated in humans[6–8]. Here, we focus on the major populations of non-haematopoietic stromal cells (BOX 1) that interact with and modulate the behaviour of tumour-infiltrating immune cells. Specifically, we discuss known and possible immunological hallmarks of BECs and LECs, as well as tumour-associated cells of mesenchymal origin including MSCs, CAFs and pericytes, that influence the cancer-immunity cycle.

Regulation of immune cell infiltration

The trafficking of newly activated antigen-specific T cells from the blood into the tumour bed is thought to be dysfunctional in cancer, and many obstacles, including stromal cells, impair this process (FIG. 2).

Structural dysfunction of the tumour vasculature. Owing to the tortuous and leaky vessels that are typical of tumour vasculature, blood flow and leukocyte extravasation are markedly impaired. An imbalance

Pericytes
Contractile cells of mesenchymal origin that are tightly wrapped around the endothelial cells to form blood vessels.

Box 2 | **Stromal cell support of tumour cells**

Stromal elements of the tumour microenvironment (TME) represent an integral part of tumours, and they establish a dynamic interaction with tumour cells that can influence tumour growth, metastasis and chemoresistance. Many aspects of this synergistic interaction between stroma and tumour cells have been described elsewhere (reviewed in REFS 120,127,128), thus we mention only a few examples here. One of the most extensively documented properties of the tumour stroma is its ability to support tumour cell growth and proliferation. In addition to global delivery of nutrients through newly formed blood vessels, stromal cells (especially cancer-associated fibroblasts (CAFs) and mesenchymal stem cells (MSCs)) nurture tumour cells and cancer stem cells by providing essential growth factors, such as hepatocyte growth factor (HGF) and fibroblast growth factor (FGF)[129]. In addition, other stroma-derived factors such as insulin growth factor 1 and insulin growth factor 2 contribute to tumour evasion of apoptosis[130,131]. The abundant extracellular matrix (ECM) of solid tumours has itself also been correlated with increased tumour growth through various mechanisms, including activation of pro-survival phosphoinositide 3-kinase (PI3K) signalling pathways downstream of integrin receptors[132].

Metastatic dissemination is also supported by tumour stroma. For instance, the secretion of transforming growth factor-β (TGFβ) by stromal cells surrounding the tumour is known to induce epithelial-to-mesenchymal transition of neoplastic cells and thus favours invasiveness[120]. Furthermore, enhanced lymphangiogenesis in solid tumours positively correlates with metastatic dissemination potential[127]. Accordingly, the expression of vascular endothelial growth factor C (VEGFC) and VEGFD, which are crucial for the growth of new lymph vessels, is often upregulated in aggressive tumours and leads to increased lymph node metastasis. Interestingly, some tumour cells express vascular endothelial growth factor receptor (VEGFR) and thus may follow a gradient of VEGFC and VEGFD towards lymphatic vessels[133].

Another way in which stromal cells support tumour cell survival is by creating a physical barrier to chemotherapies and increasing tumour cell chemoresistance[3,134]. Stromal regulation of interstitial fluid pressure has been shown to regulate transcapillary transport of drugs, reducing efficacy of therapeutics. This effect has been well studied in pancreatic ductal carcinoma and ovarian cancer, which are highly desmoplastic tumours that are notoriously chemoresistant[128,135–137]. Furthermore, stromal cells have been shown to directly reduce the sensitivity of cancer cells to chemotherapeutic drugs, including tyrosine kinases inhibitors. For example, HGF-mediated activation of the AKT– mitogen-activated protein kinase (MAPK) survival pathways in BRAF-mutant tumour cells has been related to resistance to RAF inhibitors[136]. Overall, these studies indicate that tumour stroma supports the growth, survival, metastasis and chemoresistance of tumour cells. These features, in conjunction with the immunosuppressive abilities of stromal cells, strongly suggest that targeting multiple functions of stromal cells would aid in anticancer therapies.

of pro- and anti-angiogenic factors in solid tumours contributes to such vascular aberrations[9–10]. Structural abnormalities of the underlying stroma have been associated with the irregular features of the tumour vasculature. BECs in cancer lesions are irregularly shaped with loose intercellular connections and form luminal projections. Furthermore, most tumour vessels lack the normal monolayer of endothelial cells and therefore do not have proper barrier function[9]. This dysfunctional endothelium develops in response to the overexpression of pro-angiogenic factors in tumours, including vascular endothelial growth factor A (VEGFA), which not only stimulates pathological vascularization but also acts as a potent vasodilator, generating small fissures within BECs[10].

Pericyte coverage is also abnormal in tumour vessels. In the TME, pericytes are loosely associated with BECs and the basement membrane, thereby contributing to the increased leakiness of tumour vasculature[11]. The exact cause of the aberrant vessel coverage by tumour pericytes is unknown; however, increased VEGFA in the TME may hinder pericyte function by suppressing platelet-derived growth factor receptor-β (PDGFRβ) signalling, which is crucial for pericyte survival[12]. Another molecular mediator that has been suggested to play a part in angiogenic alterations of tumour blood vessels is regulator of G-protein signalling 5 (RGS5), which is a marker for a subgroup of PDGFRβ$^+$ perivascular cells. Notably, RGS5 is overexpressed in tumour pericytes in the RIP1–TAG5 mouse model (rat insulin promoter 1–SV40 large T antigen mouse model) of pancreatic carcinoma, similar to what has been documented for several human tumours, including kidney, liver, and head and neck cancers[13,14]. Tumour pericytes in RGS5-deficient RIP1–TAG5 mice displayed a more mature pericyte phenotype, which led to an enhanced T cell infiltration and antitumour immunity, supporting the idea that targeting RGS5 signalling in pericytes may represent a suitable strategy to achieve vascular normalization and augment T cell responses in cancer[14].

Leakiness of tumour blood vessels has long been recognized as a major obstacle to therapeutic access, and preclinical and clinical studies have shown that vascular normalization can augment drug delivery to tumours[15]. Based on the findings that the disorganized nature of tumour blood vessels is also an obstacle to tumour-infiltrating lymphocytes[9], it is possible that similar approaches might enhance antitumour immunity.

Modulation of immune cell diapedesis by BECs. Dysfunctional extravasation of T cells caused initially by structural abnormalities of the tumour vasculature is further exacerbated by changes in the adhesive properties of tumour endothelial cells. Reduced expression of E-selectin by tumour-associated BECs has been reported, and leads to impaired lymphocyte recruitment[16]. In addition, adhesive receptors such as intercellular adhesion molecule 1 (ICAM1), ICAM2 and vascular cell adhesion molecule 1 (VCAM1), which facilitate integrin-mediated T cell diapedesis, are often poorly expressed by tumour-associated BECs[16]. Likewise, the nitric oxide (NO) pathway has been implicated in the prohibitive nature of the tumour vasculature. During homeostatic conditions, basal production of NO by BECs inhibits lymphocyte tissue infiltration by maintaining the vascular endothelium in a resting state[17]. Several molecules in tumours can upregulate NO production by BECs: angiogenic factors such as VEGFA and fibroblast growth factor 2 (FGF2; also known as bFGF) can efficiently stimulate NO synthase to catalyse NO production by BECs, thereby inhibiting endothelium activation and expression of adhesive molecules[16]. Furthermore, VEGF-dependent NO production by BECs can lead to a defective clustering of ICAM1 and VCAM1 and a reduced affinity for their integrin receptors on T cells[18]. Other factors have been associated with a decreased adhesion molecule expression on BECs and reduced vascular–lymphocyte associations in tumours. Binding of endothelin 1 (ET1) to the endothelin B receptor on BECs, for example, triggers NO release and suppresses T cell adhesion to the endothelium through

Cancer-immunity cycle
The multi-step process by which T cells are activated in tumour-draining lymph nodes, traffic into tumours and kill tumour cells. There are many points along this cycle that are inhibited by immunosuppressive cells and molecules.

Peripheral tolerance
The control of self-reactive T cells in the periphery.

Extravasation
The process by which an immune cell exits capillaries to enter peripheral tissues.

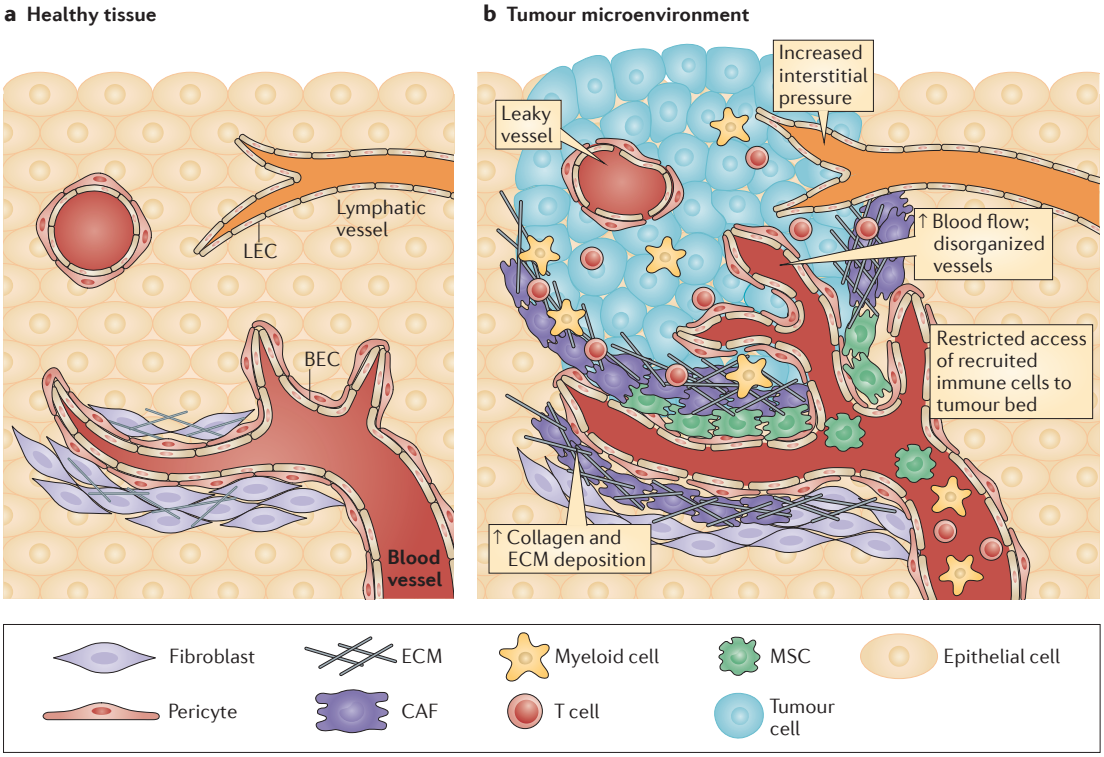

Figure 1 | **Cellular and architectural changes in the tumour microenvironment.** **a** | Stromal cells present in interstitial spaces surrounding the parenchyma of various organs promote tissue integrity by providing growth factors and structural support. Blood endothelial cells (BECs) and pericytes maintain the integrity of blood vessels and ensure the supply of oxygen and other nutrients to the tissue. Interstitial fluid is drained by lymphatic vessels. Fibroblasts are constantly remodelling the extracellular matrix (ECM) to cope with mechanical stress within connective tissue. **b** | Neoplastic transformation is often accompanied by the formation of a tumour bed and profound alterations in the surrounding connective tissue and stroma, a process that culminates in the establishment of a pathological tumour microenvironment (TME). An imbalance between pro- and anti-angiogenic factors results in the formation of aberrant vasculature, characterized by numerous leaky blood vessels. Increased interstitial pressure and inadequate drainage by the lymphatic vessels is also observed. The increased hydrostatic load, along with tumour-secreted molecules (not shown), induces the recruitment of circulating mesenchymal stem cells (MSCs), the activation of cancer-associated fibroblasts (CAFs) and a marked accumulation of ECM. Finally, various chemokines and cytokines in the TME (see FIG. 2) attract activated T cells and myeloid cells to the tumour lesion, but tortuous blood vessels and dense ECM often hinder their access to the tumour nest. Although the makeup of the cellular and extracellular milieu can differ between tumour types and stages of growth, it is becoming clear that changes in the cellular architecture of the TME can influence tumour growth, metastasis and drug resistance. LEC, lymphatic endothelial cell.

