Targeting the tumour stroma to improve cancer therapy

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Abstract

Cancers are not merely composed of cancer cells alone and, instead, are complex 'ecosystems' comprising many different cell types and noncellular factors. The tumour stroma is a critical component of the tumour microenvironment, where it has crucial roles in tumour initiation, progression, and metastasis. Most anticancer therapies target cancer cells specifically, but the tumour stroma can promote resistance of cancer cells to such therapies, eventually resulting in fatal disease. Therefore, novel treatment strategies should combine anticancer and antistroma agents. Herein, we provide an overview of the advances in understanding the complex cancer cell–tumour stroma interactions, and discuss how this knowledge can result in more effective therapeutic strategies, which might ultimately improve patient outcomes.

Introduction

Despite the increasing availability of therapeutic options, cancer remains the second leading cause of death in the USA (>600,000 estimated deaths in 2017)[1]. However, the rationale for most anticancer therapies is to target malignant cancer cells while largely ignoring the surrounding noncancer cells components of the tumour, or tumour microenvironment (TME). The TME is comprised of all the nonmalignant host cellular and noncellular components of the tumor niche, including, but not limited to, the immune system, blood cells, endothelial cells, fat cells, and the stroma. Over the past decades, the role of the TME in determining disease progression and treatment outcomes has become increasingly evident. Models that describe the effect of the TME on cancer behaviour have been inspired in a number of ecological paradigms, including Paget's 'seed and soil' hypothesis, ecosystems networks, and the optimal foraging theory[2–6]. These models highlight the complexity of cellular and noncellular interactions within a tumour, many of which support tumour growth and confer resistance to therapies targeting cancer cells. Studies in

experimental cancer models have provided ample evidence to support these theories and emphasize the need for therapeutic agents that target the TME.

As a critical component of the TME, the tumour stroma has a profound effect on many hallmarks of cancer[7]. The stroma is comprised of acellular and noncellular connective tissue that supports functional tissue. Though this paradigm took decades to gain acceptance, the stroma has been demonstrated to have crucial roles in tumorigenesis, cancer progression, metastasis, and therapy resistance. These effects are achieved through the intrinsic properties of the stroma and through additional tumour-promoting properties gained as part of an adaptive response to therapeutic intervention. The combination of cancer cell-autonomous mutations (and other alterations) coupled with changes to the tumour stroma drives tumorigenesis and, ultimately, results in fatal disease. As such, cancer therapeutic strategies that do not take the stroma into account are inadequate. The curative effects of such therapies would be greatly enhanced by combining them with strategies to inhibit the tumour-promoting properties of the stroma. Extensive work has been done to explore the interactions between cancer cells and the stroma, but these advancements remain to be translated into anticancer therapy design. Herein, we address the current state of tumour stroma research and efforts to target the tumour stroma.

Components of the stroma

In any tissue, the main function of stromal factors is to structure and remodel functional tissue. These actions require a variety of macromolecules and cells, each contributing in different ways; understanding the physiological roles of each component is critical to understanding how they affect tumour behaviour. The stroma is composed of specialized connective-tissue cells, including fibroblasts, mesenchymal stromal cells, osteoblasts, and chondrocytes, and the extracellular matrix (ECM) (FIG. 1). Other researchers in the TME field occasionally include other specialized cell types, such as endothelial cells, pericytes, adipocytes, and immune cells, as members of the stromal compartment, but we posit that these cells are more accurately defined as nonstromal cells within the TME; although we define these cells as nonstromal, they substantially influence tumour growth, metastasis, and therapeutic resistance. For example, endothelial cells provide nutrients for tumour growth, constitute routes for metastatic dissemination through angiogenesis, and contribute to resistance to chemotherapies and radiation[8–10]. Pericytes also contribute to angiogenesis and confer resistance to antiangiogenic therapy[11,12]. Adipocytes support cancers mainly through the secretion of growth factors and cytokines, and have also been shown to have roles in resistance to chemotherapies, radiotherapy, hormone therapy, and targeted therapies[13]. Immune cells influence protumorigenic phenotypes (epithelial-to-mesenchymal transition, angiogenesis, and therapy resistance) and antitumour phenotypes (immune surveillance) through diverse and complex mechanisms[11,14–16]. We recognize the importance of these and other cells in cancer progression and therapy; however, their function is beyond the scope of this Review, in which we focus on the ECM, fibroblasts, mesenchymal stromal cells, osteoblasts, and chondrocytes.

The extracellular matrix

The ECM provides structure and support for the cellular components in the extracellular space of tissues and organs, and contributes to paracrine cellular signalling[17]. The ECM is comprised of highly organized interactions of fibrous molecules, proteoglycans, glycoproteins, glycosaminoglycans, and other macromolecules. Approximately 300 different proteins have been catalogued as ECM components[18]. During embryonic development, the composition of the ECM becomes specialized in each individual organ to suit its unique needs and functions[19].

The ECM is slowly and constantly degraded by enzymes, such as collagenases and matrix metalloproteases (MMPs)[19,20], and replaced by fibroblast secretions[21]. This constant remodelling process provides the tissue with a continuous supply of growth factors, which supports tissue homeostasis and wound repair.

The ECM has two main compartments: the basement membrane and the interstitial ECM[19]. The basement membrane separates epithelial cells from interstitial ECM and other tissues. Epithelial cell attachment to the basement membrane involves integrin binding[25]. Stromal fibroblasts are usually unable to penetrate the epithelial compartment though the basement membrane, but other cell types (such as immune cells or nerve-cell protrusions) can penetrate this barrier. The interstitial ECM occupies the extracellular space between the epithelium and other tissues. Collagen is the most abundant protein in the interstitial ECM, although many noncollagenous macromolecules, including elastin, fibronectin, tenascin, or laminin, have critical roles within this compartment. All components of the interstitial ECM contain binding domains for other macromolecules and cell-membrane receptors, enabling them to fulfill their structural and signalling roles.

Fibroblasts

Fibroblasts have multiple functions that shape the ECM in which they reside. For example, they build the ECM by secreting collagens and other fibrous macromolecules, but also degrade this network by releasing proteolytic enzymes, such as the MMPs, which enables increased cell mobility throughout the ECM (insert reference from list: Alexander et al 2016). The interactions between fibroblasts and the ECM or surrounding cells, mediated by integrin signalling, affects collagen-fibre alignment and tension (insert reference from list: Alexander et al 2016). Likewise, collagen fibres have an effect on the distribution of fibroblasts throughout the ECM (insert reference from list: Laurent et al. 2007).

Fibroblasts also have an important role in tissue maintenance and homeostasis by expressing enzymes from the cytochrome P450 (CYP) family, which degrade foreign and potentially toxic molecules. These cells also recruit and regulate leukocyte infiltration and inflammation via secretion of cytokines, chemokines, and growth factors[26,27]. Apart from these canonical functions, fibroblasts might have unique functions depending on the tissue in which they reside[28–30].

Fibroblasts exert many of their functions in a nonproliferative state, in which they are able to migrate and secrete ECM components and signalling protein. Tissue wounds and certain pathological conditions can activate fibroblasts in such a way that they proliferate and

secrete higher levels of proteins in an aberrant way. As such, fibroblasts act as sentinel cells in these conditions, in which the accumulation of fibroblasts and stroma can be one of the first symptoms. Myofibroblasts are further differentiated fibroblasts that are present in the reactive stroma during wound healing or in other processes[34–36]. Reactive stroma refers to the process of stromal cell infiltration into a wound site or tumour and the resulting deposition of proteins like collagens, fibrin, and fibronectin (insert reference from list: Dvorak 1986). In cancer, and owing to the presence of an activated stroma, fibroblasts typically resemble myofibroblasts[37,38].

