5 Role of the Extracellular Matrix in Tumor Stroma: Barrier or Support?

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Abstract

Extensive evidence exists to functionally implicate stromal cancer-associated fibroblasts in tumor progression. Data from experimental cancer models has questioned the exclusive tumor-supportive function of the tumor stroma and suggested that the stroma might also act as a barrier to inhibit tumor metastasis. With consideration of this shift in dogma, we discuss the role of a specific part of the tumor stroma, the insoluble extracellular matrix (ECM), in tumor growth and spread. We summarize data from experimental tumor models on the role of fibrillar collagens, the fibronectin EDA splice form, proteoglycans and the matricellular proteins, periostin and tenascins, which are all major components of the tumor stroma. In addition to the composition of the ECM being able to regulate tumorigenesis via integrin-mediated signaling, recent data indicate that the stiffness of the ECM also significantly impacts tumor growth and progression. These two properties add to the complexity of tumor-stroma interactions and have significant implications for gene regulation, matrix remodeling, and tumor metastasis. The role of the tumor stroma is thus extremely complex and highlights the importance of relating findings to tumor-type-, tissue-, and stage-specific effects in addition to considering inter-tumor and intra-tumor heterogeneity. Further work is needed to determine the relative contribution of different ECM proteins to the tumor-supporting and tumor-inhibiting roles of the tumor stroma.

Take-Home Lessons

- The extracellular matrix (**ECM**) is a meshwork of macromolecules which in the tumor microenvironment (**TME**) is present in **interstitial matrix** and **basement membranes**.
- Major producers of interstitial ECM rich in fibrillar collagens are the **cancer-associated fibroblasts (CAFs)** whereas tumor basement membranes are dependent on endothelial cells for production of the laminin and collagen IV networks which are major structural components in basement membranes.
- Except for **fibrillar collagens**, key ECM molecules in the interstitial TME include **EDA fibronectin** (tumor progression, TGF-β activation), **periostin** (metastatic niches, extravasation stage of metastasis), **tenascin-C** (metastasis, seeding stage of metastasis) and **proteoglycans** (inhibit or stimulate tumor growth, depending on proteoglycan type). Many of these effects are mediated via **integrins** or **toll-like receptors.**
- One controversial issue in the field concerns the role of the **ECM** in the **TME**. It is becoming clear that identical ECM molecules might have diametrically different functions in different tumor types at distinct stages of tumorigenesis. Except for a *structural support* of the tissue the interstitial **TME ECM** can develop into a *protective barrier* (prevent access of immune cells and therapeutic drugs), in other instances it can *lead the way for metastasizing tumor cells*, but also be involved in establishing the pre-metastatic niche.

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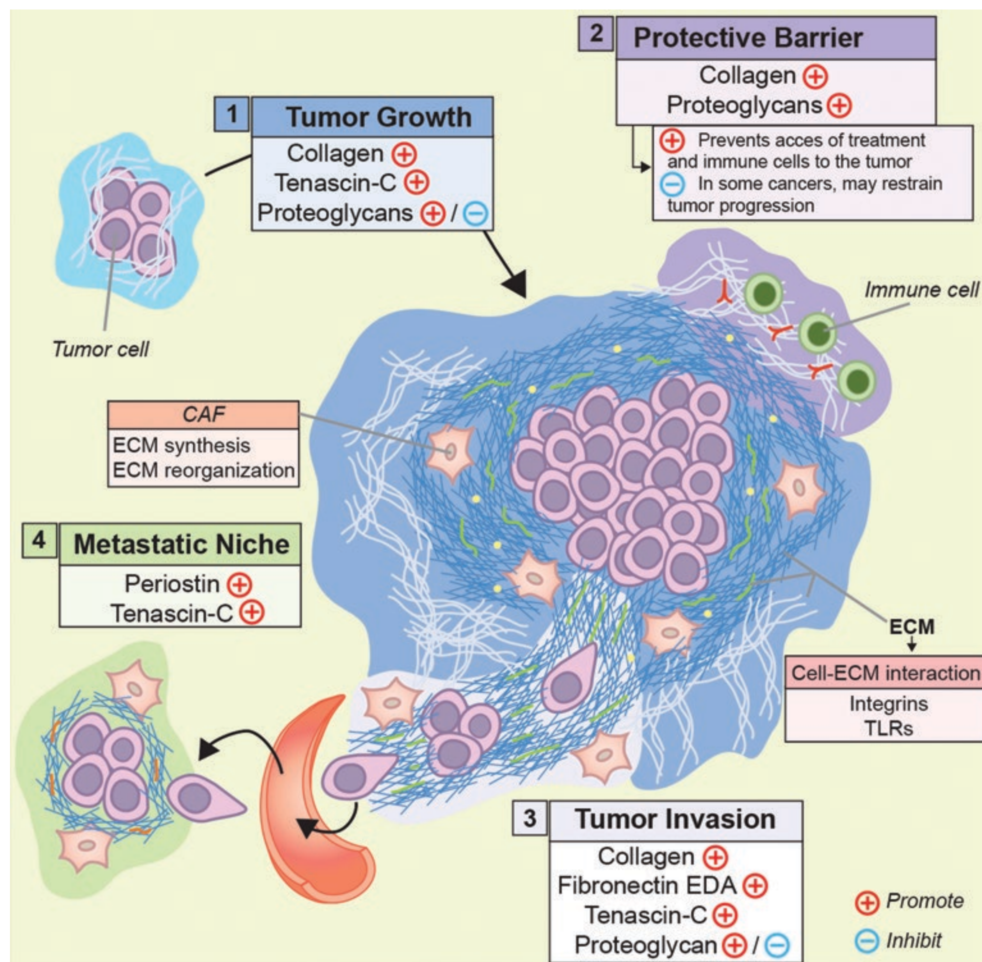

Schematic illustration of the role of extracellular matrix in the tumor microenvironment. The schematic summarizes some of the effects seen for extracellular matrix (ECM) molecules in different forms of cancer. Cancer-associated fibroblasts (CAFs) play a major role in ECM synthesis and ECM reorganization

Introduction

How one views a solid tumor depends on which "glasses" one uses. One can thus look at a tumor from a pathologist's point of view, from a cell biologist's point of view or from a molecular biologist's point of view. These different approaches provide different perspectives and information. A pathologist might note different aspects related to encapsulation, vascularization, and the amount of stroma. A cell biologist might distinguish signs of inflammation, degree of vascularization and choose to isolate cells to study their phenotypes in vitro. A molecular biologist aims to understand the molecular and genetic mechanisms involved in tumor pathogenesis, and designs experiments accordingly. No matter which "glasses" you have on, developments in the field of tumor cell-tumor stroma interactions highlight the importance of the tumor microenvironment (TME), and it is becoming increasingly clear that one needs to pay close attention to the tumor stroma when analyzing tumors.

The understanding that the tumor microenvironment influences tumor cell growth, also has implications for the design and interpretations of in vitro experiments. It has become clear that simple two-dimensional (2D) in vitro co-culture experiments are not sufficient to recapitulate the complex interactions that take place in the tumor in situ. Thus, in order to understand the cellular dynamics in the tumor, one needs to create model systems where the interactions between multiple types of cells as well as their three-dimensional (3D) compositions are incorporated. In molecular studies, inter-cellular communication, amount and properties of the extracellular matrix (ECM) and paracrine signaling, which all influence the signaling within cells, have to be taken into consideration when interpreting the data. New innovative strategies to study the influence of ECM in tumorigenesis are needed, hetero-spheroids [1, 2] and 3D organoid cultures are being recent methodological developments with great potential. 3D organoid cultures of patient tumors have recently generated great interest as novel in vitro cancer models that have several

advantages over and are complementary to established cell lines and patient-derived mouse xenograft models [3]. Unlike cell lines, organoids are both genetically and phenotypically stable during prolonged periods of cell culture and maintain the genomic representation of high-frequency gene alterations found in primary tumors [4]. Organoid models have been successfully developed for multiple tumor types, including pancreas [5] and breast cancer [6].

When discussing different mechanisms in the tumor microenvironment, it is important to avoid generalizations and to always relate the findings to a certain tumor and to the specific experimental conditions. The reasons to avoid such generalizations are:

- The TME can vary greatly between different tumors. Part of this heterogeneity is due to the source and nature of the stromal fibroblasts [7–9].
- The composition of the TME varies with the dynamics in, and stage of, the tumor: initiation, growth, and metastasis phases, all contain a TME with specific characteristics (e.g., differences in amounts of immune cells, fibroblast activation states, proteolytic activity, and stiffness).
- Matrix stiffness is another critical feature for tumor growth and for tensional homeostasis in the tumor [7, 8, 10]. Matrix stiffness has been shown to be intimately linked to posttranslational modifications of the matrix proteins, such as glycation, citrullination [9] and cross-linking, but also to ECM organization, which will vary in different regions within the tumor. In addition to the complexity in the assembly and structure of the ECM, the finding that tumor-derived exosomes affect cellular interactions in the TME introduces yet another level of complexity. Provocative data have described roles for exosomes in chemoresistance, miRNA-directed effects on gene silencing, and even in mediating changes in integrin repertoire affecting metastasis of tumor cells [11, 12].

The function of collagen in the tumor stroma is tightly linked to stromal fibroblasts, which in the solid tumor context are called cancer-associated fibroblasts (CAFs) [13–16]. CAFs have multiple roles in the tumor stroma in addition to ECM-related functions discussed below, including paracrine signaling [13] and chemoresistance [17]. A major function of CAFs is to serve as producers of ECM proteins like fibrillar collagens, and act as mechano-sensitive cells performing integrin-mediated reorganization of the matrix, resulting in changes in stromal stiffness [8]. In order for CAFs to take on this contractile function, they need to become activated. A prime signal for CAF activation is TGF-β. Data has demonstrated that integrin αvβ6 on the tumor cells is involved in TGF-β activation by binding to an RGD sequence in the latency-associated peptide (LAP) of the TGF-β/LAP complex, resulting in increased TGF-β bioavailability. Activation of TGF-β results in CAF activation [18]. Moreover, antibodies to αvβ6 in vivo have been shown to reduce growth and metastasis of the 4T1 murine breast cancer cell line [19]. Data in fibrosis and in vitro models further suggest that myofibroblasts themselves can play a vital role in activating TGF-β, by pre-straining the matrix and sensitizing latent TGF-β (LTGF-β) to activation [20–22]. In studies with dermal fibroblasts, the EDS fibronectin splice variant (EDA FN) has been shown to be induced by stiffness and to bind LTGF-β, in this way concentrating LTGF-β and enabling further activation by integrins. Integrin αvβ1 is increasingly becoming recognized for its role in TGF-β activation of myofibroblasts [23, 24].

Additionally, the finding that PDL-1/PD-1-based immunotherapy varies greatly between tumor types has resulted in an increased interest in alternative/supportive strategies that can abrogate immunosuppression [25]. In this context integrin αvβ8 has entered the spotlight. Whereas previous studies suggested that intestinal T-cells expressed αvβ8, and that this expression correlated with TGF-β activation [26], more recent studies suggest that most T-cell types lack detectable levels of αvβ8 and instead in most solid tumors the roles are reversed, i.e., tumor cells express high levels of αvβ8 and T-cells express inactive LTGF-β on their cell surface (anchored at cell surfaces via the membrane protein GARP) [27]. Further studies in mouse tumor models revealed that αvβ8 in this setting can activate TGF-β and by reducing immune cell activity help tumor cells evade host immunity. Interestingly, in this scenario, the activation of TGF-β appeared to occur independent of MMP-14. Later studies using cryo-EM confirmed the MMP-14 independency of the activation and instead demonstrated that in this context αvβ8 can bind to TGF-β while still bound to LTGF-β, without the release and diffusion of TGF-β to its receptor [28]. Another argument for considering αvβ8 as a cancer target comes from studies of pancreatic ductal adenocarcinoma where β8 was found to be over-expressed. When cultured human PDAC cells were irradiated, β8 expression protected cells from autophagy, which is also the major suggested mechanism for its radiochemoresistance effects [29].

At the stage of metastasis, CAFs have been reported to generate migratory paths in the stroma that facilitate collective cell invasion in an integrin-, caveolin-1-, RhoA-, Rab21-, and YAP-dependent manner [30, 31]. Interestingly, two highly cited reports have challenged the dogma that the tumor stroma plays a supportive role in tumor growth and metastasis [32, 33]. Both studies take advantage of advanced genetic techniques to ablate stromal cells in experimental models for pancreatic cancer after the tumors had formed. Contrary to what was expected, the tumors became more aggressive in the absence of the stroma. When analyzing these data, a number of caveats with these studies have been mentioned. However, a detailed update on the role of the tumor stroma in pancreatic ductal adenocarcinoma (PDA) using clinical patient PDA material as well as the use of transgenic mouse models, support a barrier role of the PDA

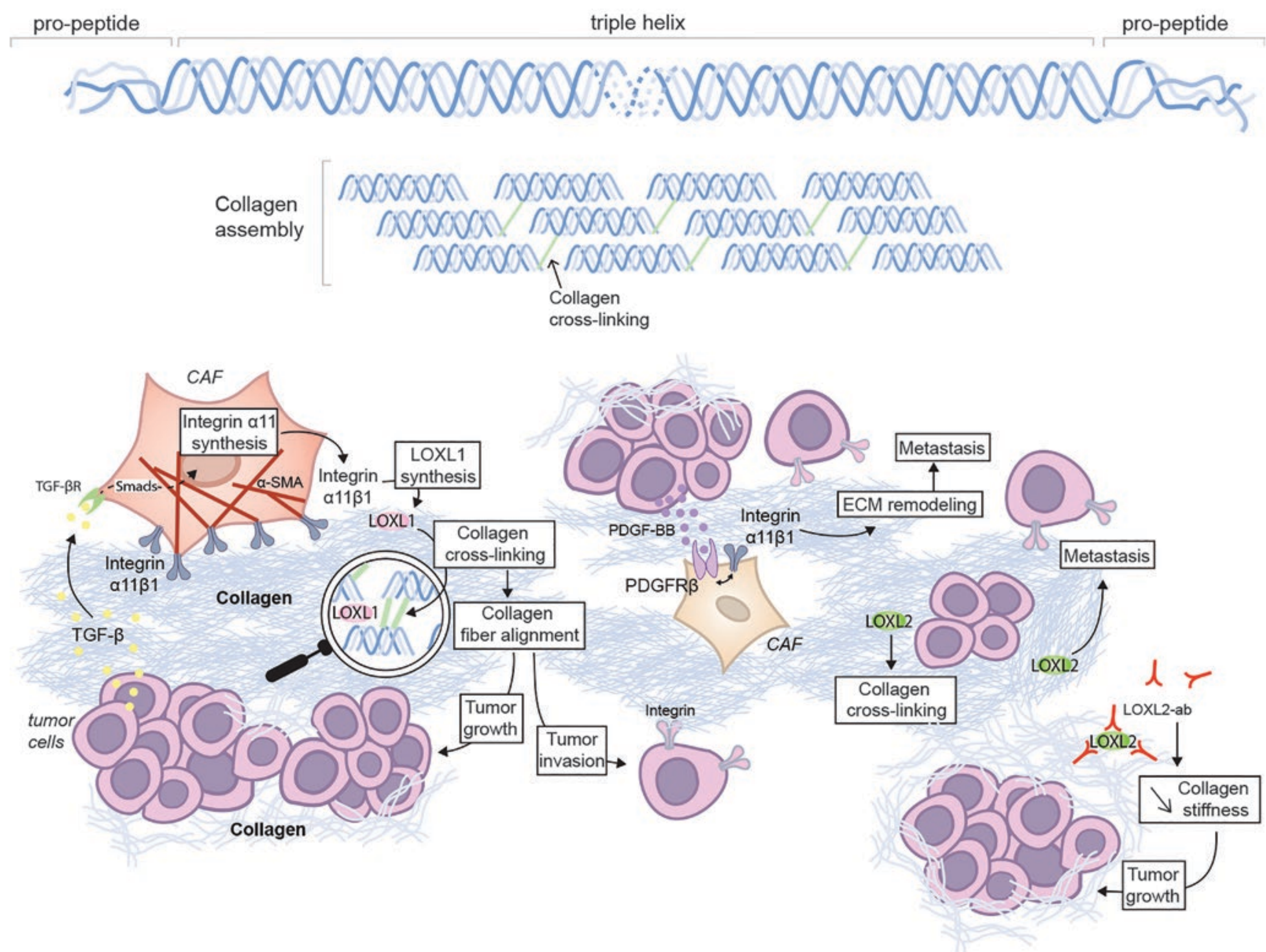

Fig. 5.1 Fibrillar collagen in cancer. Fibrillar collagens are composed of three chains that form a triple helix. The pro-peptides are cleaved for collagen assembly into fibrils. TGF-β signaling induces fibroblast differentiation into contractile myofibroblasts. The myofibroblasts express and deposit collagen, express α11β1 collagen-binding integrin, which mediates collagen remodelling. Integrin α11 induces LOXL1 expression in cancer-associated fibroblast (CAF). Secreted LOXL1 cross-links collagen fibers that enhances integrin-mediated collagen matrix reorganization and alignment of collagen fibers to support tumor growth and tumor invasion. Breast tumor cells releases PDGF-BB that activates PDGFRβ on CAFs. PDGFRβ interacts with integrin α11β1 to mediate metastasis. LOXL2, like LOXL1, mediates collagen cross-linking and is involved in metastasis of breast cancer. However, blocking LOXL2 activity using anti-LOXL2 antibody (ab) in pancreatic ductal adenocarcinoma reduces collagen stiffness that favors tumor aggressiveness and progression. This suggests that fibrillar collagens play different roles in different cancer types

stroma [34]. When the authors analyzed tumor-stromal density, patients with high tumor-stromal density enjoyed a longer survival and stromal content showed a negative correlation with overall survival. A PDA model in mouse where collagen content was modified by anti-Loxl2 monoclonal antibody (mAb) treatment confirmed that stromal depletion promoted, rather than inhibited, PDA development (Fig. 5.1). Interestingly, no striking changes in CAF subtypes, endothelial cell number, T-cell or myeloid cell infiltration were observed. In this transgenic mouse model, pharmacologic depletion of stroma thus decreased tissue tension and increased tumor aggressiveness [34]. The study suggests that the stroma has an important protective barrier role in PDA that outweighs any hypothetical pro-tumorigenic influence it may have in PDA tumor biology. The study highlights the need to firmly establish whether a fibrotic stroma in a particular tumor model is tumor promoting or tumor impeding and based on this to identify CAF subsets that are tumor promoting or tumor impeding.

Although the overall role of CAFs most likely differs between tumor types and CAF heterogeneity differs in different tumors, this does not mean that all fibroblast-targeted

therapy approaches are doomed to fail in tumors, but it highlights the complexity of tumor-stroma interactions and points to the potential need to target specific subsets of fibroblasts or even specific signaling pathways in fibroblasts, which are central to the tumor-promoting aspect of the stroma. In summary, a global targeting of all CAFs may not be the best anti-stroma therapeutic strategy [16, 35] since both tumor-supportive CAFs and tumor-inhibitory CAFs appear to exist in the tumor stroma. Continued cell lineage tracing and RNA single-cell profiling will be critical to unravel these mechanisms and provide useful insight into new CAF-associated therapies for treating tumors.

The Extracellular Matrix of the Tumor Stroma

Fibrillar Collagens in the Stroma

Fibrillar Collagen Types in the Tumor Stroma

The collagen family is composed of 28 trimeric triple helical proteins [36, 37]. The most abundant collagens are the fibrillar collagens (collagens I, II, III, V, XI, XIV, and type XXVII), which together with a subset of fibril-associated collagens with interrupted triple helices (FACIT collagens) are present in interstitial tissues [37]. In interstitial tissues, collagen I dominates with lesser amounts of collagen III being present. Collagens I and III form heterotypic fibrils where the minor collagens collagen V and XI are present in the core of these heterotypic fibrils. Collagen V in some studies has been suggested to constitute less than 5% of interstitial matrices, whereas collagen XI is present only in specialized matrices under physiological conditions [36, 37]. In carcinomas, the fibrillar collagens I/III dominate, and relatively little information is available on the status or roles, if any, of collagens V and XI [38]. The tumor stroma has been likened to a *wound that does not heal*, representing the tumor stroma in a sense as a granulation tissue, which is rich in fibrillar collagens [39, 40]. In the granulation tissue, collagen III is replaced with collagen I as the wound heals [41], but in the tumor stroma, the ratio of collagen I and III is determined by tumor type as well as the stage of the tumor and tissue-specific factors.

