Available online at www.sciencedirect.com
ScienceDirect
Current Opinion in Pharmacology

The good and bad of targeting cancer-associated extracellular matrix

Sabina Sangaletti[1], Claudia Chiodoni[1], Claudio Tripodo[2] and Mario P Colombo[1]

The maintenance of tissue homeostasis requires extracellular matrix (ECM) remodeling. Immune cells actively participate in regenerating damaged tissues contributing to ECM deposition and shaping. Dysregulated ECM deposition characterizes fibrotic diseases and cancer stromatogenesis, where a chronic inflammatory state sustains the ECM increase. In cancer, the ECM fosters several steps of tumor progression, providing pro-survival and proliferative signals, promoting tumor cell dissemination via collagen fibers or acting as a barrier to impede drug diffusion. Interfering with processes leading to chronic ECM deposition, as occurring in cancer, might allow the simultaneous targeting of both primary tumors and metastatic lesions. However, a note of caution comes from data showing that defective ECM deposition is associated with an exacerbated inflammatory and autoimmune phenotype and to lymphomagenesis. Immune cells display ITIM-inhibitory receptors recognizing collagens as counter ligands, which negatively regulate the immune response. This is in line with the idea that ECM components can provide homeostatic signals to immune cells to regulate and prevent unwanted activation, a concept particularly relevant in cancer where these mechanisms could be in place to keep infiltrating immune cells in a suppressive pro-tumoral state. In this context, the pharmacological targeting of myeloid cells, for which both direct and indirect roles in ECM deposition have been shown, can be a relevant option to this purpose.

Addresses
[1] Molecular Immunology Unit, Department of Experimental Oncology and Molecular Medicine, Fondazione IRCCS Istituto Nazionale dei Tumori, Milano, Italy
[2] Tumor Immunology Unit, University of Palermo, Palermo, Italy

Corresponding authors:
Sangaletti, Sabina (sabina.sangaletti@istitutotumori.mi.it),
Colombo, Mario P (mariopaolo.colombo@istitutotumori.mi.it)

Current Opinion in Pharmacology 2017, **35**:75–82

This review comes from a themed issue on **Cancer**

Edited by **Antonio Sica**

For a complete overview see the Issue and the Editorial

Available online 19th July 2017

http://dx.doi.org/10.1016/j.coph.2017.06.003

Tumor evolution proceeds through ECM remodeling

Tumor growth involves ECM deposition and remodeling. This occurs through mechanisms that are common to non-malignant processes of wound healing. The ECM comprises different components including proteins, glycoproteins, proteoglycans, structural proteins such as collagens, laminin and tenascin, and matricellular proteins (i.e. SPARC, osteopontin (OPN), thombospondins [1–3]). Depending from tumor origin, differentiation and grading, the amount of ECM deposition could vary. Indeed, there are tumors characterized by a strong fibrotic response with the formation of a dense and thick collagenic capsule (e.g. follicular carcinomas of the thyroid), by the formation of bundles around nests of packed tumor cells (e.g. neuroendocrine malignant tumors), by intense desmoplasia associated with dispersed tumor cell infiltration (e.g. some breast or gastric carcinoma histotypes), or by evident stromal reaction with increased ECM confined to the invasive edges (e.g. some colon adenocarcinomas). The type and distribution of ECM stromal reaction to malignant proliferation is related with the capability of malignant clones to undergo epithelial-to-mesenchymal transition, thought the mechanisms regulating the relationship between malignant clone differentiation, ECM deposition and EMT have been poorly elucidated.

The relevance of the ECM for tumor progression has been shown in pre-clinical mouse models and in human specimens starting from breast cancer where large epidemiological studies have shown that mammographic density is an independent risk factor for breast cancer development [4]. Mammographic density depends from different factors including epithelial and stromal cells, collagen and fat. All these elements are interconnected and may affect each others increasing the risk for and the progression of breast cancer [4]. More recently in high-grade breast cancers a gene signature enriched in ECM genes has been show to be capable of predicting response to therapy and clinical outcome [5]. Similar ECM gene clusters have been used to stratify colon cancer patients, also correlating with histopathological parameters and overall surgical staging [6]. ECM gene clusters have been also described in ovarian cancer and in hematologic tumors including diffuse large B cell lymphomas (DLBCL) and non-Hodgkin lymphomas (reviewed in Sangaletti *et al.* Cancer Immunology Immunotherapy 2017, [7]). Notably, it should be mentioned that some genes are common among the different ECM clusters,

such as COL1A1, LOX, SPARC and TIMP3 or belong to the same gene family (i.e. MMPs, Collagen, FN or tissue inhibitors of metalloproteinases) suggesting that common 'wound healing' mechanisms may be activated by tumors of different tissue origin to progress. Furthermore this means that common targets could be potentially identified within ECM molecules that could be used to implement current therapies, irrespective from tumor histotype.

Mouse and human data suggest the importance of collagen deposition and cross-linking for tumor development. For example the presence of fibrotic foci has been associated with a higher relapse rate and a worse overall survival in breast cancer [8,9]. Using the 4T1 triple negative breast cancer model, Cox and coll. showed that metastasis formation occurs at the site of hypoxia-driven fibrotic response and that abrogation of lysyl oxidase (LOX)-mediated collagen cross-linking prevent lung metastases [10]. The same authors profiled a cohort of 344 lymph node — negative primary breast cancer patients identifying that an hypoxic signature, originally described by Chi *et al.* [11], was closely associated with poor survival and metastasis in estrogen receptor negative (ER^-) breast cancer patients [12]. Indeed, the hypoxic secretome from bone-tropic and parental ER^- human breast cancer cells identified the ECM modifying LOX as the protein more significantly associated with bone metastases in ER^- patients, but not in ER^+ patients [12].

