ABSTRACT

In addition to malignant cells, solid tumours comprise supporting stromal tissue that consists of Extra Cellular Matrix (ECM), connective tissue cells, inflammatory cells and blood vessels. The stromal compartment and the malignant cells together shape the tumour microenvironment that in turn determines tumour progression and efficacy of anti-tumour treatments. It is now recognized that the host microenvironment undergoes extensive change during the evolution and progression of cancer. This involves the generation of Tumour-Associated Fibroblasts (TAFs), which, through release of growth factors and cytokines, lead to enhanced angiogenesis, increased tumour growth and invasion. It has also been demonstrated that TAFs may modulate the Cancer Stem Cell (CSC) phenotype, which has therapeutic implications. Understanding the various components in the tumour microenvironment may afford us the opportunity to develop new drugs that target these reversible nonmutational events in the prevention and treatment of cancer.

INTRODUCTION

Carcinomas or other solid tumours are comprised of transformed malignant cells embedded in a stroma that consists of Extra Cellular Matrix (ECM), blood vessels, inflammatory and mesenchymal cells[1]. In recent years the tumour microenvironment has become the focus of intense research, with the understanding that the alterations that occur in the stroma around a tumour might prove useful in prognosis and generate new therapeutic targets. The idea that the stromal cells might promote cancer development was first recognized in 1863 when Rudolph Virchow observed leukocytes in the stroma of neoplastic tissue and hypothesized that the malignancy originated at sites of chronic inflammation [2].

In contrast to the normal tissue, the stroma in solid tumours is activated in a way that is reminiscent of chronic inflammatory conditions[3]. The stroma is therefore said to be reactive. Reactive has been shown to facilitate tumour growth and progression. The extensive stroma reaction that is found in many types of carcinomas is referred to as desmoplasia. Even though many tumours are immunogenic, the inflammatory environment in the desmoplasic stroma rather supports tumour growth instead of inhibit it [4]. Like almost all other tissues, tumours need a vascular supply. Blood vessels are formed through angiogenesis, but due to the deregulated signalling in the desmoplasic stroma these have an impaired function and do not provide enough oxygen and nutrients. This will lead to hypoxia, which elicits even stronger demand for angiogenesis, and hypoxia may also aggravate the malignancy of the tumour cells. The outcome of malignant disease will depend on tumour growth rate, metastases formation and the efficacy of the treatment. These features are dependent on all constituents of the tumour microenvironment [3].

The microenvironment of solid tumours

Solid tumours, like carcinomas, simultaneously display similarities with both acute inflammations and fibrotic lesions, and are sometimes referred to as wounds that do not heal [5]. The carcinoma stroma is characterized by a substantial inflammatory infiltrate, comprised of immune cells representing both the innate and adaptive immune system. Further, the interstitial fibroblasts display an activated phenotype, in many ways reminiscent of myofibroblasts. Angiogenesis is also constantly ongoing within a growing tumour, which leads to activated vessels with poor function. The ECM in the tumour stroma is composed of extra-vascular plasma proteins, due to the leaky vasculature, but also dense collagen scaffolds are present, due to the fibrotic reaction. However, when treating the brain carcinoma it shows uptake of drugs Paclitaxel and Doxorubicin by tumour lesions was lower in brain carcinoma because of leaky vasculature [6]. These features of the tumour stroma profoundly contribute to the nature of the neoplastic disease, and many aspects of the progression and treatment of cancers are dependent on the non-malignant cell component of the tumour [7]. In fact, a high expression of ECM and stromal related genes in breast carcinoma predicts an increased resistance to pre-operative (neo adjuvant) chemotherapy[8], which indicates that a large stromal component either correlates with a more malignant cancer phenotype or that a large stromal component protects the cancer cells. It has also been shown that chronic inflammations can lead to the development of cancers, mainly through the deteriorating effects of a prolonged exposure to immune activity [4].

