Exploiting ECM remodelling to promote immune-mediated tumour destruction

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Abstract

Cancer immunotherapy represents a significant breakthrough in cancer treatment mainly due to the ability to harness the activities of cancer-specific T cells. Despite this, most cancers remain resistant to T cell attack. Many reasons have been proposed to explain this, ranging from a lack of antigenicity through to the immunosuppressive effects of the tumour microenvironment. In this review, we examine the relationship between the immune system and a key component of the tumour microenvironment, namely the extracellular matrix (ECM). Specifically, we explore the reciprocal effects of immune cells and the tumour ECM and how the processes underpinning this relationship act to either promote or restrain tumour progression.

Introduction

The extracellular matrix (ECM) is a key regulator of tissue function and homeostasis. Alterations in the ECM arise from diverse remodelling mechanisms that include ECM deposition, post-transcriptional modifications, proteolytic degradation and physical remodelling induced by force [1]. Each mechanism affects the ECM differently, either through modifying its biochemical properties by releasing biologically active ECM fragments and ECM-bound factors, or by modifying its mechanical properties through altering fiber alignment; all of which influence cellular signalling networks. It is clear that tumour cells and tumour stromal cells can hijack ECM remodelling processes in order to create their own tumour supporting matrix which contributes to disease progression [1,2].

During tumour development, cells within the tumour microenvironment (TME), such as tumour cells and cancer associated fibroblasts (CAFs) promote ECM stiffness resulting in fluid and solid stress in the TME. This, in turn, results in activation of mechanotransduction pathways [3,4] which drive changes to the intrinsic properties of cancer cells. An important characteristic of the tumor ECM is high levels of proteolytic degradation. This process breaks down barriers between cells facilitating invasion of malignant cells as well as migration of endothelial cells. Proteolytic degradation also promotes the activation and release of cryptic peptides named matrikines. These have been shown to exert a broad range of activities which, as described below, can contribute to either progression or suppression of tumour growth. Another consequence of ECM degradation is the exposure of hidden integrin-binding sites which play a significant role in tumour progression [3,5,6].

While CAFs are an important source of both ECM proteins and ECM remodelling enzymes [7], recent studies have demonstrated that immune cells can also play an important role in TME remodelling. Macrophages, for example, have been shown to produce collagen, Tenascin C (TNC) and versican [8,9]. Other immune cells have been shown to produce matrix-degrading enzymes; cytotoxic T lymphocytes (CTLs) upregulate the expression of matrix metalloproteinases (MMPs) once in the TME [10], neutrophils can secrete neutrophil elastase (NE) and MMP-9 [11,12], and natural killer (NK) cells can produce heparanase (HPSE) [13]. Thus, whilst is it known that the ECM can influence immune function [14], it is becoming

increasingly clear that the immune system also impacts the composition of the ECM with both components serving to either promote or restrain tumour progression.

ECM Remodelling and Tumour Progression

ECM remodelling is largely thought of as a process which drives tumour progression. Accumulation of ECM proteins, matrix degradation and fiber rearrangement, promote metastasis formation, intrinsic cell alterations and poor lymphatic drainage, which are associated with poor prognosis in patients [3,9,15-18]. There is also accumulating evidence demonstrating that the ECM impinges on immune cell behaviour potentially driving tumour progression by suppressing anti-tumour immune responses.

ECM Affects the Cellular Composition of the TME

ECM content can affect the composition of the TME by favouring the infiltration of some cell types over others. Several studies indicate that an abundance of ECM proteins and a greater extent of matrix stiffness are associated with increased macrophage infiltration (Figure 1.1) [8]. Once in the TME, tumour associated macrophages (TAMs) can modify the composition and organisation of the ECM directly, by synthesising collagen [8] and indirectly, by releasing cytokines, such as transforming growth factor β (TGFβ), that not only promote myofibroblast transformation, but also regulate CAF activity (Figure 1.2) [5,11]. These activities serve to further fortify the tumour matrix whilst facilitating the immunosuppressive and tumour-promoting effects of CAF [19].

As mentioned above, proteolytic degration of ECM proteins results in the generation of small peptides, named matrikines. Matrikines exhibit biological activity in the ECM by binding specific cell surface receptors, activating intra-cellular signalling pathways. Acetylated proline-glycine-proline is an example of such a matrikine; released during collagen degradation, it has a similar structure to certain CXC chemokines and serves to promote neutrophil influx and inflammation [20].