Further relevance of ECM and ECM-related cluster (including SPARC, COL1A1, COL3A1) variation has been shown to occur during tumor progression including their down-modulation in certain hemathologic malignancies, like CD5+ DLBCL and B-CLL, characterized by abnormal expansion of CD5+ B cells [13]. Accordingly, we have recently shown that the lack of SPARC in secondary lymphoid organ (SLO) microenvironment of autoimmunity-prone mice favors the development of a B-CLL like disease [14]. On the contrary, crossing lymphoma-prone Trp53-deficient with Sparc-deficient mice, protects them from the development of DLBCL and follicular lymphomas [15]. These studies suggest that either the increase or the decrease of the ECM can impact on tumor progression.

Myeloid cells and ECM remodeling

Myeloid cells participate in ECM organization at different levels, being able of producing enzymes/mediators capable of ECM remodeling or of producing directly ECM molecules.

Within the tumor microenvironment myeloid cells that are skewed towards a pro-tumoral phenotype (being MDSCs, M2 macrophages or N2 neutrophils) are the main source of matrix metalloproteinases (MMPs), enzymes involved in collagen remodeling [16]. In this context, zoledronic acid (ZA)-treatment of transgenic mice carrying the mutated HER-2 protein and developing mammary tumors, reduces local MMP-9 production and results in impaired expansion of myeloid cell compartment, enrichment and differentiation to tumor-associated macrophages (TAMs) at the tumor site. These events also impair tumor stroma formation and collagen deposition [17]. In line, the growth of mammary tumors in MMP-9-deficient mice is associated to defective collagen fibers formation and reduced tumor growth (Sangaletti, unpublished).

The matricellular protein SPARC is a collagen chaperone and a master stromal regulator [18]. Studies in Drosophila and mouse have shown that SPARC is required for collagen type IV fibers production and assembly into basal membranes [19,20], as well as for fibronectin-induced integrin-linked kinase activation and collagen deposition in fibrotic condition [21,22]. Genetic deletion of *Sparc* affects tissue remodeling and tumor-stroma deposition with consequence on primary tumor growth and/or metastasis, which can vary depending on the tumor histotype [23]. In a model of breast cancer, we have demonstrated that the absence of SPARC reduces primary tumor growth and lung metastases, an effect due to the incapacity of *Sparc*-deficient macrophages to sustain collagen deposition and stroma formation [20], as well as tumor cell migration on fibronectin and collagen fibers [24]. These data suggest that macrophages can be a relevant source of ECM proteins during the critical process of tumor growth. A formal demonstration that TAMs can produce ECM molecules has been obtained from integrating transcriptomic and proteomic analysis on these cells [25••]. Combining these two approaches, Afik and coll. have shown that TAMs are the source of molecules associated with collagen synthesis, stability, assembly, and cross-linking. Among them the α1 chains of collagen I and collagen XIV, the three α chains of collagen VI, the glycoprotein PCOLCE, the enzyme P4HA1, collagen cross-linkers PLOD1 and 3, the glycoprotein SPARC, and the proteoglycan biglycan. Their integrative analysis also highlighted the TAMs produce the ECM covalent cross-linker enzymes TGM2 and F13A1, the complement C1q complex, and THBS1. Overall these data showed that TAMs directly contribute in building specific types of collagenous ECM. Supporting this finding the same authors showed that colorectal cancer grown in TAM-deficient Ccr2−/− mice had aberrant deposition of collagen fibers. Furthermore, TAMs can regulate collagen production by cancer-associated fibroblasts (CAFs), whose number outcompeted that of macrophages in the analyzed colon cancer model [25••]. The mechanisms for such a cross-talk was not described. It can be speculated that IL-4 and IL-13, produced by TAMs, mediate such interaction, similarly to what has been described in a model of skin repair [26••].

In such skin injury model the authors demonstrated that IL-4/IL-13 signaling through the cognate IL-4Rα

receptor initiates a repair program ultimately controlling the architecture of collagen fibrils and the biochemistry of collagen cross-linking, which in the wounded skin is operated by fibroblasts [26••]. The authors identified Relm-α as the mediator that, released by IL-4/IL-13-stimulated macrophages, promotes the production of collagen cross linker LH2 in fibroblasts.

Overall these data suggest that targeting myeloid cells/macrophages within the tumor microenvironment could be a strategy to interfere with the excessive collagen deposition that often characterizes the tumor microenvironment.