Cells can adhere to collagen matrices, either directly or indirectly via proteins bound to collagens. Direct binding occurs via collagen receptors such as the integrins α1β1, α2β1, α10β1 and α11β1 [42, 43]. Indirect binding is mediated via collagen-integrin bridging molecules (COLINBRIs), which typically bind RGD-binding integrins like α5β1, αvβ1, αvβ3 and αvβ5 [42, 44]. Interestingly, the discoidin domain receptors (DDRs) have been shown to affect the function of collagen-binding integrins by supporting integrin activation [45–47]. The role of fibrillar collagens in the tumor TME for tumor growth and metastasis is receiving increasing attention. Some of the most provocative studies have addressed the role of collagen composition, processing, and posttranslational modifications including cross-linking in regulating stiffness, tumor growth, tumor invasion, and metastasis [48–52].

The ability of fibroblasts to produce and remodel the collagen matrix is in turn affected by interactions with other cell types in the TME such as the tumor cells themselves, different types of inflammatory and vascular cells [53]. Cell-mediated collagen remodeling can be mediated by direct binding of collagen-binding integrins and indirect binding of COLINBRI-binding integrins [44, 54]. The main integrin-collagen receptors for direct binding to the fibril form of fibrillar collagens are α2β1 and α11β1 [55]. These two integrins are both efficient in remodeling the collagen matrix, as assessed in collagen gel contraction assays [56]. Although in vitro experiments have largely failed to demonstrate a direct binding of α1β1 to collagens fibrils, α1β1 has been postulated to bind indirectly to the fibrillar forms of collagens I/III via FACIT collagens [57].

Integrin α11β1 is a receptor for fibrillar collagens and is expressed on subsets of fibroblasts and mesenchymal stem cells [58–61]. In an α11-positive subset of non-hematopoietic bone marrow-derived mesenchymal stem cells, α11 expression correlated with osteogenic potential of these cells [62]. The potential role of α11 bone marrow expression for leukemia development remains to be determined. Recent screening of tumor tissue array revealed expression of α11 in CAFs in multiple solid tumors [63]. Importantly, studies using animals deficient in α11 expression in the tumor stroma reveal a major attenuation of tumor growth and metastasis in non-small cell lung cancer and breast cancer in the absence of α11 [64–66]. In the breast cancer model, we have shown that stromal integrin α11 displays a pro-tumorigenic and pro-metastatic activity in breast cancer and strongly associates with a PDGFRβ+ CAF subset [64] (Fig. 5.1). Integrin α11 expression is strongly upregulated in the stromal compartment during mammary tumor progression. Histological analyses revealed a strong association between integrin α11 and PDGFRβ, both in clinical breast cancer samples and in the pre-clinical transgenic mouse MMTV-PyMT model. Among several tested stromal markers (PDGFRα, PDGFRβ, αSMA, FAP, FSP1, and NG2), this collagen-binding integrin was mostly associated with a PDGFRβ$^+$ CAF subpopulation at late stages of invasive tumors. Genetic ablation of integrin α11 in the PyMT model drastically reduced not only tumor growth and metastasis, but also the desmoplastic reaction in these tumors, further highlighting the contribution of this specific α11$^+$ CAF subset to tumor progression through ECM regulation. This is further supported by the fact that myofibroblastic CAFs (mCAFs) are thought to derive from resident fibroblasts, as well as from integrin α11/PDGFRβ$^+$ CAFs. Mechanistically, this study revealed that integrin α11/PDGFRβ cross-talk in CAFs endows breast cancer tumor cells with pro-invasive features through the deposition of tenascin-C protein (TN-C) (Fig. 5.2). TN-C was strongly

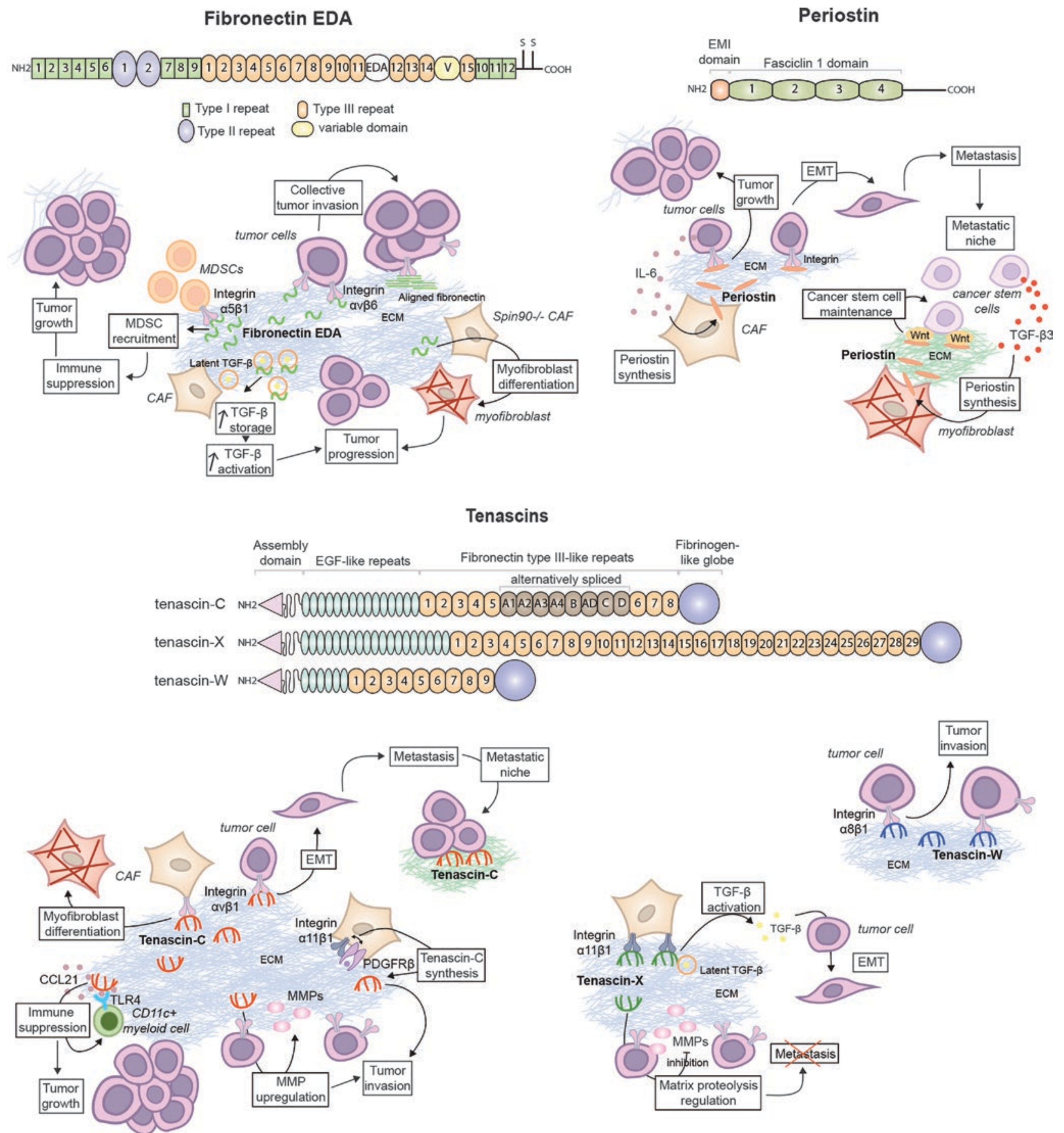

Fig. 5.2 Structure of EDA fibronectin, periostin and tenascins and their role in tumorigenesis. Fibronectin (FN) presents alternatively spliced domains, EDA and the variable domain. FN dimerizes through two disulfide bonds in the C-terminal part of the protein. EDA has been shown to reduce immune response in cancer models and to direct collective tumor cell migration. EDA FN increases recruitment and activation of the latent TGF-β in the fibroblast matrix. EDA FN induces myofibroblast differentiation of SPIN90-deficient fibroblasts to promote tumor progression. Periostin is composed of an EMI domain and four fasciclin 1 domains. Tumor cells induce periostin synthesis in CAF via IL-6. Periostin induces tumor growth and EMT. Periostin synthesis is stimulated in metastatic niches through secretion of TGF-β3 by cancer stem cells, where periostin is needed to support the metastatic colonization. Members of the tenascin family display an assembly domain at the N-terminal to form hexamers. Tenascin-C presents an alternatively spliced region within the fibronectin type III-like repeats. Tenascin-C has been shown to contribute to EMT through integrin αvβ1, promoting metastasis. Tenascin-C is required in metastatic niches to support the metastatic colonization. Tenascin-C also mediates immune suppression by immobilizing CD11c+ myeloid cell in the stroma, cancer cell invasion and myofibroblast differentiation of CAF. Tenascin-X activates TGF-β through interaction with α11β1 leading to EMT. In contrast, tenascin-X has been shown to reduce invasion and metastasis by inhibiting MMPs. Tenascin-W mediates cancer cell migration via interaction with α8β1 integrins. (For more details, please refer to the main text)

expressed by the same subset of CAFs expressing integrin α11 and PDGFRβ in the late-stage PyMT tumors, as well as in clinical samples of invasive breast cancer. Overall, this study discloses an example of a collaborative cross-talk between an integrin and a growth factor receptor in CAFs, which acts as a driver of tumor invasiveness in breast cancer. In support of a role for integrin α11 in human breast cancer, a careful analysis of α11 integrin levels in a larger cohort of breast cancer patients demonstrated that high α11 expression level was associated with aggressive breast cancer phenotypes [67]. In addition to the direct role of collagen-binding integrins in mechanotransduction to remodel the matrix, a role of matrix metalloproteinases (MMPs) to help and facilitate remodeling of the collagen matrix has also been demonstrated [68, 69]. This aspect is developed in a latter section of this chapter.

In the tumor context, the organization of the collagen matrix has been suggested to serve as an optical biomarker for metastatic propensity [70]. For this purpose, the term "Tumor associated collagen signatures (TACS)" has been introduced: TACS-1 (normal stage): anisotropic, wavy collagen fibrils, similar to normal quiescent tissue; TACS-2 (predisposed stage): prealigned collagen fibrils; TACS-3 (desmoplastic stage): aligned collagen fibrils [70]. It is important to remember that the tumor ECM is complex, and although collagen might align in specific patterns, cellular interactions during tumor spread might occur via many mechanisms, both collagen-dependent and collagen-independent. As such, the TACS signature may have to be combined with other biomarkers to be clinically useful.

Although there is some tendency to consider the biological effects of all stromal collagens to be equivalent, a recent study suggests that different fibrillar collagens have divergent functions. Whereas collagen I in the tumor stroma, according to the dogma, was considered to be pro-carcinogenic (increased density and increased stiffness, which promotes tumor growth and invasion), another independent study suggests that fibrillar collagen III has opposite effects [71]. In that study using collagen III heterozygous knockout mice (*Col3a1*$^{+/-}$ mice, $^{-/-}$ mice rarely survive perinatal age) it was demonstrated that mammary carcinomas grown in these mice were larger, more invasive and contained thicker, more organized and linearized collagen stroma. It is likely that in this model, several indirect mechanisms were operative, which need to be elucidated, including characterization of possible changes in integrin repertoire concomitant with collagen ratio switches. In the TME, the epithelial-derived carcinoma cells are, to varying degrees, surrounded by collagen IV containing basement membrane structures. As cells de-differentiate and go through epithelial-to-mesenchymal transition (EMT), they are exposed to fibrillar collagens in the tumor stroma. Multiple studies have highlighted the importance of collagens and the MMPs in this process [72–74].

Collagens Affecting Tumor Cell Growth

A number of studies have demonstrated that a collagen matrix promotes tumor growth. In the MMTV-PyMT breast tumor model, crossing the MMTV-PyMT mice with transgenic mice expressing a collagen α1 chain in which the collagenase cleavage site has been mutated, resulted in increased breast tumor growth and collagen accumulation at the tumor site [75]. In other experiments, collagen synthesis was blocked by inactivating certain enzyme isoforms, such as the intracellular enzymes prolyl 4- hydroxylase [76] and lysyl hydroxylase [77]. Blocking these enzymes in the stroma, resulted in reduced collagen accumulation and reduced collagen stiffness. These results are supported by data from tumor models inhibiting the collagen receptor α11β1, which implicate a role of cell-collagen interaction in non-small cell lung cancer and breast cancer growth and metastasis [64–66]. In these models, the decreased α11β1 function resulted in an attenuation of breast and lung tumor progression and metastasis, thus supporting a role of fibrillar collagens in supporting rather than restraining tumor growth. In yet other studies, fibrillar collagens have been shown to induce apoptosis of tumor cells [78]. In one study, MMP-14 was demonstrated to protect invading mammary carcinoma cells from collagen-induced apoptosis once they entered the fibrillar collagen I matrix [79, 80]. In experiments taking advantage of the model expressing collagen I with mutated collagenase cleavage site, pharmacologic depletion of stroma and decreased tissue tension increased tumor aggressiveness [34]. In summary, the disparate results on the varying roles of stromal collagens demonstrate the complexity of the stromal collagen interactions but probably also hint that the role of fibrillar collagens varies with the tumor type. It will be interesting to determine if the pancreatic desmoplastic tumors are the norm or the exception with regard to collagen function in the tumor stroma.

Collagens Affecting Cell Migration

Several studies have also reported MMP-dependent changes in collagen fibril diameters. In one study, MMTV-PyMT mice crossed with mice deficient in MMP-13 protein demonstrated no effect of MMP-13 depletion on breast tumor progression and lung metastasis [81]. Conversely, another study using a similar model observed a modest increase of lung metastasis in the absence of endogenous MMP-13 activity [82]. In the latter study, monitoring of breast tumors revealed that in the absence of MMP-13, the collagen content was not increased, but was comprised of thinner fibrillar collagen fibrils and a different organization of collagen at the tumor-stroma interface [82].

Two interesting explanations from the last study were proposed to explain the effects of the thinner fibrillar collagen structures. First, the normal cleavage of telopeptides from collagen I by MMP-13 may affect lateral fibril growth. Thus, if cleavage is reduced, fibrillar growth would be inhibited [83]. Alternatively, MMP-13 can also cleave collagen III, which acts to regulate fiber diameter, offering another possible mechanism for the observed thinner fibrils in the absence of MMP-13 [84]. Interestingly, a study of wound healing in zebrafish revealed that increased level of MMP-9 leads to larger fibril diameter. The authors suggest that this might be due to a switch in synthesis from collagen III to collagen I [85], offering more indications that MMP levels can have unpredictable effects on collagen fibril diameter. Although the effect of MMPs is complex due to multiple targets, the effects on fibril diameter are interesting and warrant further studies in the context of tumor growth and spread. Finally, in a study by Herchenhan et al., lysyl oxidase (LOX) inhibition in artificial tendon cultures also resulted in irregular fibril diameters, suggesting a role for LOX enzymes in regulating fibril diameter [86]. So far, corresponding effects have not been reported in the tumor context, but one might expect similar results in dense tumor matrices. The findings of different collagen fibril diameters might mainly be relevant for tumor cell migration. Previous elegant studies have demonstrated that cells can switch between protease-dependent and -independent migration, in 3D matrices, depending on the matrix pore diameter [87, 88]. A detailed study of collagen organization in aging tissues has revealed that collagen organization can also affect immune cell migration [89]. Studies of collagen in the aged skin paired with proteomic analysis of fibroblasts from young and aged individuals identified hyaluronan proteoglycan link protein 1 (HAPLN1) as a candidate protein involved in changed matrix collagen organization and increased collagen contractility of the aged cells. In the young ECM, HAPLN was suggested to take part in organizing collagen into an anisotropic basket weave structure, which could readily support T-cell migration. With regard to melanoma, the collagen organization in young skin was suggested to suppress invasion of melanoma cells, whereas in the aged skin (low HAPLN), an invasion-permissive microenvironment for melanoma cells was created, which was also characterized by a hampered immune cell infiltration.

Collagen Stiffness Regulating Tumor Growth

The stiffness of the tumor stroma has also been recognized as being able to influence tumor growth. Since collagens are major constituents of the tumor stroma, they might also play a major role in this regard. There are different mechanisms that can affect stiffness, including glycation [9]. A landmark paper in this area demonstrated that artificially forced expression of LOX in CAFs in a xenograft breast tumor model increased stiffness of the tumor with enhanced β1 integrin/FAK/ERK signaling in tumor cells, resulting in increased tumor growth [10]. It is worth noting that in non-experimental tumors, LOX is produced by different cell types, not only by CAFs [90]. Moreover, the role of LOX has also received considerable attention in relation to the metastatic niche and tumor metastasis [52, 91, 92]. These studies have demonstrated that LOX is deposited and crosslinks the basement membrane collagen IV at future sites of metastasis. In addition to collagens, other important ECM components of the metastatic niche stroma include periostin, fibronectin, EDA, and tenascin-C [93–95].

LOX expression has also been associated with poorer patient prognosis in lung adenocarcinoma [96]. For example, it has been shown that down-regulation of LOXL1, which belongs to the LOX family oxidases (LOXL 1–5), in xenograft tumors of both established and primary non-small cell lung cancer lines in integrin α11 knockout in SCID background as compared to wild-type SCID mice leads to decreased tumor growth [97]. The decrease in tumor growth was closely associated with reduced organization and stiffness of fibrillar collagen matrices (Fig. 5.1) [66].

In summary, in some tumor types, collagen matrices that are rich in collagen I and comprised of large diameter fibrils seem to be required for optimal support of tumor growth and metastasis. Current data from pancreatic cancer models suggest a barrier function of fibrillar collagens. Furthermore, stiffer matrices comprised of linear fibrils around the tumor can provide routes for invasion. Stromal collagen organization is dependent on: (1) CAFs, which produce the majority of the ECM and express cell surface integrins able to reorganize the collagen matrix, (2) LOX enzymatic activity for matrix cross-linking and (3) MMPs to facilitate ECM reorganization.

As already mentioned, experiments using two different experimental model systems that severely restrict production of mouse pancreatic tumor stroma together with more recent studies in a mouse model where mAbs to LOXL2 was used to decrease collagen levels and stroma tension have demonstrated that global obliteration of the stroma can result in tumors becoming more aggressive (Fig. 5.1) [32–34]. One way of interpreting these data is that in desmoplastic pancreatic tumors, the stroma acts as a barrier, the removal of which facilitates tumor cell migration and invasion. In light of these findings, it becomes critical to reconcile the data suggesting that linearized fibrillar collagen acts as a highway for tumor invasion [70, 75] with the multiple studies suggesting that a stiff dense matrix promotes tumor growth and tumor metastasis [10, 76, 77, 98]. The most obvious explanation is that fibrillar collagens play different roles in different tumor types. These questions will need to be addressed in order to fully delineate, which pathways involved in collagen biosynthesis, posttranslational modifications or collagen remodel-

ing, represent attractive future therapeutic targets in the tumor stroma.

Methods for Measuring Fibrillar Collagen Stiffness

Structural alterations of the ECM during tumor initiation and progression have been shown to occur in several epithelial tumors [99, 100]. As mentioned earlier, TACS signatures predict that collagen fibers in normal tissue are curly and non-oriented, which is different from the highly linearized fibers of intra-tumoral collagen [10, 66]. The fibrotic reaction observed in the stroma of many cancers, characterized by an excess accumulation of some fibrillar collagens (especially type I, III, V, XI) as a result of desmoplasia, is considered to be a hallmark of cancer [91, 101, 102]. There are multiple collagen receptors in addition to collagen-binding integrins, such as DDRs, leukocyte-associated Ig-like receptors (LAIRs), and glycoprotein VI [103]. These receptors are: (1) not necessarily expressed on tumor cells or stroma cells (LAIRs on immune cells, GPVI on platelets); and (2) unlike integrins their role as mechanoreceptors with the ability to reorganize collagen has not been established.