Mesenchymal stromal cells

The definition of the characteristics of mesenchymal stromal cells have evolved over time, and their characterization remains an active area of research[39]. This cell population was first isolated from bone marrow and, owing to being distinct from the haematopoietic lineage, referred to as colony-forming unit fibroblasts[40–43]. Further preclinical studies showed that these bone marrow stromal cells could differentiate into osteoblasts, chondrocytes, adipocytes, or myocytes depending on the culture conditions[44–46]. Currently, multipotent stromal cells are commonly referred to as mesenchymal stem cells (MSCs), although this moniker is often replaced with the broader term mesenchymal stromal cells (also abbreviated MSCs) because of the uncertainties regarding their stem-cell nature[47,48] — the defining characteristics of MSCs are not consistent in the literature. A further source of uncertainty comes from the fact that fibroblasts and MSCs express similar cell-surface markers and have a similar phenotype[48,49]. Moreover, both fibroblasts and MSCs have been shown to have multipotent and immunomodulatory functions[50–52].

To avoid confusion, in this Review we define MSCs as cells that are able to adhere to plastic surfaces, have the capacity to differentiate into osteoblasts, chondrocytes or adipocytes in culture, and express the cell surface markers CD73, CD90, and CD105, but not any leukocyte markers[53,54]. These criteria distinguish MSCs from fibroblasts, although some similarities between both cell types remain[49,54]. While both MSCs and fibroblasts occupy the stroma of many tissues, MSCs are capable of migrating through the body via blood vessels, whereas less evidence exists that fibroblasts migrate via circulation[55–57]. Relative to fibroblasts and other stromal cell types discussed herein, MSCs are rare[58]. Their primary functions are to regulate the immune response and to promote tissue regeneration[44,59–62]. They are also the source of osteoblasts and chondrocytes in bones and joints, respectively.

Osteoblasts

Osteoblasts are unique stromal cells responsible for building bone, a highly specialized ECM[63] consisting of a mixture of collagen and noncollagenous proteins that is subsequently calcified with hydroxyapatite[64,65]. Osteoblasts become embedded within this specialized matrix, eventually becoming osteocytes, which form a network of cells within bone[66]. During bone formation, osteoblasts secrete soluble molecules that activate osteoclasts (bone-resorbing cells). Bone formation then takes place in pockets of osteoclast-mediated resorption[67]. Thus, bone is continually formed and resorbed in a tightly controlled process[68].

Chondrocytes

Chondrocytes, also differentiated from MSCs, are a major stromal component. Chondrocytes produce cartilage, the specialized ECM present in joints and other cartilaginous tissues[69]. Cartilage also provides a structure for bone formation during embryonic development[70]. Chondrocyte are thought to have a lower proliferation rate than other stromal cell types[71].

Stroma–cancer interactions

Tumours are composed of cancer cells and surrounding stromal cells (FIG. 1). Tumorigenesis, cancer progression and metastasis are strongly dictated by cell-autonomous genetic and epigenetic changes, but tumour–stroma interactions have also been demonstrated to be critical in these processes[7,72]. Paget's description of the ability of cancer cells ('seeds') to grow only in certain secondary sites depending on their microenvironment ('soil')[85] remains accurate. Herein we will focus on carcinomas, which arise from the epithelium and represent 90% of all cancers, as well as on haematological malignancies, in which most of the research into the roles of the tumour stroma on cancer therapy has been conducted, rather than on sarcomas, which are cancers that originate directly from stromal cells.

Stating that the stroma is always tumour-promoting would be inexact; indeed, the stroma is tumour-suppressing in some situations. Tumours behave as an ecosystem within which stromal cells in the TME dynamically interact with cancer cells, and can either compete or cooperate with them — resulting in suppression or promotion of further tumour progression. These interactions have been shown to alter the genotype and phenotype of cancer cells[86]. Herein, we will discuss the main advances in this area of intense investigation, mostly concerning fibroblasts.

Tumour-suppressing actions of the stroma

Stromal cells usually exist in a nonproliferative state, and therefore have historically been referred to as 'bystanders' during cancer development[87]. In most nonmalignant tissues, the stroma is tumour-suppressing by nature. Indeed, the stroma has crucial roles in tightly regulating the ability of differentiated epithelial cells to proliferate, move, and invade the ECM, in order to maintain organ size and structure[88,89]. Because of this regulatory action, the nonmalignant stroma also suppresses tumorigenic hallmarks, such as cell proliferation and invasion. The pancreatic stroma, in particular, is recognized as having tumour suppressive effects in the premalignant context[90].

One of the ways stromal cells regulate epithelial cell function is through the secretion of growth factors. TGFβ, commonly secreted by fibroblasts, can either suppress or promote tumorigenesis depending on the context (insert references from list: Bhowmick et al 2004; Keight et al 2012; and Calon et al 2012). In a mouse model, inactivation of one of the TGFβ receptors in fibroblasts resulted in intraepithelial neoplasia of the prostate, a lesion that can precede cancer formation, and in invasive squamous cell carcinoma of the stomach[92].

During tumour development, an inactive stroma can also have suppressive properties. In certain cancer types (such as pancreatic ductal adenocarcinoma, or PDAC), stromal cells can prevent existing tumours from progressing to advanced-disease stages. In mice genetically

engineered to develop PDAC via conditional deletion of *p53* and Sonic hedgehog *(Shh)* and simultaneous expression of oncogenic *Kras*, it was observed that more aggressive tumours, measured by tumour size, metastasis, and survival, had less stromal content, measured by alpha-smooth muscle actin (ACTA2) expression[93]. Depletion of cancer-associated fibroblasts (CAFs) in PDAC has also been shown to cause immunosuppression[90].

The expression of proteins from the metalloproteinase inhibitor (TIMP) family in fibroblasts controls the structural organization of the ECM and stromal cells[94]. These proteins are endogenous negative regulators of MMP activity; many cancers have aberrant expression of TIMPs and/or MMPs[95]. Loss or reduction of expression TIMPs causes an increase in MMP function and enables activation of the stroma and subsequent tumour progression[96,97]. Overexpression of TIMPs, on the other hand, reduces tumorigenesis, growth, angiogenesis, and metastasis in cancer models, such as those of the pancreas[98].

In metastatic disease, tissue-specific stromal phenotypes might suppress metastasis. As Paget described[85], cancer cells only grow in secondary organs with which they are compatible, implying that certain TMEs do not support the outgrowth of disseminated tumour cells while others are permissive[99,100]. In general, the stroma can be considered tumour-suppressive in the early stages of the natural history of cancer or metastasis. When tumours within an epithelium or secondary cancer site get an advanced-stage phenotype, forming an acidic and hypoxic TME[2], the signalling context leads to activation of the stromal cells.

Tumour-promoting actions of the stroma

A publication from 1986 described the physiological similarity between the stroma of a wound and that of a tumour[101]. This activated stroma is called desmoplasia or fibrosis, and is frequently observed by pathologists in sections of tumour tissue. In advanced-stage tumours, the activated stroma promotes the further acquisition of genetic and epigenetic changes in cancer cells[102–104], and supports cancer progression[84]. Other researchers propose that cell-autonomous changes in the stroma can induce tumorigenesis, posing a 'chicken or egg' quandary, although most of the evidence points to cancer cells activating the stroma, and not the other way around. Herein we present specific examples of the tumour-promoting effects of the stroma with the caveat that it is impossible to discuss every facet of this complex field.

CAFs are perhaps the best studied cell type in the TME[105]. Unlike fibroblasts in nonmalignant tissue, CAFs are not quiescent, inert supportive cells in the ECM but, instead, are proliferative, migratory, and highly secretory cells[87,106]. Because they have an altered shape (multispindled rather than single-spindled[105]) and express ACTA2 and prolyl endopeptidase (FAP), CAFs are often referred to as myofibroblasts[106]. MSCs are also sometimes included in the CAF population[49]. CAFs secrete ECM factors that are different to those secreted by nontransformed fibroblasts, including tenascin, periostin, SPARC, and collagens[105,107–113]. The tumour ECM has an altered organization, is more rigid and contractile than that in the nonmalignant stroma and, among other effects, can downregulate the expression of the tumour suppressor PTEN in cancer cells[114]. This altered ECM promotes the growth, survival, and migration of cancer cells, and drives angiogenesis[115–119].