The bi-directional cross-talk between ECM and myeloid cells

Other than participating in ECM organization, ECM molecules are endowed of regulatory capacity over myeloid cell functions. ECM bioactive fragments, generated by MMPs digestion of collagens and laminins (also termed matricryptins or matrikines [27,28]) can be chemotactic for myeloid cells, suggesting that in the tumor microenvironment an altered ECM deposition associated with the production of MMPs can generate a condition promoting tumor infiltration by myeloid cells. Interestingly other than releasing chemotactic fragments, collagen can contribute to myeloid cell recruitment at the tumor site by regulating other pathways including the CXCR4-COX-2 axis. In this context we have recently shown that increased collagen deposition and SPARC expression in breast tumors, activate COX-2 that in turn promotes a CXCR4-mediated recruitment of MDSCs at the tumor site [29•]. Myeloid cells are not the only cell type that can be affected by collagen fibers; T lymphocytes and macrophages also use collagen fibers to migrate into inflamed tissues [30,31]. Considering the mechanism used by myeloid cells and T lymphocytes to migrate through the ECM, Wolf and coll. have shown that migration of T cells and other leukocytes within 3D collagen matrices occurs through an amoeboid process implicating the crawling along collagen fibrils (contact guidance) [32] and not involving proteases. This process can involves integrin receptors; indeed it has been shown that the crawling of effector T cells on ECM in inflamed tissues requires αvβ1 and αvβ3 activation [33].

The current idea is that immune cells can use different mechanisms to migrate across or through the ECM depending from the composition and organization of the ECM itself.

Other than regulating leukocyte migration, recent evidence indicates that the ECM can generate signals within immune cells capable of interfering with their state of activation.

Immune inhibitory receptors are a class of ITIM-bearing receptors whose engagement inhibits cellular activation [34]. Among ITIM-bearing receptors, LAIR-1 is of special interest for those studying the immune implication of ECM [35]. Indeed LAIR-1 binds to and is inhibited by collagens, corroborating the hypothesis of a bi-directional regulation between ECM molecules and immune cells [36]. LAIR-1 is expressed by the majority of hematopoietic cells, although its expression varies according to the state of differentiation and activation of the different immune cells. It is expressed by myeloblasts and promyelocites, but not by bone marrow or circulating neutrophils in which its re-expression occurs upon activation (inflammatory cytokines, LPS, PMA, [37]). In activated neutrophils LAIR-1 engagement by collagens has been shown to inhibit neutrophil extracellular trap (NET) extrusion, upon IFNγ + C5a stimulation [14]. Considering that abnormal neutrophil activation and death through NETosis is a relevant step in autoimmunity, it could be suggested that blocking ECM-mediated neutrophil inhibition could promote an autoimmune reaction. On this line, we demonstrated that the absence of the matricellular protein SPARC, in Fas-deficient mice, was associated to an altered SLO collagen remodeling leading to an unwanted neutrophil activation and NETosis, exacerbated autoimmunity and lymphomagenesis. The last was linked to NET stimulation of abnormal and clonal proliferation of CD5+ B cells towards a B-CLL like disease [14]. Notably, among human lymphomas, B-CLL is characterized by a reduced ECM deposition as compared to follicular, mantle cell and diffuse large B-cell lymphomas, all showing higher deposition of collagen matrix [14]. A similar contribution of NETs in malignant progression has been shown in solid tumors, as exemplified by breast cancer cell stimulation of neutrophil NETosis and subsequent NET support of metastasis formation [38••]. Accordingly, NETs stimulate the invasion and migration of breast cancer cells *in vitro*. Inhibiting NET formation or digesting the NET DNA thread using deoxyribonuclease I (DNase I) blocks the metastatic process. These mechanisms have been shown in the murine model of triple negative breast cancer, 4T1. Interestingly, this model is widely used to study MDSC function and expansion [39], suggesting the possibility that MDSCs and NET-prone neutrophils could co-exist or, alternatively, that NETs could be extruded by MDSCs. In another breast cancer tumor model (SN25ASP, [29•]), we found that splenic MDSCs isolated from tumor-bearing mice and seeded onto poly-D-lysine coated glasses become able of extruding NETs if treated with the canonical PMA stimulus (Sangaletti, Unpublished). Melero and coll. have recently shown that IL-8 attracts both monocytic (M-MDSCs) and granulocytic (G-MDSCs) human MDSCs that, respectively, suppress the proliferation of autologous T cells or extrude NETs under IL-8 stimulation [40•]. These findings are apparently contradictory with the common interpretation of NET induction associated to autoimmune [41] or infection conditions, whereas in case of MDSCs they should

result from a suppressive condition. A possible reconciling explanation could come from considering NET extrusion as an ancestral prerogative of myeloid cells, conserved along differentiation. Indeed NETs are extruded also by immature myeloid cells (Sangaletti, unpublished observation) and are stimulated by specific cytokines that could be present in both suppressive and activated immune microenvironment (i.e. TNF and IFNγ), consistently with their dual role. Considering the relevance of LAIR-1 in inhibiting NETosis, we evaluated the expression of LAIR-1 in the two subset of MDSCs infiltrating mouse mammary tumors and found that LAIR-1 is expressed in both subsets of MDSCs, although at different level, suggesting that in the tumor microenvironment G-MDSCs and M-MDSCs could be differently regulated by ECM collagens. For example we can hypothesize that collagens, as LAIR-1 ligands, in the tumor microenvironment can participate in keeping M-MDSCs with a suppressive phenotype or in limiting NET extrusion.

