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Obstacles to T cell migration in the tumor microenvironment

Alba Nicolas-Boluda[a,b,c], Emmanuel Donnadieu[a,b,c,*]

[a] Inserm, U1016, Institut Cochin, Paris, France
[b] Cnrs, UMR8104, Paris, France
[c] Université Paris Descartes, Sorbonne Paris Cité, Paris, France

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ABSTRACT

These last years, significant progress has been made in the design of strategies empowering T cells with efficient anti-tumor activities. Hence, adoptive T cell therapy and the use of monoclonal antibodies against the immunosuppressive surface molecules CTLA-4 and PD-1 appear as the most promising immunotherapies against cancer. One of the challenges ahead is to render these therapeutic interventions even more effective as a still elevated fraction of cancer patients is refractory to these treatments. A frequently overlooked determinant of the success of T cell-based immunotherapy relates to the ability of effector T cells to migrate into and within tumors, as well as to have access to tumor antigens. Here, we will focus on recent advances in understanding T cell trafficking into and within tumors. Both chemoattractant molecules and structural determinants are essential for regulating T cell motile behavior along with cellular interactions-mediated antigen recognition. In addition, we will review evidence that the microenvironment of advanced tumors creates multiple obstacles limiting T cells from migrating and making contact with their malignant targets. We will particularly focus on the extracellular matrix and tumor-associated macrophages that make tumors a hostile environment for T cell ability to contact and kill malignant cells. Finally, we will discuss possible strategies to restore a tumor microenvironment more favorable to T cell migration and functions with a special emphasis on approaches targeting the dysregulated extracellular matrix of growing tumors.

1. Introduction

A number of facts - increased frequency of cancer in immunocompromised individuals, abundant presence of anti-tumoral lymphocytes in the blood, good prognosis associated with intratumoral lymphocyte infiltrates, especially for Th1 and CD8 T cells - show that the immune system is able to respond against cancer and, in some conditions, even capable of clearing tumors.

Given the effectiveness of T cells in mediating anti-tumor immune response, T cell-based immunotherapy is considered an important and promising therapeutic approach against cancer [1]. Among the different novel strategies, two appear particularly efficient [2]. Adoptive cell therapy, using either endogenous anti-tumor T cells or cells genetically engineered to express anti-tumor receptors, has been shown to mediate a good percentage of complete regressions in patients with metastatic cancer [3]. In addition, the development of antagonists of T cell checkpoint molecules, namely anti-PD1 and anti-CTLA-4, is giving promising results in a significant proportion of cancer patients even with late stage cancer [4].

Although some patients treated with T-cell based immunotherapies achieve long-term disease-free survival, a still elevated percentage of them do not respond clinically and it is thus crucial to understand the reason of such a failure.

Several mechanisms have been proposed to explain the paradoxical growth of immunogenic tumors in the presence of an active immune response. Among those that received much attention, a large number of data suggest that T cell function into the tumor is impaired by multiple immunosuppressive mechanisms [5].

Besides the ability of T cells to respond adequately to tumor antigens, T lymphocytes need to make direct physical contact with tumor cells and to have access to tumor antigens. This cell-cell interaction is the end result of a series of stepwise events that go from acquisition of T cell migratory function in the draining lymph node, to homing of lymphocytes into the tumors, followed by the locomotion of T cells within the malignant site (Fig. 1). In cancer patients, one or several of these steps do not operate optimally, resulting in an overall defect in T

Abbreviations: TAM, tumor-associated macrophages; CAF, carcinoma-associated fibroblasts; FAK, focal adhesion kinase; LOX, lysyl oxidase; MMP, matrix metalloproteinases; ROCK, Rho-associated protein kinase; TGF-β, transforming growth factor beta

* Corresponding author at: Institut Cochin, INSERM U1016, 22 rue Méchain, 75014 Paris, France.
E-mail address: Emmanuel.donnadieu@inserm.fr (E. Donnadieu).