Kaukonen *et al.* showed that breast cancer cells cultured with ECM derived from nonactivated fibroblasts proliferated significantly less than those cultured with an ECM derived from CAFs[120]. Similarly, in comparison with nontransformed fibroblasts, CAFs were able to promote their proliferation of co–cultured SV40 T-immortalized prostate epithelial cells *in vitro* and to induce tumour growth *in vivo* when transplanted together in the renal capsule of mice[121].

The expression of MMPs and other enzymes that degrade and metabolize the ECM is increased in CAFs, enabling cell penetration through the ECM[122–124]. In comparison with non-activated fibroblasts, CAFs also secrete higher amounts of growth factors, cytokines, and chemokines, which promote cancer cell-intrinsic hallmarks of cancer in autocrine and paracrine fashions. Tumour-promoting factors secreted by CAFs include, among others, TGFβ, FGFs, HGF, PDGF, VEGF, transcription factor p65 (commonly known as NF-κB), TNFα, IFNγ, SDF-1α, IL-6, CTGF, EGF, growth arrest-specific protein 6 (GAS6), galectin-1, secreted frizzled-related protein 1, sonic hedgehog (SHH), and BMPs[92,116,125–146]. For example, when media from TGFβ2 receptor-null fibroblasts was added to breast cancer cells in culture, the cancer cells migrated and scattered[147]. This migratory phenotype was largely induced by secretion of HGF by fibroblasts, which signalled through hepatocyte growth factor receptor (MET) and macrophage-stimulating protein receptor (Ron) on breast cancer cells. These findings indicate a complex signalling regulatory mechanism whereby inhibition of one signalling pathway in the cancer cells lead to secretion of a different growth factor by fibroblasts and subsequent activation of the cognate receptor on the cancer cells. A breast cancer xenograft study showed that the invasive tumour stroma contains chemokines differentially throughout tumour progression. Early in tumour development, cancer cells were positive for SDF1 (also known as CXCL12) and its receptor CXC-chemokine receptor 4 (CXCR4), while the stroma was negative for these markers. When the tumour became invasive, however, the stromal cells expressed SDF1 and CXCR4, suggesting that cancer–stroma interaction became more permissive to invasion[148]. A systems biology approach to determining the tumorigenic properties of secreted fibroblast factors has been undertaken in a breast cancer setting, in which three out of five selected secreted factors promoted tumorigenicity, while the remaining two had little impact on tumour growth[149]. Moving forwards, this type of systemic analysis will be important to elucidate targetable stromal factors, as well as to determine which combinations of factors has synergistic effect on tumour growth.

With increasing human age, CAFs can convert into a senescent state. Senescent fibroblasts acquire alternative phenotypes that promote cancer progression, a phenomenon known as senescence-associated secretory phenotype (SASP), which is characterized by the secretion of molecular factors such as cytokines, chemokines, and metabolites that drive malignant transformation[150,151]. For example, senescent osteoblasts drive increased osteoclastogenesis and tumour cell seeding in the bone by secreting high levels of IL-6; thus, senescence-induced changes of the bone stroma can change the ability of tumour cells to seed and/or grow in this milieu[152]. Osteopontin, secretion of which undetectable in proliferating fibroblasts, is upregulated in senescent fibroblasts and necessary and sufficient to drive tumorigenesis in preneoplastic cells in *in vitro* and murine models[153].

Similarly to circulating tumour cells (CTCs), CAFs seem to circulate in the blood[154]. Circulating CAFs (based on FAP and ACTA2 expression) were found in 88% of patients with metastatic breast cancer and in 23% of patients with nonmetastatic disease[154]. Similarly, circulating fibroblast-like cells (positive for vimentin expression and negative for cytokeratin expression) were found in 58% of patients with metastatic prostate cancer, but were absent in patients with nonmetastatic disease[155]. These vimentin-positive/cytokeratin-negative cells might be cancer cells that have undergone epithelial-to-mesenchymal transition, although the absence of cytokeratin expression suggests that they are likely to be fibroblast-like cells (CTCs are typically defined as cytokeratin-positive). Interestingly, stromal annexin A2 expression was higher in the tumours of patients who had a majority of CTCs with a mesenchymal phenotype than in those with a majority of epithelial-like CTCs[156]. These data suggest that circulating CAFs might have a role in the metastatic process, as they are found in patients with metastatic disease at a higher rate than those with localized disease. Some have suggested that CAFs play a role in preparing a premetastatic niche for cancer cells, although this has yet to be firmly established[157].

The bone marrow is an important source of stromal cells that can promote primary tumour growth and progression and, ultimately, metastasis to bone. The results of several studies[138, 158–160] have shown that bone marrow-derived stromal cells circulate to primary tumour sites where they promote tumorigenic activities and properties. Other researchers argue that the tumour stroma is primarily derived from precursor cells present within the local primary tumour, while bone marrow-derived circulating precursor cells are rare[161]. In a metastatic setting, MSCs and osteoblasts attract tumour cells to the bone marrow by secreting a local gradient of chemoattractants, such as SDF1 or GAS6[143,162–164]. Once in the bone marrow, breast cancer cells can sequester SDF1 and affect the response of haematopoietic cells to this signalling factor in *in vitro* co-culture assays[165]. Similarly, prostate cancer cells compete with haematopoietic stem cells (HSCs) for space and nutrients within the osteoblastic niche of the bone marrow[164,166]. Cancer cells of bone-invasive tumours also secrete ligands, such as SHH, which up-regulate RANKL expression in osteoblasts, leading to osteoclast activation and bone resorption[167,168].

Tumour-induced bone resorption leads to expression and release of factors such as TGFβ, which can induce tumour growth and proliferation and further bone resorption in mouse models[169]. This signalling interaction is an example of the so-called 'vicious cycle' whereby tumour cells, osteoblasts, and osteoclasts secrete stimulatory factors that promote the activation of each cell type, typically leading to net osteolysis and tumour growth and invasion[170,171]. Osteoblasts themselves might be able to initiate this cycle[172]. In osteoclasts, expression of RUNX2 has been linked in experimental models and patient samples to the upregulation of factors that promote cancer metastasis to the bone, such as the BMPs[173]. Tumour-induced pressure causes increased osteocyte secretions (for example, of CCL5 and MMPs), which promote the growth of prostate cancer-derived bone metastasis in mouse models[174].

Some of the earliest studies of the profound effect of the stroma on the epithelium were conducted by Cunha and collaborators in the context of prostate development and tumorigenesis[175–179]. These investigators first found that, depending on the type of

urogenital mesenchyme used in *in vivo* epithelial-mesenchymal tissue recombination experiments, epithelial cells would differentiate and grow in vastly dissimilar ways[176–179]. For example, they showed that when the testicular feminization syndrome (Tfm) mesenchyme was recombined with wild-type mouse epithelium and grown in the mouse renal capsule, female sex organs developed; alternatively, when wild-type mesenchyme was recombined with Tfm epithelium, male sex organs developed [175]. These results indicate that the stroma can drive the phenotypic determination of the epithelium, even when the latter is genetically predisposed to display a different phenotype. Similarly, when fully differentiated bladder cells were combined with a neonatal rat seminal vesicle mesenchyme and placed into mice, it resulted in prostate glandular and acinar differentiation[180]. Similarly, nonmalignant prostate cells can be transformed into permanently malignant cancer cells by mixing them with CAFs in both *in vitro* and *in vivo* experiments[121,181]. Moreover, the irradiated mammary gland stroma promoted tumour growth of non-irradiated epithelial cells[182].