Neutrophils are not the only immune cell subset expressing LAIR-1 in the tumor microenvironment. T lymphocytes, according to their state of activation, can express LAIR-1 at different levels. In such a context it has been shown that naive CD4+ and CD8+ T cells, as well as CD8 + effector T cells, express higher levels of LAIR-1 than memory T cells [42]. *In vitro* T cell stimulation decreases LAIR-1 expression, a finding that needs to be confirmed *in vivo*. Furthermore, crosslinking of LAIR-1 on primary T cells results in an inhibition of T cell function [42]. These pieces of evidence suggest that microenvironments differently enriched in collagens can be detrimental for local T cell activation but also for antigen presentation, being LAIR-1 also expressed by professional antigen presenting cells. In such a context, it has been shown that LAIR-1 can limit DC differentiation and activation, although through the binding of C1q and not collagens [43]. It could be argued that local interference with ECM remodeling can putatively ameliorate antigen-mediated immune responses. More difficult is the prediction of whether this approach could be beneficial in case of tumors infiltrated by myeloid cells, since, on one hand, M-MDSCs could be reverted from their suppressive function, whereas on the other, G-MDSCs, could be excessively stimulated to undergo NETosis and therefore contribute to metastases (Figure 1).

On the basis of these considerations it could be argued that, as occurring for immune check-point inhibitor-based therapy, the use of collagen-interfering strategies should be considered only after a precise assessment of the composition of the tumor immune infiltrate.

Strategies to target ECM deposition in the tumor microenvironment

Both direct and indirect strategies to target ECM deposition can be envisaged. The first approach can include molecules able to interfere directly with ECM deposition or remodeling, that is, anti-fibrotic agents, whereas the second strategy aims at targeting the ECM indirectly by affecting the function or the recruitment of myeloid cells.

TGF-β is certainly a relevant possible target to affect ECM deposition in cancer. Indeed it has been directly involved in collagen matrix deposition by activated fibroblasts or in EMT induction. Moreover TGF-β act as an immune regulator decreasing tumor immune surveillance [44].

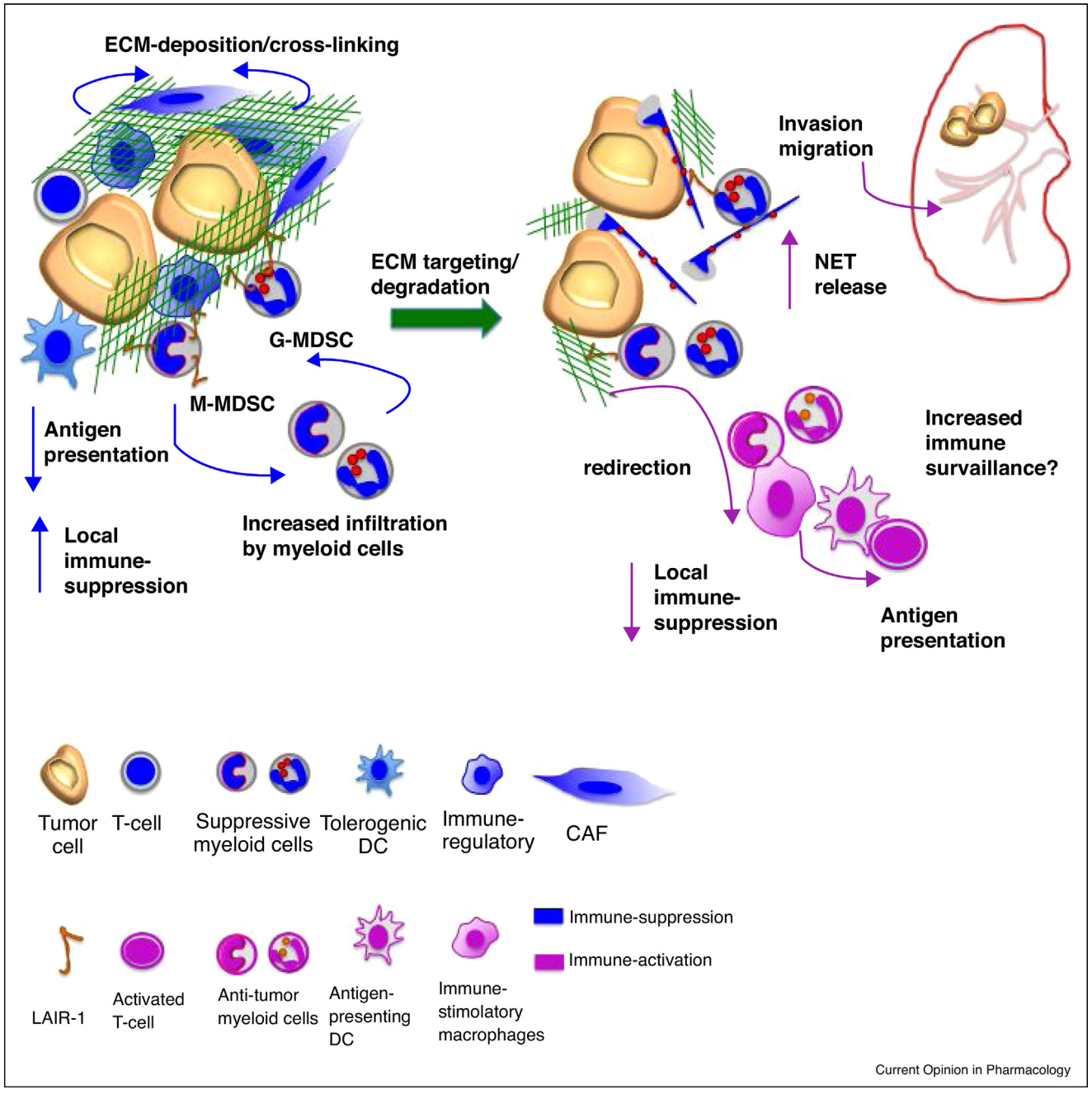
Results obtained *in vitro* or in mouse models with TGF-β inhibitors showed that these compounds poorly affected tumor cells while exerted their anti-tumoral activity mainly on stromal and immune cells in the tumor microenvironment This suggests that these compounds could be more effectively used in combination with chemotherapies. For example, this approach could be relevant in setting in which fibrosis precedes tumor development, such as in breast cancer. However, treatments affecting TGF-β signaling should take into consideration the dual role of TGF-β in cancer progression, especially for nascent tumors. Indeed, different mouse models have shown that genetic deletion or down-regulation of TGF-β signaling worsened the tumor phenotype. This effect is due to the fact that TGF-β suppresses tumor initiation and early development through inhibition of cell cycle progression, induction of apoptosis, and suppression of growth factor, cytokine and chemokine expression. The goal of TGF-β inhibition-based therapies is now to abolish the tumor-promoting effect of TGF-β, while maintaining its tumor suppressive properties.