RIP1–TAG5 mouse model
A mouse model of pancreatic cancer in which the expression of SV40 large T antigen (TAG) is expressed under the control of the rat insulin promoter 1 (RIP1). This results in the inhibition of tumour suppressor genes, which leads to the formation of tumours as the mice age.

Diapedesis
A more specific term for extravasation referring to leukocytes exiting intact blood vessels. Ligands expressed on the surface of blood endothelial cells interact with receptors on immune cells, allowing them to adhere to the vessels and squeeze through.

ICAM1 downregulation[19]. Accordingly, overexpression of endothelins and endothelin B receptor is observed in many human tumours and is associated with paucity of intratumoural T cells and poor prognosis[19]. Elevated expression of epidermal growth factor-like domain 7 (EGFL7; also known as VE-statin) in mouse and human tumours is also associated with ICAM1 and VCAM1 downregulation on BECs and limits leukocyte infiltration[20].

The inefficiency of lymphocyte infiltration into tumours is compounded by the expression of endothelial receptors that favour trafficking of immunosuppressive cells. Expression of the common lymphatic endothelial and vascular endothelial receptor 1 (CLEVER1), for instance, is upregulated in BECs from human hepatocellular carcinoma and facilitates the transmigration of T cells across the endothelium[21]. Importantly, this study showed that CLEVER1 preferentially mediates the extravasation of $FOXP3^+CD4^+$ regulatory T cells (T_{Reg} cells), suggesting that it may be responsible for an accumulation of T_{Reg} cells in tumours. Similarly, increased expression of molecules such as mucosal vascular addressin cell adhesion molecule 1 (MADCAM1) and CD166 by BECs and their ligands by T_{Reg} cells has been shown to mediate selective transmigration of T_{Reg} cells through the tumour endothelium in human pancreatic tumours[22].

Endothelial cell receptors responsible for the transmigration of specific myeloid populations have also been identified. CD99, for example, has an important role in monocyte–BEC interactions, and its blockade *in vitro* has been shown to inhibit leukocyte diapedesis[23]. Given the exacerbated infiltration of immunosuppressive myeloid cells observed in tumours[24], it is possible that 'gated' trafficking mediated by molecules such as CD99 may also exist for specific myeloid cell populations.

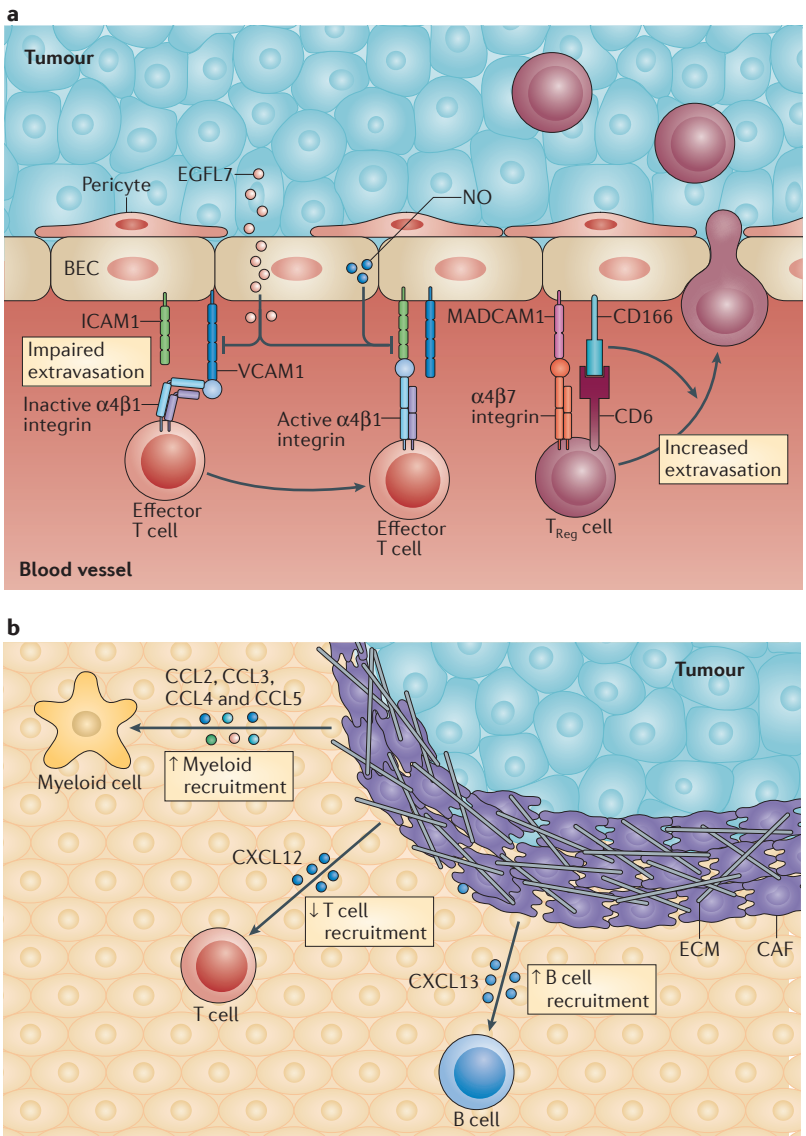

Figure 2 | **Regulation of immune cell infiltration into the tumour microenvironment.** **a** | The structural and molecular abnormalities of the tumour vasculature represent one of the major obstacles for immune cell infiltration. Decreased pericyte maturation and aberrant coverage contribute to vessel leakiness, creating a barrier to lymphocyte recruitment. Molecules such as epidermal growth factor-like domain 7 (EGFL7) and nitric oxide (NO) hinder expression of intercellular adhesion molecule 1 (ICAM1) and vascular cell adhesion protein 1 (VCAM1) by tumour blood endothelial cells (BECs), resulting in reduced lymphocyte adhesion and extravasation. In contrast, tumour-associated BECs upregulate mucosal vascular addressin cell adhesion molecule 1 (MADCAM1) and CD166 expression, which bind to α4β7 integrin and CD6, respectively, on regulatory T (T_{Reg}) cells and promote their preferential extravasation through the activation of α4β7 integrin. **b** | Several chemokines secreted by cancer-associated fibroblasts (CAFs) modulate the stromal landscape: CXC-chemokine ligand 12 (CXCL12) repels T cells, CXCL13 recruits B cells, and CC-chemokine ligand 2 (CCL2), CCL3, CCL4 and CCL5 recruit myeloid cells, including macrophages and myeloid-derived suppressor cells (MDSCs). ECM, extracellular matrix.

By disrupting infiltration of tumour-specific cytotoxic T cells, whereas selectively permitting transmigration of immunosuppressive cells into the tumour nest, the blood endothelium impairs an important step in the cancer-immunity cycle.

Fibroblasts shape the leukocytic infiltrate. Fibroblasts in healthy tissues can actively modulate immune cell trafficking by producing chemoattractants with diverse specificities. In one of the first studies that aimed to define CAF function *in vivo*, vaccine-induced killing of fibroblast activation protein (FAP)$^+$ cells by the immune system led to reduced tumour growth and metastatic dissemination in mouse models of colon and breast cancer[25,26]. This depletion of FAP$^+$ cells was associated with a shift in the polarization of T cells from a T helper 2 (T_H2) to a T_H1 phenotype, marked by increased expression of interleukin-2 (IL-2) and IL-7 and decreased IL-4 and IL-6 levels[26]. This effect was attributed to a reduced recruitment of M2 macrophages, which are key producers of type 2 cytokines, and correlated with infiltration of CD8$^+$ T cells into tumours and enhanced cytolytic activity. Indeed, the stromal expression of chemokines such as CC-chemokine ligand 2 (CCL2), CCL3, CCL4 and CCL5 may influence the macrophage composition of tumours by recruiting blood monocytes and other immature myeloid cells into the TME[27]. CCL5 production by CAFs (identified as FAP$^+$αSMA$^+$ stromal cells) in mammary carcinoma has also been shown to preferentially recruit T_{Reg} cells, owing to a higher expression levels of the CCL5 receptor, CC-chemokine receptor 1 (CCR1), by T_{Reg} cells compared with effector T cells[28].

In some tumours, CXC-chemokine ligand 12 (CXCL12) is highly and selectively expressed by FAP$^+$ CAFs. In addition to its role in promoting tumour cell survival and mobilizing bone marrow cells, CXCL12 has recently been implicated in the disadvantageous T cell compartmentalization within the TME in mouse models of pancreatic and lung carcinoma[29]. Based on studies demonstrating that a specific inhibitor of the CXCL12 receptor CXCR4 had antitumour efficacy, it was proposed that CAF-derived CXCL12 blocks intratumoural infiltration of CXCR4$^+$ lymphocytes[29]. Blockade of the CXCL12–CXCR4 axis restored sensitivity to checkpoint blockade therapy[29], suggesting that FAP$^+$ CAFs have an immunosuppressive role by modulating the chemokine milieu in tumours.

Recent studies suggest that CAFs may also have a pivotal role in recruiting B cells to the TME. In mouse models of prostate cancer, for example, CAFs were shown to respond to hypoxia and castration-induced tissue damage by upregulating CXCL13 production, which aided the recruitment of B cells and immunosuppressive plasma cells that promoted malignant tumour progression[30,31]. Thus, CAFs represent an important source of various chemokines that can shape the immune landscape within the TME.

ECM modulates T cell trafficking. Tumour progression is often accompanied by increased tissue desmoplasia, and CAFs are the major cell type in the tumour responsible for the synthesis of ECM proteins such as collagens, laminin and fibronectin[32]. Importantly, lymphocyte–ECM interactions can crucially influence leukocyte motility and localization. Using *ex vivo* tumour tissue from patients with lung cancer, Salmon and colleagues[33] demonstrated that the position and migratory behaviour

of tumour-infiltrating T cells are profoundly affected by the matrix architecture they encounter. Specifically, the authors showed that lymphocyte displacement is reduced in stromal regions containing densely packed matrix fibres, supporting the idea that ECM deposition may alter antitumour immune responses by limiting T cell motility. What these data also suggest is that specific forms of ECM may anchor T cells in stroma-rich regions rather than in the tumour bed itself. Indeed, multiple studies present evidence that prognosis worsens in proportion to the ECM content of the tumour, and drugs targeting ECM proteins have yielded promising results in preclinical animal studies[34,35]. Similarly, CAR T cells engineered to express heparanase, which degrades polymeric heparan sulfate molecules, were recently shown to possess greater tumour infiltration potential and antitumour activity, as a result of their improved ability to degrade ECM proteins[36]. This report suggests that targeting the tumour ECM holds great value not only to boost natural antitumour immunity, but also to improve efficacy of immunotherapeutics. Conversely, two recent publications suggested that a reduction in desmoplasia, following depletion of αSMA$^+$ cells or inhibition of sonic hedgehog signalling, accelerated tumour progression in a mouse model of pancreatic adenocarcinoma, possibly owing to enhanced T_{Reg} cell infiltration and increased angiogenesis[37,38]. These studies suggest that targeting the entire tumour stromal compartment may have different net effects compared with targeting specific stromal entities in the TME. New experimental approaches that enable the contributions of individual stromal cell subsets to be analysed will undoubtedly help to resolve these opposing views and more importantly establish a deeper understanding of stromal cell heterogeneity and function in cancer.