The stroma has also been shown to promote the acquisition of stem-like properties by cancer cells, providing a fertile niche in which primary cancer cells can grow, and metastatic cancer cells can colonize[183,184]. The early stages of colon cancer are thought to be driven by the stem cell niche: stromal fibroblasts secrete Wnt ligands that promote β-catenin-dependent signalling in colon epithelial cells, driving a cancer stem cell-like phenotype[185]. In this setting, colon cancer cells located at longer distances from the stroma had lower β-catenin activity and a more differentiated phenotype than those in close vicinity of the stroma[185]. In a metastatic setting, the lung stroma has also been shown to provide a fertile niche for colonization by cancer cells[186].

In addition to their reactive nature, and similarly to cancer cells, stromal cells can undergo genetic changes[187]. Mutations in the tumour suppressor genes *TP53* and *PTEN* have been found in the stromal compartment of human cancers (separated from epithelial cells using laser microdissection)[188,189]. Depletion of *APC* in the stromal compartment can lead to endometrial cancer tumorigenesis in a mouse model[190]. An activating β-catenin mutation in osteoblasts precedes acute myelogenous leukaemia (AML) in approximately 38% of patients[191]. These data indicate that mutations might occur in stromal cells before they affect the cancer cells, subsequently driving tumorigenesis. Further efforts to determine the molecular underpinnings on the conversion of non-activated fibroblasts to CAFs in both patient-derived tumour samples and mouse models have revealed several signalling mechanisms, including activation of the Hippo pathway (reference is Calvo 2013), loss of p53 (reference is Procopio 2015), or activation of heat shock factor 1 (HSF1) (reference is Scherz-Shouval 2014), that enhance tumour-promoting properties of CAFs in several tumour types (Tyekucheva 2017 is a general reference to the molecular undrpinnings of stromal-epitehlial interactions)[192–195].

Epigenetic mechanisms can also lead to phenotypic changes in CAFs; the differential gene expression observed in CAFs relative to non-activated fibroblasts[196–199] is largely the result of changes in DNA methylation and other epigenetic alterations[187,200–204]. A specific example has been shown in the conversion of non-activated lung fibroblasts into CAFs via p300-histone acetyltransferase-mediated acetylation of STAT3, which causes an invasive

phenotype in CAFs after exposure to the cytokine leukaemia inhibitory factor (LIF), derived from either tumour cells or other stromal cells[204,205]. Global hypomethylation, commonly observed in epithelial cells in carcinoma, has also been observed in CAFs from gastric cancer[206].

Many stromal cell types have been clearly shown to have a role in supporting, and potentially initiating, tumour growth, with most of the evidence coming from studies of CAFs, but also of MSCs and osteoblasts. Of note, little to no work has been done on the roles of chondrocytes in tumorigenesis[158].

Effects of tumour stroma on therapy

After receiving any modality of anticancer therapy, cancers tend to recur, even in patients who had a favourable response. The degree to which a patient with cancer will respond to a therapy depends strongly on the extent to which the stroma has become activated[207]. For example, the presence of myofibroblasts can enable prediction of biochemical recurrence in patients with prostate cancer[208], and stromal expression of FAPα is prognostic of resistance to chemotherapy and recurrence in patients with ovarian cancer[209]. Herein, we discuss examples of how specific therapies are hampered by the tumour stroma, with the caveat that this area of study needs additional exploration.

Limiting drug access

The tumour stroma can limit access of therapeutic agents to their target tissues in three ways: fibrosis, high interstitial pressure, and degradation of drugs by stromal enzymes (FIG. 2a). The buildup of a rigid ECM (fibrosis) around and throughout a tumour creates a physical barrier that reduces diffusion of therapeutic agents to cancer cells. A dense ECM can reduce blood vessel density and lead to vessels embedding within the matrix, creating a tough barrier that drugs cannot perfuse, as has been observed in PDAC[211]. In addition, cancer cells can strongly adhere to ECM proteins in order to evade chemotherapy in a process known as cell adhesion-mediated drug resistance (CAM-DR)[212,213]. CAM-DR operates chiefly through direct binding of cancer cells to fibronectin, which is associated with changes in other signalling proteins[214–217]. Interstitial pressure is higher in the TME than in nonmalignant tissue [218–221]. This difference can affect drug diffusion and delivery, as demonstrated in PDAC[222,223], melanoma[224] or glioma[225].

Fibroblasts, particularly in the bone marrow stroma, express CYPs that metabolize a variety of potentially toxic molecules, including chemotherapeutic drugs such as docetaxel[226]. CYP3A4 is expressed by stromal cells in the bone marrow niche, commonly seen as a chemoprotective microenvironment. Upon docetaxel treatment, tumours co-cultured with primary human bone marrow fibroblasts negative for CYP3A4 expression (via shRNA knockdown) reached smaller sizes than those co-cultured with fibroblasts from the same source expressing CYP3A4[227]. In a mouse model of multiple myeloma, bone marrow fibroblast expression of CYP26 resulted in a microenvironment with low expression of retinoic acid, which promoted a bortezomib-resistant phenotype[228].

Resistance to cytotoxic chemotherapy

Classic cytotoxic chemotherapy targets rapidly proliferating cells. Stromal secretion of ligands promoting proliferation is one of the reasons why cancer cells proliferate rapidly. Chemotherapy cannot eliminate 100% of cancer cells partially due to intrinsic resistant phenotypes within the cancer cell population. Moreover, the tumour stroma can promote cancer cell survival and proliferation in a treatment-naïve setting and in response to treatment, which does not eliminate nonproliferating stromal cells[105]; this intrinsic resistance might be at least partially the result of induction of autophagy rather than apoptosis in response to external agents[229] and enables stromal cells to divert their response to a nonlethal stress response.

Chemotherapy-induced DNA damage in the TME causes a stress response in stromal cells, which then secrete many factors that promote cancer cell survival, proliferation, invasion, and metastasis (FIG. 2b). For example, in a prostate cancer model, DNA damage induced by docetaxel or mitoxantrone caused increase expression of GDNF in stromal cells, which promoted tumour cell proliferation, invasion, and chemotherapy resistance in a paracrine fashion[230]. Docetaxel and mitoxantrone also caused increased stromal secretion of WNT16B, which promotes prostate cancer cell survival in the presence of chemotherapeutic agents[231]. Similarly, in a lymphoma model, doxorubicin led stromal cells to secrete IL-6 and TIMP-1, both of which supported cancer cell survival in the thymus[232]. In other *in vitro* studies, the addition of conditioned media from an immortalized fibroblast cell line to head and neck squamous cell carcinoma (HNSCC)-derived cells increased resistance to cisplatin two-fold relative to cells not treated with conditioned medium[233]. Along the same lines, chemotherapy can cause non-activated fibroblasts to develop a CAF-like phenotype, secreting factors that promote a stem-cell-like phenotype in breast cancer cells[234]. In a colon cancer model, a similar secretory phenotype was observed in fibroblasts treated with 5-fluorouracil or oxaliplatin, which induced a stem-cell-like, chemotherapy-resistant phenotype in cancer cells via a mechanism involving exosomes[235]. Finally, the release of cysteine and glutathione from primary CAFs isolated from ovarian cancer patients was described to lead to ovarian cancer cell resistance to platinum-based therapy by preventing accumulation of platinum in the cell nuclei[236].