Inflammatory cells in the tumour microenvironment

The inflammatory infiltrate in carcinomas is reminiscent of that found in chronic inflammations and fibrosis. Apart from stimulating their own growth and activation, tumour cells secrete a vast range of growth factors and cytokines that stimulate other cells in the microenvironment, which in turn augment an inflammatory response. Altered inflammatory cells like tumour associated macrophages (TAMs) are observed in the tumour stroma. TAMs have been reported to express M1 and M2 cytokines which contributes to angiogenesis. The TAMs constitute a large part of many clinical and experimental carcinomas, and their extent have a positive correlation with bad prognosis in lung carcinoma [9]. A study by Calorini and Biancini critically addresses experimental evidence that macrophages, fibroblasts, endothelial cells, and other types of stromal cells control and alter the tumoural microenvironment by inducing changes facilitating the tumour cells local and distant dissemination [10]. TAMs relates to their pro-angiogenic capacities. TAMs generally accumulate in hypoxic areas of the tumour and hypoxia in turn triggers a pro-angiogenic program in these cells. Thereby, TAMs promote the angiogenic switch and neovascularization as well as malignant transition of the tumour cells by secretion of specific proangiogenic factors (VEGF, IL-1β, TNF-α, angiogenin), or indirectly through the release of MMP-9 [11]. The tumour microenvironment inhibits maturation of dendritic cells, which affects antigen presentation. Dendritic cells that are not sufficiently activated when they present antigens to T-cells will not stimulate an activation that elicits a specific immune response. Instead they will signal tolerance, and this is a regulatory mechanism that prohibits autoimmunity [4]. Nowadays dendritic cells are using immunotherapy. There is evidence...
that dendritic cells transduced with Adenoviral Vectors (ADV) have a prolonged survival and resistance to spontaneous and Fas-mediated cell death, suggesting their utility in delivering immunotherapy more efficiently and robustly. ADV transduction itself can also augment the capacity of dendritic cells to induce protective antitumour immunity [12].

In conclusion, tumour cells not only enroll the immune system to serve as microenvironment modifiers that will support their growth and progression, they also suppress the anti-tumour effects of the immune system.

**The tumour Angiogenesis**

Carcinomas as well as other malignant cells often have higher glucose utilization than normal tissues, which even further emphasize the need for a rich vascular supply. Indeed, tumour masses often contain an abundance of vascular structures, but these vessels are poorly perfused and do not contain a correct hierarchy with regards to arteries, veins and capillaries. The reason for the poor vascular function stems from the cytokine and growth factor environment in tumours, which induce a constant vascular activation and angiogenesis, but at the same time this hampers vascular maturation. Carcinoma cells and infiltrating myeloid cells, like TAMs, secrete large amounts of growth factors that affect the vasculature, e.glergy-VEGF-A, PDGFs and FGFs. TAMs also contribute to producing proteases that can degrade ECM in order to facilitate angiogenesis. Moreover, the constant angiogenesis give rise to fragile nascent vessels that are prone to rupture, which can lead to intravascular coagulation and fibrin deposition. PDGF-B seems to have a dual effect, in retina PDGF-B can induce both pericyte dissociation from activated vessels and recruitment of pericytes to nascent vessels, which in turn can induce endothelial and vessel maturation [13]. The effect of PDGF-B on the tumour vasculature is probably dependent on the immediate microenvironment. IL-1α and TNFα (Alpha) induces the activation of both pericytes and endothelial cells. In conclusion, tumours contain abundant vascular structures, but not all are perfused vessels due to impaired regulation of vascular tone and intra-vascular coagulation. Thus, tumours are capable of inducing angiogenesis, but since the vessels produced are of poor quality, there is a demand to increase the quantity [14]. Anti-angiogenic therapies used to inhibit angiogenesis diminish tumour growth and also permit an increase in tumour cell apoptosis. The use of two angiogenic inhibitors like VEGFR2 inhibitor and PDGFR-β together has shown very promising results in a glioma model. They target the pericytes and endothelial cells causes diminish vascular supply to the tumour [15].

**Influence of Tumour-associated fibroblasts on tumour behaviour**

The interstitium of carcinomas contain not only inflammatory cells, but also fibroblasts. These fibroblasts often resemble the myofibroblasts seen in maturing scars, chronic inflammations and fibrosis. All fibroblasts found in the tumour microenvironment are referred to as Tumour Associated Fibroblasts (TAFs), independent of the extent of myofibroblast marker expression. It has been shown in various experimental models where tumour cell lines has been co-cultured or co-inoculated in mice with different types of fibroblasts, that the stromal cells contribute to an increased tumourogenicity [16]. TAFs in carcinomas are induced to perform similar tasks as fibroblasts in granulation tissue and myofibroblasts in fibrosis. They interact with carcinoma cells and inflammatory cells, providing factors for reciprocal signaling loops. TAFs has been implicated to express growth factors like Epidermal Growth Factor (EGF) and transforming growth factor (TGF), Hepatocyte Growth Factor (HGF), and FGF causes carcinoma cell proliferation [15].