Regulation of antitumour immune responses

Suppression of T cell function by BECs. In addition to influencing T cell extravasation, models of inflammation have shown that BECs can function as antigen-presenting cells that display peptide–MHC complexes and express several immunoregulatory co-receptors[39,40]. There is now evidence that these same processes may also occur in cancer: tumour BECs have been shown to express the PD1 ligands PDL1 and PDL2 (also known as B7-DC and CD273) as well as ligands for other receptors of the CD28–CTLA4 family, such as B7-H3 (also known as CD276) and B7-H4 (also known as VTCN1), which induce CD8$^+$ T cell tolerance and correlate with poor prognosis in patients with cancer[41–43]. In addition, BECs isolated from lymph nodes of patients with lymphomas were found to express the co-inhibitory molecule T cell immunoglobulin and mucin-containing domain molecule 3 (TIM3), which promoted the growth and dissemination of lymphomas by inhibiting T_H1 cell polarization[44]. BECs can also express CD137 (also known as 4-1BB)[45], a glycoprotein expressed by activated T cells, particularly CD8$^+$ T cells, that generally functions as a co-stimulatory molecule. Interestingly, tumour BECs express a soluble form of CD137 (REF. 45) that antagonizes membrane-bound CD137, which limits its co-stimulatory effects and thus contributes to a suppressive environment for T cells (FIG. 3). More recently, platelet endothelial cell adhesion molecule 1 (PECAM1; also known as CD31) has been implicated in immune escape of tumours; the homophilic interaction between PECAM1 on T cells and on DCs impairs proximal T cell receptor (TCR) signalling, and tumour growth is reduced in *Pecam1*$^{-/-}$ mice[46]. As PECAM1 is highly expressed by endothelial cells (TABLE 1), it is likely that BECs in the TME may also promote T cell tolerance through the regulation of TCR signalling.

In addition to co-stimulatory ligands, tumour BECs can express molecules that induce apoptosis of cytotoxic lymphocytes, including tumour necrosis factor (TNF)-related apoptosis inducing ligand (TRAIL)[47] and CD95 ligand (CD95L; also known as FasL)[48]. In this instance, tumour-derived factors, including VEGFA, IL-10 and prostaglandin E_2 (PGE_2), cooperatively induced CD95L expression in BECs in human ovarian tumours and prompted effector T cell apoptosis in *in vitro* cultures. Notably, tumour-infiltrating T_{Reg} cells were refractory to CD95L-mediated cell death owing to their constitutively high expression of the anti-apoptotic cellular FLICE-inhibitory protein (c-FLIP). Finally, in a mouse model of ovarian cancer, genetic or therapeutic blockade of CD95–CD95L signalling restored CD8$^+$ T cell infiltration, cytotoxic function and immune-mediated control of tumour growth[48].

BECs can also release soluble factors that modulate T cell responses, especially upon exposure to inflammatory stimuli from the TME. For instance, exposure to tumour cells drives the secretion of PGE_2, IL-6, transforming growth factor-β (TGFβ), and VEGFA by BECs[49–51]. Furthermore, supernatant from cultured lung tumour-derived BECs disrupted CD3-mediated T cell activation *in vitro*[50]. Finally, BECs from patients with high-grade tumours have been shown to express the immunomodulatory enzyme indoleamine 2,3-dioxygenase (IDO)[52]. As IDO produced by tumour-associated macrophages has been shown to halt T cell growth by inhibiting tryptophan degradation and causing a build-up of immunosuppressive metabolites, it is possible that endothelial IDO may have a similar function in the TME. Recent studies have revealed high expression of tryptophan-2,3-dioxygenase (TDO), another immunosuppressive enzyme, in several cancers[53], and it is therefore possible that endothelial cells may constitute one of its sources in the TME.

Altogether, these studies suggest that the integration of signals emanating from the TME and tumour cells trigger several immunosuppressive pathways in BECs that shape the outcome of antitumour immune responses.

Immunomodulation by LECs. Tumour-associated lymphangiogenesis has long been recognized to have a role in metastasis by providing a conduit for tumour cells to migrate to lymph nodes (BOX 2). More recently, new studies have highlighted that the association between lymphatic vessels in the TME and tumour dissemination may also be related to the direct modulation of adaptive

M2 macrophages
A subset of macrophages that are involved in wound healing and type 2 immune responses, such as allergic immune responses. They are known to generally contribute to a tumour-supporting and immunosuppressive environment in tumours.

Desmoplasia
The growth of dense connective tissue that often occurs as tumours progress from neoplasms. They are marked by high levels of fibroblasts and fibrotic tissue.

Sonic hedgehog
A signalling molecule that is highly upregulated in several solid tumours. Upon its secretion by tumour cells, it can act on cancer-associated fibroblasts and other surrounding cells to promote desmoplasia.

Indoleamine 2,3-dioxygenase
(IDO). An enzyme implicated in the catabolism of tryptophan, and its expression by myeloid cells correlates with immunosuppression by tryptophan deprivation and exposure to the tryptophan catabolite kynurenine.

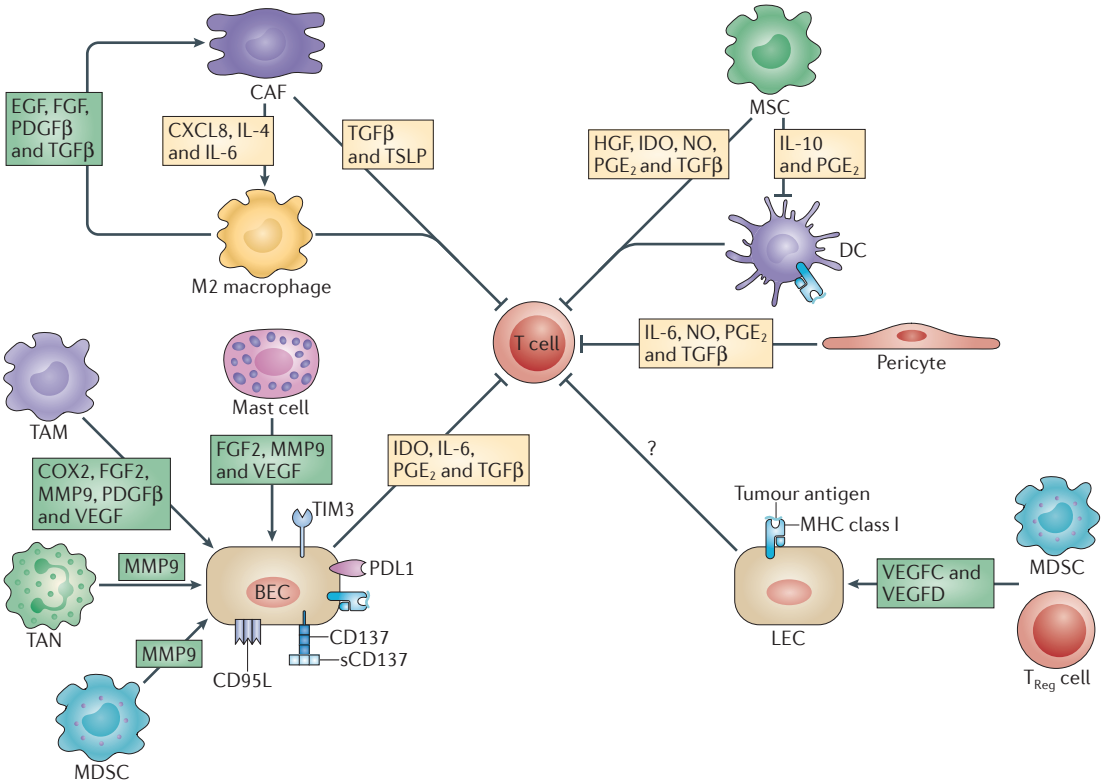

Figure 3 | **Stromal cell–immune cell crosstalk in the tumour microenvironment.** Stromal cells in the tumour microenvironment (TME) express numerous surface and secreted molecules that directly suppress $CD4^+$ and $CD8^+$ T cells and activate immunosuppressive myeloid cells. For instance, blood endothelial cells (BECs) express programmed cell death ligand 1 (PDL1), T cell immunoglobulin and mucin-containing domain molecule 3 (TIM3) and CD95L (also known as FASL) on their surface, and they secrete indoleamine 2,3-dioxygenase (IDO), interleukin-6 (IL-6), prostaglandin E_2 (PGE_2) and transforming growth factor-β (TGFβ). BECs also express a soluble form of CD137 (sCD137) that inhibits the co-stimulatory potential of the membrane-bound CD137. In addition, lymphatic epithelial cells (LECs) and BECs can cross-present tumour antigens and promote the development of a tolerizing, rather than an immunostimulatory, environment. Pericytes also secrete various factors with immunosuppressive properties, such as IL-6, nitric oxide (NO), PGE_2 and TGFβ. Mesenchymal stem cells (MSCs) secrete hepatocyte growth factor (HGF), IDO, NO, PGE_2 and TGFβ, which inhibit cytotoxic activity and differentiation of T helper 1 (T_H1) cells. MSC-secreted IL-10 and PGE_2 also impair dendritic cell (DC) maturation, which contributes to less efficient T cell activation. Cancer-associated fibroblasts (CAFs) secrete TGFβ and thymic stromal lymphopoietin (TSLP), which inhibit T cells and promote T cell skewing towards a T_H2 phenotype, respectively. CAFs also secrete inflammatory cytokines, including CXC-chemokine ligand 8 (CXCL8), IL-4 and IL-6, which support M2 macrophages and further suppress T cell activity. Notably, a deep crosstalk exists between immune cells and stromal cells in the TME. M2 macrophages secrete factors such as epidermal growth factor (EGF), fibroblast growth factor (FGF), platelet-derived growth factor-β (PDGFβ) and TGFβ, which support CAF survival and activation, including increased extracellular matrix (ECM) production. Additionally, tumour-associated macrophages (TAMs), tumour-associated neutrophils (TANs), myeloid-derived suppressor cells (MDSCs), and mast cells can all secrete matrix metalloproteinase 9 (MMP9) to release mitogenic factors stored in the ECM that induce BEC survival and angiogenesis. These immune cells also secrete soluble mediators, such as cyclooxygenase 2 (COX2), FGF2, PDGFβ and vascular endothelial growth factor (VEGF), to directly promote BEC survival. Finally, MDSCs and regulatory T (T_{Reg}) cells secrete VEGFC and VEGFD to support LEC survival and lymph vessel formation.

Cross-presentation
A process by which antigens taken up through endocytosis enter the cytosol and into the MHC class I presentation pathway. This allows for the presentation of extracellular antigens, such as tumour antigens, to $CD8^+$ T cells.

Peripheral tissue antigens
Certain antigens for which expression is generally restricted to a specific tissue, such as the kidney or lung, in an adult animal. These antigens are also expressed by stromal cells in the thymus and peripheral lymph nodes to allow for negative selection of self-reactive T cells.

Mechanotransduction
A process by which cells convert mechanical stimuli into biological signalling processes. This process allows cells to sense changes in the stiffness of the surrounding tissue and respond accordingly by activating various signalling cascades.

Myofibroblasts
A specialized type of fibroblast that has high levels of α-smooth muscle actin and the ability to strongly contract extracellular matrix in its surrounding tissue.

immune cells by LECs. A recent study, for example, demonstrated that melanoma cells genetically engineered to express VEGFC hindered antitumour immunity by promoting cross-presentation of tumour antigens by LECs, which led to apoptosis of antigen-specific $CD8^+$ T cells and thus promoted tolerance[54]. A similar phenomenon was previously described for the presentation of peripheral tissue antigens by LECs in lymphoid organs[55,56], suggesting that tumours may exploit pathways involved in the maintenance of peripheral tolerance to avoid immune detection.