Since fibrillar collagen has a non-centrosymmetric structure, it can be readily visualized with second harmonic generation (SHG) two-photon confocal microscopy both in vivo and ex-vivo (i.e., histology sections) and its organization can be probed with SHG polarization measurement [104–106]. In SHG, an excitation wavelength of 840 nm is applied to a sample, the resultant SHG signal is then measured, which is exactly one-half of the excitation wavelength (i.e., 420 nm). SHG has multiple advantages such as it enables optical sectioning and 3D imaging. Also, SHG does not require staining and absorption for signal generation, therefore, sample photobleaching is reduced. Overall, the intensity and polarization of the SHG signal depends on the sample structure and organization. Polarimetric SHG microscopy (P-SHG) allows the structural details of collagen organization in the tissue to be studied. In the use of P-SHG, the orientation of incoming laser polarization relative to a set of outgoing SHG polarizations is measured (polarization-in, polarization-out (PIPO) SHG), revealing the second-order susceptibility component ratio in each pixel of the image. These measurements reflect the hierarchical organization of collagen in the tissue [107]. The SHG polarization measurement is influenced by several factors, including the amino acid composition and sequence of the collagen triple-helix, organization of the triple helices in the collagen fibrils, arrangement of these fibrils in the fibers and finally fiber orientation with respect to the tissue section plane [106]. In addition, The SHG analysis renders an average fiber orientation in each pixel of the image, and provides information on the orientation related to the helical pitch angle of the polypeptide chain of the collagen triple-helix in the tissue [108]. Hence, polarization SHG is a promising technique to detect collagen alterations in the ECM during cancer progression [109]. SHG enables pathologists to perform a live biopsy, for example, in the endoscopic setting, or provides a quick histopathology investigation possibility that does not require staining. SHG microscopy presents unique advantages compared to conventional optical techniques to investigate the 3D heterogeneous accumulation of fibrillar collagen during fibrotic pathologies [110]. Another way to analyze the fibril orientation distribution is to measure the degree of waviness or alignment and orientation of collagen by an Image J plug-in method [111]. In this way, the local collagen fiber orientation was derived from the angle of the oriented collagen structure. The shape of the distribution indicated the degree of alignment within the image, where wide and broad shapes suggested little coherence in alignment and tight peaks implied aligned structures. In another study, the collagen fiber arrangement in NSCLC tumor xenografts was measured by a novel relative linearity index [66]. The combination of SHG polarimetric analysis and texture analysis revealed significant differences in the collagen structure between NSCLC and normal lung tissue and could quantify the structural alteration of collagen in stage-I, -II, and III-NSCLC tissue (PMID: 32341852). Therefore, the combination of polarimetric SHG microscopy and histopathology may lead to more accurate cancer diagnostics and staging.

Another method of studying the collagen linearity on a nanometer scale is electron microscopy, which involves measuring how straight or “curly” an individual fiber is [66]. Accordingly, the linearity on this scale would correlate to the stiffness of individual fibers. The advantage of the SHG images is that they show collagen arrangement on a larger scale (the images are 0.5 mm × 0.5 mm), which is indicative of the stiffness or stretchiness of tissue on the micron-to-mm scale.

In a more advanced way, the self-assembly of the native collagen fibrils in vitro could be characterized by the use of atomic force microscopy (AFM) [112, 113]. AFM elasticity measurements are a powerful tool to directly assess mechanical stiffness on the level of individual, or groups of, fibers. In fact, AFM can be used as a microdissection tool to study the inner assembly of the collagen fibrils. The AFM technique is based on detection of forces acting between a sharp probe, known as AFM tip, and the sample’s surface [114]. To determine the elastic properties of collagen fibrils, the tip of the AFM (cantilever) was used as a nanoindentor by recording force-displacement curves [115]. It has been shown that a new variant of AFM, which is called in situ atomic force indentation microscopy [116], is capable of measuring stiffness changes in mammary gland tissue as it evolves from normal to malignant with exquisite spatial detail. Based on this method, in a mouse model of human breast cancer that metastasizes to the lungs, the extracellular

matrix at the tumor boundary turned out to be the stiffest of all the tumor's components. In this study, AFM was applied to measure the stiffness of the surrounding extracellular matrix as a prognostic indicator for tumor development and aggressiveness [117].

Another technique of interest for measuring ECM and tissue stiffness at the macroscopic level is shear rheology [118]. At its simplest, this approach provides high-resolution determination of the matrix and tissue elasticity by measurements of mechanical compression and nano-indentation [118]. Shear rheology is a commonly applied means of testing the mechanical properties of materials by indenting the test material with a diamond tip while measuring the force-displacement response [118]. Although the techniques described above provide accurate and useful quantitative data on the biomechanical properties of matrix and tissue, most are generally considered invasive and/or destructive methodologies [119]. Hence, there is a need to develop methods to measure elastic properties and stiffness of tissues and matrix in a non-invasive manner for clinical application. Magnetic resonance and ultrasound elastography are routinely used tools in the clinic that provides the image contrast of elastic properties of tissues [120]. Clinical in vivo imaging by elastography shows that malignant breast tumors tend to appear stiffer than benign breast tumors; in particular, the stiffer tissue is frequently observed at the tumor margin or the invasive edge of the tumor [120]. Newer technologies based on fluorescence resonance energy transfer (FRET) [121], magnetic resonance imaging (MRI), positron emission tomography (PET) and single-photon emission computed tomography (SPECT) [122] are being developed to image the dynamic status of ECM remodeling [123]. Advances in μ-ultrasound, optical coherence tomography (OCT), optical acoustic microscopy and scanning acoustic microscopy (SAM) [118] are under development to facilitate imaging and quantitative measurement of stiffness at the microscopic scale [124]. In addition, increasing the resolution of many of the above techniques will be possible with improved contrast agents, such as so-called "smart probes," which are MRI contrast agents that can be used to study ECM components [125–127]. More information on these techniques is available in other reviews and reference materials.

In summary, new techniques that image the dynamics of cell-ECM interactions to non-invasively quantify remodeling of the ECM at the sub-millimeter level will ultimately provide additional resources for basic research and in the clinic. Therefore, increased understanding of the molecular basis of mechanotransduction may lead to identification of an entirely new class of molecular targets for anticancer therapy.

Role of EDA Fibronectin in the Tumor Stroma

Fibronectin (FN) is a large modular extracellular matrix protein composed of type I, type II and type III repeats [128] (Fig. 5.2). FN RNA is alternatively spliced at three conserved regions EIIIA (EDA), EIIB (EDB) and V (CS-1). The FN gene structure and splicing have been described in detail elsewhere [129]. The EDA and EDB domains display 29% sequence identity, but are each highly conserved among vertebrates [129]. Whereas a number of receptors have been described for EDA (described later), the cellular receptor(s) for the EDB domain remains largely unknown. Therefore, most of the focus has been on the EDA isoform.

The EDA and EDB isoforms are both highly expressed during embryonic development, especially in developing blood vessels [130], but are almost absent in the adult organism, where vascularization and tissue reorganization are quiescent. During wound healing [131], fibrosis and in solid tumors [132], the EDA/EDB embryonic splice variants are re-expressed [133], leading to the term "oncofetal" splice variants. Some studies suggest that these embryonic splice forms in tumors are mainly expressed in neo-vasculature [134], whereas other studies demonstrated their presence in the fibrotic stroma associated with myofibroblasts [135, 136].

The EDA domain is composed of 7 antiparallel beta strands separated by loops [129]. Early studies suggested that the presence of EDA in intact FN indirectly influenced the exposure of the RGD sequence in the tenth FN type III repeat leading to higher binding affinity for integrin α5β1 to FN EDA [137]. In later studies, it was demonstrated that integrin α9β1 and α4β1 bound directly to a cryptic loop region in an EDA containing fragment, but not to the intact FN EDA [138]. Binding of these integrins to the cryptic site would thus require proteolytic cleavage of fibronectin. α4β7 integrin on lung fibroblasts has also been shown to bind directly to FN EDA [139]. Similarly, Toll-like receptor 4 (TLR4) has been reported to be activated upon binding to the isolated EDA fragment, but not upon binding to the intact fibronectin EDA [140]. Importantly, FN EDA enhances TLR4 response, which in turn has been reported to augment TGF-β signaling [141]. α9β1 on basal keratinocytes co-localizes with EDA at the dermal-epidermal junction in skin wounds, but in dermal wounds, some dermal fibroblasts also express α9β1 [131]. Endothelial cells on developing and adult lymphatic vessels also express α9β1 [142]. Depending on the relative levels of different receptors, the effect of EDA FN is thus likely to vary.

Upon gross examination, mice deficient in either EDA or EDB appear normal, suggesting a redundancy for these splice forms during development [143, 144]. In contrast,

mice lacking both isoforms die at E9–10, due to cardiovascular defects and leaky blood vessels [145]. Careful analysis of fibronectin EDA$^{-/-}$ mice reveals some mild phenotypes including a mild lymph vessel impairment, due to a transient role for α9β1/fibronectin EDA during lymphangiogenesis [142]. However, other data suggests that EMILIN1 might play a more prominent role than FN EDA as an α9β1 ligand during lymph vessel development, especially in mature lymph vessels [146]. Whereas the expression of FN EDA clearly is a marker for certain biological processes such as wound healing, fibrosis, and a reactive tumor stroma, the exact role of EDA in these events is more complex [53].

Function of EDA Fibronectin Domain in Wound Healing

The role of EDA in wound healing has been studied in great detail. In a much-cited study, an essential role of EDA in TGF-β stimulated myofibroblast differentiation of rat dermal fibroblasts in vitro was determined using neutralizing antibodies [147]. In another study, EDA induced a pro-fibrotic effect in in dermal fibroblasts via binding to α4β1-mediated without affecting myofibroblast differentiation [148]. Similarly, studies of wound healing in EDA knockout mice failed to detect any major myofibroblast differentiation defects in the granulation tissue, though reduced epithelial migration was observed at the epidermal-dermal border along with some defects in granulation tissue [144, 149]. A role for integrin α9 and EDA in keratinocyte migration was further supported by experiments where α9 was conditionally deleted on keratinocytes, resulting in epithelial thinning [149]. Independent studies using EDA blocking antibodies in vivo resulted in mild effects on granulation tissue. The authors of these studies suggest that the less dense granulation tissue observed in these experiments was due to defective migration of dermal fibroblasts into the wounds, rather than defective myofibroblast differentiation [150].

Function of EDA Fibronectin Domain in Fibrosis

In the last 5–10 years, the role of fibronectin and the EDA FN isoform have attracted considerable interest in fibroblasts biology and accumulating data now attest to the biologic importance of the EDA FN isoform in tissue and tumor pathology. An in vitro study suggests that integrin α4β7 on lung fibroblasts stimulates myofibroblast differentiation [139]. In a mouse model, EDA FN deficiency prevented bleomycin-induced lung fibrosis [151]. Mechanistic analyses suggested an effect related to TGF-β activation in the lungs in this fibrosis model. Studies of infarcted hearts have also revealed reduced cardiac fibrosis and myofibroblast differentiation in the absence of EDA FN [152].

More and more studies are being published on the role of non-integrin receptors taking part in mediating the effects of EDA FN. In dermal fibroblasts both α4β1 integrin and the non-integrin receptor TLR4 have been shown to cooperate to induce fibrotic gene expression [153]. In smooth muscle cells, both receptors cooperate to mediate phenotype switching in Akt/mTOR (TLR4- mediated) and FAK/ERK/NF-kB mediated Il-1β release (integrin α4β1-mediated) [154]. Given the finding in Jain et al. [154], it would be interesting to determine the role of TLR4 in collagen remodeling under conditions when EDA FN is present. It has been suggested that EDA FN associated with TLR4 may play a role in keloids to couple a fibrotic response with an inflammatory response in the skin [141].

In a detailed in vitro study using fibroblasts, the group of Boris Hinz has convincingly demonstrated that EDA FN is increasingly produced under stiff conditions and enhances the recruitment of latent TGF-β-binding protein-1 (LTBP-1) to the ECM matrix [155]. In the context of fibrosis, EDA FN is important in myofibroblast activation (suggested to occur via integrins α4β [148], α9β1 [156] and α4β7 [139] in different experimental systems), but prior to this careful study, the link between EDA FN and TGF-β activation/storage had been elusive. Although the study was performed using skin fibroblasts, the data is probably of high relevance to different forms of tissue- and tumor fibrosis.

Function of EDA Fibronectin Domain in Tumorigenesis

In the context of tumors, in vitro and in vivo experiments have suggested different roles for EDA FN (Fig. 5.2). For fibronectin fibrillogenesis, integrins α5β1 and αvβ3 seem to cooperate to assemble a fibrillar EDA FN matrix and to direct tumor cell migration [157, 158]. The appearance and organization of fibronectin are thus closely associated with the behavior of integrin fibronectin receptors in CAFs. Elegant studies have demonstrated a role for αvβ5 in regulating α5β1 endocytosis and function in CAFs [159]. In another study, CD93 was shown to promote integrin activation and fibronectin fibrillogenesis during tumor angiogenesis [160].

In one cancer-related study, it was suggested that EDA FN, indirectly, by increased binding of α5β1 to RGD and induction of arginase-1, inhibits the immune response in cancer [161]. The elaborate mechanism worked out in this study involved α5β1- mediated increase in myeloid differentiation followed by an arginase-1-mediated suppression of the immune response, in turn potentiating enhanced tumor growth and reduced fibrosis. In yet another study, a role for CAF-produced EDA FN in directing the collective migration of HNSCC was demonstrated to depend on both α9β1 and αvβ6 in HNSCC cells [162].

Studies in spheroids using MDA-MB-231 cells demonstrated that under stiff conditions (12kP), the actin modulatory protein Mena was upregulated to potentiate α5β1 integrin-mediated assembly of EDA FN, which in turn was found to further stimulate integrin α4β1-mediated EDA

FN-dependent invasion in this spheroid context [163]. The study is intriguing given that it was performed with homospheroids, composed only of tumor cells, and it will be compelling to determine if similar data can be obtained in heterospheroids containing co-cultures of tumor cells and CAFs, since CAFs are the main contributing producers of EDA FN in the TME.

A detailed study on the role of the SH3 and NCk-binding protein Spin90 in α4β1 integrin signaling in CAF-like MEF cells in vitro and in vivo experiments demonstrates a role of SPIN90 in regulating EDA FN synthesis and fibrillogenesis as well as myofibroblast activation, in turn regulating breast cancer cell proliferation, migration and invasion [164]. The increased EDA FN synthesis in the Spin90$^{-/-}$ CAF-like MEFS could be reversed in the *Spin90* rescued cells. Interestingly, in the studies of Kwon et al, α4β1-mediated binding to EDA FN is able to reorganize a collagen matrix in *Spin90*$^{-/-}$ cells, whereas wild-type MEF cells failed to demonstrate a contribution of a cell-fibronectin interaction to the collagen matrix remodeling. In addition to the intracellular protein SPIN90 being able to control the mechanism of collagen matrix remodeling under very specific gene deletion conditions, it is likely that the integrin repertoire is a more general determinant of collagen reorganization. In conditions of high levels of collagen-binding integrins (i.e., α2β1 and α11β1) and low fibronectin synthesis, collagen-binding integrins would dominate [55]. Whereas under conditions with low levels collagen-binding integrins, high levels of fibronectin synthesis, high levels of fibronectin-binding integrins (i.e., α4β1 or α5β1), fibronectin-binding integrins would mediate collagen reorganization. The study of Kwon et al demonstrates a role for α4β1/EDA FN in collagen remodeling in genetically modified cells, and it will be interesting to determine if this interaction also can be demonstrated under more physiological conditions and in the tumor TME.

In colon carcinoma, EDA FN sustained tumor cell proliferation and induced lymphangiogenesis through VEGF-C secretion in mouse xenograft models [165, 166]. EDA FN has also been shown to induce EMT in lung and colon carcinomas, thus promoting metastasis [167, 168]. In a radiotherapeutic aspect, the presence of EDA FN reduced radiation sensitivity in head and neck carcinoma by inhibiting apoptosis of tumor cells [169]. Despite these findings, the absence of either EDA or EDB did not affect tumor growth, tumor angiogenesis, α-SMA expression in the tumor stroma, or tumor metastasis in either the Rip1- Tag2 tumor model or a xenograft model [130].

In summary, EDA FN is highly expressed in granulation tissue, in fibrotic lesions and in the tumor stroma. Critical analysis in genetic models demonstrated a moderate effect of EDA FN in wound healing, but with new methods and more careful analyses in new experimental genetic models, important contributions to fibrosis and tumorigenesis are also increasingly being recorded.

Matricellular Proteins: Tenascins and Periostin

Matricellular proteins are secreted macromolecules that do not play a primary role in matrix structure, but are able to modulate cell interactions and functions [170]. In cancer, matricellular proteins are involved in different steps of tumorigenesis due to their ability to bind different cell receptors [171]. The matricellular protein family includes thrombospondins, tenascins, SPARC, periostin, osteopontin and CCN proteins. In this chapter, we focus on the role of tenascins and periostin in cancer progression (Fig. 5.2).

Tenascins

The tenascin family is composed of four members in vertebrates, expressed in different tissues with a common role in modulation of cell adhesion and spreading [172]. Although all tenascin isoforms are expressed in different cancer forms, TN-C has been studied the most. TN-C is absent or lowly expressed in adult tissues, in contrast to the strong expression observed in cancer. TN-C is dynamically expressed during embryogenesis and pathological disorders but mice carrying a null mutation in the *Tnc* gene display no phenotype [173]. A continued interest in this molecule has, however, indicated important biological roles for TN-C, which thus is a completely different scenario compared to the largely negative results obtained in these initial challenging experiments using *Tnc*$^{-/-}$ mice. Although TN-C is highly expressed in fibrotic conditions in tissues and the tumor stroma as well as tumor metastases, several studies failed to reveal a functional role of TN-C in these fibrotic matrices. This included studies of TN-C in the PyMT breast cancer model [174]. In contrast to data from the PyMT model in *Tnc*$^{-/-}$ genetic background which suggested a very mild phenotype with macrophage filled *Tnc*$^{-/-}$ stroma with little consequences for tumor cell proliferation or lung metastasis in the absence of TN-C, continued studies in a number of models have more recently confirmed a functional role of TN-C in specific fibrotic and tumorigenesis events (please see below for details).

Just as Toll-like receptor 4 (TLR4) has emerged as a receptor mediating pro-fibrotic signal for EDA FN, TLR4 has also emerged as a receptor for different tenascin isoforms. A detailed study focusing on different motifs in TN-C has identified a structure in fibrinogen-like globe domain (FBG) of TN-C that is predicted to be active in TLR4 binding also in tenascin R- and tenascin-W, but notably not in TN-X [175]. A number of studies suggest that tenascin-C effects are mediated by both integrins and TLR4 receptors, often creating a complex interaction network involving para-

crine signaling. Experiments using cell cultures and experimental fibrosis in *Tnc*$^{-/-}$ mice have demonstrated a role for TLR4 in TN-C-dependent skin and lung fibrosis [176]. A recent study demonstrates an interesting role of TN-C in heart fibrosis following experimental myocardial infarction suggesting involvement of TIMP-3 in the reduced fibrosis observed in the absence of TN-C. In an independent study, the transcriptional regulators twist and paired-related homeobox1 (Prrx1) were identified in a positive feedback loop together with TN-C to be involved in regulating fibroblast activation both under physiological and fibrosis/wound healing responses [177]. Such fibrogenic niches composed of TN-C in has been shown to be active in kidney fibrosis [178].

Tenascin-C expression is induced in several solid tumors and is often associated with poor prognosis (for review, see [179]). It is now clear that TN-C promotes tumorigenesis, acting at different steps of this process, with the metastasis step probably being the most prominent step. TN-C can stimulate tumor growth by abolishing the cell proliferation-suppressing effect of fibronectin [180, 181]. TN-C has also been demonstrated to compete with fibronectin for syndecan-4 binding, thus weakening breast carcinoma cell adhesion and spreading on fibronectin [182]; this cell adhesion inhibition leads to cell rounding that enhances tumor cell proliferation. TN-C can reduce apoptosis of pancreatic cancer cells, by activating the anti-apoptotic Bcl-2 and Bcl-xl and inhibiting cleavage of caspase-3 [183]. Tenascin-C also stimulates EMT of breast cancer cells, in an αvβ1- and αvβ6- dependent manner [184, 185]. The Wnt/β-catenin signaling pathway, which is known to induce EMT [186], is enhanced in the presence of tenascin-C via the down-regulation of the Wnt inhibitor Dickkopf 1, which stabilizes β-catenin [181, 187]. It is interesting to note that the *TNC* gene was identified as a β-catenin signaling target in colorectal cancer, suggesting a feed-forward loop that could stabilize the EMT phenotype and influence invasion in this tumor type [188].

