Chapter 4
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 proper-

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ties 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.

Keywords Tumor microenvironment • Tumor stroma • Extracellular matrix Fibrillar collagen • Tumor growth • Tumor metastasis • Tumor stiffness • Lysyl oxidase • Fibronectin EDA • Periostin • Tenascins • Proteoglycans

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 and degree of vascularization and choose to isolate cells to study their phenotype *in vitro*. A molecular biologist aims to understand the molecular and genetic mechanisms involved in tumor pathogenesis and design 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.

With the understanding that the tumor microenvironment influences tumor cell growth, this also has implications for the design and interpretations of *in vitro* experiments. It is becoming obvious that simple 2D *in vitro* coculture 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 3D aspects as well as multiple cell type aspects are incorporated. In molecular studies, intercellular 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, e.g. heterospheroids [1, 2] being one recent methodological development with great potential.

When discussing different mechanisms in the tumor microenvironment, it is important to avoid generalizations and always relate the findings to a certain tumor and 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 [3].

– 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, fibroblasts activation states, proteolytic activity, and stiffness).
– Matrix stiffness is another critical feature for tumor growth and for tensional homeostasis in the tumor [4, 5]. Matrix stiffness has been shown to be intimately linked to posttranslational modifications of the matrix such as glycation and cross-linking but also to collagen organization and appears to vary between different regions within the tumor [6].
– In addition to the complexity in the assembly and structure of the ECM, the recent findings that tumor-derived exosomes affect cellular interactions in the TME introduce yet another level of complexity. Provocative data have described roles for exosomes in chemoresistance, miRNA-directed effects on gene silencing, and even mediating changes in integrin repertoire affecting metastasis of tumor cells [7, 8].

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) [3, 9, 10]. CAFs have different roles in the tumor stroma (including paracrine signaling [10] and chemoresistance [11]), which will not be discussed in this chapter. CAFs 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. 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). This activation of TGF-β results in CAF activation [12]. Moreover, antibodies to αvβ6 *in vivo* have been shown to reduce growth and metastasis of the 4T1 murine breast cancer cell line [13]. Data in fibrosis and *in vitro* models further suggest that myofibroblasts themselves can play an active role in activating TGF-β, by prestraining the matrix and sensitizing TGF-β to activation [14–16]. Another integrin, αvβ1, has also been shown to directly take part in TGF-β activation of myofibroblasts [17].

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 [18, 19]. Interestingly, two reports have challenged the dogma that the tumor stroma plays a supportive role in tumor growth and metastasis [20, 21]. Both studies take advantage of advanced genetic techniques to ablate stromal cells in experimental models for pancreatic cancer (in a form of genetic stroma-targeting strategy) after the tumors had formed. Contrary to what was expected, the pancreatic tumors became more aggressive in the absence of the stroma. Since the source of CAFs can vary, 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. Another

study suggests that a minor perivascular Gli-positive stem cell population in the lung stroma is the main producer of a fibrotic ECM, and careful analyses of dermal fibroblasts have revealed different origins of reticular and papillary fibroblasts [22–24]. Analogous to these studies demonstrating fibroblast heterogeneity in tissues, different mesenchymal cell populations in the tumor stroma might have different roles. In summary, a global targeting of all CAFs may not be the best therapeutic strategy [3, 25] since both tumor-supportive CAFs and tumor-inhibitory CAFs appear to exist in the tumor stroma. Cell lineage tracing 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

Figure 4.1 schematically illustrates the structure of the ECM molecules that we describe below.

Fibrillar Collagens in the Stroma

Fibrillar Collagen Types in the Tumor Stroma

The collagen family is composed of 28 trimeric triple-helical proteins [26, 27]. The most abundant collagens are the fibrillar collagens, which together with a subset of fibril-associated collagens with interrupted triple helices (FACIT collagens) are present in interstitial tissues [26]. In interstitial tissues, collagen I dominates with lesser amounts of collagen III being present. Collagen V in some studies has been suggested to constitute less than 5% of interstitial matrices, and collagen XI, under physiological conditions, is present only in specialized matrices [26, 27]. 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 [28]. 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 [29, 30]. In the granulation tissue collagen III is replaced with collagen I as the wound heals [31], 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 [32, 33]. Indirect binding is mediated via collagen-integrin bridging molecules (COLINBRIs), which typically bind RGD-binding integrins like α5β1, αvβ1, αvβ3, and αvβ5 [32, 34]. Interestingly, the discoidin domain receptors (DDRs) have recently been shown to affect the function of collagen-binding integrins by supporting integrin activation [35–37].