The aberrant blood flow and disorganized lymphatic system that are characteristic of solid tumours can lead to impaired fluid flow and interstitial pressure. Recent evidence suggests that the unique mechanobiology of tumours can also contribute to immunosuppression both locally and at distal locations[57]. Fibroblasts are sensitive to small changes in matrix stiffness and, through mechanotransduction, can respond to the high interstitial pressure by expressing TGFβ and differentiating into myofibroblasts[58]. Matrix stiffening induced by myofibroblast contraction can then activate latent stores of

Table 1 | **Phenotypic markers of mouse stromal cells**

Marker*	Description	Expression in the TME	Expression in healthy tissues
Surface glycoproteins			
Endoglin (CD105)	Type I glycoprotein, part of the TGFβ receptor complex	MSCs, BECs, pericytes and CAFs	MSCs, FRCs, activated endothelium, vascular smooth muscle cells and monocytes
3G5 antigen	A ganglioside (glycolipid containing sialic acids) identified by an antibody generated to recognize microvasculature pericytes	Pericytes	Pericytes and dermal mast cells
FAP	Membrane-bound gelatinase (serine protease)	CAFs and some tumour-infiltrating leukocytes	Myofibroblasts and FRCs
CSPG4 (NG2)	Chondroitin sulfate proteoglycan	Pericytes	Cartilage, bone and muscle during development; adipocytes, neuronal progenitors and pericytes
Podoplanin (GP38 in mice)	Mucin-type transmembrane glycoprotein that controls cell motility and contraction	CAFs, LECs and tumour cells	FRCs, LECs and alveolar epithelial cells
Adhesion molecules			
PECAM1 (CD31)	Member of the immunoglobulin superfamily involved in leukocyte transendothelial migration	BECs, LECs and some myeloid cells	BECs, LECs, platelets, monocytes, neutrophils and DCs
VCAM1 (CD106)	Cell surface sialoglycoprotein; ligand for α4β1 integrin on lymphocytes, which mediates adhesion of lymphocytes to blood vessels	MSCs, CAFs, pericytes and BECs	Activated endothelium, BECs and FRCs
ICAM1 (CD54)	Transmembrane protein of the immunoglobulin superfamily, ligand for αLβ2 integrin on leukocytes.	BECs and pericytes	BECs, pericytes, macrophages and lymphocytes
THY1 (CD90)	GPI-anchored cell surface protein involved in cell adhesion, extravasation, migration	MSCs, CAFs, T cells and tumour cells	Thymocytes, peripheral T cells, myoblasts, epidermal cells, keratinocytes and neurons
β1 integrin (CD29)	Involved in adhesion and migration	MSCs, CAFs and tumour cells	Widely expressed
Growth factor receptors			
VEGFR1 (FLT1)	Tyrosine protein kinase, high affinity receptor for VEGFA; critical for vasculature development	BECs, TAMs and MDSCs	BECs
VEGFR2 (KDR)	Tyrosine protein kinase, high affinity receptor for VEGFC; important for angiogenesis and artery formation	Pericytes and MDSCs	Pericytes
VEGFR3 (FLT4)	Tyrosine protein kinase, high affinity receptor for VEGFC and VEGFD; important for lymphangiogenesis and adult neovascularization	LECs	LECs
PDGFRα (CD140α)	Receptor tyrosine kinase for PDGFs	CAFs, pericytes and some tumour cells	FRCs and MSCs
PDGFRβ (CD140β)	Receptor tyrosine kinase for PDGFs	CAFs and pericytes	FRCs, LECs, pericytes and MSCs
Intracellular structural proteins			
Vimentin	Structural protein (type III intermediate filament) widely expressed by mesenchymal cells	MSCs, CAFs, pericytes, BECs and tumour cells that have undergone EMT	FRCs, pericytes and other mesenchymal cells
αSMA (ACTA2)	Structural protein critical for cell contraction	CAFs and pericytes	Smooth muscle cells, activated fibroblasts, FRCs and myoepithelial cells
Desmin	Structural protein (type III intermediate filament) widely expressed by mesenchymal cells	CAFs and pericytes	Myofibroblasts, FRCs and pericytes
Other proteins			
5NT (CD73 and NT5E)	Enzyme that converts AMP to adenosine	MSCs	MSCs and lymphocytes
RGS5	Regulates heterotrimeric G proteins by promoting GTPase activity	Pericytes	Pericytes
Endosialin (CD248)	C-type lectin transmembrane protein	Pericytes and CAFs	Vascular smooth muscle cells, pericytes, myofibroblasts, FRCs and T cells
FSP1 (S100A4)	Belongs to family of cytoplasmic calcium binding proteins	Some CAFs	FRCs and fibroblasts

αSMA, α-smooth muscle actin; 5NT, 5′-nucleotidase; BEC, blood endothelial cell; CAF, cancer-associated fibroblast; CSPG4, chondroitin sulfate proteoglycan 4; DC, dendritic cell; EMT, epithelial-to-mesenchymal transition; FAP, fibroblast activation protein; FRC, fibroblastic reticular cell; FSP1, fibroblast-specific protein 1; GPI, glycosylphosphatidylinositol; ICAM1, intercellular adhesion molecule 1; LEC, lymphatic endothelial cell; MDSC, myeloid-derived suppressor cell; MSC, mesenchymal stem cell; PDGFR, platelet-derived growth factor receptor; PECAM1, platelet endothelial cell adhesion molecule 1; RGS5, regulator of G protein signalling 5; TAM, tumour-associated macrophage; THY1, thymocyte differentiation antigen 1; TME, tumour microenvironment; VCAM1, vascular cell adhesion protein 1; VEGFR, vascular endothelial growth factor receptor. *Alternative name shown in brackets.

TGFβ in the ECM, leading to a further increase in TGFβ levels[59,60]. TGFβ has many effects in the TME, such as promoting cancer cell growth and suppressing anti-tumour immunity both directly and by enhancing myofibroblast differentiation (see below). In addition to these local effects, increased lymphatic drainage of tumour antigens can affect antitumour immunity by promoting a tolerogenic and immunosuppressive environment in sentinel lymph nodes[54]. Consistent with these observations, immune responses in tumour-draining lymph nodes are often greatly impaired compared with distal lymph nodes or with lymph nodes draining healthy adjacent tissue[57].

Collectively, these studies suggest an important role for tumour vasculature in modulating antitumour immunity. Given that BECs and LECs in the TME are thought to dampen T cell infiltration and/or activity, targeting pro-angiogenic and pro-lymphangiogenic pathways could hold promise for enhancing antitumour immunity, in particular when combined with other immunotherapeutic approaches.

Modulation of immune cell activation by pericytes. Immunomodulation by pericytes has been observed in various conditions, including cancer. However, the site where pericyte interactions with immune cells have been most extensively characterized is in the liver, where they are referred to as hepatic stellate cells (or Ito cells)[61]. Although hepatic stellate cells can function as antigen-presenting cells, they express negative co-stimulatory molecules and are thus predominantly tolerogenic[62,63]. These tolerogenic functions are necessary to maintain immune homeostasis in the liver but could potentially establish a permissive environment for cancer growth. Indeed, in an animal model of hepatocellular carcinoma, the co-transplantation of hepatic stellate cells with cancer cells accelerated tumour progression by augmenting T_{Reg} cell recruitment to the tumours[64]. Similarly, in orthotopic hepatocellular carcinoma models, it was reported that liver tumours tripled in size when cancer cells were injected together with hepatic stellate cells, concomitant with an expansion of T_{Reg} cells and myeloid-derived suppressor cells (MDSCs)[65].

The immunomodulatory potential of pericytes has also been documented in other tissues. Activation of brain pericytes through a gain-of-function mutation in PDGFRβ was found to prompt the expression of immunoregulatory genes, including those involved in antigen presentation and interferon signalling[66]. Furthermore, an analysis of human malignant glioma, a tumour characterized by extensive vascularization, revealed a substantial accumulation of pericytes in high-grade brain tumours, which negatively correlated with the presence of cytotoxic lymphocytes[67]. These pericytes suppressed allogeneic and mitogen-activated T cell responses *ex vivo* through the production of PGE_2, TGFβ and NO. Another study has recently demonstrated that pericytes isolated from syngeneic mouse melanoma and colon carcinoma models hindered the proliferation, activation and cytokine production of $CD4^+$ T cells[68]. This immunoregulatory function of pericytes was enhanced by exposure to IL-6 in the TME[68], suggesting that tumour-derived factors may convert pericytes into immune regulators.

We are only beginning to appreciate the varied mechanisms by which pericytes can modulate immune responses in tumours and in other inflammatory lesions. Particularly relevant is the finding that, during acute tissue injury, perivascular precursors expressing a disintegrin and metalloproteinase 12 (ADAM12) are recruited to wounds and give rise to profibrotic myofibroblasts[69]. As collagen deposition by fibroblastic cells has been suggested to alter inflammatory responses in tumours (see below), it is possible that a similar pericyte-to-myofibroblast differentiation may occur during malignant progression and may contribute to impaired antitumour immunity.

Research on pericytes has recently gained momentum and our understanding of their definitive functions in cancer and other inflammatory diseases will quickly evolve in the coming years. The discovery of new immunomodulatory functions for pericytes is likely to have implications for cancer immunotherapy.

MSCs and antitumour immunity. Several studies have shown that MSCs can directly support the growth and metastasis of solid tumours (BOX 2). Recent reports have now suggested that MSCs can indirectly have pro-tumorigenic functions by interfering with antitumour immune responses (FIG. 3). In one example, the rejection of allogeneic tumours in mice was prevented by co-inoculating tumour cells with as few as 1 MSC per 100 tumour cells[70]. MSCs have been shown to inhibit cytotoxic T cell killing of tumour cells in an IL-10-dependent manner and induced MHC class I down-regulation on tumour cells[71]. Another study found that *in vitro* pre-treatment of MSCs with interferon-γ (IFNγ) and TNF induced the expression of inducible nitric oxide synthase (iNOS) and increased MSC-mediated support of melanoma cell growth *in vivo* through the suppression of splenocyte proliferation[72]. However, *in vivo* blockade of iNOS following MSC injection only partially abolished the tumour-supporting functions of MSCs, suggesting that additional MSC-derived factors are involved. Thus, although these studies demonstrated that MSCs have the potential to suppress antitumour immunity, the extent to which they do so in different tumour types and the underlying mechanisms involved remain to be determined.

Given the striking similarity between cancer lesions and wounds, it has been postulated that MSCs in the TME may possess some of the immunomodulatory potential they exhibit in injured tissues. Upon the formation of a wound and the onset of inflammation, MSCs are mobilized and recruited from blood to damaged tissues where they promote wound resolution by either differentiating into activated fibroblasts or other tissue-resident stromal cells, or by directly modulating the action of inflammatory cells in the lesion. In particular, MSCs secrete platelet-derived growth factor (PDGF) and VEGF family members, which increase the

Sentinel lymph nodes
The first set of lymph nodes that drains a tumour. These lymph nodes can potentially contain a high concentration of tumour antigens and inflammatory molecules secreted by the tumour.

Hepatic stellate cells
A population of pericytes present in the liver in close association with the sinusoidal endothelial cells.