Resistance to radiation therapy

Approximately 50% of patients with cancer receive radiation therapy[237], which, like chemotherapy, leads to DNA damage. Similarly to tissue wounding or injury, radiation therapy also results in fibrosis. This response leads to survival and expansion of the number of stromal cells and ECM, which in turn promotes survival and radiation resistance of cancer cells, in addition to providing signals that stimulate their proliferation and invasion (FIG. 2c). Integrin expression has been shown to be consistently upregulated in fibroblasts after exposure to ionizing radiation in *in vitro*, *in vivo*, and *ex vivo* (from cancer patients) studies[238–243]. For example, pancreatic stellate cells (a specialized stromal cell type) upregulate β1 integrin in response to radiation exposure in a FAK-dependent manner, protecting PDAC cells from radiation[241]. β1 integrin expression, along with AKT signaling, is also associated with protection of breast cancer cells from ionizing radiation[242]. Finally,

integrin $\alpha_v\beta_6$ expression increases in lung cancer cells following radiation exposure and precedes the secretion of TGFβ and the development of lung fibrosis[243].

Irradiated fibroblasts also have an increased ability to secrete factors that induce chemoresistance[244]. *In in vitro* models, squamous cancer cell lines co-cultured with irradiated fibroblast cell lines were more proliferative than those co–cultured with non-irradiated fibroblasts [245]. Irradiated fibroblasts also increased the activation of proliferation signalling mechanisms (RAS and the mitogen-activated protein kinase cascade), invasion pathways (MMPs, laminin 5, and filamin A), and TGFβ signalling in cancer cells[245]. In a similar model of PDAC, irradiated fibroblasts increased HGF/MET signalling in neighbouring cancer cells, which enhanced their mobility [246].

Resistance to targeted therapies

Therapies based on agents that inhibit specific molecules or pathways are associated with two major issues: cancers are heterogeneous and thus, only certain cancer cells might be addicted to a particular oncogene; and targeting one protein or pathway typically results in the upregulation of a another pathway, resulting in adaptation, resistance, and recurrence. The stroma assists cancer cells in this adaptation (FIG. 2d).

Bevacizumab is an antibody that targets VEGFA. In a mouse model of lung cancer, treatment with bevacizumab led to acquired resistance, at least partially via upregulation of VEGFA, FGF2, FGFR2, and PDGFRA in stromal cells[248]. In addition, in patients with lung adenocarcinoma, the number of FSP-1-positive fibroblasts in tumours was higher in those who received bevacizumab than in those who did not[248]. Treatment of mice with subcutaneous syngeneic myeloma tumours with anti-VEGF antibody resulted in tumours with therapeutic resistance with CAFs present in the resistant tumours reactivating angiogenesis via PDGFC signalling[133].

Receptor tyrosine kinases (RTKs) are common targets of inhibitory therapies because they are frequently upregulated in cancer cells and promote several hallmarks of cancer. In lung cancer, EGFR is one such receptor, and many patients respond to EGFR inhibitors. However, as is the case of most tyrosine kinase inhibitors (TKIs) of RTKs, resistance develops. In a study of co-cultured lung cancer cells, resistance to EGFR-TKIs was driven by CAFs expressing podoplanin, although the molecular mechanism underlying resistance were unclear[250]. In a breast cancer model, resistance to TKIs was the result of HGF secretion by fibroblasts[251]. HGF also caused resistance to BRAF TKIs in melanoma, colorectal, and glioblastoma-derived cell lines, indicating consistency across cancer types[252]. A seminal study conducted with a melanoma mouse model showed that BRAF-driven tumours develop rapid resistance to anti-BRAF therapies primarily owing to the paradoxical activation of CAFs by such therapies. CAF activation, in turn, signalling downstream of BRAF in resistant cancer cells and promoted their survival[253]. Interestingly, a therapeutic response was observed when CAFs were targeted with an inhibitor of FAK, indicating that cancer cell resistance to targeted therapies can be overcome using fibroblast-targeting strategies.

Resistance to hormone antagonists

Therapies that target hormone signalling are used to treat patients with prostate or breast cancers, which are usually dependent on androgens or oestrogen, respectively. In prostate epithelium, androgen receptor (AR) activity promotes proliferation[254], but in the stroma, AR expression and activation by androgen binding inhibits prostate epithelial proliferation, whereas loss of AR in the stroma correlates with prostate cancer progression, although it is unknown how this observed phenomenon is manifested (please insert reference: Singh et al. 2014). This phenomenon suggests that bipolar androgen therapy (introduction of supraphysiological levels of androgen after androgen deprivation therapy) could be an effective therapeutic strategy[255]. Notably, stromal AR expression decreases with prostate cancer progression, and is associated with increased epithelial cell proliferation and poor outcomes in patients (FIG. 2e)[256,257].

Co-culturing with CAFs has been shown to reduce oestrogen receptor (ER) expression in breast cancer cells, which precedes a hormone-independent state and, therefore, resistance to hormone antagonists (FIG. 2e)[258]. Analysis of conditioned media from co-cultured cancer cells showed a >5-fold upregulation of 46 proteins (including MMPs and TGFβ) compared with media from non co-cultured breast cancer cells[258]. Other studies have shown that the loss of caveolin 1 expression in breast cancer stromal tissue enables prediction of a lack of response to tamoxifen, an ER antagonist[259,260]. Most mechanisms driving resistance to hormone therapy are thought to be cancer cell-autonomous, but these data indicate that, in prostate or breast tumours, the stroma could have important, clinically relevant roles; further studies need to be conducted.

Resistance to immunotherapy

The goal of cancer immunotherapy is to induce a T_H1 immune response, primarily driven by cytotoxic T cells and macrophages (referred to herein as effector immune cells), to eliminate tumour cells that present mutation-associated neoantigens[261]; an effective response to immunotherapy relies on effector immune cells. Tumour-associated ECM and fibroblasts have immune modulatory effects [262,263] (FIG. 2f). One example is the fact that ECM-sequestered tenascin can prevent the infiltration of effector immune cells, which has been evidenced in mice null for tenascin expression, which have higher effector immune cell infiltration in tumours than wild-type mice[264]. The organization of the molecular components of the tumour ECM also has a key role in regulating the localization and migration of effector immune cells throughout the tumour stroma (insert reference from list: Gajewski et al. 2013). For instance, activated T cells were observed in regions of loose fibronectin and collagen, but were not abundant in denser regions of tissue sections from human non-small cell lung cancer tumours[265]. In addition, T and B cell abundance was directly correlated with vessel density in tumour stroma in tissue sections from cancer patients with solid tumours[266].

Inoculation of human tumours into mice has long been known to be more effective in the presence of human stromal factors, an interaction that was thought to be caused by the immunosuppressive functions of the tumour stroma[267]. This effect was confirmed in a lung cancer mouse model, in which FAP-positive fibroblasts suppressed effector immune cell

infiltration into tumours[268]. Stromal cells express the immune-checkpoint protein programmed cell death 1 ligand 1 (PD-L1). The effect of stromal PDL1 expression on immunotherapy outcomes is currently unclear, with some studies showing an association with a better prognosis for patients with breast cancer[269], but also a poor prognosis for patients with adult T-cell leukaemia[270].

Tumour-stroma targeting strategies

Cancer therapies should include strategies to target and constrain the tumour stroma, which can both suppress and promote tumorigenesis. In situations in which the tumour stroma promotes cancer hallmarks and induces resistance to anticancer therapy, the use of therapies targeting the stroma could have curative outcomes. However, such a strategy would be counter-productive in a context in which the stroma is tumour-suppressive and thus, caution must be taken[90]. Reliable biomarkers of stromal activity and tumour-promoting properties will guide these critical therapeutic decisions.

When considering therapeutic strategies to eliminate cancer, stroma-targeting agents must be considered (TABLE 1), although targeting the stroma alone will likely not eliminate the entire tumour. Cancer cells would retain their genetic and epigenetic alterations and, therefore, would likely revert the phenotype of a reactive stroma that might have been rendered completely inert. Agents targeting the tumour stroma should therefore be administered as combination therapies with cytotoxic agents (FIG. 3).