Reports indicate that ECM proteins are more abundant at tumour periphery, exhibiting a well organised fibrillar pattern compared to a more chaotic and less abundant pattern in the tumour centre [18]. This pattern of fiber alignment at the periphery is thought to form conduits that direct cancer cell migration promoting cancer dissemination [21]. This pattern of ECM deposition can also affect T cell movement/infiltration (Figure 1.1). Salmon and colleagues showed that T cells have a reduced capacity to infiltrate tumour nests (clusters of tumour cells surrounded by stroma) when these are surrounded by ECM, migrating preferentially towards the ECM-poor tumour-surrounding stroma [22,23]. Moreover, whilst T cell migration into the tumour tissue was facilitated by chemokines, T cell movement at the tumour margins was restricted to the direction of fiber alignment [22]. Pruitt and colleagues corroborated this work, showing that $CD8^+$ T cells move faster in aligned than disorganised fibers, restricting T cell migration into the tumour core [24].

Immunosuppressive Effects of the ECM

ECM remodelling can also alter immune cell behaviour through remodelling enzymes. Hyaluronan (HA) is a glucosaminoglycan present in the ECM that can be degraded into different sized fragments, with different functions. High molecular weight HA fragments have been shown to promote immunosuppression by increasing Treg activity [6]. This occurs as the HA fragments cross-link CD44 on Tregs leading to upregulation of Foxp3 expression [25] and increased suppressive capacity (Figure 1.3) [26].

Matrix remodelling enzymes may also promote immune evasion e.g. MMP-driven shedding of the adhesion molecules, ICAM-1 and B7-H6, results in reduced tumour cell killing by NK and cytotoxic T cells (Figure 1.4) [27]. NK cell-mediated cytotoxicity can also be impaired via binding of collagen to the collagen receptor, leukocyte-associated immunoglobulin-like receptor 1 (LAIR-1). Studies *in vitro* showed that the overexpression of collagen XVII drives inhibitory signals via LAIR-1, reducing the capacity of NK cells to lyse tumour cells (Figure 1.5) [28]. Further to this work, a study by Peng and colleagues showed that collagen-binding to LAIR-1 on CD8+ T cells enables resistance to PD-1/PD-L1 blockade through SHP-1 signalling [29]. This pathway may explain the findings from two groups who demonstrated that markers of collagen turnover in the peripheral blood of melanoma patients correlated with reduced overall survival after treatment with PD-1 or CTLA-4 blocking antibodies [30,31].

ECM Remodelling and Tumour Destruction

Whilst the tumour promoting effects of ECM remodelling are well known, recent reports indicate that this process can also play a role in tumour destruction either through removing barriers to immune infiltration or by releasing matrikines with immune-activating potential.

The Inverse Relationship between ECM Proteins and a Successful T Cell Response

Using a mouse model of carcinogen (3-methylcholanthrene) induced fibrosarcomas, our studies show that depleting Tregs results in control of tumour growth in approximately half of the tumour-bearing mice [32,33]. These mice, termed “responders” are distinguished from non-responders by the presence of specialised vasculature (high endothelial venules) and a significantly higher number of tumour-infiltrating T cells in their TME when compared to non-responders. Moreover, an unbiased comparison of the TME of untreated, responders and non-responders, showed an inverse relationship between expression of some matrix genes and T cell infiltration [18]. The most differentially expressed ECM gene in non-responder compared to responder tumours was Tenascin-C (TNC). Using TNC as an exemplar, all tumours prior to treatment were rich in ECM and thus ECM did not act as a barrier to tumour rejection. However, an effective T cell response following Treg-depletion dramatically altered the density of ECM in the TME (Figure 1.6).

Analysis of RNAseq datasets from primary tumours in The Cancer Genome Atlas (TCGA) revealed a similar pattern; by interrogating 14 cancers where survival was improved with a

CTL gene signature, a link between a favourable CTL gene signature and expression of TNC was observed [18]. Overall, these findings imply an extended role for an effective immune response, not just in direct killing of tumour cells, but in widescale remodelling of the TME to favour loss of ECM and propagation of anti-cancer T cell responses.

