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Extracellular matrix mediated resistance in solid tumors against cell-based immunotherapy

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Layman's summary

Immunotherapy is a powerful weapon in the fight against cancer. Often, the immune system of a patient diagnosed with cancer is not able to recognize and kill malignant tumor cells. The principle of immunotherapy is to enhance/restore the immune system in such a way, that the immune system can recognize and eradicate tumor cells. Cell-based immunotherapies is one of the variants of immunotherapy. A well-known cell-based immunotherapy is the Chimeric Antigen Receptor (CAR) T-cell therapy. This therapy utilizes the patients' own T-cells. The T-cells are isolated from the blood and are subsequently modified in the lab in such a way that it can bind and kill tumor cells. These T-cell get then re-infused back into the patient. CAR T-cell have been mainly successful in the treatment against liquid tumors like acute lymphoblastic leukemia. Unfortunately, patients with solid tumors, don't respond as well as patient with liqiud tumors. As solid tumors account for 90% of all the cancers, the need is high to unravel the cause of the poor response of cell-based immunotherapy in solid tumors. The Extracellular matrix (ECM) plays a major role in the resistance against immunotherapies. The ECM is a complex network of molecules that provides structural and biochemical support for cells and tissues. During tumor progression, the tumor degrades the healthy ECM and forms a new ECM which supports the tumor progression. The tumor ECM is characterized by increased deposition of all kinds of ECM components. The ECM mediates immunotherapy resistance through multiple mechanism. For instance, the tumor ECM creates a physical shield around the tumor to prevent immune cells from reaching the tumor. Moreover, it can influence immune cells to become resistant. Fortunately, research effort is being made to develop therapeutics that target the tumor ECM. Combining cell-based immunotherapies with ECM targeting therapeutics has the potential to be a successful treatment against solid tumors.

Abstract

Cell-based immunotherapies like immune checkpoint blockade and CAR T-cell are powerful weapons in the battle against cancer. Despite the clinical successes in treating hematological cancers, most solid tumors show resistance against immunotherapies for reasons we do not fully understand. The extracellular matrix (ECM) plays a pivotal role in cell-based immunotherapy resistance. Essentially, the ECM is important for all tissues and organs, as it fulfills important structural and functional roles in tissue organization. However, once tumors reach the invasive stage, the normal ECM gets degraded and tumor ECM specific ECM is formed. In this paper we try to elucidate the role of the ECM in cell-based immunotherapy resistance. For instance, the tumor ECM is able to mediate immunotherapy resistance through multiple mechanisms. Increased collagen deposition and crosslinking, which is a characteristic of tumor ECM, is crucial for mediating immunotherapy resistance. Fortunately, research effort is being made to target ECM components to overcome immunotherapy resistance in solid tumors.

1. Introduction

The start of immunotherapy development dates back all the way to the nineteenth century [1]. Wilhelm Busch and Friederich Fehleisen discovered that a superficial skin infection, erysipelas, causes spontaneous regression of tumors [1]. Eventually it was William Coley that demonstrated that erysipelas was associated with better outcome in patients with sarcoma by treating patients with extracts of heat-inactivated S. pyogenes and Serratia marcescens to boost immunity in sarcoma patients, this is a form of antibody-based immunotherapy [1,2]. Since then, immunotherapy has come a great way in the battle against cancer. The major advantage over other therapies like radiation and chemotherapy is, is that immunotherapies are targeted therapies and thus limiting the collateral damage and side effects. The recent development of immunotherapies like immune checkpoint blockade (ICB) and chimeric antigen receptor (CAR)-T cell therapy has improved the survival of patients diagnosed with cancer [3,4]. The goal of immunotherapy is to enhance/restore the ability of the patients' own immune system to overcome the mechanism that the tumor develops to evade the immune system, and to enable the immune system to recognize and eradicate tumor cells [5]. In this paper, we are primarily focusing on cell-based immunotherapies. CAR T-cell treatment has proven its efficacy in treating hematological malignancies like acute lymphoblastic leukemia and lymphoma [6,7]. Despite the great success of CAR T-cell therapy, most solid tumors show poor response [8]. Solid tumors account for 90% of all tumors, so the need to elucidate resistance mechanisms in solid tumors is high [9]. Poor response in solid tumors is multifactorial, one of them is the immune exclusion of solid tumors caused by the extracellular matrix (ECM) [10]. For cell-based immunotherapies to exert therapeutic response, immune cells, such as T-lymphocytes, needs to infiltrate the tumor microenvironment (TME), and this is often does is not observed in a wide range of solid tumors[7]. The ECM of the tumor plays an important role in the formation of an immune excluded tumor. In addition, the ECM also can influence immune cells to mediate cell-based immunotherapy resistance. In this paper, we will dive deeper into the role of the extracellular matrix in cell-based immunotherapy resistance.

2. The extracellular matrix

All tissues and organs are surrounded by a well-organized three-dimensional network composed of non-cellular components called the extracellular matrix (ECM) [11,12]. The ECM forms the scaffold for solid organs and fulfils important structural and functional roles in tissue

organization and remodeling [12,13]. Moreover, it regulates cellular processes like differentiation, migration, homeostasis, apoptosis, and cell survival [11–13]. The ECM is mainly composed of a large variety of macromolecules like collagens, elastin, fibronectin, laminins, glycoproteins, proteoglycans and glycosaminoglycans [11]. Components within the ECM are post translationally modified by remodeling enzymes such as oxidases and proteases [14]. In addition, ECM components can bind soluble factors such as growth factors and other ECM associated proteins [14]. Receptors located on the cell surface can interact with ECM components and factors bound to the ECM to mediate cell signaling, thereby regulating the cellular processes mentioned previously [14].