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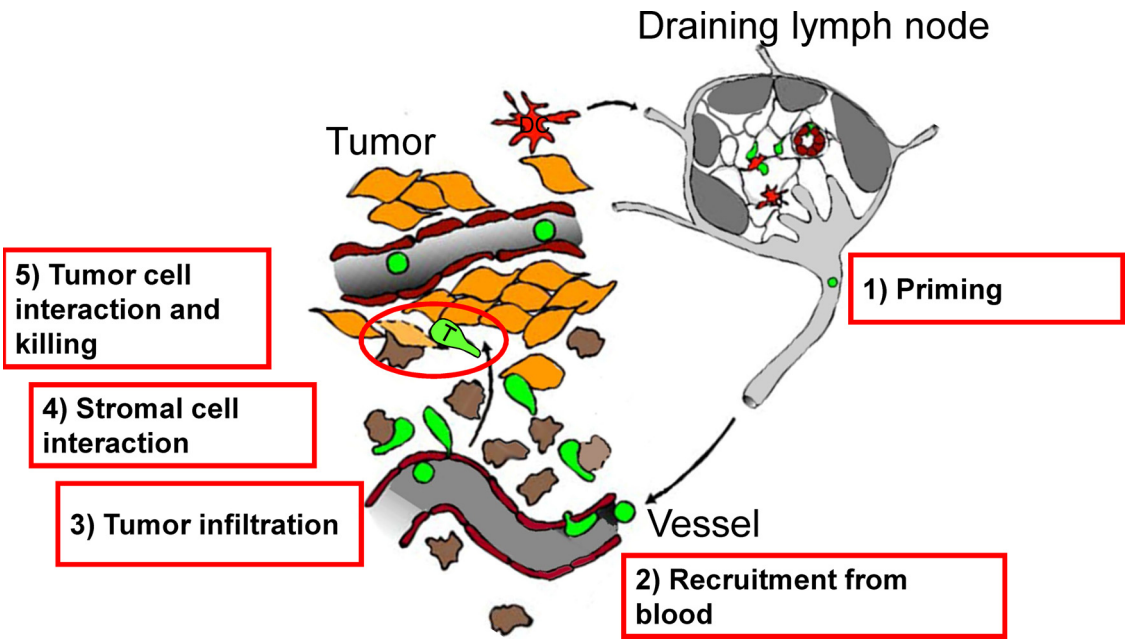

Fig. 1. T cell migration during an anti-tumor response. To reach cancer cells, T cells need to go through different steps during which T lymphocytes actively migrate. (1) During the first step, T cells are activated in the tumor-draining lymph node, acquire their effector functions and their abilities to migrate towards the tumor. (2–3) Then, T cells extravasate and enter into the malignant site. (4) In the tumor stroma, T lymphocytes interact with stromal cells and components such as the ECM and TAM. (5) Finally and once all prior steps have been reached successfully, T cells make contact and kill tumors cells. Modified from [96].

cell trafficking into and within tumors [6]. This failure represents a major obstacle to T-cell based immunotherapy and one important goal is to convert the tumor microenvironment into a tissue that supports T cell infiltration, migration and effector functions.

It is clearly established that the homing of anti-tumoral lymphocytes does not always occur normally and several alterations in the tumor blood vessels affecting the normal entry of T cells have been reported [7]. Nevertheless, even when T cells succeed in crossing the blood vessels, they are usually not in direct contact with tumor cells, but rather remain concentrated into the stroma surrounding the tumor epithelial region (Fig. 2). Recent evidence suggests that the dysregulated environment of growing tumors limits T cells from contacting tumor cells. Several levels of alterations have been demonstrated, including a lack of chemoattractant molecules, as well as an extensive remodeling of the extracellular matrix (ECM). In addition, the presence of myeloid cells, including tumor-associated macrophages (TAM), in the tumor stroma has been proposed to negatively regulate the activation and migration of T lymphocytes.

Here, we review our current understanding of the different obstacles hindering T cells from migrating to and within tumors. We also discuss

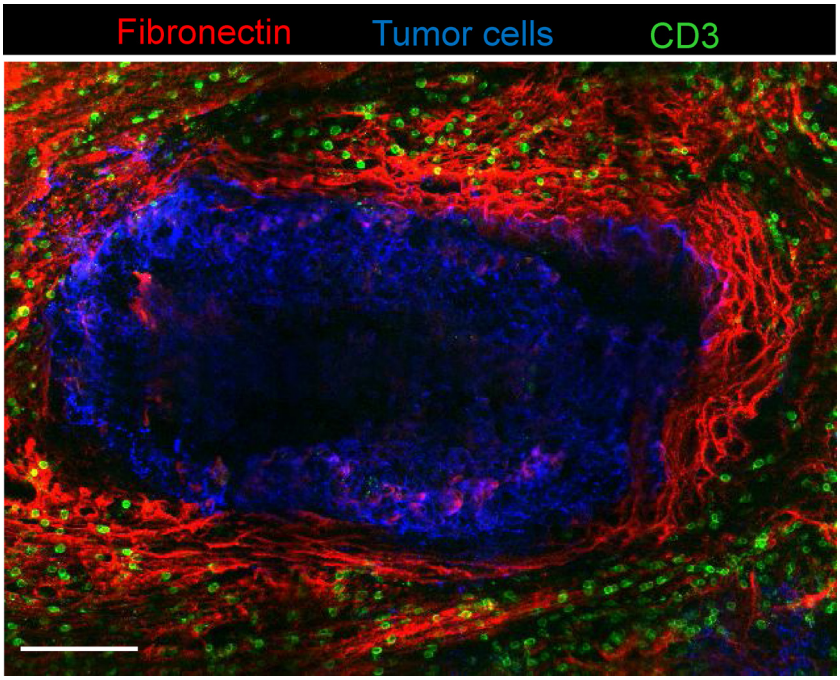

Fig. 2. Distribution of T cells in a human lung tumor. Tumor islets (blue, stained for EpCAM) are surrounded by dense and tight fibronectin fibers (red, stained for fibronectin). T cells (green, stained for CD3) are sparse in regions immediately adjacent to the tumor mass but concentrated in light matrix fiber areas. Bar, 100 μm.

possible therapeutic interventions targeting the tumor stroma, especially the dysregulated ECM in order to reinstate a microenvironment more favorable for T cells to migrate and contact tumor cells.