Immune-mediated remodelling of the TME

There is evidence that immune cells remodel the TME as an immunosurveillance mechanism. Putz and colleagues showed that activated tumour-infiltrating NK cells which upregulate expression of HPSE are capable of degrading heparan sulphate. The significance of this activity was clear as the absence of HPSE in mouse NK cells resulted in impaired $CD8^+$ T cell infiltration of tumours and was associated with poorer control of tumour development and growth. The efficacy of immunotherapies, namely anti-CTLA4 and anti-PD1 was also compromised in these mice [13].

Studies to date suggest that T cells secrete ECM remodelling enzyme as part of an anti-tumour immune response (Figure 1.7). Ahrends and colleagues demonstrated that $CD4^+$ T cells can help CTLs by empowering them with the ability to increase their motility and migration capacity. They demonstrated that CTLs upregulated the transcription of several ECM degrading enzymes, whose function was validated when the T cells were shown to exhibit higher migratory capacity through collagen-coated transwells compared to non-helped CTL [10].

Activation of the anti-tumour immune response by ECM remodelling

As well as promoting immunosuppression as described above, there is evidence that certain matrikines can stimulate effective anti-tumour immune responses. Hope and colleagues demonstrated *in vitro* that versikines, peptide products released as a consequence of versican degradation by ADAMTS-type versicanases, are able to induce type I IFN signatures, as well as IL-12 production in macrophage-like cells [34]. The group further demonstrated, in human colorectal cancer samples, that versikines promote the generation of Batf3-dendritic cells (DCs) from mobilised bone marrow progenitors [35]. There is evidence that Batf3 DCs are essential for enabling trafficking of effector T cells into the tumour [36] (Figure 1.9).

Low molecular weight (LMW)-HA may also activate DCs. Alaniz and colleagues found that LMW-HA treated mice exhibited increased expression of MHC-II and CD80 on DCs, favouring antigen presentation in secondary lymphoid organs. This was associated with enhanced T cell infiltration in the tumour and significantly reduced tumour growth [37]. A subsequent study conducted on DCs isolated from colorectal cancer showed that after treatment with LMW-HA, DC CCR7 expression as well as MMP activity was increased. Treated DCs demonstrated increased migration *in vitro* towards CCL21 and decreased migration towards IL-8, whilst *in vivo* they exhibited selective migration to the lymph node than the tumour [38] (Figure 1.8). Other groups have reported different anti-tumoural roles for this matrikine, including inducing angiogenesis [39], inhibiting fibrocyte differentiation [40], and stimulating pattern-recognition receptors [6].

ECM targeted therapies

It is clear that unique features of the tumour ECM can be exploited for therapeutic gain and that the ECM can be effectively used as a target to deliver therapeutic agents. A recent study showed that TNC antibody-drug conjugates (Figure 2.1), activated only within the TME, allowed successful delivery of chemotherapeutic agents [41]. In another study, systemic administration of nanoparticles guided by ECM-binding peptides (Figure 2.2) resulted in their selective accumulation in mouse tumour xenografts. These nanoparticles enabled tumour detection and imaging, as well as serving as tumour-seeking carriers for the specific delivery of proapoptotic payloads [42]. Super-affinity ECM-binding peptides can also be directly conjugated with checkpoint blockade antibodies (Figure 2.3); their use was shown to increase T cell infiltration in the TME contributing to delayed primary tumour growth and metastasis, and increased survival [43].

Studies have shown that indiscriminately targeting the ECM may not be effective. MMP inhibitors are a good example, since early broad-spectrum MMP inhibitors have failed in most clinical trials, mainly due to toxicity arising from blocking of MMP activity essential for maintaining normal physiology [44]. New approaches that avoid indiscriminate targeting of the ECM allow targeting of individual enzymes with increased activity at the tumour site only (Figure 2.4). Juric and colleagues showed that targeting MMP-9 increased the traffic of effector/memory T cells into the TME, and resulted in a more diverse TCR repertoire within the TIL population [45]. ECM remodelling enzymes can also be targeted. By inhibiting lysyl oxidase (LOX) in five different mouse models, Nicolas-Boluda and colleagues successfully reduced ECM stiffening which improved T cell migration and the efficiency of anti-PD1 blockade [46]. Other approaches to disrupt the ECM could include the use of anti-fibrotic agents (Figure 2.5) [47], photothermal therapy [48] and siRNAs to block ECM production (Figure 2.6) [49]. Moreover, CAR-T cells that were engineered to express HPSE (Figure 2.7) exhibited improved capacity to degrade the ECM both *in vitro*, where they also had improved cytolytic function when compared to control CAR-T cells, and *in vivo* in neuroblastoma xenograft models, where mice inoculated with these cells survived longer [50].