Targeting the ECM

Several strategies exist to prevent the ECM from acting as a barrier by reducing the density of its components and enable diffusion of therapeutic agents. Halofuginone inhibits the synthesis of type I collagen, which has been shown to reduce desmoplasia[271–273]. In a melanoma xenograft model, halofuginone reduced osteolysis and bone metastasis[273]. Likewise, PEGylated hyaluronidase and 4-methylumbelliferone inhibited the secretion of hyaluronic acid in the stroma, thereby decreasing interstitial pressure and enabling the uptake of therapeutic agents[223,274–276]. Lysyl oxidase (LOX) catalyzes collagen crosslinking, and has high levels of activity in the tumour stroma[277,278]. Agents targeting LOX activity could decrease collagen crosslinking, thereby reducing the density of the tumour stroma and increase the effectiveness of anticancer therapies[279–282]. The vitamin D receptor ligand calcipotriol [283] and all-trans retinoic acid (ATRA)[284] are other agents that reduced fibrosis in experiments with PDAC models.

Some therapeutic strategies are focused on penetrating the ECM rather than degrading it. Nanoparticle albumin-bound paclitaxel (nab-paclitaxel) is a taxane conjugated to albumin, which increases the solubility of paclitaxel in the tumour stroma[285]. When combined with gemcitabine, nab-paclitaxel has substantial antitumour activity in patients with PDAC[286]. A novel strategy is the use of amniotic MSCs to deliver cytotoxic drugs to the tumour site[287]. MSCs from other origins can also be used; for example, a prodrug in which the active toxin Leu12ADT is released upon cleavage by prostate-specific antigen (PSA) was bound to microparticles that were coated onto bone marrow-derived MSCs and injected into mice,

resulting in tumour shrinkage[288]. These strategies might be effective in clinical settings because MSCs tend to migrate towards areas of wound healing or tumour growth[289,290].

ECM-based approaches to target the TME

Instead of targeting either cancer cells or the stroma directly, some strategies exploit stromal components for the therapeutic agent to be delivered at the tumour site. One of the oldest examples of this strategy is the use of bisphosphonates to treat bone metastases. Bisphosphonates do not target cancer cells directly (although evidence of their antitumour effects has become available over time[291]) but, instead, uncouple bone turnover, thereby halting the vicious cycle in the bone metastases and depriving cancer cells of bone marrow-derived growth factors[291]. Bisphosphonates are taken up by osteoclasts and inhibit their ability to resorb bone, which also reduces the activity of osteoblasts in the bone stroma[292]. While bisphosphonates decrease the overall number of skeletal-related events in patients with bone metastasis, their administration is rarely associated with improvements of overall survival. Some investigators hypothesize that, by the time bone metastases are clinically relevant and detectable, it is too late for bisphosphonates to be effective. Rather, if these agents were administered to patients before tumour cells colonize the bone pre-metastatic niche, overt metastatic lesions might be preventable (insert new reference: Dhesy-Thind, S, et al. Use of adjuvant bisphosphonates and other bone-modifying agents in breast cancer: a cancer care Ontario and American Society of Clinical Oncology Practice Guideline. J Clin Oncol 2017).

[223]Ra is a radioisotope used to treat patients with bone metastases because it mimics calcium, and therefore substitutes for calcium in the bone mineral matrix during bone formation[295]. Once enmeshed in the bone, [223]Ra releases alpha particles that eliminate surrounding cells, including cancer and stromal cells, in the bone metastatic microenvironment. Thus, this strategy exploits the unique properties of the bone ECM and the high bone turnover of bone metastases to exert cytotoxic effects with a lower risk of adverse effects than with other therapies. This FDA-approved agent increases overall survival in patients with metastatic castration-resistant prostate cancer[296].

Targeting proteins expressed by stromal cells

Similarly to targeted therapy of cancer cells, the tumour stroma can be modulated by targeting specific proteins expressed by stromal cells that are involved in their proliferation, protein secretion, and ECM formation. FAP is a major focus for targeted therapy of stromal cells[297]. In the late 1990s and early 2000s, the anti-FAP monoclonal antibody F19 was tested in clinical trials for the treatment and imaging (via ^{131}I–mAbF19) of colorectal tumours but failed to impart a clinical benefit, likely because the antibodies used did not mediate antibody-dependent cellular toxicity of FAP-expressing stromal cells[298–301]. Subsequently, this strategy evolved and anti-FAP antibodies were conjugated to drugs. FAP5–DM1 is one of such conjugates using the cytotoxic drug maytansine (DM1). The anti-FAP antibody portion delivers the conjugate to FAP-positive fibroblasts, which internalize the compound, cleave the chemical linker, and free the drug portion that induces cell death[302]. FAP5–DM1 induced long-lasting tumour regression with substantial reduction and reorganization of the stromal compartment in xenograft models of pancreatic, lung,

colorectal, and head and neck cancers when compared to mice treated with vehicle or the antibody alone[303]. Anti-FAP antibodies labelled with radioactive tumoricidal ^{177}Lu had a similar action in the stroma of melanoma xenograft mice[304].

Another strategy exploits the endogenous protease activity of FAP to cleave and subsequently activate the prodrug promelittin to release the cytolytic toxin melittin, that eliminates neighbouring cells, including CAFs[305]. This strategy is less specific to CAFs, but the broader effects of this compound might have greater tumoricidal properties; however, the possibility of widespread toxicity is also a concern. ATRA is currently used to treat multiple cancers and, in addition to other activities, it inhibits FAP, αSMA, and TGFβR expression in CAFs, thereby reducing ECM and cytokine secretion[306,307], and subsequently, cancer cell proliferation and invasion. ATRA reverses chemoresistance through multiple mechanisms, but is metabolized by CYP26, which reduces its effects in multiple myeloma[228,309]; inhibition of CYP26 with R115866 reversed the bortezomib-resistant phenotype of multiple myeloma-derived cells in pre-clinical co-culture and xenograft models[228]. Similarly, CYP3A4 metabolizes docetaxel, thereby reducing its cytotoxic activity, and inhibition of stromal CYP3A4 with clarithromycin reversed the chemoprotective effect of this enzyme on multiple myeloma cells co-cultured with primary human bone marrow cells expressing CYP3A4[227]. This strategy could be beneficial to treat patients with prostate cancer bone metastasis, because docetaxel is one of the treatments approved for this population, but cancer recurrence after treatment with this agent is virtually universal[310].

Targeting cancer cell–stroma signalling interactions

The targeted inhibition of cell surface receptors for stromal-secreted factors in cancer cells can be considered an indirect mechanism of targeting the stroma; although, owing to the expression of these receptors on cancer cells, such agents are in fact targeting cancer cells. Moreover, mutation or overexpression of many RTKs leads to tumour cell growth independent of stromal cell-secreted growth factors. Nonetheless, this strategy can be considered to be inhibitory of the effects of the tumour stroma.

In many cancers, CAFs secrete FGFs. Many therapeutic strategies target FGF receptors, from RTK inhibitors (such as dovitinib, which targets FGFR3) to monoclonal antibodies (such as GP369, which targets FGFR2-IIIb) [311–315]. Targeting the FGF2/FGF2R axis in xenograft models has been shown to prevent resistance to bevacizumab[248]. Another novel strategy is to prevent stromal cell-secreted FGFs from binding to their receptors on cancer cells; for example, with a ligand trap for FGF using a soluble fusion protein (FGFR1-IIIc fused to the Fc domain of IgG1)[312].

Combination therapies can generate considerably greater tumour responses than monotherapies. For example, in a mouse model of breast cancer[316], high levels of TGFβ were found in the blood after radiation or chemotherapy. In these mice, treatment with a TGFβ-neutralizing antibody led to a reduction in the numbers of CTCs and lung metastases[316]. In another example, the chemokine SDF1, abundantly secreted by osteoblasts, was shown to act as a chemoattractant for prostate cancer cells in the bone marrow HSC niche[140,164]. Treatment with AMD3100, an inhibitor of the SDF1 receptor CXCR4, resulted in efflux of prostate cancer cells from the bone marrow niche and into the

blood circulation, where they are more sensitive to therapy[164,317,318]. In addition, targeting of CXCR4 in PDAC stroma caused increased immune cell infiltration into the tumour and enhanced responses to immunotherapy in syngeneic mouse models[319]. Thus, the combination of AMD3100 and cytotoxic therapy or immunotherapy holds great promise.