2. Basic rules of T cell migration

T cells can be regarded as explorers as they sample the environment in search of their cognate antigen. In order to perform this task effectively, T cells possess a high migratory potential that enable them to traffic actively in most organs and tissues [8]. The past decade has seen important progress in imaging technologies that have increased our knowledge on the dynamics of T cells in various organs, including tumors [9]. These studies have not only revealed the remarkable motility of T cells, but also identified some of the determinants that regulate this process [10].

2.1. Chemokines

There is accumulating evidence for a role of chemokines in the recruitment and migration of T cells into and within tissues [10]. By binding to their receptors, chemokines trigger a signaling machinery that leads to the formation of a ruffling leading edge and a trailing uropod, required for T cell trafficking. Notably, most effective chemokines for T cells do not act as soluble factors but through their binding to proteoglycans [11]. For example, in the T zone of lymphoid organs, CCL21 produced by fibroblastic reticular cells is retained at the surface of these fibroblasts thus creating a road onto which T cells actively migrate [12–14].

2.2. The extracellular matrix

Chemokines are not the only factors that regulate T cells through their interstitial displacements. The architecture of the tissue is also important for both facilitating and creating environmental obstacles to T cell trafficking [15]. Unlike a number of cells including macrophages and tumor cells, T lymphocytes do not produce proteases that can degrade the ECM permitting cell migration through dense tissues. Conversely, T cells use a mode of migration characterized by reorganization of their cytoskeleton leading to marked cellular deformations [16]. Experiments performed *in vitro* in 3D ECM gel initially described the ability of T cells to migrate through narrow spaces [17]. However, when the matrix becomes too dense, T cells are impeded in their migration and are forced to change direction in order to migrate towards looser ECM network [18]. Substrates created by the fibrillar ECM can also be used by T cells to migrate although evidence mostly come from experiments performed in 3D collagen gel and not yet in intact tissues. In summary, the physical characteristics of the ECM, namely its density, stiffness and orientation will have a profound impact on T cells and their displacements.

2.3. Interaction with antigen-presenting cells

In tissues, T cells also encounter a variety of cells that influence their migration. Antigen recognition by T cells has a strong influence on the motile behavior of T cells. Experiments performed *in vitro* and corroborated *in vivo* provided the evidence that presentation of the cognate antigen leads to a stable T cell - APC interaction that functions as a “tether” restricting entrapped T cells from migrating [19]. Several factors are instrumental in provoking this arrest, including an increase in intracellular Ca^{2+} and the activation of adhesion molecules, mainly integrins [20]. Moreover, this cell-cell interaction is strongly dependent on the strength of the signal received by the T lymphocyte. Accordingly, the engagement of the inhibitory receptors CTLA-4 and PD-1 at the T cell surface reduces the signal triggered by the TCR, and thus limits the duration of T cell interacting with the APC during an acute immune response [21,22].

3. Migration of T cells within the tumor

Once entered into a malignant site by crossing tumor blood vessels, T cells encounter a complex environment composed of a variety of cells organized in a specialized microenvironment, referred to as the stroma. The stroma is enriched in fibroblasts and blood vessels surrounding tumor cells in cancers of epithelial origins. T cells infiltrate the tumor through blood vessels localized in the stroma. Thus, for T cells to reach tumor cells, several principles must be fulfilled, including the presence of chemoattractants, as well as a matrix structure permissive for T cell migration.

3.1. Role of chemokines and adhesion molecules in controlling T cell migration within tumors

Chemokines have been found instrumental in the entry and accumulation of T cells into tumors. Indeed, tumor enriched in inflammatory chemokine such as CCL5, CXCL9 and CXCL10 usually contain numerous T cells and are therefore of good prognosis [23–25]. The nature of the cells within the tumor environment producing T cell-attracting chemokines remains to be established. Tumor cells, as well as CD103 DC, recently shown to attract T cells into tumors through the production of CXCR3 ligands, are good candidates [26]. Conversely, non-inflamed tumors devoid of chemokines and CD8 T cells are associated with a bad outcome. Of interest, during tumor progression a number of chemokines have been shown to be either downregulated or inactivated, a process limiting T cells from migration into malignant sites [27]. In addition, non-inflamed tumors frequently show evidence of activation of the Wnt/β- catenin pathway [28]. Expression of an active β-catenin in a genetically engineered mouse melanoma model leads to loss of inflammatory chemokines and consequently of T cells in tumors [28]. Epigenetic modifications in tumor cells can also influence the number of T cells found within a tumor. For example, histone modifications and DNA methylation in ovarian cancer cells resulted in silencing of chemokines CXCL9 and CXCL10 as well as incapacity of T cells to infiltrate the tumor [29].