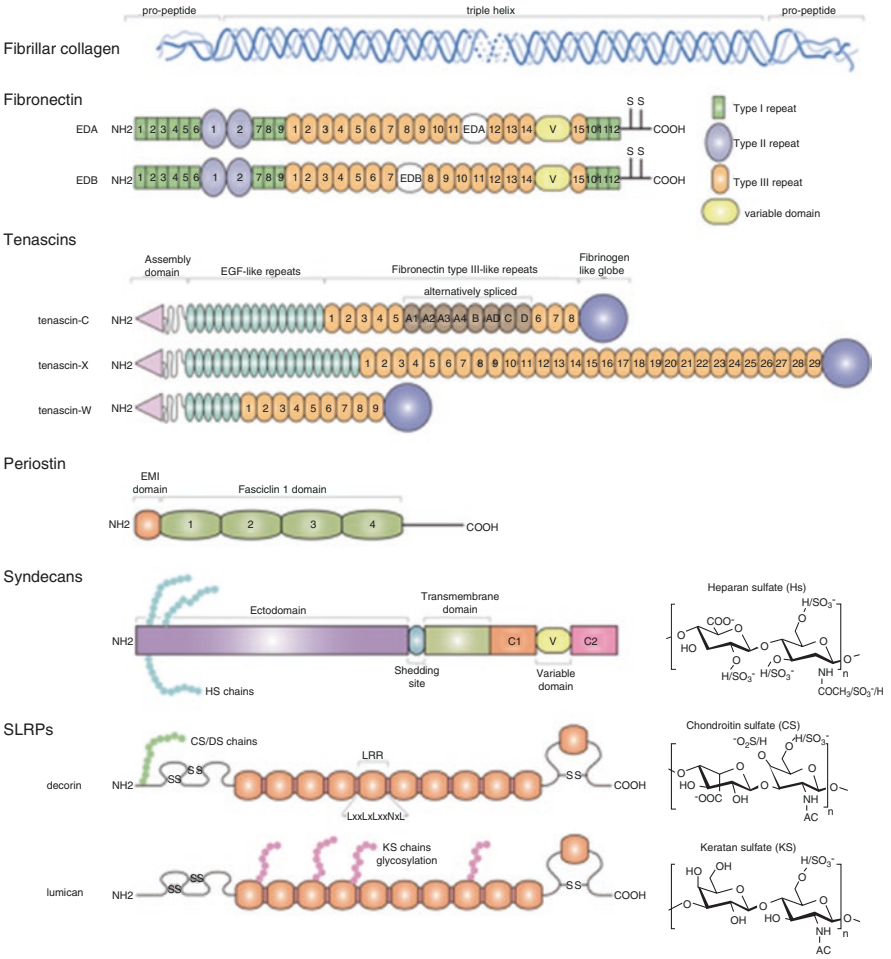

Fig. 4.1 Structure of some major stromal ECM proteins. Fibrillar collagens are composed of three chains that form a triple helix. The pro-peptides are cleaved for collagen assembly into fibrils. Fibronectin presents alternatively spliced domains, EDA, EDB, and the variable domain. Fibronectin dimerizes through two disulfide bonds in the C-terminal part of the protein. Members of the tenascin family display an assembly domain at the N-terminal to form hexamers (tenascin-C) or trimers (tenascin-X). Tenascin-C presents an alternatively spliced region within the fibronectin type III-like repeats. Periostin is composed of an EMI domain and four fasciclin 1 domains. 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 syndecan-3 have chondroitin sulfate (CS) chains in the ectodomain part close to the transmembrane domain. Small leucine-rich proteoglycans (SLRPs) display a tridimensional "banana" shape structure possible through the presence of two N-terminal and one C-terminal disulfide bonds. 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 or polylactosamine chains. Heparan sulfate, chondroitin sulfate, and keratan sulfate chains are composed of repeats of disaccharide units that could be sulfated (SO_3^-) at different locations as indicated in the figure

Due to the critical role of the TME in tumor growth and metastasis, attention must also be given to the role of fibrillar collagens in the tumor stroma. Some of the most provocative studies have addressed the role of collagen composition and processing and posttranslational modifications including cross-linking in regulating stiffness, tumor growth, tumor invasion, and metastasis [38–42].