Furthermore, TN-C plays a role in tumor cell migration and invasion [189, 190]. In a study of invasive melanoma, tenascin-C was found to form, in addition to fibronectin and collagen I, tubular structures that were proposed to serve as channels for melanoma cell invasion [191]. Interestingly, TN-C can also up-regulate MMP-9 and MMP-13 expression in breast cancer, thus promoting cancer cell invasion [192, 193]. Knockdown of tenascin-C in the MDA-MB-435 melanoma cell line decreased the number of lung metastasis in nude mice, demonstrating that tenascin-C may stimulate metastatic progression [194]. Another publication demonstrated that in lung metastatic sites, TN-C is over-expressed by *S100A4*$^{+}$ stromal cells, most likely fibroblasts, supporting metastatic colonization [195]. In the same study, *Tnc*$^{-/-}$ mice injected with 4T1 murine breast cancer cells displayed fewer and smaller metastatic lung nodules [195]. Another interesting study initiated by Oskarsson et al. showed that TN-C secretion by breast cancer cells is required to form a metastatic niche for the establishment of lung metastases [93].

A detailed careful study of ECM proteins induced in lung fibrosis, in lung cancer and in lung cancer metastases using mass spectrometry technology and various mouse models identified TN-C as being induced in all these conditions [196]. However, additional experiments in knockout models and in transgenic overexpressing mice revealed a functional role of Tn-C restricted to metastasis, which is still in stark contrast to the early experiments in PyMT mice, where no effects on lung metastasis were seen (Fig. 5.2). With regard to the cellular mechanisms, a study of human mammary fibroblasts, as a model of breast cancer CAFs, suggest that TN-C treatment increase collagen gel contraction and increased synthesis of TN-C and integrin αvβ1, in turn leading to increased TGF-β activation [197]. This is suggested to be a mechanism promoting increased matrix stiffness. It will be interesting to pursue how actually TN-C mediates this effect on collagen gel contraction. Since this process ultimately depends on a stable link between cells and the collagen matrix, it is possible that the cell-TN-C interaction creates a stimulatory autocrine signal strengthening the link between collagen-binding integrins and the collagen matrix.

In a detailed study of a mouse model of head and neck cancer, TN-C was demonstrated to be present in tumor TME to contribute to shape an immunosuppressive pro-tumoral microenvironment [198]. When TN-C was depleted in this tumor model, tumor growth and lymph node invasion were affected. The observed TN-C effects were shown to be mediated by α9β1 integrin on endothelial cells acting via CCL21 secretion and TLR4 on *CD11c*+ myeloid cells acting via CCR7. It will be interesting to determine if these immunosuppressive systemic effects also are operational in other tumor types.

Tenascin-W was the last tenascin member to be described, and relatively little is known about this tenascin family member. The expression of tenascin-W has been shown to be regulated by TGF-β [199], and was initially observed to be strongly upregulated in the tumor stroma of breast and colon cancer patients [200, 201]. In the context of breast cancer, tenascin-W has been shown to promote the migration of breast tumor cells through interaction with α8β1 integrin [202]. In later studies, Brellier et al. determined that tenascin-W expression was also induced in melanoma and in pancreatic, kidney and lung carcinomas; the authors suggested that tenascin-W might be a useful cancer biomarker in several solid tumors [203].

Tenascin-X is expressed in several tissues, with high expression in skin and skeletal muscle [204]. Deficiency or mutation in tenascin-X gene leads to a form of Ehlers-Danlos syndrome, characterized by skin and joint hyperextensibility [205]. In contrast with other tenascins, tenascin-X was first predicted to be anti-tumorigenic: its expression was strongly

decreased in malignant melanoma [206], and mice deficient in tenascin-X displayed increased melanoma invasion and metastasis [207]. This was explained by an induction of MMPs, including MMP-2, in the absence of tenascin-X through JNK signaling, indicating a role of this tenascin in matrix proteolysis regulation [208]. Alcaraz et al. have suggested a different role of tenascin-X in breast cancer progression. In their study, tenascin-X was curiously enough suggested to contribute to TGF-β activation via its interaction with α11β1 integrin, thus promoting EMT [209]. It will be interesting to determine if the binding of tenascin to α11β1 is direct, and if so, which part of integrin α11β1 binds to tenascin-X.

Periostin

Periostin is a matricellular protein, which is highly expressed in mesenchymal tissues during development [210]. Periostin is a homodimeric matricellular protein belonging to fasciclin family (Fig. 5.2). Like TN-C, periostin is induced in the tumor stroma. Detailed studies have revealed complex interactions with αv integrins (αvβ1, αvβ3, αvβ5) [211]. Genetic deletion of periostin leads to tooth defects and a periodontal-like disease, which result in dwarfism [210]. Wound healing studies suggest a promoting effect of periostin in dermal myofibroblast differentiation and collagen gel contraction [212]. A pro-fibrogenic role for periostin in cardiac and skeletal muscle fibrosis has also been reported [213, 214]. Interestingly, periostin has been observed to interact with fibrillar collagen and in the absence of periostin the collagen fibrillar diameter increases [215, 216].

In the tumor context, an early study reported reduced numbers of activated CAFs and less collagen in capsule and TME, leading to increased growth of grafted mouse tumor cell lines in postn$^{-/-}$ mice [217]. Later studies have focused on the presence of periostin in the tumor stroma of gastric cancer, melanoma, glioblastoma and in metastatic niches [218–220]. In one study, the ability of periostin to bind Wnt was suggested to be the mechanism underlying the ability of periostin to support cancer stem cell maintenance and tumor metastasis (Fig. 5.2) [94, 221]. Periostin was shown to induce EMT in cholangiocarcinoma through α5β1 integrin and the TWIST-2 axis [222]. In colorectal cancer, periostin secreted by stromal fibroblasts promotes YAP/TAZ activation and IL-6 expression in tumor cells, which in turn activates myofibroblasts and periostin synthesis to facilitate tumor progression [223]. In a study of B16F10 melanoma model, chemotherapy treatment with cisplatin was found to increase periostin levels, in turn suggested to contribute to liver metastasis by enhancing metastatic niche formation [224].

Stromal Proteoglycans

Proteoglycans (PGs), abundant at cell surfaces and in the extracellular matrix, belong to a group of glycoproteins in which the core protein is substituted with one or more polysaccharide chains (called glycosaminoglycans; GAGs). PGs play important roles during different aspects of cancer progression (for review, see [225–227]). Heparan sulfate (HS) PGs execute their function by binding to a variety of molecules including members of several growth factor families, chemokines, morphogens, serine protease inhibitors, and extracellular matrix proteins [228]. Protein binding is generally mediated by their sulfated GAG chains, but may in a few cases involve interaction with core proteins [229]. Examples of proteins that depend on binding to HSPGs for function include members of the FGF-family and their corresponding receptors, VEGF, members of the transforming growth factor-β family, Wnt proteins, pleotropin and the serin protease inhibitor antithrombin [230]. Depending on the molecule, the activity of the bound factors is mostly enhanced, although there are few examples of activities that are inhibited by the binding to HSPGs. The morphogen Wnt is sequestered by HS chains at the cell surface and becomes available for receptor activation only following enzyme-catalyzed release of specific sulfate groups from the HS chains [231].

In addition to the direct effect of HSPGs on growth factor signaling, the HSPG bound factors are protected from proteolytic degradation and can be released and activated under different physiological or pathological conditions like cancer [232]. Sequestration of chemokines and cytokines plays a critical role in regulating the shape of morphogen gradients and in inducing a signal for cell migration, a first step for invasion and metastasis [233, 234]. The major PGs are subclassified into three groups depending on their localization; intracellular PGs (serglycin), cell surface-associated PGs (syndecans, glypicans) and secreted PGs (hyalectans, small leucine-rich proteoglycans, perlecan) [235]. In this chapter, we focus on the stromal PGs the most characterized in the tumor context, shed syndecans and small leucine-rich proteins/proteoglycans (SLRPs) and summarize how their presence in tumor stroma influences cancer progression (Fig. 5.3).

Syndecans

Syndecans are transmembrane HSPGs with four members in vertebrates, syndecan-1 to -4. When present at the cell surface, they are formally not part of the tumor ECM, but since they can be shed into the ECM they are discussed in the context of TME, both for roles of unshed and shed forms. They are involved in diverse biological processes, such as regulating cell adhesion, cell migration and differentiation, as well

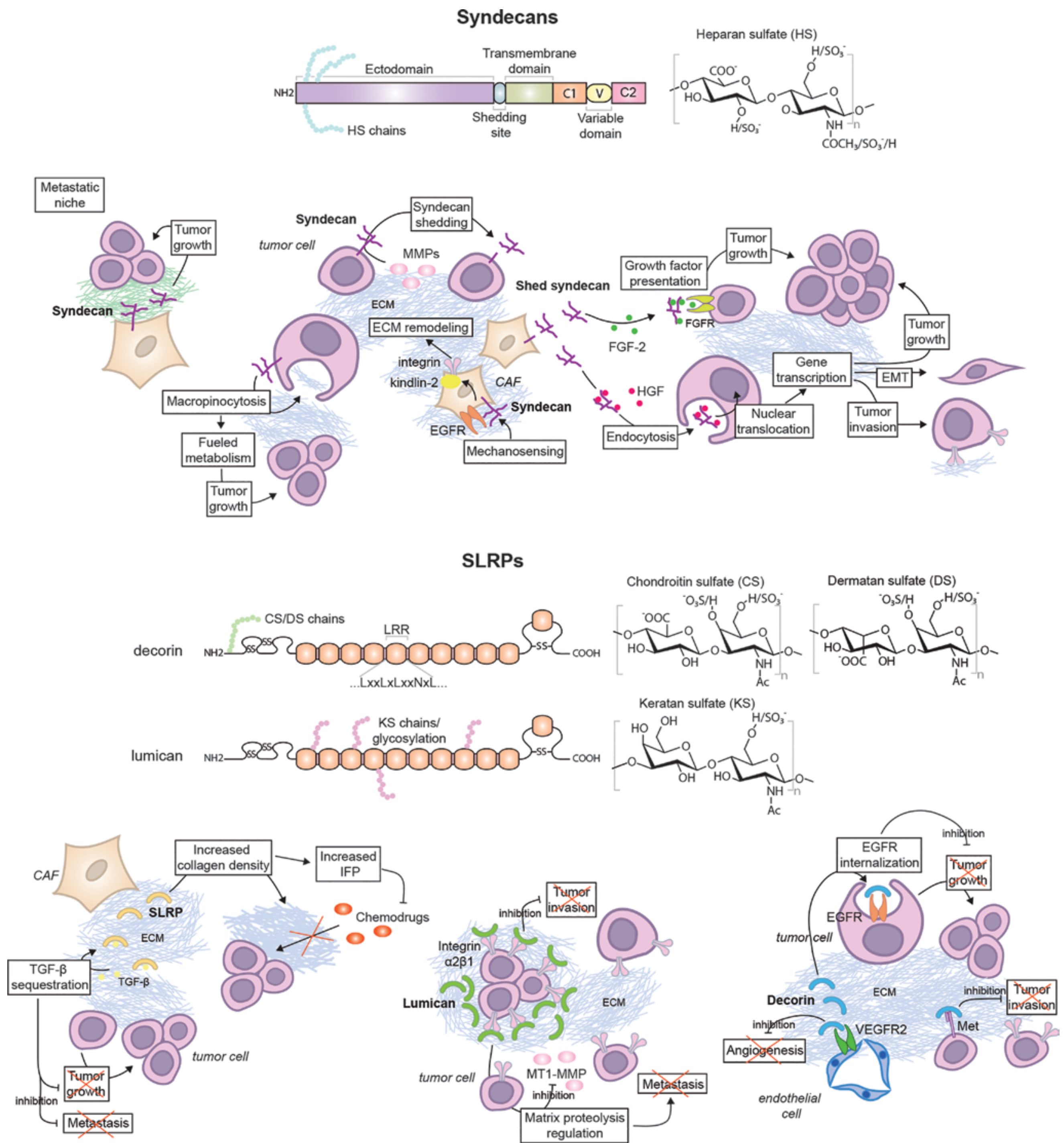

Fig. 5.3 Structure of stromal proteoglycans and their role in tumorigenesis. Syndecans is a family of four members that differ by the size of the ectodomain and the variable domain. All syndecans exhibit heparan sulfate (HS) chains, but only syndecan-1 and -3 have chondroitin sulfate (CS) chains in the ectodomain part close to the transmembrane domain. Syndecans at the cell surface can be shed by MMPs to induce its effect on cancer cells. Syndecans as co-factor for FGF receptor (FGFR) stimulate tumor growth by delivering FGF-2. Syndecan-1 could be endocytosed to deliver growth factors into the nucleus leading to increased gene transcription. Syndecan-1 can regulate macropinocytosis to fuel cancer cell growth. Syndecan-1 is also required in metastatic niches to support the metastatic colonization. Force on syndecan-4 induces activation of integrins involving kindlin-2 with the potential to reorganize the ECM. Small leucine-rich proteoglycans (SLRPs) are composed of leucine-rich repeats (LRR) that contain the LxxLxLxxNxL motif (L, leucine; N, asparagine; x, any amino acid). Decorin could exhibit one chain of chondroitin sulfate (CS) or dermatan sulfate (DS), whereas lumican could exhibit one to four keratan sulfate (KS) chains. HS, DS, CS, and KS chains are composed of repeats of disaccharide units that could be sulfated (SO_3^-) at different locations as indicated in the figure. As shown for fibromodulin, SLRPs could increase thickness of collagen fibers resulting in increased interstitial fluid pressure (IFP). Some SLRPs, such as asporin, have ability to bind and sequester TGF-β resulting in cancer growth and metastasis inhibition. Lumican has been shown to inhibit cancer cell migration by interaction through α2β1 integrin and by inhibiting MT1-MMP. Decorin has been shown to interact with tyrosine kinase receptors. Binding to EGF receptor (EGFR) leads to the receptor internalization and tumor growth inhibition. Binding to c-met inhibits cancer cell migration. Binding to VEGFR2 in endothelial cells inhibits angiogenesis. (For more details, please refer to the main text)

as participating in the organization of ECM and the cytoskeleton [236]. Syndecans can serve as co-receptors in various signaling pathways on the cell surface and also provide a link between the ECM and the cytoskeleton by directly interacting with the cytoskeleton or via other molecules [237]. In pancreatic cancer, localization of syndecan-1 at the cell surface of PDAC cells regulates macropinocytosis, which consists of uptaking proteins from the extracellular matrix to fuel cell metabolism, promoting tumor growth [238]. As described for tenascin-C and periostin (see above), stromal fibroblast-derived syndecan-1 is required in metastatic niche to promote metastases outgrowth. Syndecan-1-mediated lung metastases of breast carcinoma cells is a temperature-dependent process [239].

Syndecan-4 is ubiquitously expressed at low levels. Although integrins are the canonical mechanotransducing cell surface receptors, syndecans have also been regarded to take part in mechanosensing through their role as co-receptors for collagen- and fibronectin-binding integrins (Fig. 5.3) [240, 241]. Recent studies suggest that syndecan-4 rather than only being present with integrins in the same adhesion sites, also can generate signals in response to tension (at subcellular sites separate from integrin adhesions) that activates kindlin-2/β1 integrin/RhoA axis in a PI3K -dependent manner [242]. These new results have been obtained in an advanced experimental in vitro system using fibronectin- and collagen-coated magnetic beads. It will be interesting to see if the proposed model of syndecan-4 mechanosignaling activating integrins is also valid under more in vivo-like 3D conditions.

One interesting feature of syndecans is the shedding of the extracellular domain that enables syndecans to act as soluble factors [243], which plays an important role in tumorigenesis (Fig. 5.3). The shedding occurs next to the plasma membrane and is processed by different MMPs: MMP-7 is involved in syndecan-1 and -2 shedding, MMP-2 and -9 can cleave syndecan-1, -2 and -4, whereas MMP-14 can cleave syndecan-1 and -4 [244–246]. The shedding is regulated by different growth factors and cytokines present in the tumor microenvironment, such as FGF-2 and TNF-α [247, 248]. In addition, heparanase, an enzyme that cleaves the HS chains, regulates syndecan-1 expression and promotes syndecan-1 shedding, resulting in increased myeloma tumor growth [249].

In general, shed syndecans promote tumor progression and it was described earlier that highly soluble syndecan-1 was associated with poor outcome in non-small cell lung cancer [250]. This correlation was also observed in myeloma and bladder carcinoma [251, 252]. In breast carcinoma, shedding of syndecan-1 from CAFs stimulates tumor cell proliferation via FGF-2; shed syndecan-1 thus serving as a paracrine mediator [253, 254]. However, another study demonstrated an inhibitory effect of shed syndecan-1 on breast adenocarcinoma cell proliferation [255]. The study interestingly suggested the duality of membrane-bound and soluble syndecan-1. In a study by Nikolova et al., transmembrane syndecan-1 promoted cell proliferation and inhibited invasion, whereas shed syndecan-1 inhibited proliferation but increased invasiveness, suggesting that both syndecan forms contributed to breast cancer progression, but at different stages [255]. More recently, shed syndecan-2 has been shown to contribute to colorectal tumor growth and metastasis by up-regulating MMP-7, suggesting a positive regulatory loop between these two proteins [256].

Another study suggests that shed syndecan-1 translocates to the nucleus of tumor cells, indicating that syndecan-1 may deliver growth factors (e.g., HGF) to the nucleus, and also down-regulates histone acetylation, leading to increased gene transcription [252]. Nuclear translocation is believed to involve endocytosis of syndecan-1 growth factor complex from the cell surface and transport to the nucleus, but the exact mechanism of nuclear import has not been elucidated.

It has been reported that chemotherapeutic drugs, used in myeloma treatment, stimulate the shedding of syndecan-1, thus contributing to increased tumor growth [251]. Additionally, shed syndecan-1 contributes to chemotherapy resistance in colon cancer [257]. Targeting shed syndecans could be an effective strategy to control cancer progression; however better understanding of the molecular mechanisms of action is needed in order to avoid any potential adverse side effects.

Small Leucine-Rich Proteoglycans

SLRPs are extracellular matrix proteins rich in leucine-rich repeats, conferring a "banana" shape structure with a concave face involved in protein-protein interactions. Most SLRPs bind to fibrillar collagen and regulate collagen fibrillogenesis and matrix assembly [258]. Among the many biological processes regulated by SLRPs, tumor growth is one of the most well studied. The SLRP family encompasses 18 members, grouped into five classes (I–V) [235]. In this section, we will focus on the role of four SLRPs from classes I and II in tumor progression (Fig. 5.3).

Decorin is a chondroitin/dermatan sulfate SLRP that is expressed in several tissues. Although one study associated high expression of decorin with metastasis and poor survival in breast cancer [259], decorin is often described as having anti-tumor properties, as listed below. Decorin expression is down-regulated in bladder cancer [260], prostate cancer [261], lung cancer [262] and breast cancer [263, 264], where a reduced expression is associated with poor survival [265]. Consistent with these observations, liver carcinogenesis was promoted in decorin-null mice [266]. Moreover, overexpression of decorin was shown to inhibit metastasis of prostate cancer [267], inhibit proliferation of bladder tumor cells [260], and inhibit colorectal carcinoma cell growth and

migration [268]. Systemic injection of decorin in MDA-231 triple negative breast carcinoma xenografts induced expression of cellular adhesion molecules and promoted tumor suppressor genes, whereas inflammatory and immune response genes were down-regulated [269].

From a mechanistic point of view, decorin can affect tumor progression via its interaction with tyrosine kinase receptors. It has been demonstrated that decorin can bind to the EGF receptor and mediate internalization and degradation of the receptor and induce expression of $p21^{WAF}$, an inhibitor of the cell cycle and apoptosis [270]. Decorin can also antagonize Met, a receptor for hepatocyte growth factor, via degradation of β-catenin, leading to reduced cell migration and invasion [271]. The decorin/Met axis appears to be required for the induction of an oncostatic mitochondrial protein, mitostatin [272]. In addition, decorin has been shown to bind and antagonize VEGFR2, inhibiting angiogenesis through endothelial cell autophagy [273, 274] and to bind IGF-IR to inhibit tumor cell migration and invasion [275].

Based on these observations, decorin is considered as a promising therapeutic protein in cancer progression treatment [267]. However, similar to syndecan-1, decorin has also been reported to induce resistance to some chemotherapeutics [276, 277]. Moreover, P-cadherin expression induces decorin secretion that is required to realigned collagen fibers to promote collective cell migration in breast tumor [239].

Biglycan, like decorin, is a chondroitin/dermatan sulfate proteoglycan, which belongs to the class I of SLRPs. Available data indicates that high expression levels of biglycan correlate with poor prognosis in pancreatic adenocarcinoma and esophageal carcinoma [278, 279]. Moreover, biglycan was shown to promote migration and invasion of gastric carcinoma through FAK signaling activation [280]. However, biglycan also displays anti-tumor activity, inhibiting bladder carcinoma and pancreatic carcinoma cell proliferation [281, 282].