Orimo et al., showed that fibroblasts derived from primary human invasive breast carcinomas significantly enhanced tumour growth in xenograft models as compared to their normal counterparts. They demonstrated that these TAFs produced higher levels of stromal-derived factor (SDF)-1, which mediated the recruitment of endothelial progenitor cells into the tumour mass, leading to enhanced angiogenesis as well as directly promoting tumour cell growth [17]. In turn, carcinoma cells and inflammatory cells express growth factors that activate TAFs. Ectopic over expression of active TGF-β1 by cancer cells leads to an increased content of TAFs and ECM showing the importance of TGF-β1 as an inducer of reactive stroma in cancer. Firstly Xu et al., proposed that TGF-β1 produced by BM stromal cells promotes the survival and chemoresistance of leukemia cells via direct cell-to-cell interactions. They showed that the blockade of TGF-β signaling by LY2109761, which effectively inhibited the pro-survival signaling, could enhance the efficacy of chemotherapy against myelomonocytic leukemia cells in the bone marrow microenvironment [18].

In addition to promoting the growth of established tumours, there is strong evidence that altered fibroblast signalling may be critical in the initiation of carcinogenesis, in regulating its phenotype and in mediating metastatic spread of tumours. TAFs can also respond to PDGFs present in the tumour microenvironment, although not all TAFs express PDGF receptors. The PDGF-B in tumours can be derived from both malignant cells and inflammatory cells, mostly Tumour Associated Macrophages (TAMs). Macrophages secrete most of the produced PDGF-B, whereas some carcinoma cells retain PDGF-B on their cell surface, and subsequent stimulation of Platelet Derived Growth Factor Receptor (PDGFR β). Ectopic overexpression of PDGF-B by non-malignant human keratinocytes grafted onto mouse dermis showed an increase in extravascular PDGFR β expression in conjunction with increased α-SMA expression in the same compartment. Moreover, if these mice were maintained for a longer period, the keratinocytes underwent a malignant progression, which emphasize the role of a reactive stroma in tumour formation [19].

TAFs secreted FGF-2 and FGF-7, out of which FGF-2 turned out to be crucial for tumour angiogenesis. The role of FGF-2 was confirmed in another study in which murine melanoma cells overexpressed PDGF-C, which induced increased TAF content in tumours. The TAFs expressed FGF-2 and the matricellular protein osteopontin [20]. The TAFs promoted increased tumour growth and angiogenesis, through production of the chemokine stromal derived factor 1α (SDF-1α. a.k.a. CXCL12). SDF-1α stimulated carcinoma cells directly in addition to recruiting endothelial progenitor cells, which facilitate angiogenesis [21].

**The ECM scaffold in tumours**

The ECM of carcinomas is characterized by a collagen scaffold composed reticular and collagen fibers. The collagen fibrils from tumours often display an altered morphology compared to fibrils from normal loose connective tissue. Cross sections of fibrils found in tumours are not always smooth and circular, instead they can be multi-lobular and coarse. Moreover, collagen fibrils in tumours display a heterogeneous distribution in diameters, often are large diameter fibrils observed, which can further assemble into larger collagen bundles [22]. A reasonable explanation for the altered collagen scaffold in tumours is that the collagen fibril assembly is dysregulated, like most other processes in tumours. TAFs most likely regulate collagen assembly in tumours and as mentioned above they are quite different from their counterparts in normal tissues. Moreover, collagens, collagen modifying enzymes, Small Leucin-Rich Repeat Proteoglycan (SLRPs) are differently expressed in tumours compared to fibrils in normal connective tissue, which also contributes to the altered collagen fibril assembly. The expression of collagen fibrils are often up-regulated in many carcinomas [23], and this is also observed for the crosslinking enzymes, Lysyloxidase (LOX) or Lysyloxidase like enzymes (LOXLs). By modulating the activity of LOX or LOXLs has the effect of collagen crosslinking on tumour progression in experimental tumours been exposed. Excessive activity of LOX or LOXLs increases the collagen scaffold stiffness, which have been shown to increase invasiveness of tumour cells in vitro and in vivo. Inhibition of LOX in a transgenic muscle model of mammary carcinoma, resulted in less crosslinked collagen, prolonged tumour latency, decreased tumour incidence and volume [24]. Moreover,
the fibrogenic response in tumours can elicit expression of proteins that are not normally expressed in loose connective tissue, such as fibromodulin, which normally is expressed in tendons and other tissues that are exposed for high tensional stress. Fibromodulin have been shown to have a profound effect on collagen fibril diameter. Fibromodulin expression was lowered by an inhibitor specific for TGF-β1 and TGF-β3, which resulted in a reduced collagen scaffold density and decreased fibril diameter, suggesting that a looser ECM was formed [23].