Besides chemotaxis, tumor-derived chemokines can also have detrimental influence on T cells, keeping them in the stroma, away from tumor cells. For example, in a pancreatic mouse tumor model, production of CXCL12 by stromal fibroblasts prevents T cells from entering tumor islets by a mechanism which is not yet understood [30].

Even if the role played by chemokines favoring the entry of T cells into tumors is clearly established, the participation of chemoattractant molecules in controlling the migration of T cells within tumors as well as their ability to have access to tumor antigens remain ill-defined. Several two-photon *in vivo* studies performed in mouse tumors have shown that, rather than a general chemokine-guided infiltration, cytotoxic T lymphocytes perform a random migration that may lead to a progressive infiltration of T cell into solid tumors [10,31,32].

In order for cytotoxic T cells to kill tumor cells, they need to make a direct contact with malignant cells. This cell-cell interaction is regulated by several adhesion molecules among which two integrins, namely lymphocyte function-associated antigen-1 (LFA-1) and CD103 (αE/β7) play a critical role. LFA-1 (also known as CD11a/CD18 and αLβ2) is an integrin expressed by T cells and other hematopoietic cells. This integrin binds to intercellular adhesion molecule 1 (ICAM-1 or CD54) expressed by antigen-presenting cells as well as tumor cells. When T cells are stimulated by any of a variety of external stimuli, including antigens and chemokines, the affinity and clustering of LFA-1 increases in an inside-out signaling process that promotes the binding of this integrin to ICAM-1. It is clearly established that tumor-infiltrating T cells need LFA-1 to adhere to cancer cells before killing them [33,34]. Interestingly, ICAM-1 expression is controlled by inflammatory cytokines including interferon gamma [35]. Hence, several human tumors, likely non-inflamed ones, lack ICAM-1 expression contributing to explain the paucity of T cells in tumor islets [36]. More recently, the role

of CD103, another integrin expressed by a subset of T cells within tumors, retains major attention. CD103 which binds to the epithelial cell molecule E-cadherin defines, together with other markers, a recently identified subtype of CD8 T cells named tissue-resident memory cells [37]. Accumulating evidence suggests that CD103 not only contributes to recruitment of T lymphocytes within epithelial tumor islets but also favors their cytotoxic activity [38].

3.2. Role of the tissue structure in controlling T cell migration within tumors

As reviewed previously, T cell migration is controlled by chemical components but is also strongly influenced by the architecture of the tissue. By imaging either plated or resident T lymphocytes in 400-μm-thick human lung and ovarian tumor slices, we reported the migration of T cells in relation to tumor cells as well as ECM components [39,40]. T cells were mainly found in stromal regions surrounding tumor islets. A close examination of the motile behavior of T cells reveals the presence of several stromal territories, each composed of different matrix architectures having different effects on T cells. Stromal regions enriched in fast migrating T cells were characterized by a loose network of collagen and fibronectin fibers. These favorable migration zones, by their physical and chemical features including the presence of chemokines, present some analogies with the reticular fiber networks of secondary lymphoid organs and it is likely that they correspond to the lymphoid-like structures recently described in several human tumors, including lung, breast and melanoma cancers [41]. Apart from these well-delineated stromal regions, other stromal zones were composed of a dense network of collagen. Such fibrotic structures were mostly observed surrounding tumor islets with the ECM fibers being parallel to the tumor-stroma interface (Fig. 2). Imaging of T cells in these peritumoral regions indicate that lymphocytes crawl onto the fibers [40]. Thus, by their densities, but also by their orientations, these structures contribute to keep T cells in the stroma, away from tumor cells. This study is consistent with a number of data showing that tumors with a dense stroma contain significantly less T cells in tumor islets compared to tumors exhibiting a loose stroma. For example, pancreatic tumors characterized by a prominent desmoplastic microenvironment adjacent to tumor cells contain very few CD8 T lymphocytes, these cells being far away from tumor nests [42]. Of interest, the presence of a dense stroma can contribute to explain the lack of response of cancer patients to immunotherapy. As a matter of fact, tumors from melanoma patients who do not respond to anti-PD-1 antibodies present a mesenchymal signature characterized by an enrichment of activated fibroblasts and other factors such as transforming growth factor β (TGF-β) usually found in fibrotic tissues [43]. Moreover, human colorectal tumors enriched in mesenchymal genes including TGF-β are associated with a poor prognosis [44].