The composition and structure of the ECM varies per tissue and can therefore be divided into two types that differ in function, composition, and location [11,14] . The interstitial matrices surround the functional tissue, while the pericellular matrices are in close contact with the cells [11]. To maintain tissue homeostasis, ECM is constantly being renewed by fibroblasts [12]. Fibroblasts synthesize ECM components while also secreting enzymes that degrade the ECM [12]. Dysregulation of the ECM homeostasis is observed in many diseases, including fibrosis, cancer, and inflammation [12]. An important enzyme for remaining ECM homeostasis, is the matrix metalloproteinase (MMP), it is a zinc dependent endopeptidase, they are responsible for the degradation of the ECM. The activity of the MMPs is inhibited by tissue inhibitors of metalloproteinases. An imbalance between these two proteins can lead to the deposition of an unusual amount of ECM proteins, which leads to remodeling of the ECM [12].

3.Main ECM components and their functions

As stated earlier, the ECM is composed of many different components, each with their distinct function and some are more present in specific organs. Around 300 matrix molecules have been identified and can be classified into collagens, glycoproteins (for instance laminins, elastin and fibronectins) and proteoglycans (for instance hyaluronic acid) [14]. The coming section will cover a selection of different ECM components.

3.1 Collagens

Most of the ECM consist of collagen, accounting around 90% of the ECM [15]. Twenty-eight types of collagens have been identified [15]. All collagen molecules are either homotrimers or heterotrimers of three polypeptides chains (α chains), build out of glycine-X-Y, X and Y usually being proline and 4-hydroxyproline respectively [15]. The trimers form a triple helix that is stabilized by hydrogen and electrostatic interactions [15]. The triple helixes subsequently form collagen fibril through crosslinking, which provides tissue intensity and tenacity [15]. Collagens provide tensile strength, support chemotaxis, adhesion and migration, and direct tissue development [16]. To interact with collagens, many cells, including T-lymphocytes, expresses collagen receptors [10]. Collagen is highly present in kinds of tissue, especially in bone tissue where it acts as scaffold for bone cells[17].

3.2 Fibronectin

Fibronectin is dimer glycoprotein, that exists in soluble and insoluble form [15]. It plays a role in cell adhesion, migration and wound healing following injury [15,18]. In the ECM, fibronectin connects structural proteins to form an integrated matrix, it can connect for example collagens to form collagen-fibril [15]. Besides, fibronectin binds to integrins located on cell membranes,

thereby acting as key player of the communication between the intercellular and extracellular environment [15,19].

3.3 Elastin

Elastin is a structural protein that is the main component of elastic fibers that is mainly found in ligaments and vascular walls [15,18]. Compared to collagen, elastin is highly elastic due to its amino constituent and 3D structure, thereby fulfilling an important role for the extensibility of and elastic recoil of cardiovascular tissue and many others [15,20]. A different tissue where elastic recoil is important, is the lung. Therefore, the ECM of lung tissue is constituted of high levels of elastin [21].

3.4 Laminin

Laminin is a heterotrimer consisting of one α chain, one β chain, and one γ chain [11,15]. Laminin plays a role in vascularization, mainly in vessel maturation [15]. During wound healing, laminin is upregulated to provide an interface for the adherence of epithelial cells to adhere and stretch [15].

3.5 Hyaluronic acid

Hylauronic acid (HA) is a high molecular-weight glycosaminoglycan/polysaccharide constituted of disaccharide repeats of N-actetylglucosamine and glucouronic acid [15]. Just like collagen, HA is a major component of the ECM [15]. HA captures water by forming hydrogen bonds with the great number of hydroxyls in HA [15]. Therefore, HA acts as "water supply" for buffering ion exchange and osmotic balance within the ECM [15]. Additionally, HA can be recognized by membrane receptors like CD44 and play an important role in biological processes including cell mobility, invasion, proliferation, and inflammation [15]. HA is highly prevalent in the ECM of the central nervous system. Moreover, HA is the major organizing polysaccharide of the brain ECM [22].

4. Tumor niche

The TME is the direct well-structured environment in which tumor cells exist. Beside the ECM, the TME is constituted of a variety of immune cells (B - T-cells, NK-cells tumor associated macrophages, myeloid derived suppressor cells), cancer-associated fibroblasts, and endothelial cells, among others. The ECM can interact with these cells in the TME to alter their function. In this part, we will dive deeper into the role of some of these cell types in cancer.

4.1 T-lymphocytes

T-lymphocytes can be divided into $CD8^+$, $CD4^+$, and regulatory T-lymphocytes.

$CD8^+$ T-lymphocytes play an important role in the anti-tumor response. $CD8^+$ T-lymphocytes are able to recognize and bind MHC I complex peptides expressed on cancer cell trough the T-cell receptor (TCR) [23]. Upon binding to the MHC I complex peptide, $CD8^+$ T-lymphocytes eliminates the target cells through apoptosis by granzyme or perforin mediated pathway [23].

In cancer, $CD4^+$ T-lymphocytes possesses both anti-tumorigenic functions as well as pro-tumorigenic functions [23]. The Th1 subtype of $CD4^+$ T-lymphocytes, supports anti-tumor activity from $CD8^+$ T-lymphocytes and B-cells by producing interferon-γ and TNF- α. However, the subtype Th2 secretes anti-inflammatory stimuli to promote pro-tumorigenic functions [23].

Regulatory T-lymphocytes are the most immunosuppressive subset of T-lymphocytes. Regulatory T-cells regulate the immune homeostasis. In cancer, regulatory T-lymphocytes suppresses anti-tumor activity through diverse mechanisms.

4.2 NK-cells

Just like $CD8^+$ T-lymphocytes, NK cells can recognize and eliminate cancer cells [24]. In contrast to $CD8^+$ T-lymphocytes, NK are able to exerts their cytotoxic functions without prior exposure to cancer antigens [24]. Higher levels of NK cells in the TME is a positive predictor for improved survival in cancer [23]. Despite the potent anti-tumoral functions of NK cells, the tumor evades NK cells through multiple mechanisms like upregulating certain inhibitory receptors to suppress NK cell activation and recruiting immunosuppressive myeloid and T-regulatory lymphocytes [23].