IL-13 is the most extensively studied Th2 cytokine in the context of idiopathic pulmonary fibrosis (IPF). Indeed, in a mouse experimental model of pulmonary fibrosis IL-13 targeting therapies successfully attenuated the fibrotic phenotype [45] and anti-IL13 antibodies have been used in clinical trial for IPF treatment [46]. Within the tumor microenvironment, this cytokine, together with IL-4, promotes the development of TAMs and their production and cross-linking of ECM molecules suggesting that IL-13-directed therapies can be a possible approach to target the ECM-rich tumor microenvironment [25••].

LOX is another potential target within the ECM. Indeed the relevance of LOX activity on metastases has suggested that his targeting could be particularly relevant in case of metastatic disease [12]. However, small LOX inhibitors are currently not yet available, because of the lack of a complete crystal structure that excludes it from classical structure-driven fragment-based drug development and screening approaches.

A possible strategy to indirectly target the ECM deposition in cancer is to use bisphosphonates [47]. These drugs

Figure 1



Effect of ECM targeting on immune cell functions in the tumor microenvironment. Within the tumor microenvironment macrophages and fibroblasts (CAF) contribute to ECM deposition. ECM components can recruit MDSCs and regulate their immunosuppressive functions [29•]. The collagen receptor LAIR-1 transduces negative signals to granulocytes/G-MDSCs that no longer undergo NETosis, which has been described to promote metastases [38••,40•]. The lack/reduction of ECM signals can switch MDSCs towards a less suppressive phenotype. We can hypothesize that ECM targeting can hamper local tumor-associated immunosuppression unleashing DCs from negative signals favoring migration to lymph node and antigen presentation activity [43]. In addition the reduction of ECM signals can redirect macrophages and myeloid cells from pro-tumoral to anti-tumoral activity. Nevertheless ECM degradation or reduction can foster NET formation by G-MDSC and in turn promote metastases.

that inhibit mevalonate metabolism are used to prevent the loss of bone mass in osteoporosis. Our group has shown in different studies performed in breast cancer models, that zoledronic-acid (ZA), the most active and clinically approved third-generation amino-bisphosphonate, can affect tumor growth and metastasis through its ability to interfere with myeloid cell expansion and suppressive function [29•]. Inhibiting MDSCs with ZA, we were able of reverting EMT and halting metastasis in high-grade, SPARC-expressing mammary tumors [29•]. Other groups have shown that ZA can affect myeloid cell differentiation. In mesothelioma ZA leads to reduced macrophage infiltration and impairs their polarization towards an M2 phenotype, a phenotype that, however, was associated with an increased number of immature myeloid cells, which, as suggested by the authors, could on the other hand diminish the effects of ZA on survival [48]. Very recently it has been shown that ZA administration modifies the BM stem cell niche inducing transient changes in the number of hematopoietic stem cells, myeloid-biased and lymphoid-biased progenitor cells. The same authors showed that BM cells from mice treated with a single, clinically relevant, dose of ZA inhibited mammary tumor outgrowth *in vivo*, when co-injected with tumor cells [49]. Overall these studies suggested that ZA can affect myeloid cell recruitment, expansion and pro-tumor phenotype. Our observation in BALB-NeuT mice treated with ZA or in SPARC-expressing high-grade mammary tumors indicated that ZA treatment also affects collagen deposition within the tumor microenvironment, as ZA-treated tumors displayed reduced collagen deposition ([17] and Sangaletti *et al.* unpublished). Interestingly, Cox *et al.* showed that ZA completely blocked the formation of focal premetastatic osteolytic lesions and almost completely eliminated the tumor burden in metastatic models of breast cancer through the targeting of the downstream activities of LOX, namely, the *de novo* generation of functionally active osteoclasts [12]. Different studies have suggested the relevance to use bisphosphonate as anticancer agents in breast cancer also in early breast cancer [50]. Overall clinical trials with bisphosphonates as adjuvant therapy for breast cancer showed contrasting results [51], suggesting that a selective rather than a broad administration of this drug could be the best approach. Indeed, the efficacy of ZA administration can be potentially dependent from different factors, including hormone status, p53 mutational status or tumor grade. Indeed, tumor cells bearing mutant p53 have enhanced mevalonate pathway activity and may be particularly sensitive to inhibition [52] and our recent studies showed that high-grade breast cancers with a specific ECM signature could benefit from ZA treatment [29•].