Conclusion

The studies described herein clearly demonstrate that by altering the TME, it may be possible to tip the balance in favour of immune-mediated cancer destruction, even when the T cell response is not optimal. Therefore, combining immunotherapy approaches designed to reinvigorate T cell response with approaches to remodel the ECM may improve the response to immunotherapy in patients who currently typically fail with treatment.

Declaration of interests: None

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Figure legends:

Figure 1. ECM remodelling can either impair immune responses contributing to tumour progression or help stimulate a potent anti-tumour immune response contributing to tumour destruction. Representation of direct and indirect ECM remodelling mechanisms that may inhibit or potentiate effector anti-tumour immune responses and/or immunosuppressive mechanisms. 1. The presence of high-density ECM (blue and green matrix) in the tumours might impair T cell (green cells) infiltration by limiting their movement, at the same time as it can promote TAM recruitment 2. Macrophages (purple cells) and B cells (blue cells) can release cytokines that will promote fibroblast (brown cells) transformation into CAFs and regulate their activity. 3. ECM fibers (blue strand) can increase Treg cells (blue cell) activity by cross-linking CD44 receptors at their surface, promoting upregulation of Foxp3 expression. 4. ECM remodelling enzymes (light blue) can shed proteins (dark blue) from the tumour cell surface, promoting immune evasion. 5. Binding of T or NK cell (green cell) receptors to ECM can induce the activation of inhibitory signals in these cells, reducing their capacity to kill tumour cells. Created with BioRender.com. 6. A strong anti-tumour immune response (green cells), reflected by a strong release of cytokines like IFNγ and TNFα, as well as ECM degrading enzymes, is associated with reduction of ECM, which in turn is associated with less physical pressure. Less pressure can induce downregulation of stemness-related genes expression as well as better lymphatic drainage. 7. CTLs (green cells) have the capacity to produce ECM degrading enzymes that will degrade the ECM in order to facilitate their infiltration into the tumour. 8. Some ECM fragments (matrikines, blue and green), are associated with T cell (purple cells) infiltration in the tumour. 9. Certain matrikines have also the power of activate specific DC subsets, which are essential for T cell infiltration. ECM, Extracellular matrix; TAM, Tumour associated macrophage; CAF, Cancer associated fibroblast; INFγ, Interferon gamma; TNFα, Tumour necrosis factor alpha; CTL, Cytotoxic T lymphocyte; HA, Hyaluronan.

Figure 2. Targeting the ECM to tip the scales in favour of tumour destruction. Representation of strategies that either use the ECM as a target to deliver drugs/immunotherapies directly to in the tumour, or which promote ECM destruction thereby removing barriers to immune infiltration. 1. Tumour ECM specific antibodies can be conjugated with drugs in order to promote drug delivery specifically in the TME. 2. Nanoparticles (yellow) guided by ECM-specific peptides (brown) can be used as carriers to deliver proapoptotic payloads in the TME. 3. ECM-specific peptides (brown) can also be associated with checkpoint blockade antibodies (green), delivering immunotherapy directly in the TME. 4. Specific ECM enzymes (blue and red) identified as promoters of tumour progression can be directly targeted and inhibited. 5. The use of anti-fibrotic agents (scissors) can help with ECM degradation to ease T cell infiltration. 6. The delivery of RNAi (pink) to the tumour (light blue) can be used to block the production of specific ECM proteins. 7. CAR-T cells (green) can be engineered to produce ECM degrading enzymes, improving T cell infiltration. Created with BioRender.com. ECM,

Extracellular matrix; TME, Tumour microenvironment; RNAi, RNA interference; CAR, Chimeric antigen receptor.