4.3 Tumor associated macrophages

Tumor associated macrophages (TAMs) are one of the most abundant cells in solid tumors [25]. TAMs can be divided into M1 and M2 macrophages. M1 macrophages are pro-inflammatory and exerts anti-tumoral activities like direct phagocytosis of cancer cells and activation of anti-tumor responses [23,26]. In contrast, M2 macrophages exerts tumor supporting functions such as angiogenesis, metastasis formation, therapy resistance and immunosuppression [23,26].

4.4 Myeloid derived suppressor cells

Myeloid derived suppressor cells (MDSCs) are a heterogenous population of myeloid cells consisting mainly of monocytes and neutrophils, which possesses immunosuppressive phenotypes [23]. MDSCs inhibits the anti-tumor activities of T-lymphocytes and NK cells through paracrine signaling and cell-cell contact mechanisms [23]. The number of MDSCs increases significantly during tumor progression and play important roles in tumor development, metastasis, and therapy resistance [27]. High presence of MDSCs in the TME are correlated with poor prognosis in patients [23].

4.5 Cancer associated fibroblast

Cancer associated fibroblasts (CAFs) are activated fibroblasts and are an important component of the tumor stroma [23,25]. CAFs interact not only with cancers cells via cell-cell adhesion and secreted stimuli, but also indirectly influence the ECM and immune cells within the TME [28]. CAFs are able to remodel the ECM by synthesizing various ECM components, thereby altering the behavior of cancer and immune cells to mediate therapy resistance [23]. In addition, therapy resistance may also be the consequence of secretion of chemokines, growth factors, metabolites, and exosomes secretion by CAFs [25]. Despite all the evidence for the tumor promoting functions of CAFs, some studies indicate that some CAFs may inhibit cancer progression as host defense mechanism against tumor formation [28]. This can be explained by the high heterogeneity of CAFs, which is dependent on the CAF precursor origin, cancer type, and tumor progression state [28].

4.6 Endothelial cells

Endothelial cells are the cells that forms a single cell layer that lines all blood vessels [23]. Endothelial cells that line the blood vessels in tumors are significantly different from

endothelial cells in healthy tissue [23]. Tumor endothelial cells have an impaired barrier function due to lower expression of adhesion molecules [23]. In addition, tumor endothelial cells also express higher levels of inhibitory immune checkpoint molecules, which leads to immunosuppression [23].

5. Immunotherapy resistance in solid tumors and the role of the ECM

From all the TME components, the tumor ECM plays a crucial role in immunotherapy resistance mediated through multiple mechanisms. As stated earlier, all immunotherapies aim to enhance the ability of immune cells to recognize and kill tumor cells. ICB and CAR-T therapy are among the immunotherapies that are most promising [29]. CAR-T cell therapy is a cell-based immunotherapy focused on redirecting the patient's own T-lymphocytes by genetically modifying them to target and kill specifically tumor cells [30,31]. CARs are genetically engineered fusion proteins constituted of an antigen recognition domain derived from a monoclonal antibody, intracellular T cell signaling, and a costimulatory domain [31]. The gene encoding the CAR can be delivered by using viral and non-viral approaches [32]. After transduction, the T-lymphocytes are reinfused back into the patient. Subsequently, the CAR T-cells recognizes and bind specific antigens present on the tumor via the CAR receptor and eliminate the tumor cell. In contrast to T-cell receptors (TCRs), CARs bind antigens that do not require peptide processing or HLA expression to be recognized, therefore it can even target tumors with down-regulated HLA expression or proteasomal antigens processing [33]. Depending on the malignancy, a different antigen is targeted [33].

Since the introduction of CARs, multiple generations have been developed to overcome the limitations of the previous generation, leading to five CAR T-cell generations [34]. It is mainly the structure, composition, and function of the intracellular part of the CAR T-cells that have changed significantly in the five generations [34]. Besides, CAR-Natural Killer (CAR-NK) and CAR Macrophages (CAR-M) therapies have been introduced as a complementary/alternative to CAR T-cell therapy for solid tumors [30]. CAR-NKs are being considered due to their advantageous immunological characteristics, multiple sources, and their minimal side effects [30]. The phagocytotic functions, antigen presenting, and their natural infiltration of the tumor makes CAR-M also a viable alternative to CAR-T [30]. CAR T-cell therapy is widely used in the treatment against a wide variety of hematological malignancies. However, antitumor response in solid tumors have not been demonstrated [9,35]. Insufficient T-cell migration and penetration into the tumor is a major obstacle for T-cell based immunotherapies [36,37]. However, efforts are being made to engineer new CAR T-cells that with an enhanced ability to infiltrate tumors. For instance, CAR T-cell migration is dependent on chemokines and chemokine receptors (CCR) [30]. So, by engineering CAR T-cells that expresses a certain chemokine receptor that can bind the chemokine that is expressed by the tumor, could enhance tumor infiltration [30,38]. However, the tumor ECM is still capable of preventing these CAR T-cells from reaching the tumor.

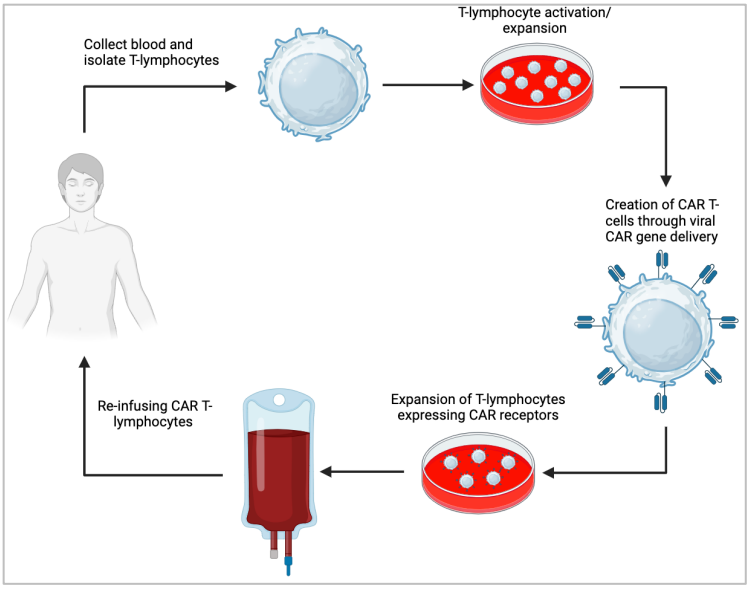

Figure 1. Schematic figure of CAR T-cell process. First the T-lymphocytes are enriched from the patients' blood. Next, the T-lymphocytes are activated and expanded. Creation of CAR T-cell happens by (non) viral delivering the gene that encodes the CAR receptor. The CAR T-cell are subsequently expanded prior to re-infusing them back into the patient (Created in Biorender).