Myeloid-derived suppressor cells
(MDSCs). A heterogeneous set of cells of myeloid origin that can suppress T cell activity.

survival of endothelial cells and fibroblasts, and factors that act on myeloid cells, such as macrophage colony-stimulating factor (M-CSF), which stimulate the uptake of cell debris by macrophages in wounds[73]. Furthermore, during wound healing, MSCs secrete several immunosuppressive cues known to inhibit T cells — including PGE_2, TGFβ, NO and hepatocyte growth factor (HGF) — and they express immunomodulatory enzymes such as IDO[72,73]. Therefore, it is possible that the immunoregulatory role of MSCs in tumours may be more substantial than currently appreciated and that MSCs in tumours may employ mechanisms of immunosuppression that are similar to those used during wound healing.

Pleiotropic functions of CAFs in immunomodulation. CAFs use a range of different mechanisms to alter the functional activity of immune cells. First, CAFs are major producers of TGFβ, which is associated with poor prognosis in patients[74]. The role of TGFβ in immune modulation in the tumour is multifaceted. Notably, TGFβ can attenuate the cytotoxicity of T cells[75] and promote hyporesponsiveness of effector memory T cells by inhibiting TCR–CD28 signalling[76]. TGFβ also contributes to immune evasion by the recruitment and retention of macrophages[77]. PGE_2 has also been linked to CAF-induced immunosuppression in the TME owing to its ability to inhibit NK cell cytotoxicity and cytokine production[78,79]. Another immunomodulatory factor produced by CAFs is thymic stromal lymphopoietin (TSLP), which favours T_H2 cell polarization in the tumour and is associated with poor prognosis in patients[80]. CAFs can also support the maintenance of immunosuppressive myeloid cells by producing the M2 macrophage-polarizing cytokines IL-4, IL-6 and CXCL8 (REFS 81,82). IL-6 also inhibits DC maturation in tumours, which would have a net result of reducing T cell activation[83].

The immunomodulatory potential of CAFs has been validated in depletion studies. For instance, genetic ablation of FAP^+ CAFs — using a bacterial artificial (BAC)-transgenic mouse line in which the primate diphtheria toxin receptor (DTR) is expressed under the control of the *Fap* promoter (FAP–DTR mice), and diphtheria toxin administration leads to acute depletion of FAP^+ stromal cells — demonstrated that FAP^+ cells can be immunosuppressive in autochthonous and transplanted tumour models[84]. Importantly, ablation of FAP^+ cells in immunogenic tumours or in combination with a therapeutic cancer vaccine correlated with impaired tumour growth. Although this impaired tumour growth was immune-mediated, the ablation of FAP^+ cells did not lead to an enhancement in $CD8^+$ T cell numbers or cytotoxicity. Instead, the authors showed that FAP^+ CAFs attenuated cytokine-induced hypoxic necrosis of tumour and stromal cells by repressing their responsiveness to IFNγ and TNF[84]. Similarly, the use of CAR T cells directed against the FAP antigen in preclinical models of mesothelioma and lung cancer has demonstrated that depletion of FAP^+ cells can augment endogenous antitumour immunity, probably by enhancing activation of T cells[85].

The production of specific ECM proteins by CAFs has also been correlated with reduced antitumour immunity. For instance, tenascin C inhibits β1 integrin-mediated adhesion of T cells to fibronectin[86] and can interfere with their activation in response to alloantigens and mitogens[87]. Thrombospondin 1 (TSP1) is another ECM molecule that is highly enriched in many solid tumours, and its expression correlates with weak T_H1 cell responses, probably owing to its binding to CD47 on macrophages and suppressing IL-12 production in the TME[88]. Furthermore, the upregulation of MHC class II and co-stimulatory molecule expression on DCs is impaired in the presence of TSP1, supporting the idea that this molecule may dampen T cell activation by maintaining DCs in an immature state[89]. As TSP1 can also bind to T cells, it is likely that this interaction may have additional immunomodulatory consequences. However, the net effect of TSP1 on lymphocyte activation remains unclear, as both inhibitory and stimulatory effects have been described[90–92]. These contrary observations are probably due to the ability of TSP1 to engage different receptors on T cells. Indeed, it has been shown that binding of TSP1 to CD47 on T cells greatly reduced lymphocyte proliferation, whereas binding to α4β1 integrin, an alternative receptor for TSP1, enhanced lymphocyte adhesion and chemotaxis[93]. Finally, the proteolytic activity of matrix metalloproteinases (MMPs), many of which are highly expressed by CAFs, promotes the turnover of the ECM and controls the activity of cytokines and chemokines[94]. Thus, CAF-derived MMPs are likely to regulate various aspects of the cancer-immunity cycle.

Collectively, these studies suggest that CAFs modulate antitumour immune responses in pleiotropic ways. Considering that many immunosuppressive molecules expressed by MDSCs and T_{Reg} cells are also expressed by CAFs (such as PDL1), it is likely that CAFs hinder T cell responses through additional, as yet uncovered, mechanisms.

Immune cell–stroma crosstalk in the TME

The increased scientific interest in the mechanisms governing stromal immunoregulation in the TME has in recent years unveiled that these modulatory pathways are not unidirectional. In fact, emerging evidence supports the notion that tumour-infiltrating leukocytes can actively shape the stromal milieu, thus highlighting a great degree of crosstalk between the two compartments (FIG. 3). One of the major areas that has been actively studied is the regulation of tumour vasculature by immune cells, through their interaction with endothelial cells. In addition, many tumour-infiltrating leukocytes secrete several factors that can directly influence stromal cell behaviour and are therefore important players in immune cell–stroma crosstalk.

Modulation of tumour vessel formation by immune cells. In recent years, several studies have established that the extensive blood vascular network in tumours originates not only from cancer-secreted factors, but it is also fostered by the action of infiltrating immune cells. In particular, bone-marrow derived myeloid cells such as

macrophages, neutrophils and MDSCs are often abundant in tumours where they can actively influence the formation and maintenance of blood vessels. Exposure to the hypoxic microenvironment and to factors such as TNF and angiopoietin 2 has been shown to stimulate a potent pro-angiogenic programme in tumour-associated macrophages (TAMs), as demonstrated by upregulation of several factors such as VEGF, FGF2, PDGFβ and cyclooxygenase 2 (COX2), which can boost BEC proliferation and angiogenesis[95]. Indeed, elevated numbers of TAMs often correlate with higher vessel density in human tumours[96–98], whereas depletion of macrophages in different animal models of cancer has been associated with reduced angiogenesis[99]. Furthermore, neutrophil depletion in the RIP1–TAG2 transgenic mouse model of pancreatic cancer led to a reduction in the density of tumour blood vessels[100]. Accordingly, tumour-associated neutrophils (TANs) are major producers of MMP9, a key factor that triggers the pro-angiogenic switch during carcinogenesis[101]. MDSCs also express high levels of various MMPs, including MMP9, and blocking MDSC recruitment lowers the formation of blood vessels in xenograft models of colorectal cancer[102]. By contrast, co-injection of MDSCs with tumour cells greatly increased blood vessel formation in mice, as compared to inoculation of tumour cells alone[103].

Considerable evidence now exists to suggest that other myeloid cells, which are usually present in lower numbers than TAMs, TANs and MDSCs, may also have a role in modulating endothelial cell activity. Mast cells, which represent a tiny fraction of the leukocytic infiltrate of most tumours, have been shown to degranulate in tumours, thus contributing to the accumulation of pro-angiogenic molecules such as VEGFA, FGF2 and MMP9 (REFS 104,105). Indeed, studies from transgenic mice lacking mast cells have demonstrated that their absence reduces angiogenesis in several tumour models[104–106]. Eosinophils also contain granules filled with VEGF and other pro-angiogenic molecules that are promptly secreted upon exposure to IL-15 (REF. 107). However, the importance of this pathway in tumour angiogenesis remains to be confirmed.

The stimulatory effects of immune cells on tumour endothelial cells are not restricted to blood vessels. Many tumour-infiltrating leukocytes, such as macrophages and MDSCs, are also capable of secreting the lymphatic-specific growth factors VEGFC and VEGFD, which induce proliferation and activation of LECs by binding to their receptor VEGFR3 (REF. 108).

Collectively, these findings highlight how signals emanating from infiltrating immune cells are integrated within the TME and, by modulating the activity of endothelial cells, cooperate with tumours in the induction of extensive vasculature networks.

Immune regulation of stromal cell function. During wound repair, inflammatory cells such as macrophages and mast cells secrete numerous cytokines and growth factors to activate local stromal cells to aid in tissue repair. Once inflammation is resolved, the abundance of such stimuli rapidly decreases, leading to the restoration of stromal quiescence and tissue homeostasis. Similarly, in cancer, infiltrating immune cells establish a chemokine and cytokine milieu that shapes the recruitment and activation of stromal cells within the tumour. In contrast to normal wound healing, however, the highly inflammatory microenvironment of tumours results in the chronic activation of inflammatory cells, particularly myeloid cells, which in turn promotes the proliferation and activation of stromal cells.

Among the immune cells that influence the tumour stromal landscape, macrophages, especially M2-polarized cells, are major drivers of inflammation and stroma activation[95]. These cells are a major source of several growth factors, including epidermal growth factor (EGF), FGF and PDGFβ[109], which, as discussed above, exert pleiotropic effects on several stromal cell populations. TGFβ is also produced by macrophages in tumours, where it triggers fibroblast activation and collagen deposition[109]. These effects are magnified by the ability of several types of leukocytes to secrete matrix proteolytic enzymes such as MMP9 that can release ECM-stored factors with stromal mitogenic potential[110]. Thus, macrophages are key players in the TME, with the ability to induce myofibroblast differentiation, increase fibrosis and support stromal cell survival. In this respect, reprogramming macrophage phenotypes could represent an effective strategy to modulate the stromal cell landscape in the TME[111]. For example, in a mouse model of pancreatic ductal adenocarcinoma, treatment with CD40-specific antibodies induced the activation and rapid accumulation of macrophages in tumours[112]. Rather than sustaining stromal activation, these macrophages promoted the elimination of desmoplastic stromal cells, leading to a marked reduction of tumour-associated fibrosis.

Although much of the literature has focused on the crosstalk between myeloid and stromal cells in tumours, other infiltrating immune cells can also influence stromal function in the TME. TGFβ, for example, is produced in large amounts by T_{Reg} cells and is therefore likely to affect the behaviour of stromal cells in the TME. In addition, T_H2, T_H17 and natural killer T (NKT) cells produce IL-13, which can augment desmoplastic activation by binding to IL-4 receptor in stromal cells[113]. Recently, it was reported that the accumulation of $CXCL13^+$ stroma and infiltration of lymphotoxin-producing B cells correlate in prostate cancer, which suggests the existence of an additional cross-regulatory pathway between immune cells and stroma[30,114]. B cell-derived lymphotoxin promotes the differentiation and survival of follicular stromal cells in lymphoid organs[115], and therefore it is possible that B cells in tumours could support the maintenance of follicle-like stromal cells through a similar pathway.

Collectively, these studies highlight a considerable level of crosstalk between stromal and immune cells, which adds to the complexity of the TME. A deeper understanding of these interactions will advance scientific progress in elucidating the immunosuppressive mechanisms that interfere with the cancer-immunity cycle and responsiveness to immunotherapy.

Box 3 | **Tertiary lymphoid structures in the tumour microenvironment**

Stromal cells have long been recognized as key structural components of many tissues. However, only recently have we begun to appreciate how far-reaching their effects are on various aspects of immunity. One of the sites where immunomodulatory stromal cells have been well characterized is lymphoid organs, which contain multiple lineages of stromal cells that reside in distinct locations and perform unique functions. In particular, numerous studies have demonstrated that these subsets of stromal cells express cytokines and chemokines that directly regulate immune cell recruitment, homeostasis and activation[138–140]. Notably, many chronic pathological conditions, including cancer, have been associated with development of tertiary lymphoid structures (TLSs) in ectopic locations, probably mediated by persistent inflammation[141].