3.3. Importance of macrophages in controlling T cell migration within tumors

As described previously, antigen recognition usually results in T cell arrest and consequently contributes to T cell positioning at the effector site of an immune response. Dynamic imaging experiments performed in mouse tumor models have highlighted different antigen recognition process with different outcomes for T cells. Indeed, imaging of ectopic thymomas (EL4) using labeled TCR transgenic T cells has revealed long-lived antigen-dependent contacts between T cells and the tumor cells during tumor rejection [31,32]. However, in this model, the introduction of tumor-specific T cells leads to tumor regression, unlike the case in typical refractory tumors. In a spontaneous tumor model of human breast cancer, T cells have been shown forming long-lasting contacts with myeloid cells sharing some analogies with both dendritic cells and TAM [45,46]. Interestingly, these interactions which fail to promote T cell effector function occur in stromal regions immediately adjacent to tumor cells. Our recent data obtained in human and murine tumors also evidenced the presence of stable conjugates formed between TAM and CD8 T cells in the stroma [47]. Remarkably, the depletion of TAM in several mouse tumor models increased the motility of CD8 T cells and their ability to reach tumor cells. This macrophage-depletion strategy, which by itself has minor effect on the tumor growth, also improved the effect of anti-PD-1 antibodies [47]. Altogether, these data suggest that TAM, very abundant cells in the tumor microenvironment, participate to the exclusion of CD8 T cells from the vicinity of cancer cells. The mechanisms by which TAM prevent CD8 T cells from reaching tumor cells is not known at the moment. Although we favor an adhesion process between both cell types triggered by an effective antigen recognition, an effect of macrophages on environmental factors controlling the motility of T cells cannot be ruled out. In some situations like breast development, macrophages actively participate to the construction of the tissue through interaction with fibroblasts [48]. In addition, evidence indicates a role of TAM in ECM production within the tumor [49]. Interestingly, a recent study demonstrates that TAM activate carcinoma-associated fibroblasts to produce excessive amount of the ECM, excluding T cells from tumor cells, through the secretion of granulin, a growth factor belonging to the epithelin family [50]. However, in the murine models we studied [47] the loss of macrophages in the tumor microenvironment though CSF1R inhibition did not significantly altered tumor architecture, at least histologically. It would be interesting to explore whether ECM remodeling in tumors can be observed with other TAM targeting therapies.

4. Therapeutic interventions

A major hurdle for T cell-based anti-tumor immunotherapy is the presence of a stromal microenvironment that alters T cell functions, including their capacity to migrate and interact with malignant cells. Therefore, strategies aimed at improving the migration of T cells to and within tumors are likely to enhance the efficacy of current immunotherapy.

As summarized in the previous section, biochemical, structural and cellular determinants by positively - chemokines - and negatively - dense ECM and TAM - controlling T cell distribution and migration within tumors appear as good targetable elements. Thus, an ideal treatment to augment intratumoral T cell trafficking would consist of increasing the production of chemokines and, at the same time, decreasing the dense fibrotic network and the amount of TAM.

4.1. Increasing the chemokine axis

The conversion of tumors into an inflamed tissue enriched in inflammatory chemokines may help to recruit anti-tumor effector T cells. For example, the introduction of TLR agonists or type I interferon in human tumor explants kept in culture has been shown to facilitate chemokine production and desirable inflammation at the tumor site [51].

However increasing the inflammation within the tumor is not always beneficial, as it can provoke in a genetically engineered mouse melanoma model the differentiation of tumor cells into a mesenchymal-like phenotype that resists T cell attack [52]. Some chemotherapeutic drugs (i.e., dacarbazine and cisplatin), originally selected for their capacity to induce cancer cell apoptosis, can also have, in melanoma mouse models, some effects favoring the migration of T cells through an induced expression of intratumoral chemokines [24]. The molecular mechanism underlying this effect is unclear at present and will require further investigations. The manipulation of fibroblasts, cells known to produce important chemokines for T cells, retains the attention too. As an example, the TNF superfamily member LIGHT has been reported to engage LTβR on stromal fibroblasts and induce chemokine production, T cell recruitment and tumor rejection in mouse tumor models, especially when combined with checkpoint inhibitors [53,54].

Another level of intervention consists of adoptively transferring T

cells that have been genetically engineered to express receptors for a chemokine abundantly produced at the tumor site. Several studies performed in preclinical mouse tumor models support the rational for such a strategy [55–57].

4.2. Targeting tumor-associated macrophages

TAM participate in suppressing T cell anti-tumor function in various ways [58]. As reviewed previously, TAM engage T cells in long-lived interaction in the stroma in unproductive manner [47]. Thus, depletion of TAM shows a great interest in cancer immunotherapy.