Finally, considering that tumor-associated fibroblasts are key regulators of ECM deposition and tumor growth, vaccine specifically targeting fibroblast activation protein (FAP), which is specifically overexpressed by fibroblasts in the tumor stroma, can be a possible strategy to halt tumor growth [53]. Indeed there are preclinical data, obtained with mouse models, showing that FAP-vaccinated mice had markedly decreased collagen type I expression and increased uptake of chemotherapeutic drugs [54].

Concluding remarks

Growing body of evidence suggests that targeting the ECM is feasible and that this new approach could improve the efficacy of anti-cancer therapies. However a note of caution stems from the less appreciated immunoregulatory role of ECM proteins. Therefore ECM targeting might, on one hand, limit tumor-associated immune-suppression unleashing immune cells from inhibitory signals and, on the other, exacerbate immune cells activation towards autoimmune-like responses.

Funding

This work was supported by Associazione Italiana per la Ricerca sul Cancro (IG 10137 to Mario P. Colombo, MFAG 12810 to Sabina Sangaletti, IG 17261 to Claudia Chiodoni], and the Italian Ministry of Health [GR-2013-02355637 to Sabina Sangaletti).

Conflict of interest statement

Nothing declared.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Frantz C, Stewart KM, Weaver VM: **The extracellular matrix at a glance**. *J Cell Sci* 2010, **123**:4195-4200.
2. Bornstein P: **Matricellular proteins: an overview**. *J Cell Commun Signal* 2009, **3**:163-165.
3. Murphy-Ullrich JE, Sage EH: **Revisiting the matricellular concept**. *Matrix Biol* 2014, **37**:1-14.
4. Martin LJ, Boyd NF: **Mammographic density. Potential mechanisms of breast cancer risk associated with mammographic density: hypotheses based on epidemiological evidence**. *Breast Cancer Res* 2008, **10**:201.
5. Triulzi T, Casalini P, Sandri M, Ratti M, Carcangiu ML, Colombo MP, Balsari A, Menard S, Orlandi R, Tagliabue E: **Neoplastic and stromal cells contribute to an extracellular matrix gene expression profile defining a breast cancer subtype likely to progress**. *PLOS ONE* 2013, **8**.
6. Hayes CJ, Dowling CM, Dwane S, McCumiskey ME, Tormey SM, Anne Merrigan B, Coffey JC, Kiely PA, Dalton TM: **Extracellular matrix gene expression profiling using microfluidics for colorectal carcinoma stratification**. *Biomicrofluidics* 2016, **10**:054124.
7. Sangaletti S, Chiodoni C, Tripodo C, Colombo MP: **Common extracellular matrix regulation of myeloid cell activity in the bone marrow and tumor microenvironments**. *Cancer Immunol Immunother* 2017 http://dx.doi.org/10.1007/s00262-017-2014-y.
8. Boyd NF, Martin LJ, Yaffe MJ, Minkin S: **Mammographic density and breast cancer risk: current understanding and future prospects**. *Breast Cancer Res* 2011, **13**:223.

9. Li T, Sun L, Miller N, Nicklee T, Woo J, Hulse-Smith L, Tsao MS, Khokha R, Martin L, Boyd N: **The association of measured breast tissue characteristics with mammographic density and other risk factors for breast cancer**. *Cancer Epidemiol Biomarkers Prev* 2005, **14**:343-349.

10. Cox TR, Bird D, Baker AM, Barker HE, Ho MW, Lang G, Erler JT: **LOX-mediated collagen crosslinking is responsible for fibrosis-enhanced metastasis**. *Cancer Res* 2013, **73**:1721-1732.

11. Chi JT, Wang Z, Nuyten DS, Rodriguez EH, Schaner ME, Salim A, Wang Y, Kristensen GB, Helland A, Borresen-Dale AL *et al.*: **Gene expression programs in response to hypoxia: cell type specificity and prognostic significance in human cancers**. *PLoS Med* 2006, **3**:e47.

12. Cox TR, Gartland A, Erler JT: **Lysyl oxidase, a targetable secreted molecule involved in cancer metastasis**. *Cancer Res* 2016, **76**:188-192.

13. Jain P, Fayad LE, Rosenwald A, Young KH, O'Brien S: **Recent advances in de novo CD5+ diffuse large B cell lymphoma**. *Am J Hematol* 2013, **88**:798-802.

14. Sangaletti S, Tripodo C, Vitali C, Portararo P, Guarnotta C, Casalini P, Cappetti B, Miotti S, Pinciroli P, Fuligni F *et al.*: **Defective stromal remodeling and neutrophil extracellular traps in lymphoid tissues favor the transition from autoimmunity to lymphoma**. *Cancer Discov* 2014, **4**:110-129.

15. Sangaletti S, Tripodo C, Portararo P, Dugo M, Vitali C, Botti L, Guarnotta C, Cappetti B, Gulino A, Torselli I *et al.*: **Stromal niche communalities underscore the contribution of the matricellular protein SPARC to B-cell development and lymphoid malignancies**. *Oncoimmunology* 2014, **3**.

16. Kessenbrock K, Plaks V, Werb Z: **Matrix metalloproteinases: regulators of the tumor microenvironment**. *Cell* 2010, **141**:52-67.