Targeting stromal antigens with immune cells

Chimeric antigen receptor-T (CAR-T) cells are T cells that have been genetically engineered to express antigen-recognizing ectodomains fused to signalling domains from T cell receptors and co–stimulatory molecules[320]. CAR-T cells have the ability to gain access to the epithelial cell compartment by invading through the ECM and evading immunosuppressive signals[321]. In preclinical studies CAR-T cells have been reprogrammed to recognize FAP-positive cells and target stromal cells specifically[322]. Such FAP-specific T cells had single-agent antitumour activity in a mouse lung cancer model, but were associated with sustained antitumour effects and prolonged survival when combined with CAR-T cells specific for the cancer cell antigen erythropoietin-producing hepatocellular carcinoma A2 (EphA2)[323]. Cancer vaccines against the FAP antigen are another option for CAF targeting[324–327]; T cell-mediated elimination FAP-positive CAFs (comparison of treatment with the vaccine or an empty vector control in tumor-bearing mice) caused a reduction in collagen density in the ECM, enabling greater uptake of several chemotherapeutic drugs[326]. FAP-specific vaccines cause tumour regression without the addition of any cytotoxic drugs by stimulation of antitumour immunity, but in combination with other agents, these vaccines might make this strategy more effective.

Macrophages have both profibrotic and antifibrotic properties[328], dependent on their status in the spectrum between M1 and M2 polarization[329]. Agonists of CD40, a co–stimulatory molecule commonly expressed by antigen-presenting cells, can activate macrophages and induce them to degrade the ECM. In several studies, activation of the MMPs in monocytes and macrophages treated with CD40 agonists led to degradation of the ECM and improved tumour infiltration of immune cells as well as response to checkpoint blockade inhibition, warranting further exploration of this strategy in the future[328,330–333].

Finally, chondrocytes, cells often excluded in discussions about the tumour stroma, can be engineered to deliver cytotoxic drugs to tumours. Fibroblasts differentiated to chondrocytes *ex vivo* were engineered to express high levels of IL-12, and irradiated to prevent their proliferation. When these cells were injected into mice bearing colon tumours, they secreted high levels of IL-12, and caused a sizable increase in T cell and natural killer cells infiltration, which eliminated cancer cells and reduced tumour angiogenesis[334]. This unique strategy might have potential in future combinatorial studies.

Conclusions

Cancer develops as a result of an accumulation of genetic and epigenetic alterations[346–348]. Increasing evidence suggests that, in addition to cancer cell-autonomous genetic mutations, stromal pathology often contributes to tumorigenesis[121,181,189–191]. We have presented ample evidence from preclinical and clinical studies that clarify the critical importance of the interactions between cancer cells and the stromal compartment in tumour progression

and therapeutic resistance. Upon exposure to therapies that target cancer cells, the tumour stroma supports cancer-cell survival and recurrence, leading to fatal metastatic disease. To combat this resistance, drugs that target stromal components are in various stages of preclinical and clinical development. In many cases, these stroma-targeting drugs reverse or abrogate resistance to cancer cell-targeting therapies, leading to strong anticancer responses. Despite these discoveries, completely curative strategies are not currently available. The substantial gap in knowledge regarding the optimal sequence and composition of combinations comprising stromal-targeting agents and cancer-targeting agents prevents such strategies from being offered to patients[349]. The order in which these agents are administered is important, because certain sequences can result in adverse effects. For example, when chemotherapy is administered after an immune response has been initiated, it could cause the death of immune cells, thereby reversing their cytotoxic effect. Ongoing clinical trials are assessing the activity and response of the tumour stroma before and after anticancer therapy (NCT03165487). Discovery studies involving laser capture microdissection of stroma[350], coupled with analyses of genetic mutations[351] and epigenetic changes[352] can uncover novel therapeutic targets. These types of studies will provide information about how to better target the stroma in the future.

An important consideration is that many of the experiments addressing cancer cell–stroma interactions are performed using imperfect models, such as co-cultures and xenograft or syngeneic mouse models. These experimental models are fundamental to preclinical cancer research, but substantial gaps exist between the results of these experiments and those obtained in clinical settings. Communication between researchers and medical professionals will be crucial in determining the next steps to bring discoveries to patients. As we continue to develop a better understanding of the complex interactions between a heterogeneous milieu of cellular and noncellular contributors in the TME, we will be able to improve stroma-targeting strategies and design more-effective anticancer therapies.

Acknowledgments

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Box 1

Biomarkers of stromal involvement

Owing to the evident involvement of the stroma in tumour progression and therapy resistance, ideally, specific cell types and/or targetable molecular lesions should be identifiable within the tumour microenvironment or in liquid biopsies that would enable targeting the stroma with therapeutic interventions or stratification of patients into treatment subgroups[335]. The stromal compartment, separate from cancer-specific alterations, might have independent prognostic value [335]. Cancer-associated fibroblasts (CAFs) typically express prolyl endopeptidase and alpha smooth muscle actin. Detection of these markers using immunostaining can enable the identification of CAFs in tumour biopsy samples to assess the extent of fibroblast involvement[336]. This approach would provide evidence on the outcomes of tumour-targeting strategies. Other markers, such as podoplanin, CD99, matrix metalloproteinases, and the transcription factor forkhead box F1 have also been associated with the tumour stroma, but none have been successfully used for clinical purposes[337–340]. An example of a marker with potential clinical utility is the loss of expression of caveolin-1 in the tumour stroma as a predictor of recurrence in patients with breast cancer[259]. In patients with pancreatic cancer, the presence of intratumoural hyaluronic acid could predict the ability of a drug to penetrate the stroma and access the tumour[276]. Beyond single protein markers, gene signatures based on the expression of selected genes could represent the most effective method[341–345]. Ideally, such signatures should be assessed with blood-based assays; similarly to cancer cells, stromal cells are detectable in the blood of patients with cancer[154]. However, little evidence supports the clinical relevance of such a biomarker at present, arguably, as a result of the scarcity of clinical data. Future studies (likely using a systemic approach) should identify clinically relevant molecules of stromal origin that would predict whether specific therapies are effective in individual patients and/or provide a readout of the outcomes of a therapy after it has been administered.

Key points

- Tumours are comprised of cancer cells as well as a stromal compartment with cellular and noncellular components

- The tumour stroma has critical roles in cancer development, progression and metastasis

- Typically, anticancer therapies predominantly target cancer cells, and their effect on the tumour stroma is not taken into account

- The tumour stroma responds to anticancer therapies by inducing therapeutic resistance, which can ultimately lead to fatal disease