Several populations of macrophage are present within the tumor microenvironment. In progressing tumors, the dominant phenotype is reported to be anti-inflammatory, immune regulatory as opposed to pro-inflammatory and tumoricidal [58,59]. Macrophages depend on the CSF-1 signaling axis for their recruitment and survival into tissues. Therefore, many studies have used CSF-1 inhibitors such as PLX3397 (pexidartinib), a clinically tested inhibitor of CSF-1 receptor, to deplete TAM and assess its consequence [60]. CSF-1R inhibition alone has usually minor influence on tumor growth, suggesting that the depletion of TAM is not sufficient to elicit tumor regression [47]. However, in preclinical mouse tumor models, CSF-1R inhibitors improved chemo- and radiotherapy, cellular therapies such as adoptive T cell transfer and immune checkpoint inhibitor immunotherapies [47,61–64]. Targeting CCR2, a chemokine receptor expressed by several myeloid cells including TAM has also been shown to prevent the recruitment of macrophages to the malignant site in mouse tumor models [65].

4.3. Targeting the dense ECM of growing tumors

4.3.1. Pharmacological approaches

As described previously, one of the hallmarks of solid tumors is the excessive accumulation and aberrant architecture of the ECM [66]. ECM remodeling results from the activation of cancer-associated fibroblasts (CAF) which consequently increase their proliferation and enhanced the secretion of ECM component such as type I collagen, fibronectin, tenascin C and hyaluronic acid [67,68]. In response to TGF-β as well as to mechanical forces [69,70] fibroblasts within tumors are activated into myofibroblasts showing a contractile activity. TGF-β is also involved in the production of ECM remodeling enzymes such as matrix metalloproteinases (MMP) or lysyl oxidase (LOX) [70]. The production of long, linear and highly reticulated collagen networks results in a considerable stiffening of the tumor ECM as shown in a spontaneous mammary mouse tumor model [71].

In order to reduce tumor stiffness and normalize ECM architecture, different pharmacological strategies have been designed which target the different ECM components or block their production by fibroblasts (Fig. 3). These strategies can roughly be classified in three categories: (i) enzymes acting directly on ECM constituents, (ii) agents reprogramming stromal cells such as CAF and (iii) other anti-fibrotic agents.

(i) Enzymes:
In order to reduce tumor ECM, enzymes targeting collagen, hyaluronic acid and MMPs have been tested. For instance, bacterial collagenase has been used to improve the efficacy of oncolytic Herpes Simplex viral therapy in a human melanoma xenograft model. This allowed the uniform distribution of viral particles, which resulted in tumor regression [72]. A similar approach with collagenase-2 was used in a solid tumor xenografted in rats [73]. Hyaluronidase, which targets hyaluronic acid has also been used in murine pancreatic ductal adenocarcinoma models, resulting in a decrease of the tumor volume [74]. In fact, a nanoformulation combining hyaluronidase and polyethylene glycol (PEGPH20) is currently in phase 2 clinical trial (ClinicalTrials.gov Identifier: NCT01839487) as an adjuvant to chemotherapeutics in pancreatic cancer [75]. Moreover, MMP, in particular MMP-1 and MMP-8, have been likewise tested as an adjuvant therapy to oncolytic viruses in human soft tissue sarcoma xenografted in immune-deficient mice. It resulted in depletion of sulfated glycosaminoglycans which consequently improved virus propagation [76]. Altering the dense network of collagen of growing tumors can not only improve the diffusion of chemotherapeutics but also the recruitment and migration of T cells. Our data indicate that the treatment of human lung tumor slices with collagenase increased the fraction of T cells in contact with tumor cells [40]. Furthermore, T cells engineered to express a chimeric antigen receptor and heparanase, an ECM-degrading enzyme, demonstrated enhanced infiltration into xenografted tumors and anti-tumor efficacy [77].