17. Melani C, Sangaletti S, Barazzetta FM, Werb Z, Colombo MP: **Amino-biphosphonate-mediated MMP-9 inhibition breaks the tumor-bone marrow axis responsible for myeloid-derived suppressor cell expansion and macrophage infiltration in tumor stroma**. *Cancer Res* 2007, **67**:11438-11446.

18. Chiodoni C, Colombo MP, Sangaletti S: **Matricellular proteins: from homeostasis to inflammation, cancer, and metastasis**. *Cancer Metastasis Rev* 2010, **29**:295-307.

19. Martinek N, Shahab J, Saathoff M, Ringuette M: **Haemocyte-derived SPARC is required for collagen-IV-dependent stability of basal laminae in Drosophila embryos**. *J Cell Sci* 2008, **121**:1671-1680.

20. Sangaletti S, Stoppacciaro A, Guiducci C, Torrisi MR, Colombo MP: **Leukocyte, rather than tumor-produced SPARC, determines stroma and collagen type IV deposition in mammary carcinoma**. *J Exp Med* 2003, **198**:1475-1485.

21. Barker TH, Baneyx G, Cardo-Vila M, Workman GA, Weaver M, Menon PM, Dedhar S, Rempel SA, Arap W, Pasqualini R *et al.*: **SPARC regulates extracellular matrix organization through its modulation of integrin-linked kinase activity**. *J Biol Chem* 2005, **280**:36483-36493.

22. Sangaletti S, Gioiosa L, Guiducci C, Rotta G, Rescigno M, Stoppacciaro A, Chiodoni C, Colombo MP: **Accelerated dendritic-cell migration and T-cell priming in SPARC-deficient mice**. *J Cell Sci* 2005, **118**:3685-3694.

23. Said N: **Roles of SPARC in urothelial carcinogenesis, progression and metastasis**. *Oncotarget* 2016, **7**:67574-67585.

24. Sangaletti S, Di Carlo E, Gariboldi S, Miotti S, Cappetti B, Parenza M, Rumio C, Brekken RA, Chiodoni C, Colombo MP: **Macrophage-derived SPARC bridges tumor cell-extracellular matrix interactions toward metastasis**. *Cancer Res* 2008, **68**:9050-9059.

25. •• Afik R, Zigmond E, Vugman M, Klepfish M, Shimshoni E, Pasmanik-Chor M, Shenoy A, Bassat E, Halpern Z, Geiger T *et al.*: **Tumor macrophages are pivotal constructors of tumor collagenous matrix**. *J Exp Med* 2016, **213**:2315-2331.

This paper provides the first formal demonstration that macrophages are source of ECM molecules in the tumor.

26. •• Knipper JA, Willenborg S, Brinckmann J, Bloch W, Maass T, Wagener R, Krieg T, Sutherland T, Munitz A, Rothenberg ME *et al.*: **Interleukin-4 receptor alpha signaling in myeloid cells controls collagen fibril assembly in skin repair**. *Immunity* 2015, **43**:803-816.

The authors demonstrated that IL-4/IL-13 signaling on myeloid cells initiates a repair program ultimately controlling the architecture of collagen fibrils.

27. Schenk S, Quaranta V: **Tales from the crypt[ic] sites of the extracellular matrix**. *Trends Cell Biol* 2003, **13**:366-375.

28. Schor SL, Schor AM: **Phenotypic and genetic alterations in mammary stroma: implications for tumour progression**. *Breast Cancer Res* 2001, **3**:373-379.

29. • Sangaletti S, Tripodo C, Santangelo A, Castioni N, Portararo P, Gulino A, Botti L, Parenza M, Cappetti B, Orlandi R *et al.*: **Mesenchymal transition of high-grade breast carcinomas depends on extracellular matrix control of myeloid suppressor cell activity**. *Cell Rep* 2016, **17**:233-248.

This paper shows that the ECM in breast cancer can regulate myeloid-derived suppressor cells recruitment and function.

30. Cougoule C, Van Goethem E, Le Cabec V, Lafouresse F, Dupre L, Mehraj V, Mege JL, Lastrucci C, Maridonneau-Parini I: **Blood leukocytes and macrophages of various phenotypes have distinct abilities to form podosomes and to migrate in 3D environments**. *Eur J Cell Biol* 2012, **91**:938-949.

31. Korpos E, Wu C, Song J, Hallmann R, Sorokin L: **Role of the extracellular matrix in lymphocyte migration**. *Cell Tissue Res* 2010, **339**:47-57.

32. Wolf K, Muller R, Borgmann S, Brocker EB, Friedl P: **Amoeboid shape change and contact guidance: T-lymphocyte crawling through fibrillar collagen is independent of matrix remodeling by MMPs and other proteases**. *Blood* 2003, **102**:3262-3269.

33. Kim CH: **Crawling of effector T cells on extracellular matrix: role of integrins in interstitial migration in inflamed tissues**. *Cell Mol Immunol* 2014, **11**:1-4.

34. Ravetch JV, Lanier LL: **Immune inhibitory receptors**. *Science* 2000, **290**:84-89.

35. Meyaard L: **The inhibitory collagen receptor LAIR-1 (CD305)**. *J Leukoc Biol* 2008, **83**:799-803.

36. Lebbink RJ, de Ruiter T, Adelmeijer J, Brenkman AB, van Helvoort JM, Koch M, Farndale RW, Lisman T, Sonnenberg A, Lenting PJ *et al.*: **Collagens are functional, high affinity ligands for the inhibitory immune receptor LAIR-1**. *J Exp Med* 2006, **203**:1419-1425.