The mechanism of ICB is based on using antibodies to block checkpoint proteins that are located on either the T-lymphocyte or the tumor cell [39]. Checkpoint proteins regulate the immune response of T-lymphocytes. For instance, programmed cell death 1 (PD-1) and cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) are co-inhibitory receptors expressed on the surface of T-lymphocytes, when they bind to their ligand, PD-L1 and CD80/86 respectively, T-cell response is suppressed [40]. The tumor expresses these ligands to evade an immune response. By blocking the receptors with antibodies (anti PD-1 and anti CTLA-4), the anti-tumor immune response can be restored (**Figure 2**) [39,40]. Despite ICB therapy does not count as a cell-based immunotherapy, combining ICB therapy with CAR T-cell treatment can create a synergistic effect [37]. ICB therapy can facilitate sustained persistence and function a of CAR T-cells [37]. However, combining ICB with CAR T-cell does not overcome the resistance in solid tumors [36,37].

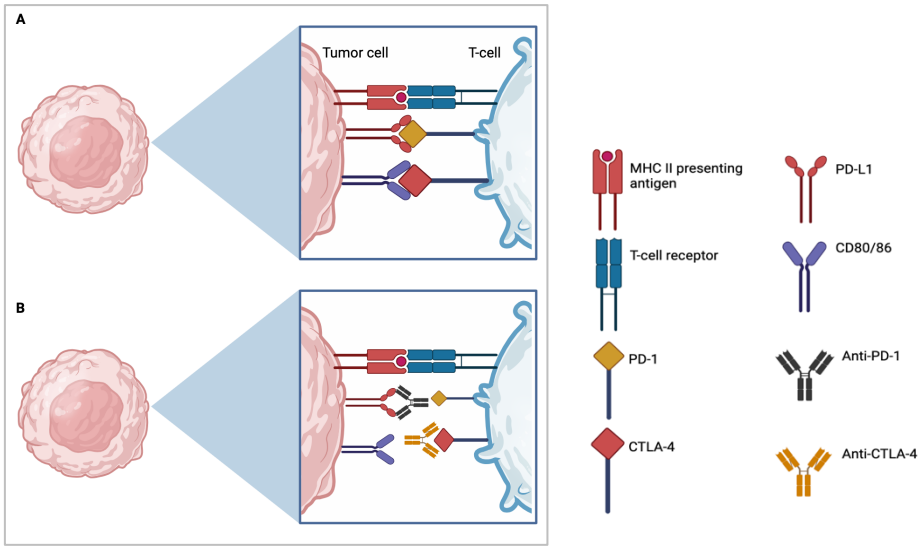

*Figure 2. Mechanism of ICB therapy. **A** T-cell receptor recognizes and binds to tumor antigen presented on MHC II. However, when PD-1 and CTLA-4 binds, located on T-cells, to PD-L1 and CD80/86 respectively, T-cell response is inhibited. **B** T-cell response can be restored by inhibiting the binding by blocking either/both PD-L1 and CTLA-4 by using antibodies (Created in Biorender).*

Remodeling of the ECM has been linked to cell-based immunotherapy resistance in solid tumors and poor prognosis [41,42]. During tumor progression, the ECM undergoes intensive remodeling mediated by the tumor [14,42]. The normal ECM is degraded, and tumor specific ECM is created. The tumor ECM is significantly different from normal ECM in terms of architecture and composition [14,42]. Healthy ECM and tumor ECM is depicted in **figure 3**. Tumor ECM is characterized by increased deposition and crosslinking of ECM components, especially collagen, which leads to a more rigid and stiff ECM [14,42]. In addition, ECM remodeling enzymes like MMPs, lysyl oxidase (LOX), lysyl oxidase-like proteins (LOXLs), and WNT1-inducible signaling pathway proteins (WISPs) are increased [14,42,43]. This leads to a wide range biophysical and biochemical changes that affects cell signaling, cell migration, tumor progression and ultimately therapy resistance [14].

It is known that remodeling of tumor ECM can regulate immune cells and form an immunosuppressive TME that prevents cytotoxic immune cells from infiltrating the tumor, and thereby hinders the efficacy of immunotherapies [41,43]. So, ECM remodeling induces immunotherapy resistance in multiple ways. The coming sections will discuss the resistance mechanisms in more detail with the focus on ECM components.