Lumican is expressed as keratan sulfate PG in the cornea, but exists as a glycoprotein substituted by non- or low-sulfated polylactosamine chains in other tissues [275]. In tumor tissues, lumican is often over-expressed by stromal cells and/or tumor cells, and the correlation of its expression to malignancy is complex [283, 284]. In advanced colorectal cancer, Seya et al. have shown that lumican expression in tumor cells is associated with poor survival [285], whereas de Wit et al. have described a correlation with good survival in stage II patients [286]. In breast cancer, lumican expression was found to decrease with the progression of disease [287]. Consistent with this observation, high expression of lumican is associated with good survival in invasive stages of breast cancer [265]. Lumican upregulates the expression of α2β1 integrin but decreases integrin signaling in the highly metastatic MDA-MB-231 breast cancer cell line, inhibiting migratory cell morphology [288]. In pancreatic cancer, patient outcome is dependent on the type of cells expressing lumican. Expression in tumor cells is associated with longer survival, whereas expression in pancreatic stromal cells is associated with poor outcome [289]. However, a recent study showed that lumican expression in pancreatic stroma was only correlated with good survival after surgery [290]. This correlation is also observed in lung adenocarcinoma patients, where patients with stromal lumican-positive tumors had longer survival than those expressing lumican in tumor cells [291]. We suggest that these differences could be related to the secretion of different glycosylated forms of lumican in different cellular contexts.

The anti-tumor properties of lumican have mainly been reported in melanoma, where lumican is expressed in the peritumoral stroma [292] and is suggested to serve as a biological barrier, controlling melanoma invasion. Lumican was shown to inhibit melanoma cell progression via interaction with α2β1 integrin and altering composition of focal adhesion complexes [293–295]. Lumican was defined as a new inhibitor of MT1-MMP in melanoma cells, thus inhibiting tumor environment proteolysis and invasiveness [296]. Anti-tumorigenic activities of lumican were also found in prostate cancer [297], in colon cancer by affecting tumor cell migration through up-regulation of gelsolin [298], and in pancreatic cancer, in which lumican reduced EGF receptor expression resulting in reduced Akt signaling and tumor cell growth inhibition [290].

Fibromodulin, like lumican, is a keratan sulfate SLRP that belongs to class II and is expressed in dense regular connective tissues. Although fibromodulin expression has been described in some types of cancer, its role has been poorly investigated. Oldberg et al. have shown that in experimental carcinomas, fibromodulin promotes the formation of a dense collagen matrix through the regulation of fibril diameter, leading to an increased interstitial fluid pressure (IFP), with possible adverse consequences for delivery of chemotherapeutics [299]. It is interesting to remember that other SLRPs also modulate collagen fibrillogenesis and could be thus involved in IFP regulation in different types of cancers, despite their anti-tumorigenic properties.

SLRPs also function to sequester TGF-β [300], a growth factor already described in this chapter, involved in EMT and fibroblast activation. A work by Maris et al. demonstrates that asporin, a member of the class I SLRPs, inhibits TGF-β activity resulting in reduced breast cancer growth and metastasis in NOD-SCID mice [301]. Interestingly, asporin expression is induced by TGF-β, thus asporin and TGF-β appear to regulate each other in an intricate feedback loop.

In summary, proteoglycans and matricellular proteins show different effects on tumorigenesis, sometimes with opposite effects in different tumor types. Table 5.1 summarizes the role of stromal proteins in tumorigenesis and the experiments we have mentioned in the text.

Table 5.1 Role of extracellular matrix proteins in the tumor microenvironment

ECM protein	Knockout phenotype mice	Potential ECM receptors in tumor stroma	Localization in tumors	Effects in tumor context
Collagen I	Embryonic lethal, severe structural defects in connective tissues [302–304]	α2β1, α11β1	Stroma	– Supports tumor growth [64–66] – Highway for metastasis [305] – Protective barrier role in pancreatic ductal adenocarcinoma [34]
Collagen III	Perinatal lethal [306]	α2β1, α11β1	Stroma	– Restricts tumor growth [71]
EDA Fibronectin	Normal, defective lymph vessels [130, 142]	α5β1 α4β1, α4β7 α9β1 TLR2/4	Stroma [130]	– No effect angiogenesis in Rip1-Tag2 model [130] – Reduces immune response in cancer models [161] – Stimulates breast cancer progression [164] – Direct collective cell migration of head and neck cancer cells [162] – Recruits latent TGF-β binding protein-1 to EDA FN fibrils [155]
Tenascin-C	Viable, subtle defects hair follicles [307, 308]	αvβ1, α4β1 [200], TLR4	Stroma	Minimal effect in pyMT model in TN-C$^{-/-}$ background, no effect on tumor growth, or metastasis [174] Stimulates metastasis in tumor models of melanoma, breast cancer and lung cancer 190, 193, 194] Role in metastatic niche formation in lung cancer [196] Role in shaping immunosuppressive TME in head and neck cancer models [198]
Periostin	Tooth eruption defect [309]	αv-integrins (αvβ1,αvβ3, αvβ5)	Stroma	– Breast cancer metastasis to lungs, concentrates Wnt in cancer stem cell niches [94, 220, 310, 311]
Decorin	Skin fragility [312]	Affect integrin expression indirectly via TGF-β pathway, TLR2/4 [313]	Stroma	– Inhibits tumor growth [314] – Affects inflammatory status of TME [315]
Lumican	Skin fragility, cornea opacity [316]	α2β1	Stroma, tumor cells	– Inhibits melanoma growth and invasion [295]
Syndecan-1	Normal	Cooperate with integrins on cell surface.	Tumor and stromal cells	– Shedding [254], increased angiogenesis [254, 317], affect tumor growth [318]
Syndecan-4	Normal	Co-receptor for certain β1 integrins	Stromal cells	– Co-receptor for integrins binding fibronectin and collagen [241, 319], cross-talks with integrins via intracellular signaling [242, 320]

Concluding Remarks/Summary

The tumor stroma is complex and dynamic during tumor growth and contains an ECM with changing composition. The exact function of the tumor stroma varies with the tumor type, the tumor stage, and it will be important to better elucidate the function of ECM molecules at different stages of tumor growth and metastasis. To determine if the tumor stroma acts as a fertile soil, providing a supportive ECM network rich in blood vessels, or if it acts as a stiff barrier, we have to consider additional components of the stroma. In this chapter, we have highlighted some aspects ascribed to the insoluble ECM of the stroma, but additional consideration of the integrated roles of the immune system, paracrine signaling and above all, intertumoral and intra-tumoral heterogeneity in tumor composition is necessary in order to fully address the central question: Tumor stroma—friend or foe? Barrier or support?

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References

1. Osterholm C, Lu N, Liden A, Karlsen TV, Gullberg D, Reed RK, et al. Fibroblast EXT1-levels influence tumor cell proliferation and migration in composite spheroids. PLoS One. 2012;7(7):e41334.
2. Lu N, Karlsen TV, Reed RK, Kusche-Gullberg M, Gullberg D. Fibroblast alpha11beta1 integrin regulates tensional homeostasis in fibroblast/A549 carcinoma heterospheroids. PLoS One. 2014;9(7):e103173.
3. Fatehullah A, Tan SH, Barker N. Organoids as an in vitro model of human development and disease. Nat Cell Biol. 2016;18(3):246–54.

4. Shi R, Radulovich N, Ng C, Liu N, Notsuda H, Cabanero M, et al. Organoid cultures as preclinical models of non-small cell lung cancer. Clin Cancer Res. 2020;26(5):1162–74.
5. Boj SF, Hwang CI, Baker LA, Chio II, Engle DD, Corbo V, et al. Organoid models of human and mouse ductal pancreatic cancer. Cell. 2015;160(1–2):324–38.
6. Sachs N, de Ligt J, Kopper O, Gogola E, Bounova G, Weeber F, et al. A living biobank of breast cancer organoids captures disease heterogeneity. Cell. 2018;172(1-2):373–86.e10.
7. Weaver VM. The microenvironment matters. Mol Biol Cell. 2014;25(21):3254–8.
8. Piersma B, Hayward MK, Weaver VM. Fibrosis and cancer: a strained relationship. Biochim Biophys Acta Rev Cancer. 2020;1873(2):188356.
9. Zeltz C, Gullberg D. Post-translational modifications of integrin ligands as pathogenic mechanisms in disease. Matrix Biol. 2014;40:5–9.
10. Levental KR, Yu H, Kass L, Lakins JN, Egeblad M, Erler JT, et al. Matrix crosslinking forces tumor progression by enhancing integrin signaling. Cell. 2009;139(5):891–906.
11. Azmi AS, Bao B, Sarkar FH. Exosomes in cancer development, metastasis, and drug resistance: a comprehensive review. Cancer Metastasis Rev. 2013;32(3-4):623–42.
12. Hoshino A, Costa-Silva B, Shen TL, Rodrigues G, Hashimoto A, Tesic Mark M, et al. Tumour exosome integrins determine organotropic metastasis. Nature. 2015;527(7578):329–35.
13. Östman A, Augsten M. Cancer-associated fibroblasts and tumor growth—bystanders turning into key players. Curr Opin Genet Dev. 2009;19(1):67–73.
14. Cirri P, Chiarugi P. Cancer associated fibroblasts: the dark side of the coin. Am J Cancer Res. 2011;1(4):482–97.
15. Ohlund D, Elyada E, Tuveson D. Fibroblast heterogeneity in the cancer wound. J Exp Med. 2014;211(8):1503–23.
16. Sahai E, Astsaturov I, Cukierman E, DeNardo DG, Egeblad M, Evans RM, et al. A framework for advancing our understanding of cancer-associated fibroblasts. Nat Rev Cancer. 2020;20(3):174–86.
17. Hu Y, Yan C, Mu L, Huang K, Li X, Tao D, et al. Fibroblast-Derived Exosomes Contribute to Chemoresistance through Priming Cancer Stem Cells in Colorectal Cancer. PLoS One. 2015;10(5):e0125625.
18. Eberlein C, Rooney C, Ross SJ, Farren M, Weir HM, Barry ST. E-Cadherin and EpCAM expression by NSCLC tumour cells associate with normal fibroblast activation through a pathway initiated by integrin alphavbeta6 and maintained through TGFbeta signalling. Oncogene. 2014;34(6):704–16.
19. Eberlein C, Kendrew J, McDaid K, Alfred A, Kang JS, Jacobs VN, et al. A human monoclonal antibody 264RAD targeting alphavbeta6 integrin reduces tumour growth and metastasis, and modulates key biomarkers in vivo. Oncogene. 2013;32(37):4406–16.
20. Klingberg F, Chow ML, Koehler A, Boo S, Buscemi L, Quinn TM, et al. Prestress in the extracellular matrix sensitizes latent TGF-beta1 for activation. J Cell Biol. 2014;207(2):283–97.
21. Hinz B. The extracellular matrix and transforming growth factor-beta1: tale of a strained relationship. Matrix Biol. 2015;47:54–65.
22. Henderson NC, Arnold TD, Katamura Y, Giacomini MM, Rodriguez JD, McCarty JH, et al. Targeting of alphav integrin identifies a core molecular pathway that regulates fibrosis in several organs. Nat Med. 2013;19(12):1617–24.
23. Reed NI, Jo H, Chen C, Tsujino K, Arnold TD, DeGrado WF, et al. The alphavbeta1 integrin plays a critical in vivo role in tissue fibrosis. Sci Transl Med. 2015;7(288):288ra79.
24. Lodyga M, Hinz B. TGF-beta1—a truly transforming growth factor in fibrosis and immunity. Semin Cell Dev Biol. 2020;101:123–39.
25. Jiang X, Wang J, Deng X, Xiong F, Ge J, Xiang B, et al. Role of the tumor microenvironment in PD-L1/PD-1-mediated tumor immune escape. Mol Cancer. 2019;18(1):10.
26. Stockis J, Lienart S, Colau D, Collignon A, Nishimura SL, Sheppard D, et al. Blocking immunosuppression by human Tregs in vivo with antibodies targeting integrin alphaVbeta8. Proc Natl Acad Sci U S A. 2017;114(47):E10161–E8.
27. Takasaka N, Seed RI, Cormier A, Bondesson AJ, Lou J, Elattma A, et al. Integrin alphavbeta8-expressing tumor cells evade host immunity by regulating TGF-beta activation in immune cells. JCI Insight. 2018;3(20):e122591.
28. Campbell MG, Cormier A, Ito S, Seed RI, Bondesson AJ, Lou J, et al. Cryo-EM reveals integrin-mediated TGF-beta activation without release from latent TGF-beta. Cell. 2020;180(3):490–501.e16.
29. Jin S, Lee WC, Aust D, Pilarsky C, Cordes N. beta8 integrin mediates pancreatic cancer cell radiochemoresistance. Mol Cancer Res. 2019;17(10):2126–38.
30. Gaggioli C, Hooper S, Hidalgo-Carcedo C, Grosse R, Marshall JF, Harrington K, et al. Fibroblast-led collective invasion of carcinoma cells with differing roles for RhoGTPases in leading and following cells. Nat Cell Biol. 2007;9(12):1392–400.
31. Sanz-Moreno V, Gaggioli C, Yeo M, Albrengues J, Wallberg F, Viros A, et al. ROCK and JAK1 signaling cooperate to control actomyosin contractility in tumor cells and stroma. Cancer Cell. 2011;20(2):229–45.
32. Ozdemir BC, Pentcheva-Hoang T, Carstens JL, Zheng X, Wu CC, Simpson TR, et al. Depletion of carcinoma-associated fibroblasts and fibrosis induces immunosuppression and accelerates pancreas cancer with reduced survival. Cancer Cell. 2014;25:719–34.
33. Rhim AD, Oberstein PE, Thomas DH, Mirek ET, Palermo CF, Sastra SA, et al. Stromal elements act to restrain, rather than support, pancreatic ductal adenocarcinoma. Cancer Cell. 2014;25(6):735–47.
34. Jiang H, Torphy RJ, Steiger K, Hongo H, Ritchie AJ, Kriegsmann M, et al. Pancreatic ductal adenocarcinoma progression is restrained by stromal matrix. J Clin Invest. 2020;130(9):4704–9.
35. Zeltz C, Primac I, Erusappan P, Alam J, Noel A, Gullberg D. Cancer-associated fibroblasts in desmoplastic tumors: emerging role of integrins. Semin Cancer Biol. 2020;62:166–81.
36. Rubashkin MG, Ou G, Weaver VM. Deconstructing signaling in three dimensions. Biochemistry. 2014;53(13):2078–90.
37. Ricard-Blum S. The collagen family. Cold Spring Harb Perspect Biol. 2011;3(1):a004978.
38. Egeblad M, Rasch MG, Weaver VM. Dynamic interplay between the collagen scaffold and tumor evolution. Curr Opin Cell Biol. 2010;22(5):697–706.
39. Dvorak HF. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. N Engl J Med. 1986;315(26):1650–9.
40. Dvorak HF. Tumors: wounds that do not heal-redux. Cancer Immunol Res. 2015;3(1):1–11.
41. Merkel JR, DiPaolo BR, Hallock GG, Rice DC. Type I and type III collagen content of healing wounds in fetal and adult rats. Proc Soc Exp Biol Med. 1988;187(4):493–7.
42. Barczyk M, Carracedo S, Gullberg D. Integrins. Cell Tissue Res. 2010;339(1):269–80.
43. Zeltz C, Gullberg D. The integrin-collagen connection—a glue for tissue repair? J Cell Sci. 2016;129(4):653–64.
44. Zeltz C, Orgel J, Gullberg D. Molecular composition and function of integrin-based collagen glues-introducing COLINBRIs. Biochim Biophys Acta. 2014;1840(8):2533–48.
45. Staudinger LA, Spano SJ, Lee W, Coelho N, Rajshankar D, Bendeck MP, et al. Interactions between the discoidin domain receptor 1 and beta1 integrin regulate attachment to collagen. Biol Open. 2013;2(11):1148–59.
46. Xu H, Bihan D, Chang F, Huang PH, Farndale RW, Leitinger B. Discoidin domain receptors promote alpha1beta1- and alpha2beta1-integrin mediated cell adhesion to collagen by enhancing integrin activation. PLoS One. 2012;7(12):e52209.

47. Abbonante V, Gruppi C, Rubel D, Gross O, Moratti R, Balduini A. Discoidin domain receptor 1 protein is a novel modulator of megakaryocyte-collagen interactions. J Biol Chem. 2013;288(23):16738–46.
48. DuFort CC, Paszek MJ, Weaver VM. Balancing forces: architectural control of mechanotransduction. Nat Rev Mol Cell Biol. 2011;12(5):308–19.
49. Gilkes DM, Semenza GL, Wirtz D. Hypoxia and the extracellular matrix: drivers of tumour metastasis. Nat Rev Cancer. 2014;14(6):430–9.
50. Malik R, Lelkes PI, Cukierman E. Biomechanical and biochemical remodeling of stromal extracellular matrix in cancer. Trends Biotechnol. 2015;33(4):230–6.
51. Cox TR, Erler JT. Molecular pathways: connecting fibrosis and solid tumor metastasis. Clin Cancer Res. 2014;20(14):3637–43.
52. Miller BW, Morton JP, Pinese M, Saturno G, Jamieson NB, McGhee E, et al. Targeting the LOX/hypoxia axis reverses many of the features that make pancreatic cancer deadly: inhibition of LOX abrogates metastasis and enhances drug efficacy. EMBO Mol Med. 2015;7(8):1063–76.
53. Kalluri R, Zeisberg M. Fibroblasts in cancer. Nat Rev Cancer. 2006;6(5):392–401.
54. Cooke ME, Sakai T, Mosher DF. Contraction of collagen matrices mediated by a2b1A and avb3 integrins. J Cell Sci. 2000;113(Pt 13):2375–83.
55. Schulz JN, Zeltz C, Sorensen IW, Barczyk M, Carracedo S, Hallinger R, et al. Reduced granulation tissue and wound strength in the absence of alpha11beta1 integrin. J Invest Dermatol. 2015;135(5):1435–44.
56. Gullberg D, Tingstrom A, Thuresson AC, Olsson L, Terracio L, Borg TK, et al. b1 integrin-mediated collagen gel contraction is stimulated by PDGF. Exp Cell Res. 1990;186(2):264–72.
57. Jokinen J, Dadu E, Nykvist P, Kapyla J, White DJ, Ivaska J, et al. Integrin-mediated cell adhesion to type I collagen fibrils. J Biol Chem. 2004;279(30):31956–63.
58. Shen B, Vardy K, Hughes P, Tasdogan A, Zhao Z, Yue R, et al. Integrin alpha11 is an Osteolectin receptor and is required for the maintenance of adult skeletal bone mass. elife. 2019;8:e42274.
59. Velling T, Kusche-Gullberg M, Sejersen T, Gullberg D. cDNA cloning and chromosomal localization of human alpha(11) integrin. A collagen-binding, I domain-containing, beta(1)-associated integrin alpha-chain present in muscle tissues. J Biol Chem. 1999;274(36):25735–42.
60. Popova SN, Rodriguez-Sanchez B, Liden A, Betsholtz C, Van Den Bos T, Gullberg D. The mesenchymal alpha11beta1 integrin attenuates PDGF-BB-stimulated chemotaxis of embryonic fibroblasts on collagens. Dev Biol. 2004;270(2):427–42.
61. Popova SN, Barczyk M, Tiger CF, Beertsen W, Zigrino P, Aszodi A, et al. Alpha11 beta1 integrin-dependent regulation of periodontal ligament function in the erupting mouse incisor. Mol Cell Biol. 2007;27(12):4306–16.
62. Popov C, Radic T, Haasters F, Prall WC, Aszodi A, Gullberg D, et al. Integrins alpha2beta1 and alpha11beta1 regulate the survival of mesenchymal stem cells on collagen I. Cell Death Dis. 2011;2:e186.
63. Zeltz C, Alam J, Liu H, Erusappan PM, Hoschuetzky H, Molven A, et al. α11β1 integrin is induced in a subset of cancer-associated fibroblasts in desmoplastic tumor stroma and mediates in vitro cell migration. Cancers. 2019;11:765. https://doi.org/10.3390/cancers11060765.
64. Primac I, Maquoi E, Blacher S, Heljasvaara R, Van Deun J, Smeland HY, et al. Stromal integrin alpha11 regulates PDGFR-beta signaling and promotes breast cancer progression. J Clin Invest. 2019;130:4609–28.
65. Zhu CQ, Popova SN, Brown ER, Barsyte-Lovejoy D, Navab R, Shih W, et al. Integrin alpha 11 regulates IGF2 expression in fibroblasts to enhance tumorigenicity of human non-small-cell lung cancer cells. Proc Natl Acad Sci U S A. 2007;104(28):11754–9.
66. Navab R, Strumpf D, To C, Pasko E, Kim KS, Park CJ, et al. Integrin alpha11beta1 regulates cancer stromal stiffness and promotes tumorigenicity and metastasis in non-small cell lung cancer. Oncogene. 2016;35(15):1899–908.
67. Smeland HY, Askeland C, Wik E, Knutsvik G, Molven A, Edelmann RJ, et al. Integrin alpha11beta1 is expressed in breast cancer stroma and associates with aggressive tumor phenotypes. J Pathol Clin Res. 2020;6(1):69–82.
68. Barczyk MM, Lu N, Popova SN, Bolstad AI, Gullberg D. alpha11beta1 integrin-mediated MMP-13-dependent collagen lattice contraction by fibroblasts: evidence for integrin-coordinated collagen proteolysis. J Cell Physiol. 2013;228:1108–19.
69. Ravanti L, Heino J, Lopez-Otin C, Kahari VM. Induction of collagenase-3 (MMP-13) expression in human skin fibroblasts by three-dimensional collagen is mediated by p38 mitogen-activated protein kinase. J Biol Chem. 1999;274(4):2446–55.
70. Provenzano PP, Eliceiri KW, Keely PJ. Shining new light on 3D cell motility and the metastatic process. Trends Cell Biol. 2009;19(11):638–48.
71. Brisson BK, Mauldin EA, Lei W, Vogel LK, Power AM, Lo A, et al. Type III collagen directs stromal organization and limits metastasis in a murine model of breast cancer. Am J Pathol. 2015;185(5):1471–86.
72. Radisky D, Muschler J, Bissell MJ. Order and disorder: the role of extracellular matrix in epithelial cancer. Cancer Investig. 2002;20(1):139–53.
73. Nistico P, Bissell MJ, Radisky DC. Epithelial-mesenchymal transition: general principles and pathological relevance with special emphasis on the role of matrix metalloproteinases. Cold Spring Harb Perspect Biol. 2012;4(2):a011908.
74. Smith BN, Bhowmick NA. Role of EMT in metastasis and therapy resistance. J Clin Med. 2016;5(2):17.
75. Provenzano PP, Inman DR, Eliceiri KW, Knittel JG, Yan L, Rueden CT, et al. Collagen density promotes mammary tumor initiation and progression. BMC Med. 2008;6:11.
76. Xiong G, Deng L, Zhu J, Rychahou PG, Xu R. Prolyl-4-hydroxylase alpha subunit 2 promotes breast cancer progression and metastasis by regulating collagen deposition. BMC Cancer. 2014;14:1.
77. Chen Y, Terajima M, Yang Y, Sun L, Ahn YH, Pankova D, et al. Lysyl hydroxylase 2 induces a collagen cross-link switch in tumor stroma. J Clin Invest. 2015;125(3):1147–62.
78. Montgomery AM, Reisfeld RA, Cheresh DA. Integrin alpha v beta 3 rescues melanoma cells from apoptosis in three-dimensional dermal collagen. Proc Natl Acad Sci U S A. 1994;91(19):8856–60.
79. Maquoi E, Assent D, Detilleux J, Pequeux C, Foidart JM, Noël A. MT1-MMP protects breast carcinoma cells against type I collagen-induced apoptosis. Oncogene. 2011;31(4):480–93.
80. Assent D, Bourgot I, Hennuy B, Geurts P, Noel A, Foidart JM, et al. A membrane-type-1 matrix metalloproteinase (MT1-MMP)-discoidin domain receptor 1 axis regulates collagen-induced apoptosis in breast cancer cells. PLoS One. 2015;10(3):e0116006.
81. Nielsen BS, Egeblad M, Rank F, Askautrud HA, Pennington CJ, Pedersen TX, et al. Matrix metalloproteinase 13 is induced in fibroblasts in polyomavirus middle T antigen-driven mammary carcinoma without influencing tumor progression. PLoS One. 2008;3(8):e2959.
82. Perry SW, Schueckler JM, Burke K, Arcuri GL, Brown EB. Stromal matrix metalloprotease-13 knockout alters Collagen I structure at the tumor-host interface and increases lung metas-