37. Verbrugge A, de Ruiter T, Geest C, Coffer PJ, Meyaard L: **Differential expression of leukocyte-associated Ig-like receptor-1 during neutrophil differentiation and activation**. *J Leukoc Biol* 2006, **79**:828-836.

38. •• Park J, Wysocki RW, Amoozgar Z, Maiorino L, Fein MR, Jorns J, Schott AF, Kinugasa-Katayama Y, Lee Y, Won NH *et al.*: **Cancer cells induce metastasis-supporting neutrophil extracellular DNA traps**. *Sci Transl Med* 2016, **8**:361ra138.

This paper demonstrate the relevance of NETs in malignant progression of breast cancer by supporting metastases.

39. Sangaletti S, Tripodo C, Sandri S, Torselli I, Vitali C, Ratti C, Botti L, Burocchi A, Porcasi R, Tomirotti A *et al.*: **Osteopontin shapes immunosuppression in the metastatic niche**. *Cancer Res* 2014, **74**:4706-4719.

40. • Alfaro C, Teijeira A, Onate C, Perez G, Sanmamed MF, Andueza MP, Alignani D, Labiano S, Azpilikueta A, Rodriguez-Paulete A *et al.*: **Tumor-produced interleukin-8 attracts human myeloid-derived suppressor cells and elicits extrusion of neutrophil extracellular traps (NETs)**. *Clin Cancer Res* 2016, **22**:3924-3936.

This paper provides the evidence that G-MDSC are able to extrude NET.

41. Smith CK, Kaplan MJ: **The role of neutrophils in the pathogenesis of systemic lupus erythematosus**. *Curr Opin Rheumatol* 2015, **27**:448-453.

42. Jansen CA, Cruijsen CW, de Ruiter T, Nanlohy N, Willems N, Janssens-Korpela PL, Meyaard L: **Regulated expression of the**

inhibitory receptor LAIR-1 on human peripheral T cells during T cell activation and differentiation. *Eur J Immunol* 2007, **37**:914-924.

43. Son M, Santiago-Schwarz F, Al-Abed Y, Diamond B: **C1q limits dendritic cell differentiation and activation by engaging LAIR-1**. *Proc Natl Acad Sci U S A* 2012, **109**:E3160-E3167.

44. Yang L, Pang Y, Moses HL: **TGF-beta and immune cells: an important regulatory axis in the tumor microenvironment and progression**. *Trends Immunol* 2010, **31**:220-227.

45. Jakubzick C, Choi ES, Joshi BH, Keane MP, Kunkel SL, Puri RK, Hogaboam CM: **Therapeutic attenuation of pulmonary fibrosis via targeting of IL-4- and IL-13-responsive cells**. *J Immunol* 2003, **171**:2684-2693.

46. Datta A, Scotton CJ, Chambers RC: **Novel therapeutic approaches for pulmonary fibrosis**. *Br J Pharmacol* 2011, **163**:141-172.

47. Gnant M, Clezardin P: **Direct and indirect anticancer activity of bisphosphonates: a brief review of published literature**. *Cancer Treat Rev* 2012, **38**:407-415.

48. Veltman JD, Lambers ME, van Nimwegen M, Hendriks RW, Hoogsteden HC, Hegmans JP, Aerts JG: **Zoledronic acid impairs myeloid differentiation to tumour-associated macrophages in mesothelioma**. *Br J Cancer* 2010, **103**:629-641.

49. Ubellacker JM, Haider MT, DeCristo MJ, Allocca G, Brown NJ, Silver DP, Holen I, McAllister SS: **Zoledronic acid alters hematopoiesis and generates breast tumor-suppressive bone marrow cells**. *Breast Cancer Res* 2017, **19**:23.

50. Santini D, Stumbo L, Spoto C, D'Onofrio L, Pantano F, Iuliani M, Fioramonti M, Zoccoli A, Ribelli G, Virzi V *et al.*: **Bisphosphonates as anticancer agents in early breast cancer: preclinical and clinical evidence**. *Breast Cancer Res* 2015, **17**:121.

51. Coleman RE, Marshall H, Cameron D, Dodwell D, Burkinshaw R, Keane M, Gil M, Houston SJ, Grieve RJ, Barrett-Lee PJ *et al.*: **Breast-cancer adjuvant therapy with zoledronic acid**. *N Engl J Med* 2011, **365**:1396-1405.

52. Thurnher M, Nussbaumer O, Gruenbacher G: **Novel aspects of mevalonate pathway inhibitors as antitumor agents**. *Clin Cancer Res* 2012, **18**:3524-3531.

53. Lee J, Fassnacht M, Nair S, Boczkowski D, Gilboa E: **Tumor immunotherapy targeting fibroblast activation protein, a product expressed in tumor-associated fibroblasts**. *Cancer Res* 2005, **65**:11156-11163.

54. Loeffler M, Kruger JA, Niethammer AG, Reisfeld RA: **Targeting tumor-associated fibroblasts improves cancer chemotherapy by increasing intratumoral drug uptake**. *J Clin Invest* 2006, **116**:1955-1962.