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 and different types of inflammatory cells and vascular cells [43]. Cell-mediated collagen remodeling can be mediated by collagen-binding integrins and COLINBRI-binding integrins [34, 44]. The main integrin-collagen receptors for direct binding to the fibril form of fibrillar collagens are α2β1 and α11β1 [45]. They are both efficient in remodeling the collagen matrix, as assessed in floating collagen gel contraction assays [46]. This is a widely used assay to monitor the ability of cells to reorganize a fibrillar collagen I matrix, a process shown to be dependent on β1-integrins [46]. 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 and III via Fibril Associated Collagens with Interrupted Triple helices (FACIT) collagens [47]. In addition to the direct role of collagen-binding integrins in mechanotransduction to remodel the matrix, a role for matrix metalloproteinases (MMPs) to help and facilitate remodeling of the collagen matrix has also been demonstrated [48, 49]. 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 [50]. 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), and aligned collagen fibrils [50]. 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 tumor density and stiffness, which promote tumor growth and invasion), another independent study suggests that fibrillar collagen III has opposite effects [51]. In that study using collagen $III^{+/-}$ ($^{-/-}$ mice rarely survive perinatal age), it was demonstrated that mammary carcinomas grown in these mice were larger and more invasive and contained thicker, more organized, 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 switch.

Role of the Stromal Collagens in Tumorigenesis

In the TME, the epithelial-derived carcinoma cells are, to varying degrees, surrounded by basement membrane structures. As cells dedifferentiate 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 matrix metalloproteinases (MMPs) in this process [52–54].

Collagens Affecting Tumor Cell Growth

A number of studies have demonstrated that a collagen matrix promotes tumor growth. In the MMTV-PyMT breast cancer 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 cancer tumor growth and in increased collagen accumulation at the tumor site [55]. In subsequent experiments, collagen synthesis was blocked by inactivating certain enzyme isoforms, such as the intracellular enzymes prolyl 4-hydroxylase [56] and lysyl hydroxylase [57]. In the stroma, blocking these enzymes resulted in reduced collagen accumulation and reduced collagen stiffness. The result was an attenuation of breast and lung tumor progression and lung metastasis, thus supporting a role of fibrillar collagens in tumor growth. Of note, fibrillar collagens have been shown to induce apoptosis of tumor cells [58]. In one study, MMP-14 was demonstrated to protect invading mammary carcinoma cells from collagen apoptosis once they entered the fibrillar collagen I matrix [59, 60]. Identification of the underlying molecular mechanism for this effect may be a critical step in the further understanding of the role of the TME in tumor progression.

Collagens Affecting Cell Migration

Several studies have also reported MMP-dependent changes in fibril diameter. In one study, MMTV-PyMT mice crossed with mice genetically deficient in MMP-13 demonstrated no effect of MMP-13 depletion on breast tumor progression and lung metastasis [61]. Conversely, another study using a similar model observed a modest increase of lung metastasis in the absence of endogenous MMP-13 activity [62]. 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 [62].

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 [63]. 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 [64]. Interestingly, a study of wound healing in zebrafish revealed that increased levels of MMP-9 lead to larger fibril diameter. The authors suggest that this might be due to a switch in synthesis from collagen III to collagen I [65], 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 [66]. 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 protease-independent migration, in 3D matrices, depending on the matrix pore diameter [67, 68].

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 [6]. 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 increases in β1 integrin/FAK/ERK signaling in tumor cells, resulting in increased tumor growth [4]. It is worth noting that in nonexperimental tumors, LOX is produced by different cell types, not only by CAFs [69]. Moreover, the role of LOX has also received considerable attention in relation to the metastatic niche and tumor metastasis [42, 70, 71]. These studies have demonstrated that LOX is deposited and cross-links 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 [72–74].