The framework and cellular composition of TLSs are deeply reminiscent of lymphoid organs, and the compartmentalization of immune cell populations and the presence of high endothelial venules are both hallmarks of TLSs. Additionally, many of the stromal cell populations inhabiting lymphoid organs have been observed in TLSs in tumours[142,143], where they establish an intimate communication with haematopoietic cells that is pivotal in the regulation of the ensuing inflammatory response.

Notably, whereas the emerging picture of stromal cells in the tumour microenvironment (TME) suggests that their presence can be detrimental to antitumour immune responses, the presence of TLSs in tumours is often associated with better prognosis, indicating that the stromal components in TLSs may aid in generating or maintaining antitumour responses[144,145]. Indeed, data have shown that stromal cells actively recruit immune cells to TLSs[146,147], by providing chemotactic cues such as CC-chemokine ligand 19 (CCL19), CCL21 and CXC-chemokine ligand 13 (CXCL13). Furthermore, they support T cell activation and effector functions, as well as induction of memory T cells[141,142]. Humoral immune responses are also initiated in TLSs, and the underlying stroma is involved in the formation of germinal centres[148]. How TLS stroma differs from peritumoural or intratumoural stroma remains to be determined, but a deeper understanding of these stromal compartments may aid in the development of efficacious therapeutics for modulating antitumour immunity.

Conclusions and future directions

Numerous studies have demonstrated that the stromal determinants of the TME nurture tumour cells and contribute to tumour growth and metastasis. Recently, we have come to understand that antitumour immunity is also influenced by the tumour stroma. To this end, targeting stromal cells has been shown to be necessary for effective adoptive T cell therapy in cancer[116], suggesting that the tumour stroma may also influence immunity elicited by therapeutic intervention. As our knowledge of the pleiotropic roles of stromal cells during cancer progression deepens, it becomes clear that the tumour stroma should no longer be considered to be an inert bystander. Consequently, there is growing interest in developing novel therapeutic strategies that target the tumour stroma. We have gained considerable knowledge about the major cellular components of the TME, the biologically active factors they produce and their interactions with tumour and immune cells.

Despite this progress, we still face tremendous challenges in developing adequate tools to modify the stromal landscape of cancer lesions. Studies that have aimed to ascribe specific functions to particular stromal compartments are confounded by our limited understanding of cellular heterogeneity within the TME and a lack of highly targeted approaches. Furthermore, stromal cells are obligate components of physiological processes, and thus any therapy aimed at limiting stromal activity in tumour lesions should be specific enough to spare stromal cells in healthy tissues and in potentially beneficial tertiary lymphoid structures (BOX 3).

The last decade has witnessed one of the most exciting phases of cancer research, as several biological agents with immunomodulatory functions have demonstrated significant clinical benefits. Based on mounting evidence that non-haematopoietic cells impede the development of proper antitumour immunity, it is reasonable to speculate that pharmacological approaches targeting the tumour stroma could potentially aid in achieving full clinical benefits of immunotherapy. Future studies must focus on determining the feasibility and tractability of stromal cell targeting to boost natural antitumour immunity, as well as immunity induced by therapeutic intervention.

1. Joyce, J. A. & Pollard, J. W. Microenvironmental regulation of metastasis. *Nat. Rev. Cancer* **9**, 239–252 (2009).
2. Polyak, K., Haviv, I. & Campbell, I. G. Co-evolution of tumor cells and their microenvironment. *Trends Genet.* **25**, 30–38 (2009).
References 1 and 2 are comprehensive reviews on the development and function of the TME.
3. Nakasone, E. S. *et al.* Imaging tumor-stroma interactions during chemotherapy reveals contributions of the microenvironment to resistance. *Cancer Cell* **21**, 488–503 (2012).
4. Chen, D. S. & Mellman, I. Oncology meets immunology: the cancer-immunity cycle. *Immunity* **39**, 1–10 (2013).
This study is a seminal review on the current state of tumour immunotherapies in the clinic.
5. Pivarcsi, A. *et al.* Tumor immune escape by the loss of homeostatic chemokine expression. *Proc. Natl Acad. Sci. USA* **104**, 19055–19060 (2007).
6. Powles, T. *et al.* MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. *Nature* **515**, 558–562 (2014).
7. Herbst, R. S. *et al.* Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature* **515**, 563–567 (2014).
8. Tumeh, P. C. *et al.* PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature* **515**, 568–571 (2014).
9. Castermans, K. & Griffioen, A. W. Tumor blood vessels, a difficult hurdle for infiltrating leukocytes. *Biochim. Biophys. Acta* **1776**, 160–174 (2007).
10. Nagy, J. A., Dvorak, A. M. & Dvorak, H. F. VEGF-A and the induction of pathological angiogenesis. *Annu. Rev. Pathol.* **2**, 251–275 (2007).
11. Jain, R. K. Molecular regulation of vessel maturation. *Nat. Med.* **9**, 685–693 (2003).
12. Greenberg, J. I. *et al.* A role for VEGF as a negative regulator of pericyte function and vessel maturation. *Nature* **456**, 809–813 (2008).
13. Furuya, M. *et al.* Expression of regulator of G protein signalling protein 5 (RGS5) in the tumour vasculature of human renal cell carcinoma. *J. Pathol.* **203**, 551–558 (2004).
14. Hamzah, J. *et al.* Vascular normalization in Rgs5-deficient tumours promotes immune destruction. *Nature* **453**, 410–414 (2008).
15. Goel, S. *et al.* Normalization of the vasculature for treatment of cancer and other diseases. *Physiol. Rev.* **91**, 1071–1121 (2011).
16. Griffioen, A. W., Damen, C. A., Blijham, G. H. & Groenewegen, G. Tumor angiogenesis is accompanied by a decreased inflammatory response of tumor-associated endothelium. *Blood* **88**, 667–673 (1996).
17. Griffioen, A. W., Damen, C. A., Martinotti, S., Blijham, G. H. & Groenewegen, G. Endothelial intercellular adhesion molecule-1 expression is suppressed in human malignancies: the role of angiogenic factors. *Cancer Res.* **56**, 1111–1117 (1996).
18. Bouzin, C., Brouet, A., De Vriese, J., Dewever, J. & Feron, O. Effects of vascular endothelial growth factor on the lymphocyte–endothelium interactions: identification of caveolin-1 and nitric oxide as control points of endothelial cell anergy. *J. Immunol.* **178**, 1505–1511 (2007).
19. Buckanovich, R. J. *et al.* Endothelin B receptor mediates the endothelial barrier to T cell homing to tumors and disables immune therapy. *Nat. Med.* **14**, 28–36 (2008).
20. Delfortrie, S. *et al.* Egfl7 promotes tumor escape from immunity by repressing endothelial cell activation. *Cancer Res.* **71**, 7176–7186 (2011).
21. Shetty, S. *et al.* Common lymphatic endothelial and vascular endothelial receptor-1 mediates the transmigration of regulatory T cells across human hepatic sinusoidal endothelium. *J. Immunol.* **186**, 4147–4155 (2011).
22. Nummer, D. *et al.* Role of tumor endothelium in $CD4^+$ $CD25^+$ regulatory T cell infiltration of human pancreatic carcinoma. *J. Natl Cancer Inst.* **99**, 1188–1199 (2007).
23. Schenkel, A. R., Mamdouh, Z., Chen, X., Liebman, R. M. & Muller, W. A. CD99 plays a major role in the migration of monocytes through endothelial junctions. *Nat. Immunol.* **3**, 143–150 (2002).
24. Talmadge, J. E. & Gabrilovich, D. I. History of myeloid-derived suppressor cells. *Nat. Rev. Cancer* **13**, 739–752 (2013).
25. Loeffler, M., Kruger, J. A., Niethammer, A. G. & Reisfeld, R. A. Targeting tumor-associated fibroblasts improves cancer chemotherapy by increasing intratumoral drug uptake. *J. Clin. Invest.* **116**, 1955–1962 (2006).
One of the first studies demonstrating that targeting CAFs affects tumour progression.