(ii) Agents reprogramming CAF:
Another way of tackling the tumor ECM is targeting directly the producing cells, namely CAF. In an attempt to modulate CAF, Zhang et al. tested the combination of gemcitabine and cisplatin encapsulated in lipid nanoparticles. This approach was tested in a murine model of advanced bladder carcinoma resulting in a decrease of α-smooth muscle actin positive fibroblasts, CAF depletion, enhanced tumor vessel permeability and the alteration of collagen deposition [78]. Likewise, metformin, normally used as glucose-lowering drug, was shown to reduce TGF-β signaling as well as hyaluronan and collagen-I production by pancreatic stellate cells in pancreatic ductal adenocarcinoma mouse models [79,80]. This alleviation of desmoplasia was associated to a reduction of ECM remodeling and in systemic metastasis. Furthermore, metformin has also been reported to have an effect on tumor inflammation by lowering the expression of IL-1β as well as the infiltration and M2 polarization of TAM, cells which can participate to a fibrotic environment [79].
Another way to decrease CAF activation is to target their cytoskeleton through the focal adhesion kinase (FAK) or the Rho-associated protein kinase (ROCK). In a pancreatic mouse tumor model, FAK inhibition substantially limited tumor growth and enhanced anti-PD-1 antibody efficiency [81]. This delay in tumor progression was associated with markedly reduced tumor fibrosis and increased numbers of CD8 T cells in tumors. Such combination strategies are ongoing in a number of human solid malignancies (NCT02758587, NCT02943317). ROCK inhibition by the drug Fasudil leads to the loss of stress fibers and focal adhesion complexes and also transient tumor stroma remodeling in a murine pancreatic cancer model. The combination of Fasudil and gemcitabine/Abraxane, was reported to improve the effect of the chemotherapy on both primary tumors and metastasis [82]. Finally, recent evidence indicates that targeting of TGF-β in mouse tumor models increases the infiltration of T cells into tumor islets and enhances responsiveness to anti-PD-1 antibodies [83,84]. These important findings pave the way for clinical trials combining TGF-β inhibitors with PD-1/ PD-L1 blockade.

(iii) Other anti-fibrotic agents:
In addition to targeting ECM producing cells and the ECM components themselves, enzymes involved in the construction of the matrix network can be targeted too. LOX, an enzyme responsible for the crosslinking of collagen molecules into fibers, is overexpressed in many metastatic tumors [85]. Kanapathipillai et al have shown that PLGA nanoparticles coated with LOX inhibitory antibodies alter the ECM, suppress mouse mammary cancer cell growth and invasion *in vitro* and also reduce tumor growth *in vivo* [86]. In addition, clinically approved anti-fibrotic agents which are routinely used such as angiostensin II inhibitors, have been shown to decrease collagen and hyaluronic acid production by regulating TGF-β activity. Lorsatan, for example, has been tested in different mouse solid tumors (melanoma, breast tumor and pancreatic adenocarcinoma) showing a reduction of solid stress which leads into decompression of blood vessels and normalization of the ECM [87]. In orthotopic pancreatic mouse tumors, Lorsatan combined

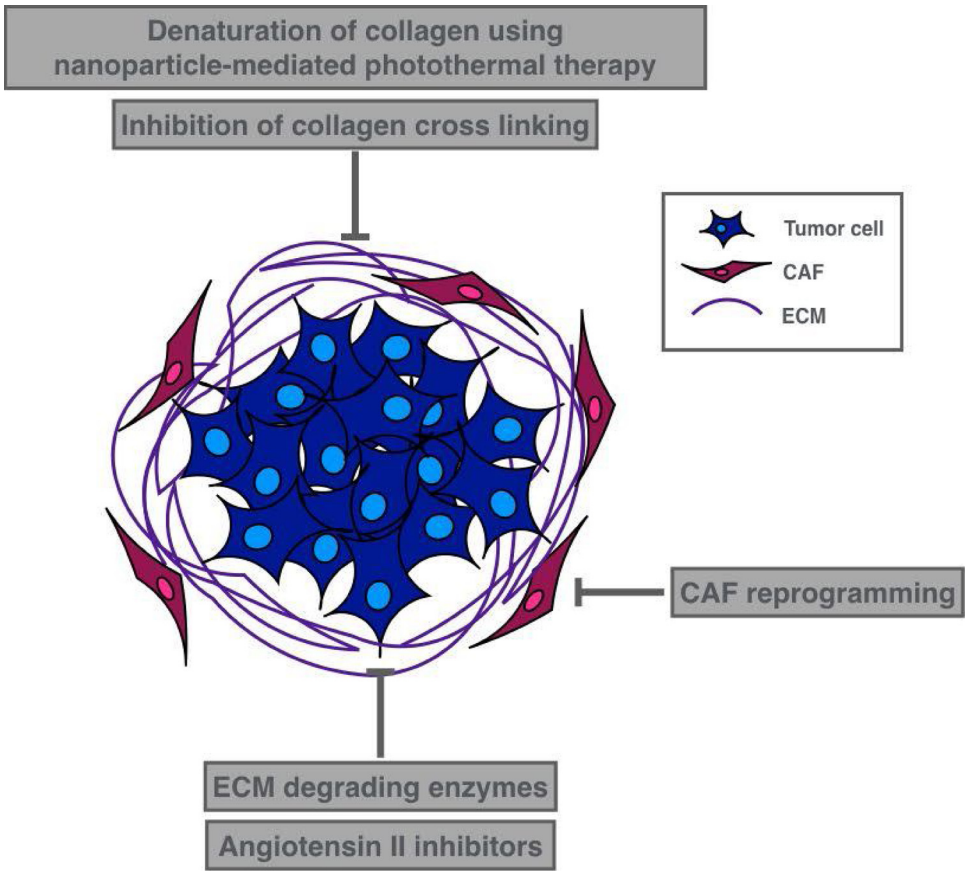

Fig. 3. Promising approaches targeting the dysregulated ECM of growing tumors. Strategies to decrease the dense ECM network include the inhibition of collagen cross-linking enzymes such as LOX, the denaturation of collagen using nanoparticle mediated photothermal (or magnetothermal therapy), CAF reprogramming, the degradation of pre-existing matrix fibers using ECM degrading enzymes and the use of other anti-fibrotic agents such as angiotensin II inhibitors.