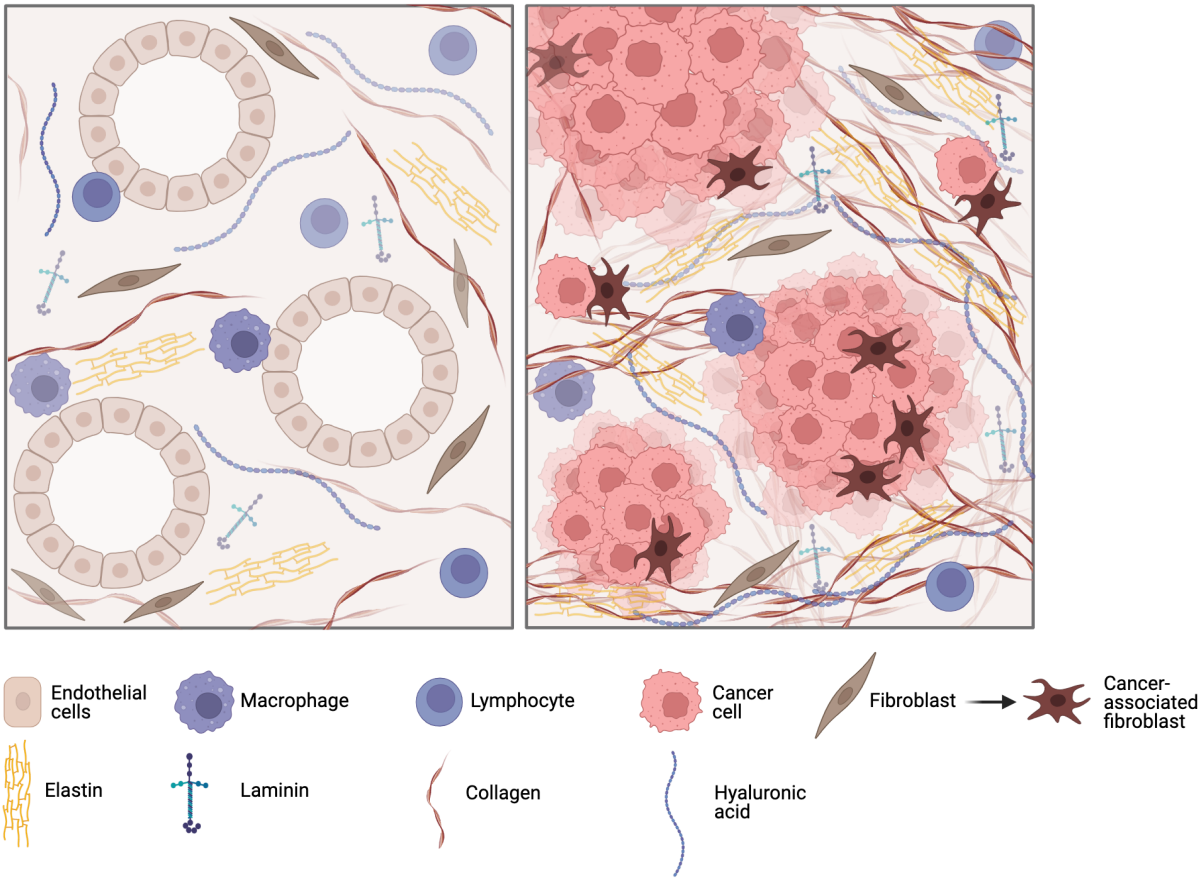

Figure 3. Healthy ECM (left picture) VS tumor EMC (right picture). The healthy ECM gets degraded, and the tumor ECM is formed. The tumor ECM is characterized by increased deposition of ECM components and stiffness. Especially increased collagen and hyaluronic acid causes the ECM to stiffen up. Moreover, fibroblast gets transformed to cancer associated fibroblasts which promotes ECM remodeling (Created in Biorender)

5.1 Immune exclusion by formation of physical barrier by tumor ECM

The main obstacle for immunotherapies in solid tumors is the poor infiltration rate of T-lymphocytes into the TME, as the level of infiltration is predictive of the response [7]. The infiltration rate is determined by the immunogenicity of the tumor and to what extent the ECM forms a protective layer around the tumor [7]. This protective layer is a physical barrier against immune cells and the checkpoint inhibitors, it is created by increased ECM stiffness through increased deposition and crosslinking of collagens that forms a dense fibril alignment around tumor cells (**figure 4**) [41,43,44]. In healthy tissue, the collagen matrix is highly porous, allowing immune cells to migrate through the ECM to facilitate immune surveillance activity within the tissue [10]. The fibril alignment formed during tumor progression is not porous, inhibiting CAR T-cells, T-lymphocytes, and checkpoint inhibitors from reaching the tumor cells, thereby inhibiting the therapeutic effect of immunotherapies.