26. Liao, D., Luo, Y., Markowitz, D., Xiang, R. & Reisfeld, R. A. Cancer associated fibroblasts promote tumor growth and metastasis by modulating the tumor immune microenvironment in a 4T1 murine breast cancer model. *PLoS ONE* **4**, e7965 (2009). **This study details the use of a FAP-targeted vaccine to deplete CAFs.**
27. Murdoch, C., Giannoudis, A. & Lewis, C. E. Mechanisms regulating the recruitment of macrophages into hypoxic areas of tumors and other ischemic tissues. *Blood* **104**, 2224–2234 (2004).
28. Tan, W. *et al.* Tumour-infiltrating regulatory T cells stimulate mammary cancer metastasis through RANKL–RANK signalling. *Nature* **470**, 548–553 (2011).
29. Feig, C. *et al.* Targeting CXCL12 from FAP-expressing carcinoma-associated fibroblasts synergizes with anti-PD-L1 immunotherapy in pancreatic cancer. *Proc. Natl Acad. Sci. USA* **110**, 20212–20217 (2013). **This study provides an example of how blocking immunosuppressive products from CAFs can impair tumour growth in an immune-mediated manner.**
30. Ammirante, M., Shalapour, S., Kang, Y., Jamieson, C. A. & Karin, M. Tissue injury and hypoxia promote malignant progression of prostate cancer by inducing CXCL13 expression in tumor myofibroblasts. *Proc. Natl Acad. Sci. USA* **111**, 14776–14781 (2014). **This study provides an example of a feedback loop between hypoxia, CAFs and B cells promoting tumour progression.**
31. Shalapour, S. *et al.* Immunosuppressive plasma cells impede T-cell-dependent immunogenic chemotherapy. *Nature* **521**, 94–98 (2015).
32. Kalluri, R. & Zeisberg, M. Fibroblasts in cancer. *Nat. Rev. Cancer* **6**, 392–401 (2006).
33. Salmon, H. *et al.* Matrix architecture defines the preferential localization and migration of T cells into the stroma of human lung tumors. *J. Clin. Invest.* **122**, 899–910 (2012). **This study is an important example of how the ECM in the TME affects T cell localization and motility in human tumours.**
34. Provenzano, P. P. *et al.* Enzymatic targeting of the stroma ablates physical barriers to treatment of pancreatic ductal adenocarcinoma. *Cancer Cell* **21**, 418–429 (2012).
35. Jacobetz, M. A. *et al.* Hyaluronan impairs vascular function and drug delivery in a mouse model of pancreatic cancer. *Gut* **62**, 112–120 (2013).
36. Caruana, I. *et al.* Heparanase promotes tumor infiltration and antitumor activity of CAR-redirected T lymphocytes. *Nat. Med.* **21**, 524–529 (2015).
37. Ozdemir, B. C. *et al.* Depletion of carcinoma-associated fibroblasts and fibrosis induces immunosuppression and accelerates pancreas cancer with reduced survival. *Cancer Cell* **25**, 719–734 (2014).
38. Rhim, A. D. *et al.* Stromal elements act to restrain, rather than support, pancreatic ductal adenocarcinoma. *Cancer Cell* **25**, 735–747 (2014). **References 37 and 38 are recent examples of how targeting FAP$^+$ cells may have negative effects for the host.**
39. Valujskikh, A., Lantz, O., Celli, S., Matzinger, P. & Heeger, P. S. Cross-primed CD8$^+$ T cells mediate graft rejection via a distinct effector pathway. *Nat. Immunol.* **3**, 844–851 (2002).
40. Savinov, A. Y., Wong, F. S., Stonebraker, A. C. & Chervonsky, A. V. Presentation of antigen by endothelial cells and chemoattraction are required for homing of insulin-specific CD8$^+$ T cells. *J. Exp. Med.* **197**, 643–656 (2003).
41. Zang, X. *et al.* Tumor associated endothelial expression of B7-H3 predicts survival in ovarian carcinomas. *Mod. Pathol.* **23**, 1104–1112 (2010).
42. Rodig, N. *et al.* Endothelial expression of PD-L1 and PD-L2 down-regulates CD8$^+$ T cell activation and cytolysis. *Eur. J. Immunol.* **33**, 3117–3126 (2003).
43. Mazanet, M. M. & Hughes, C. C. B7-H1 is expressed by human endothelial cells and suppresses T cell cytokine synthesis. *J. Immunol.* **169**, 3581–3588 (2002).
44. Huang, X. *et al.* Lymphoma endothelium preferentially expresses Tim-3 and facilitates the progression of lymphoma by mediating immune evasion. *J. Exp. Med.* **207**, 505–520 (2010).
45. Seaman, S. *et al.* Genes that distinguish physiological and pathological angiogenesis. *Cancer Cell* **11**, 539–554 (2007).
46. Ma, L. *et al.* Ig gene-like molecule CD31 plays a nonredundant role in the regulation of T-cell immunity and tolerance. *Proc. Natl Acad. Sci. USA* **107**, 19461–19466 (2010).
47. Secchiero, P. & Zauli, G. The puzzling role of TRAIL in endothelial cell biology. *Arterioscler. Thromb. Vasc. Biol.* **28**, e4, author reply e5–e6 (2008).
48. Motz, G. T. *et al.* Tumor endothelium FasL establishes a selective immune barrier promoting tolerance in tumors. *Nat. Med.* **20**, 607–615 (2014). **This study provides an example of how the tumour endothelium can contribute to immunosuppression by promoting T cell apoptosis.**
49. Mulligan, J. K., Day, T. A., Gillespie, M. B., Rosenzweig, S. A. & Young, M. R. Secretion of vascular endothelial growth factor by oral squamous cell carcinoma cells skews endothelial cells to suppress T-cell functions. *Hum. Immunol.* **70**, 375–382 (2009).
50. Mulligan, J. K. & Young, M. R. Tumors induce the formation of suppressor endothelial cells *in vivo*. *Cancer Immunol. Immunother.* **59**, 267–277 (2010).
51. Casos, K. *et al.* Tumor cells induce COX-2 and mPGES-1 expression in microvascular endothelial cells mainly by means of IL-1 receptor activation. *Microvasc. Res.* **81**, 261–268 (2011).
52. Munn, D. H. & Mellor, A. L. Indoleamine 2,3-dioxygenase and tumor-induced tolerance. *J. Clin. Invest.* **117**, 1147–1154 (2007).
53. Pilotte, L. *et al.* Reversal of tumoral immune resistance by inhibition of tryptophan 2,3-dioxygenase. *Proc. Natl Acad. Sci. USA* **109**, 2497–2502 (2012).
54. Lund, A. W. *et al.* VEGF-C promotes immune tolerance in B16 melanomas and cross-presentation of tumor antigen by lymph node lymphatics. *Cell Rep.* **1**, 191–199 (2012). **This study details how LECs can promote a tolerizing environment in the tumour and contribute to overall immunosuppression.**
55. Cohen, J. N. *et al.* Lymph node-resident lymphatic endothelial cells mediate peripheral tolerance via Aire-independent direct antigen presentation. *J. Exp. Med.* **207**, 681–688 (2010).
56. Tamburini, B. A., Burchill, M. A. & Kedl, R. M. Antigen capture and archiving by lymphatic endothelial cells following vaccination or viral infection. *Nat. Commun.* **5**, 3989 (2014).
57. Swartz, M. A. & Lund, A. W. Lymphatic and interstitial flow in the tumour microenvironment: linking mechanobiology with immunity. *Nat. Rev. Cancer* **12**, 210–219 (2012). **This review details the current state of the field of tumour lymphatics and mechanobiology.**
58. Ng, C. P., Hinz, B. & Swartz, M. A. Interstitial fluid flow induces myofibroblast differentiation and collagen alignment *in vitro*. *J. Cell Sci.* **118**, 4731–4739 (2005).
59. Wipff, P. J., Rifkin, D. B., Meister, J. J. & Hinz, B. Myofibroblast contraction activates latent TGF-β1 from the extracellular matrix. *J. Cell Biol.* **179**, 1311–1323 (2007).
60. Ahamed, J. *et al. In vitro* and *in vivo* evidence for shear-induced activation of latent transforming growth factor-β1. *Blood* **112**, 3650–3660 (2008).
61. Tiegs, G. & Lohse, A. W. Immune tolerance: what is unique about the liver. *J. Autoimmun.* **34**, 1–6 (2010).
62. Ichikawa, S., Mucida, D., Tyznik, A. J., Kronenberg, M. & Cheroutre, H. Hepatic stellate cells function as regulatory bystanders. *J. Immunol.* **186**, 5549–5555 (2011).
63. Schildberg, F. A. *et al.* Murine hepatic stellate cells veto CD8 T cell activation by a CD54-dependent mechanism. *Hepatology* **54**, 262–272 (2011).
64. Zhao, W. *et al.* Activated hepatic stellate cells promote hepatocellular carcinoma development in immunocompetent mice. *Int. J. Cancer* **129**, 2651–2661 (2011).
65. Zhao, W. *et al.* Hepatic stellate cells promote tumor progression by enhancement of immunosuppressive cells in an orthotopic liver tumor mouse model. *Lab. Invest.* **94**, 182–191 (2014).
66. Olson, L. E. & Soriano, P. PDGFRβ signaling regulates mural cell plasticity and inhibits fat development. *Dev. Cell* **20**, 815–826 (2011).
67. Ochs, K. *et al.* Immature mesenchymal stem cell-like pericytes as mediators of immunosuppression in human malignant glioma. *J. Neuroimmunol.* **265**, 106–116 (2013).
68. Bose, A. *et al.* Tumor-derived vascular pericytes anergize Th cells. *J. Immunol.* **191**, 971–981 (2013).
69. Dulauroy, S., Di Carlo, S. E., Langa, F., Eberl, G. & Peduto, L. Lineage tracing and genetic ablation of ADAM12$^+$ perivascular cells identify a major source of profibrotic cells during acute tissue injury. *Nat. Med.* **18**, 1262–1270 (2012).
70. Djouad, F. *et al.* Immunosuppressive effect of mesenchymal stem cells favors tumor growth in allogeneic animals. *Blood* **102**, 3837–3844 (2003).
71. Montesinos, J. J. *et al. In vitro* evidence of the presence of mesenchymal stromal cells in cervical cancer and their role in protecting cancer cells from cytotoxic T cell activity. *Stem Cells Dev.* **22**, 2508–2519 (2013).
72. Han, Z. *et al.* Immunosuppressive effect of bone marrow-derived mesenchymal stem cells in inflammatory microenvironment favours the growth of B16 melanoma cells. *J. Cell. Mol. Med.* **15**, 2343–2352 (2011). **This study provides an example of how MSCs function to promote immunosuppression and tumour progression.**
73. Gnecchi, M., Zhang, Z., Ni, A. & Dzau, V. J. Paracrine mechanisms in adult stem cell signaling and therapy. *Circ. Res.* **103**, 1204–1219 (2008).
74. Saunier, E. F. & Akhurst, R. J. TGF β inhibition for cancer therapy. *Curr. Cancer Drug Targets* **6**, 565–578 (2006).
75. Ahmadzadeh, M. & Rosenberg, S. A. TGF-β 1 attenuates the acquisition and expression of effector function by tumor antigen-specific human memory CD8 T cells. *J. Immunol.* **174**, 5215–5223 (2005).
76. Broderick, L. & Bankert, R. B. Membrane-associated TGF-β1 inhibits human memory T cell signaling in malignant and nonmalignant inflammatory microenvironments. *J. Immunol.* **177**, 3082–3088 (2006).
77. Byrne, S. N., Knox, M. C. & Halliday, G. M. TGFβ is responsible for skin tumour infiltration by macrophages enabling the tumours to escape immune destruction. *Immunol. Cell Biol.* **86**, 92–97 (2008).
78. Balsamo, M. *et al.* Melanoma-associated fibroblasts modulate NK cell phenotype and antitumor cytotoxicity. *Proc. Natl Acad. Sci. USA* **106**, 20847–20852 (2009).
79. Li, T. *et al.* Colorectal carcinoma-derived fibroblasts modulate natural killer cell phenotype and antitumor cytotoxicity. *Med. Oncol.* **30**, 663 (2013).
80. De Monte, L. *et al.* Intratumor T helper type 2 cell infiltrate correlates with cancer-associated fibroblast thymic stromal lymphopoietin production and reduced survival in pancreatic cancer. *J. Exp. Med.* **208**, 469–478 (2011).
81. Paunescu, V. *et al.* Tumour-associated fibroblasts and mesenchymal stem cells: more similarities than differences. *J. Cell. Mol. Med.* **15**, 635–646 (2011).
82. Kim, J. H. *et al.* The role of myofibroblasts in upregulation of S100A8 and S100A9 and the differentiation of myeloid cells in the colorectal cancer microenvironment. *Biochem. Biophys. Res. Commun.* **423**, 60–66 (2012).
83. 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).
84. Kraman, M. *et al.* Suppression of antitumor immunity by stromal cells expressing fibroblast activation protein-α. *Science* **330**, 827–830 (2010).
85. Wang, L. C. *et al.* Targeting fibroblast activation protein in tumor stroma with chimeric antigen receptor T cells can inhibit tumor growth and augment host immunity without severe toxicity. *Cancer Immunol. Res.* **2**, 154–166 (2014).
86. Hauzenberger, D., Olivier, P., Gundersen, D. & Ruegg, C. Tenascin-C inhibits β integrin-dependent T lymphocyte adhesion to fibronectin through the binding of its fnIII 1–5 repeats to fibronectin. *Eur. J. Immunol.* **29**, 1435–1447 (1999).
87. Ruegg, C. R., Chiquet-Ehrismann, R. & Alkan, S. S. Tenascin, an extracellular matrix protein, exerts immunomodulatory activities. *Proc. Natl Acad. Sci. USA* **86**, 7437–7441 (1989).
88. Armant, M. *et al.* CD47 ligation selectively downregulates human interleukin 12 production. *J. Exp. Med.* **190**, 1175–1182 (1999).
89. Demeure, C. E. *et al.* CD47 engagement inhibits cytokine production and maturation of human dendritic cells. *J. Immunol.* **164**, 2193–2199 (2000).