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Papers of particular interest have been highlighted as:
* of special interest
** of outstanding interest
(Please see bottom of the list)

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* Ref 1 – This review discusses the mechanisms behind ECM remodelling and tumour progression, as well as how such mechanisms contribute to tumour to spreading and metastasis.
* Ref 6 – This review discusses the limitations and need for biomarkers predictive of response to immunotherapy, and how ECM remodelling could provide an opportunity to identify matrix-based biomarkers.
** Ref 10 – Ahrends *et al*. show that, when helped by $CD4^+$ T cells, CTLs are able to produce ECM degrading enzymes in order to digest and migrate through the matrix.
* Ref 13 –Putz *et al*. uncover a previously unknown role of HPSE in regulating NK cell-mediated tumour immunosurveillance.
** Ref 18 – This manuscript shows an inverse relationship between an effective anti-tumour response and ECM density, also exploring effects on other components of the TME in mice. A similar trend was observed in human cancers by analysing the publicly available TCGA database.
** Ref 23 – Salmon *et al*. show how the matrix controls position and migration of T cells, affecting anti-tumour immunity.

** Ref 30 – Peng *et al*. identify LAIR1 as the collagen receptor capable of inducing $CD8^+$ T cell exhaustion. They also suggest that LAIR1 could be a biomarker for immunotherapy resistance and validate different therapeutic combinations capable of reversing LAIR1 activation effects.
* Ref 36 – In this study, Hope *et al*. show how matrix degradation can stimulate an effective anti-tumour immune response by activating a DC subset essential for T cell trafficking.
* Ref 43 – This manuscript shows that ECM-binding peptides can be used to successfully target the tumour tissue and deliver immune checkpoint blockade therapies in an efficient manner.
** Ref 50 – In this study, Caruana *et al*. demonstrate that by engineering CAR-T cells to express heparanse, it is possible to improve T cell infiltration and anti-tumour activity.

Figures:

Figure 1

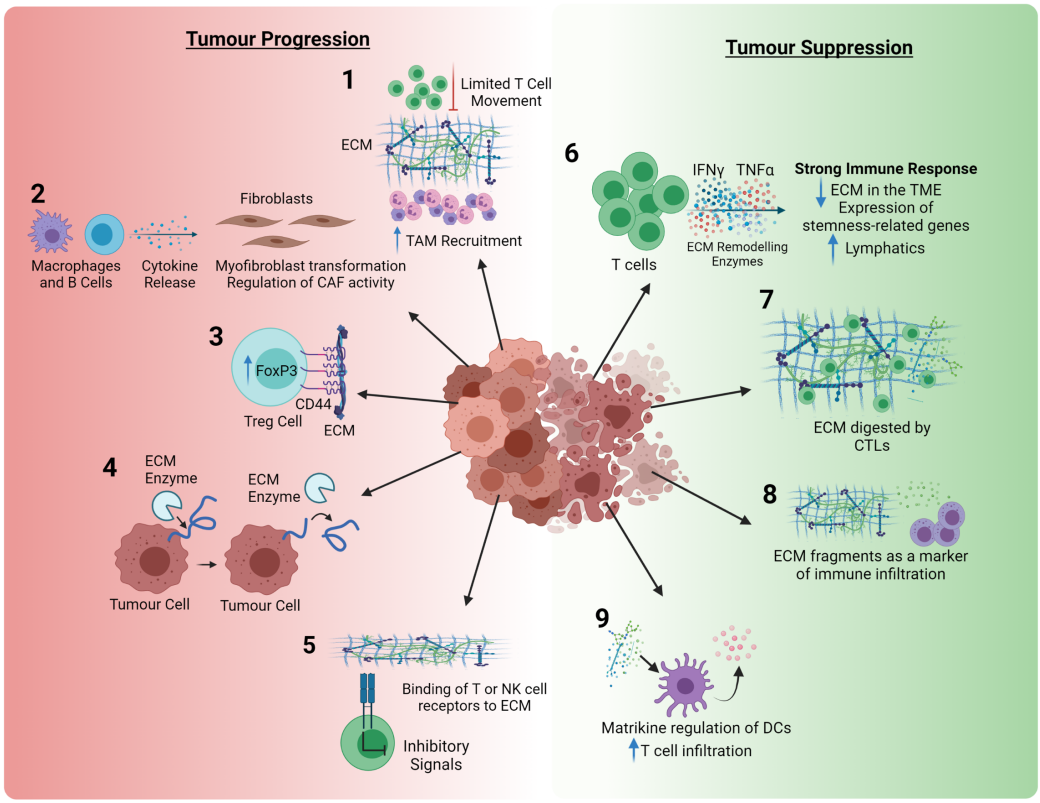

Figure 2