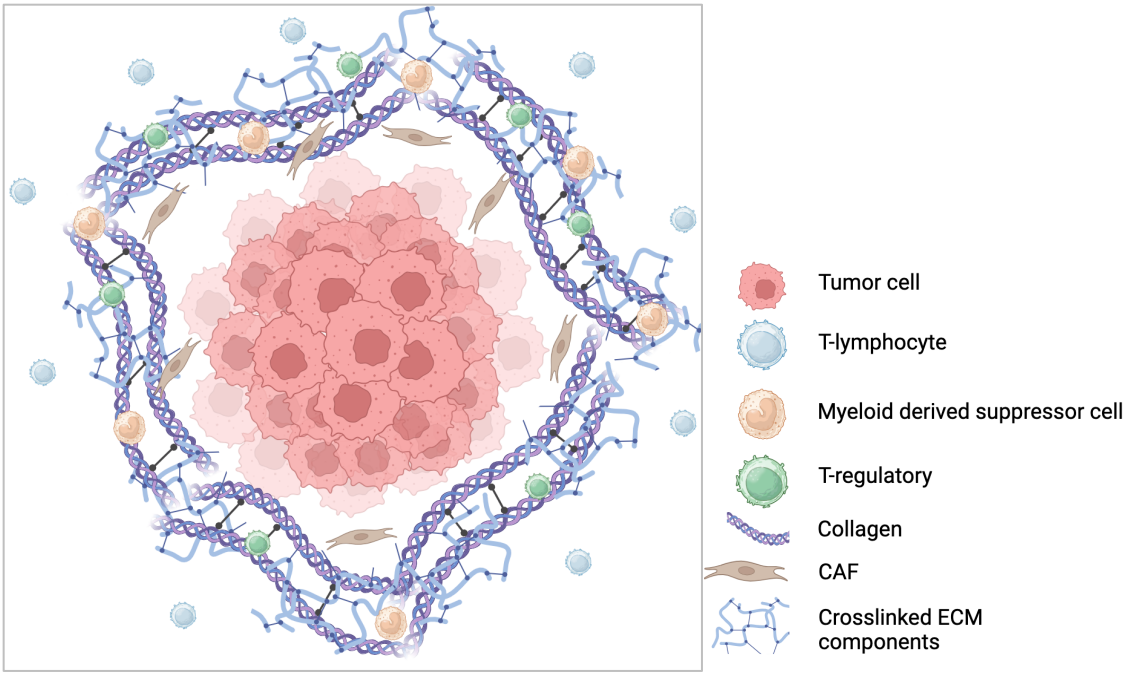

Figure 4. Remodeled tumor ECM. The tumor is shielded by increased deposition and crosslinking of ECM composition, mainly collagen. This leads to an immune excluded tumor since cytotoxic immune cells are not able to reach the tumor. Moreover, ECM components promote immunosuppressive activities by recruiting immune cells with an immunosuppressive phenotype like T-regulatory cells and myeloid derived suppressor cells. CAFs are one of main producers of ECM components (Created in Biorender).

This phenomenon is enhanced by the hypoxic environment of the tumor [14]. Tumor cells are characterized by increased proliferation and growth rate, this rapid growth leads to a certain tissue architecture, in which tumor cells are located more distant from blood vessels, resulting in cells that are poorly supplied with nutrients and oxygen [14,43]. When the metabolic energy demand exceeds the oxygen supply, oxygen deficiency in the TME will occur, eventually leading to hypoxia [45]. Despite the expression of EPO and angiogenic factors are increased to promote proliferation of vascular endothelial cells, the arrangement is severely disorganized causing the formation of non-functioning vessels [45]. Hypoxia promotes cellular signaling mediated by the hypoxia-inducible transcription factor (HIF-1), inducing upregulation of ECM enzymes such as LOX and collagen prolyl 4-hydroxylase (CP4H) [14,43,45]. LOX mediates collagen crosslinking and C-P4H is key catalyst in the biosynthesis of collagen [14,46].

The main producer of collagen and other ECM components in healthy tissue as well as in tumor tissues, are fibroblasts, especially the activated and transformed version; myofibroblasts [14,43].

The pro-inflammatory factor transforming growth factor β (TGF-β) is the most important pro-inflammatory factor that leads to the activation of myofibroblast [14]. Healthy ECM remodeling by myofibroblasts is a crucial physiological process, also their inactivation is important after restoring homeostasis [14]. However, continued inflammatory stimuli and TGF-β secretion by tumor cells can create deregulated, hyperproliferative, and overactive myofibroblasts [14]. Furthermore, tumor cells secrete pro-fibrotic factors and inflammatory factors such as TGF-α, TGF-β, fibroblast growth factor (FGF)-2, platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) to recruit fibroblasts and differentiate them into cancer-associated fibroblasts (CAFs) [15]. CAFs acts as fibroblasts with a stronger ability to proliferate and promote ECM stiffness by secreting a number of different ECM components (such as collagen, fibronectin, MMPs) and cytokines like TGF-β [14,41,44,47]. CAF activity is enhanced under hypoxic environments by HIF-1 [14,43]. So, TGF-β secretion is indispensable for formation of fibril collagen through activation of CAFs, which in his turn, secretes more TGF-β and ECM components to support the ECM remodeling [15]. In addition, HA also forms a dense structure around tumor cells that prevent immune cells infiltrating into the tumor in a similar manner as collagen and activates multiple cell receptors including TGF-β through HA-CD44 binding [7,15]

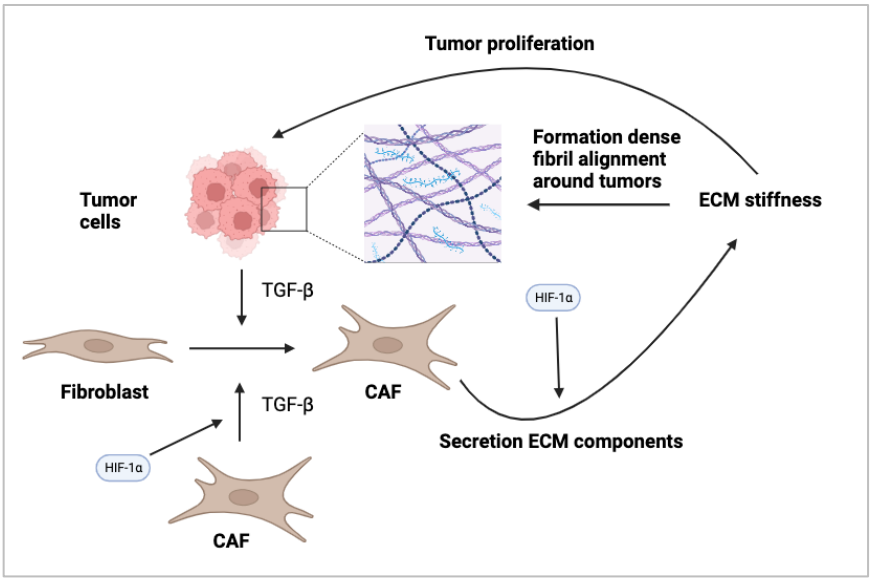

Figure 5. Tumor cells secrete TGF-β, which recruits fibroblasts and differentiate them into CAFs. CAFs themselves, can also secrete TGF-β to increase activation of fibroblast. CAFs promotes tumor ECM remodeling by producing various ECM components, including collagen, which leads to increased crosslinking and stiffness of the ECM. Increased ECM stiffness and crosslinking promotes tumor progression, which causes secretion of more TGF-β [15] *(Created in Biorender).*

To summarize, remodeling of tumor ECM leads to increase deposition of ECM components. Especially the increased deposition and crosslinking of collagens leads to the formation of fibril alignment around the tumor that act as shield to prevent immune cells to penetrate the tumor. Increased deposition of collagen is the consequence of continued secretion of TGF-β which leads to overactive myofibroblasts, which is the main producer of ECM components. CAFs, differentiated from fibroblasts through TGF-β, secreted by tumor cells, also contribute to the increase deposition of collagens (**figure 5**). CAFs are considered the main contributor of ECM remodeling [14,48].