90. Reinhold, M. I., Lindberg, F. P., Kersh, G. J., Allen, P. M. & Brown, E. J. Costimulation of T cell activation by integrin-associated protein (CD47) is an adhesion-dependent, CD28-independent signaling pathway. *J. Exp. Med.* **185**, 1–11 (1997).
91. Vallejo, A. N., Mugge, L. O., Klimiuk, P. A., Weyand, C. M. & Goronzy, J. J. Central role of thrombospondin-1 in the activation and clonal expansion of inflammatory T cells. *J. Immunol.* **164**, 2947–2954 (2000).
92. Avice, M. N., Rubio, M., Sergerie, M., Delespesse, G. & Sarfati, M. Role of CD47 in the induction of human naive T cell anergy. *J. Immunol.* **167**, 2459–2468 (2001).
93. Li, Z. *et al.* Interactions of thrombospondins with α4β1 integrin and CD47 differentially modulate T cell behavior. *J. Cell Biol.* **157**, 509–519 (2002).
94. Parks, W. C., Wilson, C. L. & Lopez-Boado, Y. S. Matrix metalloproteinases as modulators of inflammation and innate immunity. *Nat. Rev. Immunol.* **4**, 617–629 (2004).
95. Coussens, L. M., Zitvogel, L. & Palucka, A. K. Neutralizing tumor-promoting chronic inflammation: a magic bullet? *Science* **339**, 286–291 (2013).
This comprehensive review details the contributions of multiple immune cells to inflammation in the TME and provides examples of new targets.
96. Leek, R. D. *et al.* Association of macrophage infiltration with angiogenesis and prognosis in invasive breast carcinoma. *Cancer Res.* **56**, 4625–4629 (1996).
97. Onita, T. *et al.* Hypoxia-induced, perinecrotic expression of endothelial Per–ARNT–Sim domain protein-1/hypoxia-inducible factor-2α correlates with tumor progression, vascularization, and focal macrophage infiltration in bladder cancer. *Clin. Cancer Res.* **8**, 471–480 (2002).
98. Takanami, I., Takeuchi, K. & Kodaira, S. Tumor-associated macrophage infiltration in pulmonary adenocarcinoma: association with angiogenesis and poor prognosis. *Oncology* **57**, 138–142 (1999).
99. Lin, E. Y. *et al.* Macrophages regulate the angiogenic switch in a mouse model of breast cancer. *Cancer Res.* **66**, 11238–11246 (2006).
100. Nozawa, H., Chiu, C. & Hanahan, D. Infiltrating neutrophils mediate the initial angiogenic switch in a mouse model of multistage carcinogenesis. *Proc. Natl Acad. Sci. USA* **103**, 12493–12498 (2006).
101. Bergers, G. *et al.* Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. *Nat. Cell Biol.* **2**, 737–744 (2000).
102. Shojaei, F. *et al.* Bv8 regulates myeloid-cell-dependent tumour angiogenesis. *Nature* **450**, 825–831 (2007).
103. Yang, L. *et al.* Expansion of myeloid immune suppressor Gr^+CD11b^+ cells in tumor-bearing host directly promotes tumor angiogenesis. *Cancer Cell* **6**, 409–421 (2004).
104. Coussens, L. M. *et al.* Inflammatory mast cells up-regulate angiogenesis during squamous epithelial carcinogenesis. *Genes Dev.* **13**, 1382–1397 (1999).
105. Soucek, L. *et al.* Mast cells are required for angiogenesis and macroscopic expansion of Myc-induced pancreatic islet tumors. *Nat. Med.* **13**, 1211–1218 (2007).
106. Starkey, J. R., Crowle, P. K. & Taubenberger, S. Mast-cell-deficient W/Wv mice exhibit a decreased rate of tumor angiogenesis. *Int. J. Cancer* **42**, 48–52 (1988).
107. Munitz, A. & Levi-Schaffer, F. Eosinophils: 'new' roles for 'old' cells. *Allergy* **59**, 268–275 (2004).
108. Zumsteg, A. & Christofori, G. Myeloid cells and lymphangiogenesis. *Cold Spring Harb. Perspect. Med.* **2**, a006494 (2012).
109. Balkwill, F., Charles, K. A. & Mantovani, A. Smoldering and polarized inflammation in the initiation and promotion of malignant disease. *Cancer Cell* **7**, 211–217 (2005).
110. Lu, P., Takai, K., Weaver, V. M. & Werb, Z. Extracellular matrix degradation and remodeling in development and disease. *Cold Spring Harb. Perspect. Biol.* **3**, a005058 (2011).
111. Noy, R. & Pollard, J. W. Tumor-associated macrophages: from mechanisms to therapy. *Immunity* **41**, 49–61 (2014).
This review details the pleiotropic functions of macrophages in the TME.
112. Beatty, G. L. *et al.* CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans. *Science* **331**, 1612–1616 (2011).
113. Terabe, M., Park, J. M. & Berzofsky, J. A. Role of IL-13 in regulation of anti-tumor immunity and tumor growth. *Cancer Immunol. Immunother.* **53**, 79–85 (2004).
114. Ammirante, M., Luo, J. L., Grivennikov, S., Nedospasov, S. & Karin, M. B-cell-derived lymphotoxin promotes castration-resistant prostate cancer. *Nature* **464**, 302–305 (2010).
115. Gommerman, J. L. & Browning, J. L. Lymphotoxin/LIGHT, lymphoid microenvironments and autoimmune disease. *Nat. Rev. Immunol.* **3**, 642–655 (2003).
116. Textor, A. *et al.* Efficacy of CAR T-cell therapy in large tumors relies upon stromal targeting by IFNγ. *Cancer Res.* **74**, 6796–6805 (2014).
117. Achen, M. G., McColl, B. K. & Stacker, S. A. Focus on lymphangiogenesis in tumor metastasis. *Cancer Cell* **7**, 121–127 (2005).
118. Adotevi, O. *et al.* A decrease of regulatory T cells correlates with overall survival after sunitinib-based antiangiogenic therapy in metastatic renal cancer patients. *J. Immunother.* **33**, 991–998 (2010).
This study provides an example of how reducing the increased vascularization in tumours can modulate the immune landscape and reduce tumour metastasis.
119. Ko, J. S. *et al.* Sunitinib mediates reversal of myeloid-derived suppressor cell accumulation in renal cell carcinoma patients. *Clin. Cancer Res.* **15**, 2148–2157 (2009).
120. Mueller, M. M. & Fusenig, N. E. Friends or foes — bipolar effects of the tumour stroma in cancer. *Nat. Rev. Cancer* **4**, 839–849 (2004).
121. Sugimoto, H., Mundel, T. M., Kieran, M. W. & Kalluri, R. Identification of fibroblast heterogeneity in the tumor microenvironment. *Cancer Biol. Ther.* **5**, 1640–1646 (2006).
122. Henry, L. R. *et al.* Clinical implications of fibroblast activation protein in patients with colon cancer. *Clin. Cancer Res.* **13**, 1736–1741 (2007).
123. Tsujino, T. *et al.* Stromal myofibroblasts predict disease recurrence for colorectal cancer. *Clin. Cancer Res.* **13**, 2082–2090 (2007).
124. Abramsson, A., Lindblom, P. & Betsholtz, C. Endothelial and nonendothelial sources of PDGF-B regulate pericyte recruitment and influence vascular pattern formation in tumors. *J. Clin. Invest.* **112**, 1142–1151 (2003).
125. Gerhardt, H. & Betsholtz, C. Endothelial–pericyte interactions in angiogenesis. *Cell Tissue Res.* **314**, 15–23 (2003).
126. Maciag, P. C., Seavey, M. M., Pan, Z. K., Ferrone, S. & Paterson, Y. Cancer immunotherapy targeting the high molecular weight melanoma-associated antigen protein results in a broad antitumor response and reduction of pericytes in the tumor vasculature. *Cancer Res.* **68**, 8066–8075 (2008).
127. Lund, A. W. & Swartz, M. A. Role of lymphatic vessels in tumor immunity: passive conduits or active participants? *J. Mammary Gland Biol. Neoplasia* **15**, 341–352 (2010).
128. Feig, C. *et al.* The pancreas cancer microenvironment. *Clin. Cancer Res.* **18**, 4266–4276 (2012).
129. Bhowmick, N. A., Neilson, E. G. & Moses, H. L. Stromal fibroblasts in cancer initiation and progression. *Nature* **432**, 332–337 (2004).
130. Strnad, H. *et al.* Head and neck squamous cancer stromal fibroblasts produce growth factors influencing phenotype of normal human keratinocytes. *Histochem. Cell Biol.* **133**, 201–211 (2010).
131. LeBedis, C., Chen, K., Fallavollita, L., Boutros, T. & Brodt, P. Peripheral lymph node stromal cells can promote growth and tumorigenicity of breast carcinoma cells through the release of IGF-I and EGF. *Int. J. Cancer* **100**, 2–8 (2002).
132. Levental, K. R. *et al.* Matrix crosslinking forces tumor progression by enhancing integrin signaling. *Cell* **139**, 891–906 (2009).
133. Shields, J. D. *et al.* Autologous chemotaxis as a mechanism of tumor cell homing to lymphatics via interstitial flow and autocrine CCR7 signaling. *Cancer Cell* **11**, 526–538 (2007).
134. Chauhan, V. P. *et al.* Angiotensin inhibition enhances drug delivery and potentiates chemotherapy by decompressing tumour blood vessels. *Nat. Commun.* **4**, 2516 (2013).
135. Peranzoni, E., Rivas-Caicedo, A., Bougherara, H., Salmon, H. & Donnadieu, E. Positive and negative influence of the matrix architecture on antitumor immune surveillance. *Cell. Mol. Life Sci.* **70**, 4431–4448 (2013).
136. Straussman, R. *et al.* Tumour micro-environment elicits innate resistance to RAF inhibitors through HGF secretion. *Nature* **487**, 500–504 (2012).
137. Steg, A. D. *et al.* Stem cell pathways contribute to clinical chemoresistance in ovarian cancer. *Clin. Cancer Res.* **18**, 869–881 (2012).
138. Fletcher, A. L., Acton, S. E. & Knoblich, K. Lymph node fibroblastic reticular cells in health and disease. *Nat. Rev. Immunol.* **15**, 350–361 (2015).
139. Junt, T., Scandella, E. & Ludewig, B. Form follows function: lymphoid tissue microarchitecture in antimicrobial immune defence. *Nat. Rev. Immunol.* **8**, 764–775 (2008).
140. Malhotra, D., Fletcher, A. L. & Turley, S. J. Stromal and hematopoietic cells in secondary lymphoid organs: partners in immunity. *Immunol. Rev.* **251**, 160–176 (2013).
References 138–140 are comprehensive reviews of the field of lymph node stromal cell biology.
141. Peduto, L. *et al.* Inflammation recapitulates the ontogeny of lymphoid stromal cells. *J. Immunol.* **182**, 5789–5799 (2009).
142. Pitzalis, C., Jones, G. W., Bombardieri, M. & Jones, S. A. Ectopic lymphoid-like structures in infection, cancer and autoimmunity. *Nat. Rev. Immunol.* **14**, 447–462 (2014).
143. Goc, J., Fridman, W. H., Sautes-Fridman, C. & Dieu-Nosjean, M. C. Characteristics of tertiary lymphoid structures in primary cancers. *Oncoimmunology* **2**, e26836 (2013).
This review provides a great introduction to tertiary lymphoid structures in cancer and their various roles in shaping the immune landscape in the TME.
144. Wirsing, A. M., Rikardsen, O. G., Steigen, S. E., Uhlin-Hansen, L. & Hadler-Olsen, E. Characterisation and prognostic value of tertiary lymphoid structures in oral squamous cell carcinoma. *BMC Clin. Pathol.* **14**, 38 (2014).
145. Germain, C. *et al.* Presence of B cells in tertiary lymphoid structures is associated with a protective immunity in patients with lung cancer. *Am. J. Respir. Crit. Care Med.* **189**, 832–844 (2014).
146. Peske, J. D. *et al.* Effector lymphocyte-induced lymph node-like vasculature enables naive T-cell entry into tumours and enhanced anti-tumour immunity. *Nat. Commun.* **6**, 7114 (2015).
147. Martinet, L. *et al.* Human solid tumors contain high endothelial venules: association with T- and B-lymphocyte infiltration and favorable prognosis in breast cancer. *Cancer Res.* **71**, 5678–5687 (2011).
148. Cipponi, A. *et al.* Neogenesis of lymphoid structures and antibody responses occur in human melanoma metastases. *Cancer Res.* **72**, 3997–4007 (2012).

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Competing interests statement
The authors declare competing interests: see Web version for details.