tasis of C57BL/6 syngeneic E0771 mammary tumor cells. BMC Cancer. 2013;13:411.
83. Krane SM, Byrne MH, Lemaitre V, Henriet P, Jeffrey JJ, Witter JP, et al. Different collagenase gene products have different roles in degradation of type I collagen. J Biol Chem. 1996;271(45):28509–15.
84. Romanic AM, Adachi E, Kadler KE, Hojima Y, Prockop DJ. Copolymerization of pNcollagen III and collagen I. pNcollagen III decreases the rate of incorporation of collagen I into fibrils, the amount of collagen I incorporated, and the diameter of the fibrils formed. J Biol Chem. 1991;266(19):12703–9.
85. LeBert DC, Squirrell JM, Rindy J, Broadbridge E, Lui Y, Zakrzewska A, et al. Matrix metalloproteinase 9 modulates collagen matrices and wound repair. Development. 2015;142(12):2136–46.
86. Herchenhan A, Uhlenbrock F, Eliasson P, Weis M, Eyre D, Kadler KE, et al. Lysyl oxidase activity is required for ordered collagen fibrillogenesis by tendon cells. J Biol Chem. 2015;290(26):16440–50.
87. Sabeh F, Shimizu-Hirota R, Weiss SJ. Protease-dependent versus -independent cancer cell invasion programs: three-dimensional amoeboid movement revisited. J Cell Biol. 2009;185(1):11–9.
88. Wolf K, Te Lindert M, Krause M, Alexander S, Te Riet J, Willis AL, et al. Physical limits of cell migration: control by ECM space and nuclear deformation and tuning by proteolysis and traction force. J Cell Biol. 2013;201(7):1069–84.
89. Kaur A, Ecker BL, Douglass SM, Kugel CH 3rd, Webster MR, Almeida FV, et al. Remodeling of the collagen matrix in aging skin promotes melanoma metastasis and affects immune cell motility. Cancer Discov. 2019;9(1):64–81.
90. Nishioka T, Eustace A, West C. Lysyl oxidase: from basic science to future cancer treatment. Cell Struct Funct. 2012;37(1):75–80.
91. Cox TR, Erler JT. Remodeling and homeostasis of the extracellular matrix: implications for fibrotic diseases and cancer. Dis Model Mech. 2011;4(2):165–78.
92. Cox TR, Rumney RM, Schoof EM, Perryman L, Hoye AM, Agrawal A, et al. The hypoxic cancer secretome induces pre-metastatic bone lesions through lysyl oxidase. Nature. 2015;522(7554):106–10.
93. Oskarsson T, Acharyya S, Zhang XH, Vanharanta S, Tavazoie SF, Morris PG, et al. Breast cancer cells produce tenascin C as a metastatic niche component to colonize the lungs. Nat Med. 2011;17(7):867–74.
94. Oskarsson T, Massague J. Extracellular matrix players in metastatic niches. EMBO J. 2012;31(2):254–6.
95. Kaplan RN, Riba RD, Zacharoulis S, Bramley AH, Vincent L, Costa C, et al. VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. Nature. 2005;438(7069):820–7.
96. Wilgus ML, Borczuk AC, Stoopler M, Ginsburg M, Gorenstein L, Sonett JR, et al. Lysyl oxidase: a lung adenocarcinoma biomarker of invasion and survival. Cancer. 2011;117(10):2186–91.
97. Zeltz C, Pasko E, Cox TR, Navab R, Tsao MS. LOXL1 Is regulated by integrin alpha11 and promotes non-small cell lung cancer tumorigenicity. Cancers (Basel). 2019;11(5):705.
98. Erler JT, Bennewith KL, Nicolau M, Dornhofer N, Kong C, Le QT, et al. Lysyl oxidase is essential for hypoxia-induced metastasis. Nature. 2006;440(7088):1222–6.
99. Pupa SM, Menard S, Forti S, Tagliabue E. New insights into the role of extracellular matrix during tumor onset and progression. J Cell Physiol. 2002;192(3):259–67.
100. Theret N, Musso O, Turlin B, Lotrian D, Bioulac-Sage P, Campion JP, et al. Increased extracellular matrix remodeling is associated with tumor progression in human hepatocellular carcinomas. Hepatology. 2001;34(1):82–8.
101. Ronnov-Jessen L, Petersen OW, Bissell MJ. Cellular changes involved in conversion of normal to malignant breast: importance of the stromal reaction. Physiol Rev. 1996;76(1):69–125.
102. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell. 2011;144(5):646–74.
103. Leitinger B, Hohenester E. Mammalian collagen receptors. Matrix Biol. 2007;26(3):146–55.
104. Chen X, Nadiarynkh O, Plotnikov S, Campagnola PJ. Second harmonic generation microscopy for quantitative analysis of collagen fibrillar structure. Nat Protoc. 2012;7(4):654–69.
105. Tuer A, Tokarz D, Prent N, Cisek R, Alami J, Dumont DJ, et al. Nonlinear multicontrast microscopy of hematoxylin-and-eosin-stained histological sections. J Biomed Opt. 2010;15(2):026018.
106. Tuer AE, Akens MK, Krouglov S, Sandkuijl D, Wilson BC, Whyne CM, et al. Hierarchical model of fibrillar collagen organization for interpreting the second-order susceptibility tensors in biological tissue. Biophys J. 2012;103(10):2093–105.
107. Tuer AE, Krouglov S, Prent N, Cisek R, Sandkuijl D, Yasufuku K, et al. Nonlinear optical properties of type I collagen fibers studied by polarization dependent second harmonic generation microscopy. J Phys Chem B. 2011;115(44):12759–69.
108. Amat-Roldan I, Psilodimitrakopoulos S, Loza-Alvarez P, Artigas D. Fast image analysis in polarization SHG microscopy. Opt Express. 2010;18(16):17209–19.
109. Golaraei A, Cisek R, Krouglov S, Navab R, Niu C, Sakashita S, et al. Characterization of collagen in non-small cell lung carcinoma with second harmonic polarization microscopy. Biomed Opt Express. 2014;5(10):3562–7.
110. Strupler M, Pena AM, Hernest M, Tharaux PL, Martin JL, Beaurepaire E, et al. Second harmonic imaging and scoring of collagen in fibrotic tissues. Opt Express. 2007;15(7):4054–65.
111. Rezakhaniha R, Agianniotis A, Schrauwen JT, Griffa A, Sage D, Bouten CV, et al. Experimental investigation of collagen waviness and orientation in the arterial adventitia using confocal laser scanning microscopy. Biomech Model Mechanobiol. 2012;11(3-4):461–73.
112. Zhang J, Wang YL, Gu L, Pan J. Atomic force microscopy of actin. Sheng Wu Hua Xue Yu Sheng Wu Wu Li Xue Bao (Shanghai). 2003;35(6):489–94.
113. Paige MF, Rainey JK, Goh MC. A study of fibrous long spacing collagen ultrastructure and assembly by atomic force microscopy. Micron. 2001;32(3):341–53.
114. Glatzel T, Holscher H, Schimmel T, Baykara MZ, Schwarz UD, Garcia R. Advanced atomic force microscopy techniques. Beilstein J Nanotechnol. 2012;3:893–4.
115. Strasser S, Zink A, Janko M, Heckl WM, Thalhammer S. Structural investigations on native collagen type I fibrils using AFM. Biochem Biophys Res Commun. 2007;354(1):27–32.
116. Lopez JI, Kang I, You WK, McDonald DM, Weaver VM. In situ force mapping of mammary gland transformation. Integr Biol (Camb). 2011;3(9):910–21.
117. Braet F, Vermijlen D, Bossuyt V, De Zanger R, Wisse E. Early detection of cytotoxic events between hepatic natural killer cells and colon carcinoma cells as probed with the atomic force microscope. Ultramicroscopy. 2001;89(4):265–73.
118. Akhtar R, Schwarzer N, Sherratt MJ, Watson RE, Graham HK, Trafford AW, et al. Nanoindentation of histological specimens: mapping the elastic properties of soft tissues. J Mater Res. 2009;24(3):638–46.
119. Gueta R, Barlam D, Shneck RZ, Rousso I. Measurement of the mechanical properties of isolated tectorial membrane using atomic force microscopy. Proc Natl Acad Sci U S A. 2006;103(40):14790–5.
120. Barbone PE, Bamber JC. Quantitative elasticity imaging: what can and cannot be inferred from strain images. Phys Med Biol. 2002;47(12):2147–64.

121. Jiang T, Olson ES, Nguyen QT, Roy M, Jennings PA, Tsien RY. Tumor imaging by means of proteolytic activation of cell-penetrating peptides. Proc Natl Acad Sci U S A. 2004;101(51):17867–72.
122. Scherer RL, VanSaun MN, McIntyre JO, Matrisian LM. Optical imaging of matrix metalloproteinase-7 activity in vivo using a proteolytic nanobeacon. Mol Imaging. 2008;7(3):118–31.
123. Littlepage LE, Sternlicht MD, Rougier N, Phillips J, Gallo E, Yu Y, et al. Matrix metalloproteinases contribute distinct roles in neuroendocrine prostate carcinogenesis, metastasis, and angiogenesis progression. Cancer Res. 2010;70(6):2224–34.
124. Low AF, Tearney GJ, Bouma BE, Jang IK. Technology Insight: optical coherence tomography—current status and future development. Nat Clin Pract Cardiovasc Med. 2006;3(3):154–62. quiz 72
125. Spuentrup E, Buecker A, Katoh M, Wiethoff AJ, Parsons EC Jr, Botnar RM, et al. Molecular magnetic resonance imaging of coronary thrombosis and pulmonary emboli with a novel fibrin-targeted contrast agent. Circulation. 2005;111(11):1377–82.
126. Stracke CP, Katoh M, Wiethoff AJ, Parsons EC, Spangenberg P, Spuntrup E. Molecular MRI of cerebral venous sinus thrombosis using a new fibrin-specific MR contrast agent. Stroke. 2007;38(5):1476–81.
127. Miserus RJ, Herias MV, Prinzen L, Lobbes MB, Van Suylen RJ, Dirksen A, et al. Molecular MRI of early thrombus formation using a bimodal alpha2-antiplasmin-based contrast agent. JACC Cardiovasc Imaging. 2009;2(8):987–96.
128. Hynes R. Molecular biology of fibronectin. Annu Rev Cell Biol. 1985;1:67–90.
129. White ES, Baralle FE, Muro AF. New insights into form and function of fibronectin splice variants. J Pathol. 2008;216(1):1–14.
130. Astrof S, Crowley D, George EL, Fukuda T, Sekiguchi K, Hanahan D, et al. Direct test of potential roles of EIIIA and EIIIB alternatively spliced segments of fibronectin in physiological and tumor angiogenesis. Mol Cell Biol. 2004;24(19):8662–70.
131. Singh P, Reimer CL, Peters JH, Stepp MA, Hynes RO, Van De Water L. The spatial and temporal expression patterns of integrin alpha9beta1 and one of its ligands, the EIIIA segment of fibronectin, in cutaneous wound healing. J Invest Dermatol. 2004;123(6):1176–81.
132. Bhattacharyya S, Tamaki Z, Wang W, Hinchcliff M, Hoover P, Getsios S, et al. FibronectinEDA promotes chronic cutaneous fibrosis through Toll-like receptor signaling. Sci Transl Med. 2014;6(232):232ra50.
133. Rybinski B, Franco-Barraza J, Cukierman E. The wound healing, chronic fibrosis, and cancer progression triad. Physiol Genomics. 2014;46(7):223–44.
134. Rybak JN, Roesli C, Kaspar M, Villa A, Neri D. The extra-domain A of fibronectin is a vascular marker of solid tumors and metastases. Cancer Res. 2007;67(22):10948–57.
135. Matsumoto E, Yoshida T, Kawarada Y, Sakakura T. Expression of fibronectin isoforms in human breast tissue: production of extra domain A+/extra domain B+ by cancer cells and extra domain A+ by stromal cells. Jpn J Cancer Res. 1999;90(3):320–5.
136. Pujuguet P, Hammann A, Moutet M, Samuel JL, Martin F, Martin M. Expression of fibronectin ED-A+ and ED-B+ isoforms by human and experimental colorectal cancer. Contribution of cancer cells and tumor-associated myofibroblasts. Am J Pathol. 1996;148(2):579–92.
137. Manabe R, Ohe N, Maeda T, Fukuda T, Sekiguchi K. Modulation of cell-adhesive activity of fibronectin by the alternatively spliced EDA segment. J Cell Biol. 1997;139(1):295–307.
138. Shinde AV, Bystroff C, Wang C, Vogelezang MG, Vincent PA, Hynes RO, et al. Identification of the peptide sequences within the EIIIA (EDA) segment of fibronectin that mediate integrin alpha9beta1-dependent cellular activities. J Biol Chem. 2008;283(5):2858–70.
139. Kohan M, Muro AF, White ES, Berkman N. EDA-containing cellular fibronectin induces fibroblast differentiation through binding to alpha4beta7 integrin receptor and MAPK/Erk 1/2-dependent signaling. FASEB J. 2010;24(11):4503–12.
140. Okamura Y, Watari M, Jerud ES, Young DW, Ishizaka ST, Rose J, et al. The extra domain A of fibronectin activates Toll-like receptor 4. J Biol Chem. 2001;276(13):10229–33.
141. Kelsh RM, McKeown-Longo PJ, Clark RA. EDA fibronectin in keloids create a vicious cycle of fibrotic tumor formation. J Invest Dermatol. 2015;135(7):1714–8.
142. Bazigou E, Xie S, Chen C, Weston A, Miura N, Sorokin L, et al. Integrin-alpha9 is required for fibronectin matrix assembly during lymphatic valve morphogenesis. Dev Cell. 2009;17(2):175–86.
143. Fukuda T, Yoshida N, Kataoka Y, Manabe R, Mizuno-Horikawa Y, Sato M, et al. Mice lacking the EDB segment of fibronectin develop normally but exhibit reduced cell growth and fibronectin matrix assembly in vitro. Cancer Res. 2002;62(19):5603–10.
144. Muro AF, Chauhan AK, Gajovic S, Iaconcig A, Porro F, Stanta G, et al. Regulated splicing of the fibronectin EDA exon is essential for proper skin wound healing and normal lifespan. J Cell Biol. 2003;162(1):149–60.
145. Astrof S, Crowley D, Hynes RO. Multiple cardiovascular defects caused by the absence of alternatively spliced segments of fibronectin. Dev Biol. 2007;311(1):11–24.
146. Danussi C, Del Bel BL, Pivetta E, Modica TM, Muro A, Wassermann B, et al. EMILIN1/alpha9beta1 integrin interaction is crucial in lymphatic valve formation and maintenance. Mol Cell Biol. 2013;33(22):4381–94.
147. Serini G, Bochaton-Piallat ML, Ropraz P, Geinoz A, Borsi L, Zardi L, et al. The fibronectin domain ED-A is crucial for myofibroblastic phenotype induction by transforming growth factor-beta1. J Cell Biol. 1998;142(3):873–81.
148. Shinde AV, Kelsh R, Peters JH, Sekiguchi K, Van De Water L, PJ MK-L. The alpha4beta1 integrin and the EDA domain of fibronectin regulate a profibrotic phenotype in dermal fibroblasts. Matrix Biol. 2015;41:26–35.
149. Singh P, Chen C, Pal-Ghosh S, Stepp MA, Sheppard D, Van De Water L. Loss of integrin alpha9beta1 results in defects in proliferation, causing poor re-epithelialization during cutaneous wound healing. J Invest Dermatol. 2009;129(1):217–28.
150. Nakayama Y, Kon S, Kurotaki D, Morimoto J, Matsui Y, Uede T. Blockade of interaction of alpha9 integrin with its ligands hinders the formation of granulation in cutaneous wound healing. Lab Investig. 2010;90(6):881–94.
151. Muro AF, Moretti FA, Moore BB, Yan M, Atrasz RG, Wilke CA, et al. An essential role for fibronectin extra type III domain A in pulmonary fibrosis. Am J Respir Crit Care Med. 2008;177(6):638–45.
152. Arslan F, Smeets MB, Riem Vis PW, Karper JC, Quax PH, Bongartz LG, et al. Lack of fibronectin-EDA promotes survival and prevents adverse remodeling and heart function deterioration after myocardial infarction. Circ Res. 2011;108(5):582–92.
153. Kelsh-Lasher RM, Ambesi A, Bertram C, McKeown-Longo PJ. Integrin alpha4beta1 and TLR4 Cooperate to Induce Fibrotic Gene Expression in Response to Fibronectin's EDA Domain. J Invest Dermatol. 2017;137(12):2505–12.
154. Jain M, Dhanesha N, Doddapattar P, Chorawala MR, Nayak MK, Cornelissen A, et al. Smooth muscle cell-specific fibronectin-EDA mediates phenotypic switching and neointimal hyperplasia. J Clin Invest. 2020;130(1):295–314.
155. Klingberg F, Chau G, Walraven M, Boo S, Koehler A, Chow ML, et al. The fibronectin ED-A domain enhances recruitment of latent TGF-beta-binding protein-1 to the fibroblast matrix. J Cell Sci. 2018;131(5):jcs201293.
156. Liao YF, Gotwals PJ, Koteliansky VE, Sheppard D, Van De Water L. The EIIIA segment of fibronectin is a ligand for integrins