LOX expression has also been associated with poorer patient prognosis in lung adenocarcinoma [75]. For example, it has been shown that downregulation of LOXL1 in xenograft tumors of non small cell lung cancer lines grown in α11 knockout SCID background reduce tumor growth compared to growth in wild-type SCID mice. The decrease in tumor growth was closely associated with reduced organization and stiffness of fibrillar collagen matrices (Fig. 4.2) [76].

In summary, collagen matrices that are rich in collagen I and comprised of large diameter fibrils seem to be required for optimal support tumor growth and metastasis. Furthermore, stiffer matrices composed 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 matrix, (2) LOX enzymatic activity for matrix cross-linking, and (3) MMPs to facilitate reorganization.

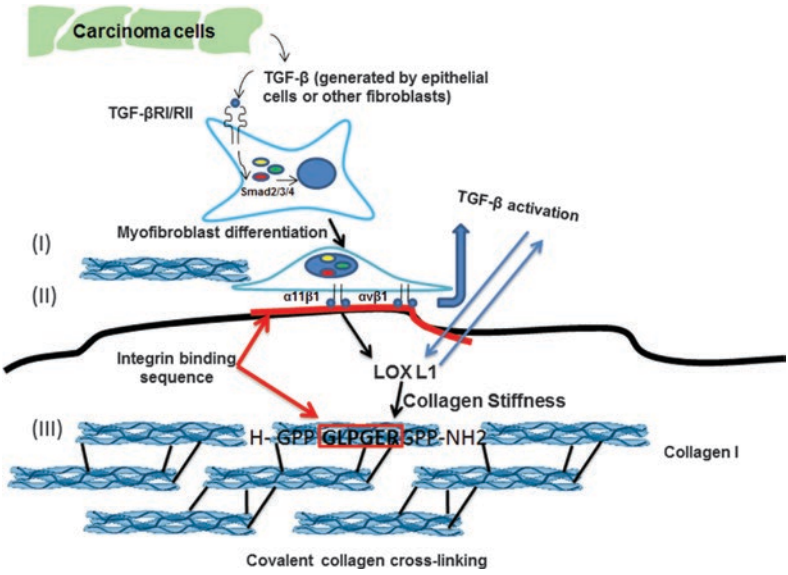

Fig. 4.2 Possible mechanism of tumor cell-stroma interactions in mediating tumorigenicity and metastasis. A number of soluble autocrine and paracrine mechanisms are likely to be involved in directly or indirectly stimulating the growth of the tumor cells. TGF-β plays a major role in tumor-stroma interactions. Excessive TGF-β activity is present in stromal, inflammatory, and cancer cells within a tumor, and the metastatic phenotype can develop when the epithelium overcomes the growth-inhibitory effect of TGF-β. TGF-β signaling induces fibroblast differentiation into contractile myofibroblasts (I). The myofibroblasts express and deposit collagen, express collagen-binding integrins (α11β1) and αv-integrins (αvβ1) that mediate collagen remodeling, and activate latent TGF-β from the matrix (II). Based on microarray differential gene expression analysis, it is possible that LOXL1, a fibrillar collagen cross-linking enzyme belonging to LOX family oxidases (LOXL 1–5), is under the regulation of integrin α11β1. Secreted LOX is responsible for the invasive properties of hypoxic human cancer cells through focal adhesion kinase activity and cell-to-matrix adhesion and is associated with collagen cross-linking and the organization and stiffness of fibrillar collagen matrices (III). MMPs collaborate with LOX to facilitate collagen maturation, and MMPs and LOX regulate the expression and activity of soluble factors such as TGF-β that regulate tumor cell behavior. TGF-β in turn regulates enzymes including LOXs (III), and TGF-β increases levels of factors that evoke inflammation, induce fibrosis, and promote metastasis