Fluid balance in the tumour stroma
Due to the impaired vasculature of tumours, an apparent lack of lymphatic vessels and an altered ECM phenotype, the fluid balance in tumours is not in balance when compared to normal loose connective tissue. Since the vasculature leaks plasma proteins, there is no colloid osmotic pressure difference between the interstitium and the blood, and the lack of functional lymphatics results in a lack of drainage of excess interstitial water [25]. The blood fluid pressure is often lower than in normal tissues, but it is still positive; tumour capillary fluid pressure is in the range of 7-31 (mean 17 ± 6) mm Hg. Altogether this would lead to a severe edema, if it was not for the fact that most solid tumours has an increased Interstitial Fluid Pressure (IFP). This results in a low transport of water and other low molecular weight molecules, since the rudimentary pressure gradient that is present in the tumour vasculature is not enough to drive transport by convection. Instead most transport is propelled by diffusion, which is far less efficient, especially for larger proteins. Further, transport of proteins in the interstitium is also hampered by the composition of the ECM. The collagen scaffolds acts as a sieve that exclude larger proteins. Treatment with a TGF-β inhibitor was successful in lowering IFP and a concomitant increase of anti-tumour treatment efficacy was observed [26].

Role of Hypoxia in tumour Progression
As tumours grow, areas of nutrient deprivation and oxygen deprivation (hypoxia) arise as a result of an insufficient blood supply. Although a limiting factor for tumour growth, hypoxia also represents a stimulus for invasion and metastasis, and a number of studies have shown that hypoxia is an independent predictor of poor prognosis. Hypoxia stimulates Hypoxia–Inducible Family (HIF) proteins, mainly HIF 1α which regulate diverse cellular processes, including metabolism, angiogenesis, cell proliferation, apoptosis and tissue remodeling [27]. Whereas the major focus on hypoxia has been its role in enhancing angiogenesis, up-regulation of HIF-1α, which leads to an angiogenic switch, enhancing expression of pro-angiogenic factors such as VEGF, PDGF and FGF, with down-regulation of anti-angiogenic Thrombospondin leads to increased angiogenesis [28]. In one key study, using a series of cell lines derived from breast, lung, cervical and ovarian cancers, among others, Pennacchietti et al showed that HIF-1α binds to the c-Met promoter, leading to over-expression of c-Met and enhanced sensitivity to HGF. This leads to an ‘invasive switch’ in the tumour cells, increasing degradation of the ECM and allowing tumour cells to move freely towards more oxygen-rich areas. This has therapeutic implications, since targeting angiogenesis alone may not be sufficient, and indeed may even aggravate, this invasive response to hypoxia [29].