alpha 9beta 1 and alpha 4beta 1 providing a novel mechanism for regulating cell adhesion by alternative splicing. J Biol Chem. 2002;277(17):14467–74.
157. Erdogan B, Ao M, White LM, Means AL, Brewer BM, Yang L, et al. Cancer-associated fibroblasts promote directional cancer cell migration by aligning fibronectin. J Cell Biol. 2017;216(11):3799–816.
158. Attieh Y, Clark AG, Grass C, Richon S, Pocard M, Mariani P, et al. Cancer-associated fibroblasts lead tumor invasion through integrin-beta3-dependent fibronectin assembly. J Cell Biol. 2017;216(11):3509–20.
159. Franco-Barraza J, Francescone R, Luong T, Shah N, Madhani R, Cukierman G, et al. Matrix-regulated integrin alphavbeta5 maintains alpha5beta1-dependent desmoplastic traits prognostic of neoplastic recurrence. elife. 2017;6:e20600.
160. Lugano R, Vemuri K, Yu D, Bergqvist M, Smits A, Essand M, et al. CD93 promotes beta1 integrin activation and fibronectin fibrillogenesis during tumor angiogenesis. J Clin Invest. 2018;128(8):3280–97.
161. Rossnagl S, Altrock E, Sens C, Kraft S, Rau K, Milsom MD, et al. EDA-fibronectin originating from osteoblasts inhibits the immune response against cancer. PLoS Biol. 2016;14(9):e1002562.
162. Gopal S, Veracini L, Grall D, Butori C, Schaub S, Audebert S, et al. Fibronectin-guided migration of carcinoma collectives. Nat Commun. 2017;8:14105.
163. Berger AJ, Renner CM, Hale I, Yang X, Ponik SM, Weisman PS, et al. Scaffold stiffness influences breast cancer cell invasion via EGFR-linked Mena upregulation and matrix remodeling. Matrix Biol. 2020;85–86:80–93.
164. Kwon A, Chae IH, You E, Kim SH, Ahn SY, Lee OJ, et al. Extra domain A-containing fibronectin expression in Spin90-deficient fibroblasts mediates cancer-stroma interaction and promotes breast cancer progression. J Cell Physiol. 2020;235(5):4494–507.
165. Ou J, Deng J, Wei X, Xie G, Zhou R, Yu L, et al. Fibronectin extra domain A (EDA) sustains CD133(+)/CD44(+) subpopulation of colorectal cancer cells. Stem Cell Res. 2013;11(2):820–33.
166. Xiang L, Xie G, Ou J, Wei X, Pan F, Liang H. The extra domain A of fibronectin increases VEGF-C expression in colorectal carcinoma involving the PI3K/AKT signaling pathway. PLoS One. 2012;7(4):e35378.
167. Sun X, Fa P, Cui Z, Xia Y, Sun L, Li Z, et al. The EDA-containing cellular fibronectin induces epithelial-mesenchymal transition in lung cancer cells through integrin alpha9beta1-mediated activation of PI3-K/AKT and Erk1/2. Carcinogenesis. 2014;35(1):184–91.
168. Ou J, Peng Y, Deng J, Miao H, Zhou J, Zha L, et al. Endothelial cell-derived fibronectin extra domain A promotes colorectal cancer metastasis via inducing epithelial-mesenchymal transition. Carcinogenesis. 2014;35(7):1661–70.
169. Ou J, Pan F, Geng P, Wei X, Xie G, Deng J, et al. Silencing fibronectin extra domain A enhances radiosensitivity in nasopharyngeal carcinomas involving an FAK/Akt/JNK pathway. Int J Radiat Oncol Biol Phys. 2012;82(4):e685–91.
170. Mosher DF, Adams JC. Adhesion-modulating/matricellular ECM protein families: a structural, functional and evolutionary appraisal. Matrix Biol. 2012;31(3):155–61.
171. Wong GS, Rustgi AK. Matricellular proteins: priming the tumour microenvironment for cancer development and metastasis. Br J Cancer. 2013;108(4):755–61.
172. Chiquet-Ehrismann R, Tucker RP. Tenascins and the importance of adhesion modulation. Cold Spring Harb Perspect Biol. 2011;3(5):a004960.
173. Forsberg E, Hirsch E, Frohlich L, Meyer M, Ekblom P, Aszodi A, et al. Skin wounds and severed nerves heal normally in mice lacking tenascin-C. Proc Natl Acad Sci U S A. 1996;93(13):6594–9.
174. Talts JF, Wirl G, Dictor M, Muller WJ, Fassler R. Tenascin-C modulates tumor stroma and monocyte/macrophage recruitment but not tumor growth or metastasis in a mouse strain with spontaneous mammary cancer. J Cell Sci. 1999;112(Pt 12):1855–64.
175. Zuliani-Alvarez L, Marzeda AM, Deligne C, Schwenzer A, McCann FE, Marsden BD, et al. Mapping tenascin-C interaction with toll-like receptor 4 reveals a new subset of endogenous inflammatory triggers. Nat Commun. 2017;8(1):1595.
176. Bhattacharyya S, Wang W, Morales-Nebreda L, Feng G, Wu M, Zhou X, et al. Tenascin-C drives persistence of organ fibrosis. Nat Commun. 2016;7:11703.
177. Yeo SY, Lee KW, Shin D, An S, Cho KH, Kim SH. A positive feedback loop bi-stably activates fibroblasts. Nat Commun. 2018;9(1):3016.
178. Fu H, Tian Y, Zhou L, Zhou D, Tan RJ, Stolz DB, et al. Tenascin-C is a major component of the fibrogenic niche in kidney fibrosis. J Am Soc Nephrol. 2017;28(3):785–801.
179. Orend G, Chiquet-Ehrismann R. Tenascin-C induced signaling in cancer. Cancer Lett. 2006;244(2):143–63.
180. Martin D, Brown-Luedi M, Chiquet-Ehrismann R. Tenascin-C signaling through induction of 14-3-3 tau. J Cell Biol. 2003;160(2):171–5.
181. Ruiz C, Huang W, Hegi ME, Lange K, Hamou MF, Fluri E, et al. Growth promoting signaling by tenascin-C [corrected]. Cancer Res. 2004;64(20):7377–85.
182. Huang W, Chiquet-Ehrismann R, Moyano JV, Garcia-Pardo A, Orend G. Interference of tenascin-C with syndecan-4 binding to fibronectin blocks cell adhesion and stimulates tumor cell proliferation. Cancer Res. 2001;61(23):8586–94.
183. Shi M, He X, Wei W, Wang J, Zhang T, Shen X. Tenascin-C induces resistance to apoptosis in pancreatic cancer cell through activation of ERK/NF-kappaB pathway. Apoptosis. 2015;20(6):843–57.
184. Nagaharu K, Zhang X, Yoshida T, Katoh D, Hanamura N, Kozuka Y, et al. Tenascin C induces epithelial-mesenchymal transition-like change accompanied by SRC activation and focal adhesion kinase phosphorylation in human breast cancer cells. Am J Pathol. 2011;178(2):754–63.
185. Katoh D, Nagaharu K, Shimojo N, Hanamura N, Yamashita M, Kozuka Y, et al. Binding of alphavbeta1 and alphavbeta6 integrins to tenascin-C induces epithelial-mesenchymal transition-like change of breast cancer cells. Oncogenesis. 2013;2:e65.
186. Ghahhari NM, Babashah S. Interplay between microRNAs and WNT/beta-catenin signalling pathway regulates epithelial-mesenchymal transition in cancer. Eur J Cancer. 2015;51(12):1638–49.
187. Saupe F, Schwenzer A, Jia Y, Gasser I, Spenle C, Langlois B, et al. Tenascin-C downregulates wnt inhibitor dickkopf-1, promoting tumorigenesis in a neuroendocrine tumor model. Cell Rep. 2013;5(2):482–92.
188. Beiter K, Hiendlmeyer E, Brabletz T, Hlubek F, Haynl A, Knoll C, et al. beta-Catenin regulates the expression of tenascin-C in human colorectal tumors. Oncogene. 2005;24(55):8200–4.
189. Nong Y, Wu D, Lin Y, Zhang Y, Bai L, Tang H. Tenascin-C expression is associated with poor prognosis in hepatocellular carcinoma (HCC) patients and the inflammatory cytokine TNF-alpha-induced TNC expression promotes migration in HCC cells. Am J Cancer Res. 2015;5(2):782–91.
190. Grahovac J, Becker D, Wells A. Melanoma cell invasiveness is promoted at least in part by the epidermal growth factor-like repeats of tenascin-C. J Invest Dermatol. 2013;133(1):210–20.
191. Kaariainen E, Nummela P, Soikkeli J, Yin M, Lukk M, Jahkola T, et al. Switch to an invasive growth phase in melanoma is associated with tenascin-C, fibronectin, and procollagen-I forming specific channel structures for invasion. J Pathol. 2006;210(2):181–91.
192. Kalembeyi I, Inada H, Nishiura R, Imanaka-Yoshida K, Sakakura T, Yoshida T. Tenascin-C upregulates matrix metalloproteinase-9 in breast cancer cells: direct and synergistic effects with transforming growth factor beta1. Int J Cancer. 2003;105(1):53–60.

193. Hancox RA, Allen MD, Holliday DL, Edwards DR, Pennington CJ, Guttery DS, et al. Tumour-associated tenascin-C isoforms promote breast cancer cell invasion and growth by matrix metalloproteinase-dependent and independent mechanisms. Breast Cancer Res. 2009;11(2):R24.
194. Calvo A, Catena R, Noble MS, Carbott D, Gil-Bazo I, Gonzalez-Moreno O, et al. Identification of VEGF-regulated genes associated with increased lung metastatic potential: functional involvement of tenascin-C in tumor growth and lung metastasis. Oncogene. 2008;27(40):5373–84.
195. O'Connell JT, Sugimoto H, Cooke VG, MacDonald BA, Mehta AI, LeBleu VS, et al. VEGF-A and Tenascin-C produced by S100A4+ stromal cells are important for metastatic colonization. Proc Natl Acad Sci U S A. 2011;108(38):16002–7.
196. Gocheva V, Naba A, Bhutkar A, Guardia T, Miller KM, Li CM, et al. Quantitative proteomics identify Tenascin-C as a promoter of lung cancer progression and contributor to a signature prognostic of patient survival. Proc Natl Acad Sci U S A. 2017;114(28):E5625–E34.
197. Katoh D, Kozuka Y, Noro A, Ogawa T, Imanaka-Yoshida K, Yoshida T. Tenascin-C induces phenotypic changes in fibroblasts to myofibroblasts with high contractility through the integrin alphavbeta1/TGF-beta/SMAD signaling axis in human breast cancer. Am J Pathol. 2020;190(10):2123–35.
198. Spenle C, Loustau T, Murdamoothoo D, Erne W, la Forest B-d, Divonne S, Veber R, et al. Tenascin-C orchestrates an immune-suppressive tumor microenvironment in oral squamous cell carcinoma. Cancer Immunol Res. 2020;8(9):1122–38.
199. Chiovaro F, Martina E, Bottos A, Scherberich A, Hynes NE, Chiquet-Ehrismann R. Transcriptional regulation of tenascin-W by TGF-beta signaling in the bone metastatic niche of breast cancer cells. Int J Cancer. 2015;137(8):1842–54.
200. Degen M, Brellier F, Kain R, Ruiz C, Terracciano L, Orend G, et al. Tenascin-W is a novel marker for activated tumor stroma in low-grade human breast cancer and influences cell behavior. Cancer Res. 2007;67(19):9169–79.
201. Degen M, Brellier F, Schenk S, Driscoll R, Zaman K, Stupp R, et al. Tenascin-W, a new marker of cancer stroma, is elevated in sera of colon and breast cancer patients. Int J Cancer. 2008;122(11):2454–61.
202. Scherberich A, Tucker RP, Degen M, Brown-Luedi M, Andres AC, Chiquet-Ehrismann R. Tenascin-W is found in malignant mammary tumors, promotes alpha8 integrin-dependent motility and requires p38MAPK activity for BMP-2 and TNF-alpha induced expression in vitro. Oncogene. 2005;24(9):1525–32.
203. Brellier F, Martina E, Degen M, Heuze-Vourc'h N, Petit A, Kryza T, et al. Tenascin-W is a better cancer biomarker than tenascin-C for most human solid tumors. BMC Clin Pathol. 2012;12:14.
204. Matsumoto K, Saga Y, Ikemura T, Sakakura T, Chiquet-Ehrismann R. The distribution of tenascin-X is distinct and often reciprocal to that of tenascin-C. J Cell Biol. 1994;125(2):483–93.
205. Chiquet-Ehrismann R, Chiquet M. Tenascins: regulation and putative functions during pathological stress. J Pathol. 2003;200(4):488–99.
206. Geffrotin C, Horak V, Crechet F, Tricaud Y, Lethias C, Vincent-Naulleau S, et al. Opposite regulation of tenascin-C and tenascin-X in MeLiM swine heritable cutaneous malignant melanoma. Biochim Biophys Acta. 2000;1524(2-3):196–202.
207. Matsumoto K, Takayama N, Ohnishi J, Ohnishi E, Shirayoshi Y, Nakatsuji N, et al. Tumour invasion and metastasis are promoted in mice deficient in tenascin-X. Genes Cells. 2001;6(12):1101–11.
208. Matsumoto K, Minamitani T, Orba Y, Sato M, Sawa H, Ariga H. Induction of matrix metalloproteinase-2 by tenascin-X deficiency is mediated through the c-Jun N-terminal kinase and protein tyrosine kinase phosphorylation pathway. Exp Cell Res. 2004;297(2):404–14.
209. Alcaraz LB, Exposito JY, Chuvin N, Pommier RM, Cluzel C, Martel S, et al. Tenascin-X promotes epithelial-to-mesenchymal transition by activating latent TGF-beta. J Cell Biol. 2014;205(3):409–28.
210. Rios H, Koushik SV, Wang H, Wang J, Zhou HM, Lindsley A, et al. periostin null mice exhibit dwarfism, incisor enamel defects, and an early-onset periodontal disease-like phenotype. Mol Cell Biol. 2005;25(24):11131–44.
211. Conway SJ, Izuhara K, Kudo Y, Litvin J, Markwald R, Ouyang G, et al. The role of periostin in tissue remodeling across health and disease. Cell Mol Life Sci. 2014;71(7):1279–88.
212. Elliott CG, Wang J, Guo X, Sw X, Eastwood M, Guan J, et al. Periostin modulates myofibroblast differentiation during full-thickness cutaneous wound repair. J Cell Sci. 2012;125(1):121–32.
213. Shimazaki M, Nakamura K, Kii I, Kashima T, Amizuka N, Li M, et al. Periostin is essential for cardiac healing after acute myocardial infarction. J Exp Med. 2008;205(2):295–303.
214. Lorts A, Schwanekamp JA, Baudino TA, McNally EM, Molkentin JD. Deletion of periostin reduces muscular dystrophy and fibrosis in mice by modulating the transforming growth factor-beta pathway. Proc Natl Acad Sci U S A. 2012;109(27):10978–83.
215. Norris RA, Damon B, Mironov V, Kasyanov V, Ramamurthi A, Moreno-Rodriguez R, et al. Periostin regulates collagen fibrillogenesis and the biomechanical properties of connective tissues. J Cell Biochem. 2007;101(3):695–711.
216. Egbert M, Ruetze M, Sattler M, Wenck H, Gallinat S, Lucius R, et al. The matricellular protein periostin contributes to proper collagen function and is downregulated during skin aging. J Dermatol Sci. 2014;73(1):40–8.
217. Shimazaki M, Kudo A. Impaired capsule formation of tumors in periostin-null mice. Biochem Biophys Res Commun. 2008;367(4):736–42.
218. Kikuchi Y, Kunita A, Iwata C, Komura D, Nishiyama T, Shimazu K, et al. The niche component periostin is produced by cancer-associated fibroblasts, supporting growth of gastric cancer through ERK activation. Am J Pathol. 2014;184(3):859–70.
219. Fukuda K, Sugihara E, Ohta S, Izuhara K, Funakoshi T, Amagai M, et al. Periostin is a key niche component for wound metastasis of melanoma. PLoS One. 2015;10(6):e0129704.
220. Zhou W, Ke SQ, Huang Z, Flavahan W, Fang X, Paul J, et al. Periostin secreted by glioblastoma stem cells recruits M2 tumour-associated macrophages and promotes malignant growth. Nat Cell Biol. 2015;17(2):170–82.
221. Malanchi I, Santamaria-Martinez A, Susanto E, Peng H, Lehr HA, Delaloye JF, et al. Interactions between cancer stem cells and their niche govern metastatic colonization. Nature. 2012;481(7379):85–9.
222. Sonongbua J, Siritungyong S, Thongchot S, Kamolhan T, Utispan K, Thuwajit P, et al. Periostin induces epithelialtomesenchymal transition via the integrin alpha5beta1/TWIST2 axis in cholangiocarcinoma. Oncol Rep. 2020;43(4):1147–58.
223. Ma H, Wang J, Zhao X, Wu T, Huang Z, Chen D, et al. Periostin promotes colorectal tumorigenesis through integrin-FAK-Src pathway-mediated YAP/TAZ activation. Cell Rep. 2020;30(3):793–806 e6.
224. Zenitani M, Nojiri T, Hosoda H, Kimura T, Uehara S, Miyazato M, et al. Chemotherapy can promote liver metastasis by enhancing metastatic niche formation in mice. J Surg Res. 2018;224:50–7.
225. Wegrowski Y, Maquart FX. Involvement of stromal proteoglycans in tumour progression. Crit Rev Oncol Hematol. 2004;49(3):259–68.
226. Theocharis AD, Skandalis SS, Tzanakakis GN, Karamanos NK. Proteoglycans in health and disease: novel roles for proteoglycans in malignancy and their pharmacological targeting. FEBS J. 2010;277(19):3904–23.