with chemotherapy resulted in an improvement of perfusion and the therapeutic efficiency of nanoparticles [88]. These findings have been translated into clinics and a phase II clinical trial is currently testing the combination of 5-fluorouracil, leucovorin and oxaliplatin with lorsatan prior to proton radiation therapy in pancreatic cancer (ClinicalTrials.gov Identifier: NCT01821729). Pirfenidone is another clinically approved anti-fibrotic drug that is generally used for the treatment of idiopathic pulmonary fibrosis. Results obtained in orthotopic mammary tumor models show that Pirfenidone significant reduces collagen and hyaluronan levels mediated by TGF-β signaling pathway [89].

Most of the strategies mentioned in this section have mainly been used to improve drug penetration and treatment efficiency in solid tumors. Nevertheless, the same strategies could also be applied to favor T cell migration and their contact with tumor cells.

However, special attention must be paid since the systemic actions of these therapeutic molecules can induce side effects in other organs. Thus, alternative strategies derived from the nanomedicine field are currently under study in order to achieve more efficient as well as safer therapies.

4.3.2. Nanomedicine approaches

In an attempt to achieve a localized effect, different strategies based on the use of nanoparticles have recently emerged to modulate tumor ECM (Fig. 3).

Certain types of energy- absorbing nanoparticles, such as magnetic and/or plasmonic metallic nanoparticles and carbon-based nanoparticles, can be activated on demand externally to induce heat dissipation. These nanoparticles activated either by near-infrared light or by an alternating magnetic field (therapy referred to as photothermal and magnetothermal therapy, respectively), are capable of producing a localized hyperthermia, sparing healthy tissue from damage. Localized hyperthermia has been shown to induce structural modification of tumor ECM. For example, studies performed using carbon nanotubes [90] or iron oxide nanocubes [91] remotely activated by a near-infrared laser and a magnetic field respectively, show that localized heating induced the denaturation of collagen fibers. This ECM denaturation significantly improved nanoparticle and drug penetration into the malignant tissue of mouse tumors [91]. Other studies performed with gold nanorods injected intravenously in a murine tumor model and activated by a near-infrared laser, report a transitory increase of cytotoxic drug within the tumor [92,93]. Similar to the pharmacological approaches aforementioned, these strategies have been initially designed to enhance cytotoxic drug diffusion and accumulation in the tumor. However, a modification of the tumor ECM, in the form of collagen fibers denaturation would also have a beneficial effect on T cell migration into the malignant site.

Finally, other nanoparticle based therapies have been designed to combine nanomedicine and immunotherapy. A recent study describes the use of a ferritin functionalized with fibroblast-activation protein (FAP) antibody to enhance cytotoxic T cell infiltration [94]. FAP is a surface protein which is over-expressed by CAF and therefore appears as a promising target [30]. The conjugation of FAP antibody onto ferritin nanocages allows nanoparticle specific targeting to CAF, which is subsequently followed by photoirradiation. The selective killing of CAF by this strategy resulted in a decrease of ECM deposition. Consequently, enhanced T cell infiltration followed by efficient tumor suppression was observed [94]. Notably, FAP is not specific to CAF and is also expressed by mesenchymal cells from normal tissues, raising issues about toxicity associated with a FAP-targeting strategy [95].

5. Concluding remarks

A dense ECM, a lack of chemoattractant molecules and the presence of TAM have detrimental effects on T cell migration and ability to

contact tumor cells. Thus, these elements are responsible to the T cell excluded profile observed in many human tumors and consequently to the resistance mechanisms of T cell-based immunotherapies. As reviewed here, an important goal is to target these different determinants in order to increase the recruitment and migration of effector T cells while at the same time avoiding the infiltration of immunosuppressive immune cells. However, we assume that approaches increasing the migration of CD8 T cells and their ability to contact tumor cells will not be effective by themselves since lymphocytes within the tumor environment present a dysfunctional phenotype. Conversely, clinical studies clearly indicate that boosting the activity of CD8 T cells is not sufficient as lymphocytes in most tumors are excluded from tumor cell regions. Thus, our review supports the need for therapies that tackle both processes, namely the migration but also the activation of T cells. We believe that targeting the dysregulated ECM of growing tumors with the numerous strategies described here combined with approaches that relieve the brake of T cell suppression would make T cells efficient in reaching and killing tumor cells.

Declaration of interest

None.

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