Therapeutic targeting [Table/Fig-1]:
Current treatments for carcinoma consist of chemotherapy associated with surgery. Most patients are chemosensitive and cancer free immediately after the treatment. However, depending on the quality of the surgery, 50% to 70% of patients will relapse within one year. When such relapse occurs, in most cases the carcinoma cells have acquired a chemoresistant phenotype. This chemoresistance can be associated with genetic alterations within the cancer cells but recent studies have proposed that it could also be associated with the tumour microenvironment [30]. Indeed, this microenvironment has become recognized as a major factor influencing the growth of cancer and impacting the outcome of therapy. While the niche cells are not malignant per se, their role in supporting cancer growth is so vital for the survival of the tumour that they have become an attractive target for chemotherapeutic agents . Meads et al. have shown that environment-mediated drug resistance is rapidly induced by signaling events from the tumour microenvironment and is likely to be reversible because removal of the microenvironment restores the drug sensitivity [31].In experimental models, inhibition of the angiogenic switch prevents progression of hyperplastic foci to in situ carcinoma, decreases cancer insurgence and reduces metastasis. Targeting tumour angiogenesis to inhibit tumour growth has now become a clinical reality. Now a day’s most common used drugs are angiogenic inhibitors. The majority of these targets the VEGF signalling pathway and can extend progression-free survival in colorectal, lung and breast cancer when used in combination with other chemotherapeutics [32]. Angiogenesis is also regulated through integrins αvβ3 and αvβ5, which are expressed on endothelial cells. Integrins have been shown to suppress tumour growth in preclinical models. The mechanism is thought to be through blocking their adhesive functions and hence preventing tumour growth by targeting the tumour cells as well as inhibiting angiogenesis. Cilengitide, a αvβ3 inhibitor, has been effective in the treatment of glioma [33]. Other aspects of the tumour microenvironment are also being targeted. The expression of ‘tumourspecific’ ECM proteins has been exploited to target delivery of bioactive molecules to tumours. These ECM components are highly abundant in tumours and are often more stable than antigens located on the cell surface of tumour cells. Radio labelled antibodies specific to TNC (Tenascin-C domain C) domains A1 and D have been used successfully in the clinic to treat glioma and lymphoma [34]. The EDB-targeting antibody L19 has been used as a vehicle for TNFα (Alpha) and has been shown to induce necrosis in tumours. Recently EDB has been targeted in lymphoma patients, using a radiolabelled antibody131I-L19SIP . Two patients treated with this antibody showed a sustained partial remission, indicating that a therapeutic dose of radioactivity can be delivered to tumours using this approach [35]. TNFα (Alpha) antagonists have been shown to induce stabilization of disease and partial responses in breast and advanced cancer and multiple myeloma is treated very successfully with combinations of drugs, including lenalidomide which suppresses the production of several inflammatory cytokines [36]. A novel small molecule of IKKβ has been developed (KINK-1), which demonstrates the ability to sensitize tumours in mice to doxorubicin and reduce tumour mass and metastases. A number of drugs in clinical trials for other diseases that target the immune system, such as non-steroidal anti-inflammatory drugs (NSAIDs; eg COX inhibitors such as celecoxib) for treating arthritis [37] have been applied to some tumours like malignant melanoma and pancreatic cancer. COX-2 inhibitors have also been shown to prevent the recurrence of sporadic and genetically predisposed adenomas [38]. Other therapeutic targetting molecules and their functions are mentioned in [Table/Fig-1].

<table>
<thead>
<tr>
<th>Micro-environmental Target</th>
<th>Molecular target</th>
<th>Molecule</th>
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<tbody>
<tr>
<td>Angiogenesis</td>
<td>αvβ3</td>
<td>Cilengitide</td>
<td>Reduces angiogenesis and increases apoptosis of TEC in vitro.</td>
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</tr>
<tr>
<td></td>
<td>αvβ5</td>
<td>Atebrin</td>
<td>Inhibits angiogenesis</td>
<td>[40]</td>
</tr>
<tr>
<td></td>
<td>αvβ1</td>
<td>Veloximab</td>
<td>Sensitive endothelial cells to radiotherapy.</td>
<td>[39]</td>
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<tr>
<td>Signaling Inhibitors</td>
<td>PDN/Ad5 and mTOR</td>
<td>In vitro siRNA</td>
<td>Inhibits fibronectin induced proliferation death</td>
<td>[8]</td>
</tr>
<tr>
<td>Cytokine inhibitors</td>
<td>SDF-1/ CXCR4</td>
<td>Brystosatin-1</td>
<td>Antagonizes CXCR4- mediated migration and metastasis/inhibits neovascularization</td>
<td>[40]</td>
</tr>
<tr>
<td>ECM degradation inhibitors</td>
<td>FAPα (Separse)</td>
<td>Sibrotumumab</td>
<td>Reduced growth and invasion</td>
<td>[41]</td>
</tr>
<tr>
<td></td>
<td>Tenascin-C</td>
<td>BR206 (131I- labelled Ab)</td>
<td>Delays tumour growth, prolonged survival</td>
<td>[41]</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>CAX</td>
<td>WX-G250</td>
<td>Induces antibody-dependent cellular toxicity</td>
<td>[42]</td>
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</tbody>
</table>

[Table/Fig-1]: Therapeutic targeting of the microenvironment
CONCLUSION

Taken together, these findings emphasize the need to further unravel the complex molecular networks and cross talk between different components of the tumour microenvironment and the tumour cells itself. A deepened knowledge on mechanisms involved in tumour progression and invasiveness toward metastasis could be essential to improve efficacy of current therapeutic interventions with significant clinical impact.

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