227. Iozzo RV, Sanderson RD. Proteoglycans in cancer biology, tumour microenvironment and angiogenesis. J Cell Mol Med. 2011;15(5):1013–31.
228. Sarrazin S, Lamanna WC, Esko JD. Heparan sulfate proteoglycans. Cold Spring Harb Perspect Biol. 2011;3(7):a004952.
229. Li JP, Kusche-Gullberg M. Heparan sulfate: biosynthesis, structure, and function. Int Rev Cell Mol Biol. 2016;325:215–73.
230. Bishop JR, Schuksz M, Esko JD. Heparan sulphate proteoglycans fine-tune mammalian physiology. Nature. 2007;446(7139):1030–7.
231. Ai X, Do AT, Lozynska O, Kusche-Gullberg M, Lindahl U, Emerson CP Jr. QSulf1 remodels the 6-O sulfation states of cell surface heparan sulfate proteoglycans to promote Wnt signaling. J Cell Biol. 2003;162(2):341–51.
232. Billings PC, Pacifici M. Interactions of signaling proteins, growth factors and other proteins with heparan sulfate: mechanisms and mysteries. Connect Tissue Res. 2015;56(4):272–80.
233. Lau EK, Paavola CD, Johnson Z, Gaudry JP, Geretti E, Borlat F, et al. Identification of the glycosaminoglycan binding site of the CC chemokine, MCP-1: implications for structure and function in vivo. J Biol Chem. 2004;279(21):22294–305.
234. Dowsland MH, Harvey JR, Lennard TW, Kirby JA, Ali S. Chemokines and breast cancer: a gateway to revolutionary targeted cancer treatments? Curr Med Chem. 2003;10(7):579–92.
235. Iozzo RV, Schaefer L. Proteoglycan form and function: a comprehensive nomenclature of proteoglycans. Matrix Biol. 2015;42:11–55.
236. Choi Y, Chung H, Jung H, Couchman JR, Oh ES. Syndecans as cell surface receptors: unique structure equates with functional diversity. Matrix Biol. 2011;30(2):93–9.
237. Couchman JR, Gopal S, Lim HC, Norgaard S, Multhaupt HA. Syndecans: from peripheral coreceptors to mainstream regulators of cell behaviour. Int J Exp Pathol. 2015;96(1):1–10.
238. Yao W, Rose JL, Wang W, Seth S, Jiang H, Taguchi A, et al. Syndecan 1 is a critical mediator of macropinocytosis in pancreatic cancer. Nature. 2019;568(7752):410–4.
239. Chute C, Yang X, Meyer K, Yang N, O'Neil K, Kasza I, et al. Syndecan-1 induction in lung microenvironment supports the establishment of breast tumor metastases. Breast Cancer Res. 2018;20(1):66.
240. Gondelaud F, Ricard-Blum S. Structures and interactions of syndecans. FEBS J. 2019;286(15):2994–3007.
241. Vuoriluoto K, Hognas G, Meller P, Lehti K, Ivaska J. Syndecan-1 and -4 differentially regulate oncogenic K-ras dependent cell invasion into collagen through alpha2beta1 integrin and MT1-MMP. Matrix Biol. 2011;30(3):207–17.
242. Chronopoulos A, Thorpe SD, Cortes E, Lachowski D, Rice AJ, Mykuliak VV, et al. Syndecan-4 tunes cell mechanics by activating the kindlin-integrin-RhoA pathway. Nat Mater. 2020;19(6):669–78.
243. Ihrcke NS, Platt JL. Shedding of heparan sulfate proteoglycan by stimulated endothelial cells: evidence for proteolysis of cell-surface molecules. J Cell Physiol. 1996;168(3):625–37.
244. Manon-Jensen T, Multhaupt HA, Couchman JR. Mapping of matrix metalloproteinase cleavage sites on syndecan-1 and syndecan-4 ectodomains. FEBS J. 2013;280(10):2320–31.
245. Choi S, Kim JY, Park JH, Lee ST, Han IO, Oh ES. The matrix metalloproteinase-7 regulates the extracellular shedding of syndecan-2 from colon cancer cells. Biochem Biophys Res Commun. 2012;417(4):1260–4.
246. Manon-Jensen T, Itoh Y, Couchman JR. Proteoglycans in health and disease: the multiple roles of syndecan shedding. FEBS J. 2010;277(19):3876–89.
247. Ding K, Lopez-Burks M, Sanchez-Duran JA, Korc M, Lander AD. Growth factor-induced shedding of syndecan-1 confers glypican-1 dependence on mitogenic responses of cancer cells. J Cell Biol. 2005;171(4):729–38.
248. Tan X, Khalil N, Tesarik C, Vanapalli K, Yaputra V, Alkhouri H, et al. Th1 cytokine-induced syndecan-4 shedding by airway smooth muscle cells is dependent on mitogen-activated protein kinases. Am J Physiol Lung Cell Mol Physiol. 2012;302(7):L700–10.
249. Yang Y, Macleod V, Miao HQ, Theus A, Zhan F, Shaughnessy JD Jr, et al. Heparanase enhances syndecan-1 shedding: a novel mechanism for stimulation of tumor growth and metastasis. J Biol Chem. 2007;282(18):13326–33.
250. Joensuu H, Anttonen A, Eriksson M, Makitaro R, Alfthan H, Kinnula V, et al. Soluble syndecan-1 and serum basic fibroblast growth factor are new prognostic factors in lung cancer. Cancer Res. 2002;62(18):5210–7.
251. Ramani VC, Sanderson RD. Chemotherapy stimulates syndecan-1 shedding: a potentially negative effect of treatment that may promote tumor relapse. Matrix Biol. 2014;35:215–22.
252. Stewart MD, Ramani VC, Sanderson RD. Shed syndecan-1 translocates to the nucleus of cells delivering growth factors and inhibiting histone acetylation: a novel mechanism of tumor-host cross-talk. J Biol Chem. 2015;290(2):941–9.
253. Su G, Blaine SA, Qiao D, Friedl A. Membrane type 1 matrix metalloproteinase-mediated stromal syndecan-1 shedding stimulates breast carcinoma cell proliferation. Cancer Res. 2008;68(22):9558–65.
254. Su G, Blaine SA, Qiao D, Friedl A. Shedding of syndecan-1 by stromal fibroblasts stimulates human breast cancer cell proliferation via FGF2 activation. J Biol Chem. 2007;282(20):14906–15.
255. Nikolova V, Koo CY, Ibrahim SA, Wang Z, Spillmann D, Dreier R, et al. Differential roles for membrane-bound and soluble syndecan-1 (CD138) in breast cancer progression. Carcinogenesis. 2009;30(3):397–407.
256. Choi S, Choi Y, Jun E, Kim IS, Kim SE, Jung SA, et al. Shed syndecan-2 enhances tumorigenic activities of colon cancer cells. Oncotarget. 2015;6(6):3874–86.
257. Wang X, Zuo D, Chen Y, Li W, Liu R, He Y, et al. Shed Syndecan-1 is involved in chemotherapy resistance via the EGFR pathway in colorectal cancer. Br J Cancer. 2014;111(10):1965–76.
258. Chen S, Birk DE. The regulatory roles of small leucine-rich proteoglycans in extracellular matrix assembly. FEBS J. 2013;280(10):2120–37.
259. Cawthorn TR, Moreno JC, Dharsee M, Tran-Thanh D, Ackloo S, Zhu PH, et al. Proteomic analyses reveal high expression of decorin and endoplasmin (HSP90B1) are associated with breast cancer metastasis and decreased survival. PLoS One. 2012;7(2):e30992.
260. Sainio A, Nyman M, Lund R, Vuorikoski S, Bostrom P, Laato M, et al. Lack of decorin expression by human bladder cancer cells offers new tools in the therapy of urothelial malignancies. PLoS One. 2013;8(10):e76190.
261. Henke A, Grace OC, Ashley GR, Stewart GD, Riddick AC, Yeun H, et al. Stromal expression of decorin, Semaphorin6D, SPARC, Sprouty1 and Tsukushi in developing prostate and decreased levels of decorin in prostate cancer. PLoS One. 2012;7(8):e42516.
262. Campioni M, Ambrogi V, Pompeo E, Citro G, Castelli M, Spugnini EP, et al. Identification of genes down-regulated during lung cancer progression: a cDNA array study. J Exp Clin Cancer Res. 2008;27:38.
263. Bostrom P, Sainio A, Kakko T, Savontaus M, Soderstrom M, Jarvelainen H. Localization of decorin gene expression in normal human breast tissue and in benign and malignant tumors of the human breast. Histochem Cell Biol. 2013;139(1):161–71.
264. Oda G, Sato T, Ishikawa T, Kawachi H, Nakagawa T, Kuwayama T, et al. Significance of stromal decorin expression during the progression of breast cancer. Oncol Rep. 2012;28(6):2003–8.

265. Troup S, Njue C, Kliewer EV, Parisien M, Roskelley C, Chakravarti S, et al. Reduced expression of the small leucine-rich proteoglycans, lumican, and decorin is associated with poor outcome in node-negative invasive breast cancer. Clin Cancer Res. 2003;9(1):207–14.
266. Horvath Z, Kovalszky I, Fullar A, Kiss K, Schaff Z, Iozzo RV, et al. Decorin deficiency promotes hepatic carcinogenesis. Matrix Biol. 2014;35:194–205.
267. Xu W, Neill T, Yang Y, Hu Z, Cleveland E, Wu Y, et al. The systemic delivery of an oncolytic adenovirus expressing decorin inhibits bone metastasis in a mouse model of human prostate cancer. Gene Ther. 2015;22(3):31–40.
268. Nyman MC, Sainio AO, Pennanen MM, Lund RJ, Vuorikoski S, Sundstrom JT, et al. Decorin in human colon cancer: localization in vivo and effect on cancer cell behavior in vitro. J Histochem Cytochem. 2015;63(9):710–20.
269. Buraschi S, Neill T, Owens RT, Iniguez LA, Purkins G, Vadigepalli R, et al. Decorin protein core affects the global gene expression profile of the tumor microenvironment in a triple-negative orthotopic breast carcinoma xenograft model. PLoS One. 2012;7(9):e45559.
270. Goldoni S, Iozzo RV. Tumor microenvironment: modulation by decorin and related molecules harboring leucine-rich tandem motifs. Int J Cancer. 2008;123(11):2473–9.
271. Buraschi S, Pal N, Tyler-Rubinstein N, Owens RT, Neill T, Iozzo RV. Decorin antagonizes Met receptor activity and down-regulates {beta}-catenin and Myc levels. J Biol Chem. 2010;285(53):42075–85.
272. Neill T, Torres A, Buraschi S, Owens RT, Hoek JB, Baffa R, et al. Decorin induces mitophagy in breast carcinoma cells via peroxisome proliferator-activated receptor gamma coactivator-1alpha (PGC-1alpha) and mitostatin. J Biol Chem. 2014;289(8):4952–68.
273. Neill T, Painter H, Buraschi S, Owens RT, Lisanti MP, Schaefer L, et al. Decorin antagonizes the angiogenic network: concurrent inhibition of Met, hypoxia inducible factor 1alpha, vascular endothelial growth factor A, and induction of thrombospondin-1 and TIMP3. J Biol Chem. 2012;287(8):5492–506.
274. Buraschi S, Neill T, Goyal A, Poluzzi C, Smythies J, Owens RT, et al. Decorin causes autophagy in endothelial cells via Peg3. Proc Natl Acad Sci U S A. 2013;110(28):E2582–91.
275. Morcavallo A, Buraschi S, Xu SQ, Belfiore A, Schaefer L, Iozzo RV, et al. Decorin differentially modulates the activity of insulin receptor isoform A ligands. Matrix Biol. 2014;35:82–90.
276. Koninger J, Giese NA, di Mola FF, Berberat P, Giese T, Esposito I, et al. Overexpressed decorin in pancreatic cancer: potential tumor growth inhibition and attenuation of chemotherapeutic action. Clin Cancer Res. 2004;10(14):4776–83.
277. Kasamatsu A, Uzawa K, Minakawa Y, Ishige S, Kasama H, Endo-Sakamoto Y, et al. Decorin in human oral cancer: a promising predictive biomarker of S-1 neoadjuvant chemosensitivity. Biochem Biophys Res Commun. 2015;457(1):71–6.
278. Zhu YH, Yang F, Zhang SS, Zeng TT, Xie X, Guan XY. High expression of biglycan is associated with poor prognosis in patients with esophageal squamous cell carcinoma. Int J Clin Exp Pathol. 2013;6(11):2497–505.
279. Aprile G, Avellini C, Reni M, Mazzer M, Foltran L, Rossi D, et al. Biglycan expression and clinical outcome in patients with pancreatic adenocarcinoma. Tumour Biol. 2013;34(1):131–7.
280. Hu L, Duan YT, Li JF, Su LP, Yan M, Zhu ZG, et al. Biglycan enhances gastric cancer invasion by activating FAK signaling pathway. Oncotarget. 2014;5(7):1885–96.
281. Niedworok C, Rock K, Kretschmer I, Freudenberger T, Nagy N, Szarvas T, et al. Inhibitory role of the small leucine-rich proteoglycan biglycan in bladder cancer. PLoS One. 2013;8(11):e80084.
282. Weber CK, Sommer G, Michl P, Fensterer H, Weimer M, Gansauge F, et al. Biglycan is overexpressed in pancreatic cancer and induces G1-arrest in pancreatic cancer cell lines. Gastroenterology. 2001;121(3):657–67.
283. Nikitovic D, Papoutsidakis A, Karamanos NK, Tzanakakis GN. Lumican affects tumor cell functions, tumor-ECM interactions, angiogenesis and inflammatory response. Matrix Biol. 2014;35:206–14.
284. Brezillon S, Pietraszek K, Maquart FX, Wegrowski Y. Lumican effects in the control of tumour progression and their links with metalloproteinases and integrins. FEBS J. 2013;280(10):2369–81.
285. Seya T, Tanaka N, Shinji S, Yokoi K, Koizumi M, Teranishi N, et al. Lumican expression in advanced colorectal cancer with nodal metastasis correlates with poor prognosis. Oncol Rep. 2006;16(6):1225–30.
286. de Wit M, Belt EJ, Delis-van Diemen PM, Carvalho B, Coupe VM, Stockmann HB, et al. Lumican and versican are associated with good outcome in stage II and III colon cancer. Ann Surg Oncol. 2013;20(Suppl 3):S348–59.
287. Panis C, Pizzatti L, Herrera AC, Cecchini R, Abdelhay E. Putative circulating markers of the early and advanced stages of breast cancer identified by high-resolution label-free proteomics. Cancer Lett. 2013;330(1):57–66.
288. Karamanou K, Franchi M, Onisto M, Passi A, Vynios DH, Brezillon S. Evaluation of lumican effects on morphology of invading breast cancer cells, expression of integrins and downstream signaling. FEBS J. 2020;287:4862–80.
289. Ishiwata T, Cho K, Kawahara K, Yamamoto T, Fujiwara Y, Uchida E, et al. Role of lumican in cancer cells and adjacent stromal tissues in human pancreatic cancer. Oncol Rep. 2007;18(3):537–43.
290. Li X, Truty MA, Kang Y, Chopin-Laly X, Zhang R, Roife D, et al. Extracellular lumican inhibits pancreatic cancer cell growth and is associated with prolonged survival after surgery. Clin Cancer Res. 2014;20(24):6529–40.
291. Matsuda Y, Yamamoto T, Kudo M, Kawahara K, Kawamoto M, Nakajima Y, et al. Expression and roles of lumican in lung adenocarcinoma and squamous cell carcinoma. Int J Oncol. 2008;33(6):1177–85.
292. Brezillon S, Venteo L, Ramont L, D'Onofrio MF, Perreau C, Pluot M, et al. Expression of lumican, a small leucine-rich proteoglycan with antitumour activity, in human malignant melanoma. Clin Exp Dermatol. 2007;32(4):405–16.
293. Brezillon S, Radwanska A, Zeltz C, Malkowski A, Ploton D, Bobichon H, et al. Lumican core protein inhibits melanoma cell migration via alterations of focal adhesion complexes. Cancer Lett. 2009;283(1):92–100.
294. Zeltz C, Brezillon S, Perreau C, Ramont L, Maquart FX, Wegrowski Y. Lumcorin: a leucine-rich repeat 9-derived peptide from human lumican inhibiting melanoma cell migration. FEBS Lett. 2009;583(18):3027–32.
295. Zeltz C, Brezillon S, Kapyla J, Eble JA, Bobichon H, Terryn C, et al. Lumican inhibits cell migration through alpha2beta1 integrin. Exp Cell Res. 2010;316(17):2922–31.
296. Pietraszek K, Chatron-Colliet A, Brezillon S, Perreau C, Jakubiak-Augustyn A, Krotkiewski H, et al. Lumican: a new inhibitor of matrix metalloproteinase-14 activity. FEBS Lett. 2014;588(23):4319–24.
297. Coulson-Thomas VJ, Coulson-Thomas YM, Gesteira TF, Andrade de Paula CA, Carneiro CR, Ortiz V, et al. Lumican expression, localization and antitumor activity in prostate cancer. Exp Cell Res. 2013;319(7):967–81.
298. Radwanska A, Litwin M, Nowak D, Baczynska D, Wegrowski Y, Maquart FX, et al. Overexpression of lumican affects the migration of human colon cancer cells through up-regulation of gelsolin and filamentous actin reorganization. Exp Cell Res. 2012;318(18):2312–23.
299. Oldberg A, Kalamajski S, Salnikov AV, Stuhr L, Morgelin M, Reed RK, et al. Collagen-binding proteoglycan fibro-

modulin can determine stroma matrix structure and fluid balance in experimental carcinoma. Proc Natl Acad Sci U S A. 2007;104(35):13966–71.
300. Hildebrand A, Romaris M, Rasmussen LM, Heinegard D, Twardzik DR, Border WA, et al. Interaction of the small interstitial proteoglycans biglycan, decorin and fibromodulin with transforming growth factor beta. Biochem J. 1994;302(Pt 2):527–34.
301. Maris P, Blomme A, Palacios AP, Costanza B, Bellahcene A, Bianchi E, et al. Asporin Is a Fibroblast-Derived TGF-beta1 Inhibitor and a Tumor Suppressor Associated with Good Prognosis in Breast Cancer. PLoS Med. 2015;12(9):e1001871.
302. Lohler J, Timpl R, Jaenisch R. Embryonic lethal mutation in mouse collagen I gene causes rupture of blood vessels and is associated with erythropoietic and mesenchymal cell death. Cell. 1984;38(2):597–607.
303. Andrikopoulos K, Liu X, Keene DR, Jaenisch R, Ramirez F. Targeted mutation in the col5a2 gene reveals a regulatory role for type V collagen during matrix assembly. Nat Genet. 1995;9(1):31–6.
304. Wenstrup RJ, Florer JB, Brunskill EW, Bell SM, Chervoneva I, Birk DE. Type V collagen controls the initiation of collagen fibril assembly. J Biol Chem. 2004;279(51):53331–7.
305. Provenzano PP, Eliceiri KW, Campbell JM, Inman DR, White JG, Keely PJ. Collagen reorganization at the tumor-stromal interface facilitates local invasion. BMC Med. 2006;4(1):38.
306. Liu X, Wu H, Byrne M, Krane S, Jaenisch R. Type III collagen is crucial for collagen I fibrillogenesis and for normal cardiovascular development. Proc Natl Acad Sci U S A. 1997;94(5):1852–6.
307. Saga Y, Yagi T, Ikawa Y, Sakakura T, Aizawa S. Mice develop normally without tenascin. Genes Dev. 1992;6:1821–31.
308. Hendaoui I, Tucker RP, Zingg D, Bichet S, Schittny J, Chiquet-Ehrismann R. Tenascin-C is required for normal Wnt/beta-catenin signaling in the whisker follicle stem cell niche. Matrix Biol. 2014;40:46–53.
309. Kii I, Amizuka N, Minqi L, Kitajima S, Saga Y, Kudo A. Periostin is an extracellular matrix protein required for eruption of incisors in mice. Biochem Biophys Res Commun. 2006;342(3):766–72.
310. Ontsuka K, Kotobuki Y, Shiraishi H, Serada S, Ohta S, Tanemura A, et al. Periostin, a matricellular protein, accelerates cutaneous wound repair by activating dermal fibroblasts. Exp Dermatol. 2012;21(5):331–6.
311. Ishiba T, Nagahara M, Nakagawa T, Sato T, Ishikawa T, Uetake H, et al. Periostin suppression induces decorin secretion leading to reduced breast cancer cell motility and invasion. Sci Rep. 2014;4:7069.
312. Danielson KG, Baribault H, Holmes DF, Graham H, Kadler KE, Iozzo RV. Targeted disruption of decorin leads to abnormal collagen fibril morphology and skin fragility. J Cell Biol. 1997;136(3):729–43.
313. Neill T, Schaefer L, Iozzo RV. Decorin: a guardian from the matrix. Am J Pathol. 2012;181(2):380–7.
314. Yamaguchi Y, Mann DM, Ruoslahti E. Negative regulation of transforming growth factor-beta by the proteoglycan decorin. Nature. 1990;346(6281):281–4.
315. Merline R, Moreth K, Beckmann J, Nastase MV, Zeng-Brouwers J, Tralhao JG, et al. Signaling by the matrix proteoglycan decorin controls inflammation and cancer through PDCD4 and MicroRNA-21. Sci Signal. 2011;4(199):ra75.
316. Chakravarti S, Magnuson T, Lass JH, Jepsen KJ, LaMantia C, Carroll H. Lumican regulates collagen fibril assembly: skin fragility and corneal opacity in the absence of lumican. J Cell Biol. 1998;141(5):1277–86.
317. Beauvais DM, Ell BJ, McWhorter AR, Rapraeger AC. Syndecan-1 regulates {alpha}v{beta}3 and {alpha}v{beta}5 integrin activation during angiogenesis and is blocked by synstatin, a novel peptide inhibitor. J Exp Med. 2009;16:691–705.
318. Theocharis AD, Karamanos NK. Proteoglycans remodeling in cancer: underlying molecular mechanisms. Matrix Biol. 2019;75-76:220–59.
319. Multhaupt HA, Leitinger B, Gullberg D, Couchman JR. Extracellular matrix component signaling in cancer. Adv Drug Deliv Rev. 2016;97:28–40.
320. Guilluy C, Dolega ME. Syndecan-4 forces integrins to cooperate. Nat Mater. 2020;19(6):587–8.