- Anticancer therapies should target both cancer cells and the stromal compartment to be effective and result in improved patient outcomes
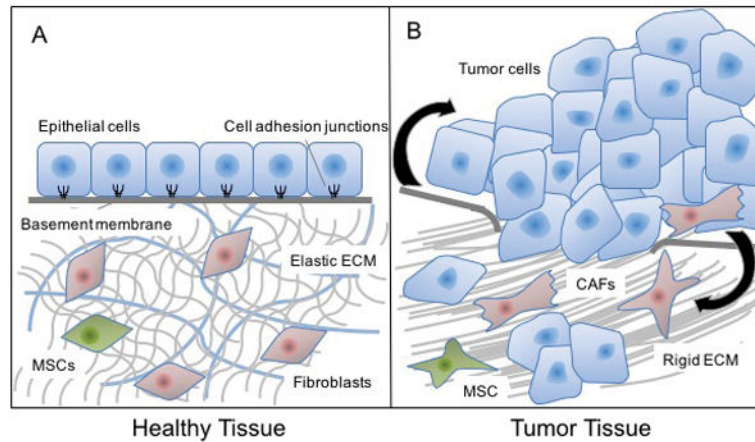
Figure 1. Comparison of nonmalignant stroma and tumour strom
a. Nonmalignant epithelial tissue is supported by a stroma composed of extracellular matrix (ECM), fibroblasts, mesenchymal stromal cells (MSCs), osteoblasts (in bone), and/or chondrocytes (in joints). Cells in the nonmalignant stroma are usually in a quiescent state and maintain homeostasis in the ECM and epithelial compartment, in part by negatively regulating the proliferation, motility, and invasion of cells in the epithelial layer. When cancer develops, the stroma undergoes vast changes to become fibrotic and activated. The ECM becomes denser and more rigid, and is composed of alternative forms of connective fibres, such as tenascin and fibronectin, which cancer cells can invade through. Fibroblasts and MSCs change shape and expression profiles and become more proliferative and secrete higher levels of growth factors, cytokines, and chemokines (black arrows). Stromal fibroblasts in the tumour microenvironment are referred to as cancer-associated fibroblasts (CAFs) or myofibroblasts. The tumour stroma promotes cancer progression and metastasis, and leads to resistance to therapy and disease recurrence.
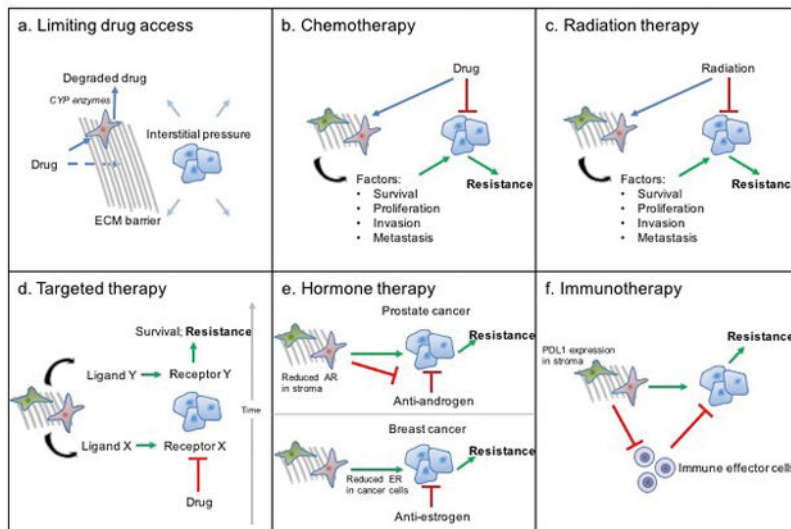
Figure 2. Tumour stroma-mediated chemoresistance

In response to anticancer therapy, the tumour stroma mediates resistance to therapy and disease recurrence. **a |** Dense fibrosis causes limited access of cancer cells to therapeutic agents in three ways: creating an extracellular matrix (ECM) barrier that such agents cannot diffuse through; promoting stromal cytochrome P450 (CYP)-mediated degradation of drugs; and increasing interstitial pressure that prevents therapeutic agents from entering the tumour. **b |** In response to chemotherapy or **c |** radiation therapy, cancer-associated fibroblasts (CAFs) and mesenchymal stromal cells (MSCs) secrete different growth factors, cytokines, and chemokines that promote cancer-cell survival, proliferation, invasion, and metastasis, leading to resistance. **d |** Targeted inhibition of a specific pathway (ligand–receptor X) results in the stromal secretion of new ligands (ligand–receptor Y), resulting in survival and resistance. **e |** In prostate cancer, decreased androgen receptor (AR) expression in the stroma leads to resistance to androgen-deprivation therapies. In breast cancer; the stroma promotes decreased oestrogen receptor (ER) expression in cancer cells, leading to resistance to antihormonal therapies. **f |** CAFs, MSCs, and ECM suppress effector immune cell activation and tumour infiltration.
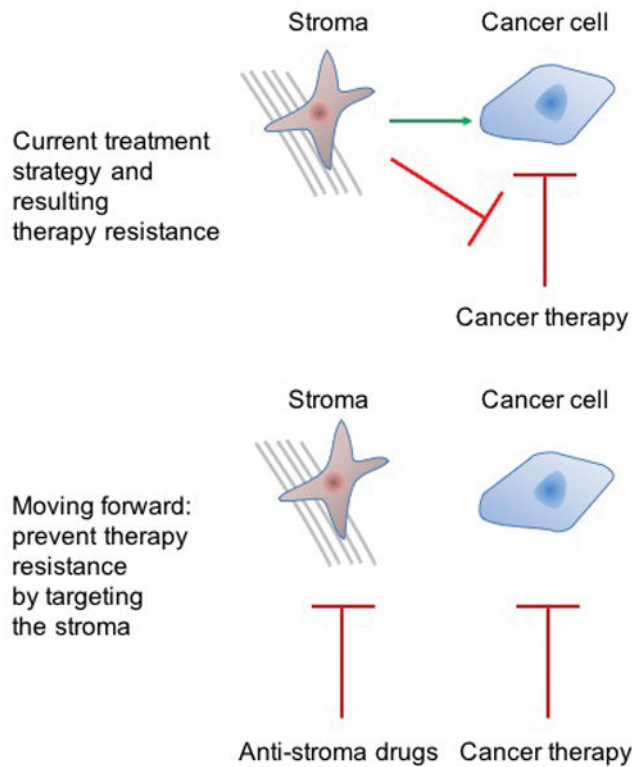
Figure 3. Targeting tumour stromal cells in addition to cancer cells
a | Currently, most antitumour therapies target and eliminate cancer cells, and are not design to directly affect the tumour stroma. **b** | However, tumour recurrence can result from the interactions of the tumour stroma with both cancer cells and anticancer therapies. Through its interaction with cancer cells, the stroma promotes the hallmarks of cancer and can induce a therapy-resistant phenotype. Through its direct interaction with anticancer therapy, the stroma can prevent the action of such therapies on cancer cells. (FIG. 2). **c** | We posit that, in addition to targeting cancer cells, anticancer therapeutic strategies should include methods to target and constrain the stroma, or to revert it to a tumour-suppressive state.

Table 1

Targeting tumor stroma for cancer therapy

Example stromal targets (Preclinical study references)	Example agents involved in cancer clinical trials (ClinicalTrials.gov identifier for representative clinical trial)
CXC-chemokine receptor 4 (Shiozawa, Y. *et al.* 2011; Domanska, U. M. *et al.* 2014; Domanska, U. M. *et al.* 2012)	Plerixafor (NCT01610999)
Cytochrome P450 3A4 (Alonso, S. *et al.* 2015)	Clarithromycin (NCT03043989) Itraconazole (NCT02157883)
FAK (Mantoni, T. S. *et al.* 2011)	Defactinib (NCT03287271)
Prolyl endopeptidase FAP (Hofheinz, R. D. *et al.* 2003; Scott, A. M. *et al.* 2003; Mersmann, M. *et al.* 2001; Welt, S. *et al.* 1994)	Sibrotuzumab (NCT02198274) RO6874813 (NCT02558140)
FGF pathway (Mitsuhashi, A. *et al.* 2015; Bai, A. *et al.* 2010; Chae, Y. K. *et al.* 2017; Katoh, M. *et al.* 2014; Bello, E. *et al.* 2011; Gozgit, J. M. *et al.* 2012)	Dovitinib (NCT01548924) AZD4547 (NCT01791985)
Hyaluronic acid (Provenzano, P. P. *et al.* 2012; Kultti, A. *et al.* 2009; Hajime, M. *et al.* 2007)	PEGPH20 (NCT01453153)
TGFβ pathway (Biswas, S. *et al.* 2007)	Galunisertib (NCT02304419) Fresolimumab (NCT02581787)