5.2 Immune modulation by tumor ECM

Beside physical exclusion of T-lymphocytes by the tumor ECM, tumor ECM is also capable of inactivating T-lymphocytes in the TME by multiple mechanisms [15]. Firstly, the infiltration movement of T-lymphocytes towards the chemotaxis gradient is disturbed by haptotaxis signals from the tumor ECM [15]. Instead of entering the TME, T-lymphocytes migrate along the tumor ECM due to adhesion molecules such as aligned collagen [15].

Secondly, poor diffusion of immune cells due to the increased stiffness of the ECM causes increase of hypoxia and metabolic stress, this leads to upregulation of immunosuppressive factors like IL-10, CCL18, CCL22, TGF-β, prostaglandin E2, and VEGF-A [7,15]. TGF-β not only plays an important role in collagen mediated immunotherapy resistance, but it also acts as immune suppressor by recruiting and differentiation of regulatory T-lymphocytes to the TME to hinder the effect of cytotoxic T-lymphocytes and NK-cells and acts as a M2- polarizing regulator for macrophages [7,15]. M2 macrophage is a subtype of macrophages that contain an anti-inflammatory/pro-tumor phenotype that promote tumor progression by secreting immunosuppressive factors [49,50]. So, collagen suppresses immune response indirectly through TGF-β, which polarizes macrophages into M2 macrophages and recruits regulatory T-lymphocytes [7].

Thirdly, T-lymphocytes can be directly regulated by some ECM components. It is observed that T-lymphocytes are expressing inhibitory receptors, also defined as exhausted T-lymphocytes, and myeloid cells (tumor associated macrophages, tumor associated DCs, and myeloid derived suppressor cells) possesses an immunosuppressive phenotype [41,51]. Exhausted T-lymphocytes are induced by collagen through leukocyte associated immunoglobin like receptor 1 (LAIR1) expressed on $CD8^+$ T-lymphocytes [15,52]. LAIR1 binds to collagen with high affinity and contains two immunoreceptor tyrosine-based inhibitory motifs (ITIMs) [53]. Upon binding of LAIR1 with collagen, the two ITIMs suppresses $CD8^+$ T-lymphocyte activity through SHP-1 signaling and promotes ICB therapy resistance (**figure 6**) [15,52,53]. LAIR1 upregulation is the consequence of collagen binding to integrin beta 2 (CD18) [15,52].

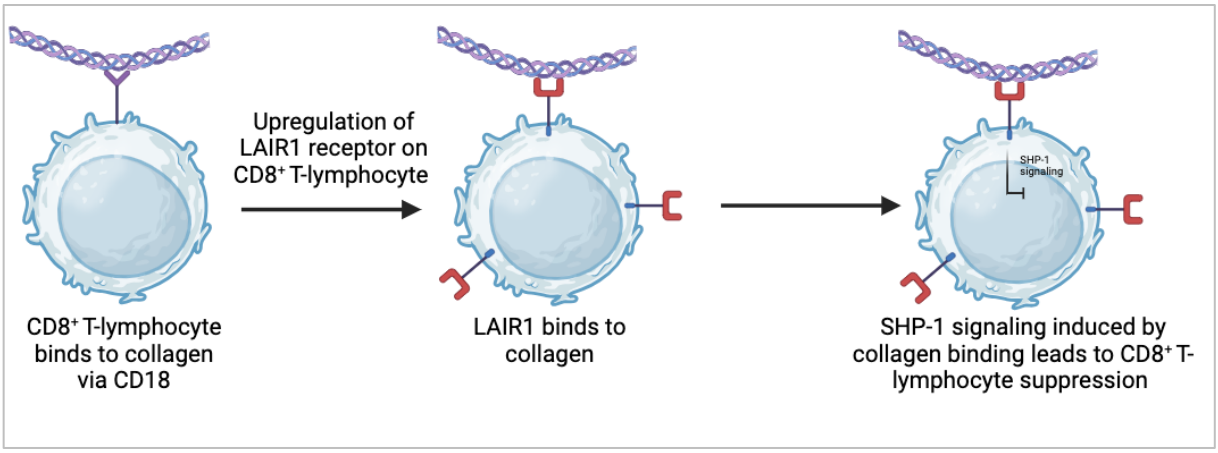

Figure 6. $CD8^+$ T-lymphocytes binds to collagen, which leads upregulation of LAIR1. LAIR1 is also able to bind collagen, upon this interaction, SHP-1 signaling is induced, which leads to $CD8^+$ T-lymphocytes suppression (Created in Biorender).

Studies showed that collagen could lead to the activation of the focal adhesion pathway (FAK) pathway, inducing depletion of $CD8^+$ T-cells and promotes the recruitment of immunosuppressive immune cells like T-regulatory cells and MDSCs into the TME [47]. An in vitro study also showed that macrophages cultured in high density collagen inhibited T-lymphocyte

proliferation compared to macrophages cultured in low density collagens [51]. Higher production of PGE_2 by macrophages cultured in high density collagen could be the cause, PGE_2 is known to affect T-lymphocyte activity [51]. Furthermore, HA could enhance the activity of T-regulatory lymphocytes to suppress the immune response [15].

6. Therapeutics targeting ECM components

Tumor ECM remodeling plays a major role in the resistance against immunotherapies. To overcome this resistance, therapeutics that (indirectly) targets the tumor ECM components could be the key to enhance patient response to immunotherapies. Multiple therapeutic strategies have been developed that each target a different aspect of the tumor ECM. Besides targeting the ECM to overcome immunotherapy resistance, it is worthy to mention that therapies targeting ECM components have the potential to acts as cancer therapy on its own, this however, won't be covered in this paper since we aim to focus on overcoming cell-based immunotherapy resistance by targeting ECM components.

6.1 Therapies targeting collagen

Collagen is a prominent target, as collagen plays a major role in immunotherapy resistance by forming fibril alignment around the tumor and modulates immune cells. Targeting collagens could facilitate the infiltration of checkpoint inhibitors and T-lymphocytes [15]. Collagen targeting therapies are mostly focused on the synthesis, degradation, and crosslinking of collagens [15].

For targeting the synthesis of collagen, TGF-β is often directly/indirectly targeted, as its signaling pathway is important for the synthesis of collagen [15]. For instance, the anti-fibrotic agent Halofugione blocks fibroblasts' Smad3 activation upon stimulation by TGF-β, has shown to reduce collagen synthesis and improved infiltration of immune cells in an animal pancreatic cancer model [15,54]. An anti-hypertensive drug, Losartan, also acts as an anti-fibrotic agent by suppressing active TGF-β to decrease collagen synthesis by preventing activation of CAFs [15,54]. However, targeting TGF-β should be studied more due to its roles in both inflammatory and tumorigenesis [15]. In addition to targeting TGF-β, CP4H is also a logical target as it is a key catalyst in the synthesis of collagen [14,46]. Several drugs have been developed that target CP4H [46].

Direct targeting of collagen can be achieved through collagen degradation by collagenases [15,54]. Several animal studies have shown that collagenase treatment improves the diffusion and uptake of macromolecules [54]. Big concerns of this therapeutic strategy are that degradation of collagen may lead to the release of growth factors and cytokines that are located in collagens, which could initiate inflammatory signals causing and tumor progression [15]. In addition, breaking down collagen could lead to tumor migration and invasion [15].

Lastly, preventing collagens from crosslinking is another strategy to target collagens. LOX enzyme is upregulated in tumor ECM and mediates the crosslinking of collagens. Inhibition of LOX and LOXLs by small molecule inhibitors or anti-LOX(L) antibodies prevents collagens from crosslinking and may improve immune infiltration. The downside of this approach is that it might not work for a tumor with an already existing mature collagen alignment [15].

6.2 Therapies targeting HA

An in vivo study showed enhanced T-lymphocyte infiltration by combining hyaluronidase with cancer nano-vaccines, which creates T-lymphocytes targeting antigens located on the tumor [55]. So, breaking down HA with hyaluronidase could also benefit the infiltration of CAR-T cells and checkpoint inhibitors [56,57].

6.3 Special engineered CARs to target ECM components

Due to the fibril alignment, CAR T-cells are not able to reach the tumor. However, functional changes in the T-lymphocytes after ex-vivo manipulation may also play a role in the poor infiltration [58]. A study demonstrated that in-vitro cultured T-lymphocytes lost the expression of the enzyme heparinase (HPSE), which degrades heparan sulfate proteoglycans which is also a main component of the ECM [58]. Specific engineered CAR T-cells that expresses the HSPE enzyme showed enhancement in ECM degradation in-vitro as well as in in-vivo, which led to promotion of T-lymphocyte infiltration and anti-tumor activity [58].

CAFs, as stated earlier, are considered one of the main contributors of ECM remodeling, they overexpress the Fibroblast activation protein (FAP) which is associated with ECM remodeling [38,43]. Therefore, FAP is a potential target to degrade the ECM. A study showed that a FAP specific CAR T-cell induced ECM degradation in pancreatic ductal adenocarcinoma [38,43].

Besides CARs for T-lymphocytes, CARs for macrophages are also in development. A great advantage of macrophages is that it can infiltrate the tumor. A study, in which they designed a CAR, constituted of an extracellular part that recognizes HER2 (an important biomarker in breast cancer) and the intracellular part of CD147, which is used to activate the expression of the ECM degrading enzymes MMPs in macrophages [59]. MMPs are enzymes that can degrade ECM components to alleviate ECM stiffness. Even though the CAR-M did not affect tumor growth, T-lymphocyte infiltration was greatly enhanced [59].

6.3 Limiting factors

A limiting factor in the development of therapies targeting the ECM is the lack of materials that can simulate the ECM in vitro [15]. Despite the use of materials such as Matrigel in 3D cultures, and the use of Gelatin Methacryloyl in 3D print, it remains difficult to recreate the biological and physiological properties and the interactions between the different ECM components [15]. In addition, the use of mice models is not accurate, as the ECM in mice is significantly different from human ECM due to scale and histology [15].

7. Conclusion

Cell-based immunotherapy has come a great way in the battle against cancer. Recent development of immunotherapies such as immune checkpoint blockade and CAR T-cell therapies have improved survival among cancer patients. Immunotherapies are enhancing the patients' own immune system to recognize and destroy malignant cells. Unlike chemotherapy and radiation therapy, immunotherapies are targeted, thereby limiting side effects and collateral damage, and have yielded great success in the treatment of hematological cancers. Despite the success of treatment in hematological cancers, the success rate in the treatment of solid tumors remains low. The poor response in solid tumors is multifactorial, but it is believed that the tumor ECM plays an important role. During tumor progression, the normal ECM is degraded, and the tumor ECM is formed. Tumor ECM is characterized by increased

deposition of ECM components like HA and collagen, and increased stiffness. The ECM can basically facilitate therapy resistance in two ways. Firstly, collagens and other ECM components undergoes crosslinking and form a dense fibril alignment around the tumor. Cytotoxic immune cells and checkpoint inhibitors are not able to penetrate this fibril alignment, creating an immune excluded tumor. Secondly, certain ECM components can regulate immune cells to create an immunosuppressive TME by recruiting immunosuppressive immune cells. Therapies targeting the ECM shows great promise in *in-vitro* studies to overcome immunotherapy resistance. Since collagen is an important player in tumor ECM remodeling, collagen is an obvious target. Other targets include HA, LOX enzymes, and TGF-β. Most therapies have shown promising results in in vitro and in vivo studies. However, more effort should be made to bring therapeutics to clinical studies. The development of ECM targeting therapeutics faces some challenges like creating models, which can accurately simulate ECM physiology and ECM interactions. When ECM therapeutics can successfully target tumor ECM components to overcome resistance, cell-based immunotherapies can become even more powerful.

8. References

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