Still other experiments using two different experimental model systems that severely restrict production of mouse pancreatic tumor stroma have demonstrated that global obliteration of the stroma can result in tumors becoming more aggressive [20, 21]. 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 [50, 55] with the multiple studies suggesting that a stiff dense matrix promotes

tumor growth and tumor metastasis [4, 56, 57, 77]. These questions will need to be addressed in order to more fully delineate which pathways involved in collagen biosynthesis, posttranslational modifications, or collagen remodeling 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 [78, 79]. 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 [4, 76]. The fibrotic reaction observed in the stroma of many cancers, characterized by an excess accumulation of some fibrillar collagens (especially types I, III, V, XI) as a result of desmoplasia, is considered to be a hallmark of cancer [70, 80, 81]. There are multiple collagen receptors in addition to collagen-binding integrins, such as DDRs, leukocyte-associated Ig-like receptors (LAIRs), and glycoprotein VI [82]. These receptors are (1) not necessarily expressed on tumor cells or stroma cells (LAIRs on immune cells, Glycoprotein VI (GPVI) on platelets), and (2) unlike integrins their role as mechanoreceptors with the ability to reorganize collagen has not been established.

Fibrillar collagens 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 [83–85]. In SHG, an excitation wavelength of 840 nm is applied to a sample, and the resultant SHG signal is then measured, which is exactly one-half of the excitation wavelength (i.e., 420 nm). SHG polarization microscopy allows the structural details of collagen organization in the tissue to be studied, whereby for each orientation of incoming laser polarization, a set of outgoing SHG polarizations is measured revealing the second-order susceptibility component ratio in each pixel of the image. These measurements reflect the hierarchical organization of collagen in the tissue [86]. 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 [85]. 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 [87]. Hence, polarization SHG is a promising technique to detect collagen alterations in the ECM during cancer progression [88]. 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 [89]. 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 [90]. 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 [76].

Another method of studying the collagen linearity on a nanometer scale is electron microscopy, measuring how straight or "curly" an individual fiber is [91]. 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) [92, 93]. 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 [94]. To determine the elastic properties of collagen fibrils, the tip of the AFM (cantilever) was used as a nanoindenter by recording force-displacement curves [95]. It has been shown that a new variant of AFM, which is called *in situ* atomic force indentation microscopy [96], 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 [97].

Another technique of interest for measuring ECM and tissue stiffness at the macroscopic level is shear rheology [98]. At its simplest, this approach provides high-resolution determination of the matrix and tissue elasticity by measurements of mechanical compression and nanoindentation [98]. 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 [98]. 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 [99]. Hence, there is a need to develop methods to measure elastic properties and stiffness of tissues and matrix in a noninvasive manner for clinical application. Magnetic resonance and ultrasound elastography are routinely used tools in the clinic that provide the image contrast of elastic properties of tissues [100]. 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 [100]. Newer technologies based on fluorescence resonance energy transfer (FRET) [101],

magnetic resonance imaging (MRI), positron emission tomography (PET), and single-photon emission computed tomography (SPECT) [102] are being developed to image the dynamic status of ECM remodeling [103]. Advances in μ-ultrasound, optical coherence tomography (OCT), optical acoustic microscopy, and scanning acoustic microscopy (SAM) [98] are under development to facilitate imaging and quantitative measurement of stiffness at the microscopic scale [104]. 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 [105–107]. 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 noninvasively quantify remodeling of the ECM at the submillimeter 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 Fibronectin EDA in the Tumor Stroma

Fibronectin (FN) is a large modular extracellular matrix protein composed of type I, type II, and type III repeats [108]. 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 [109]. The EDA and EDB domains display 29% sequence identity but are each highly conserved among vertebrates [109]. 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 [110], but are almost absent in the adult organism where vascularization and tissue reorganization are quiescent. During wound healing [111] and fibrosis and in solid tumors [112], the EDA/EDB embryonic splice variants are reexpressed [113], leading to the term “oncofetal” splice variants. Some studies suggest that these embryonic splice forms in tumors are mainly expressed in neo-vasculature [114], whereas other studies demonstrated their presence in the fibrotic stroma associated with myofibroblasts [115, 116].

The EDA domain is composed of seven antiparallel beta strands separated by loops [109]. Early studies suggested that the presence of EDA in intact FN indirectly influenced the exposure of the RGD sequence in the 10th FN type III repeat leading to higher binding affinity for integrin α5β1 to FN EDA [117]. In later studies, it was demonstrated that integrins α9β1 and α4β1 bound directly to a cryptic loop region in an EDA-containing fragment but not to the intact FN EDA [118]. 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

[119]. 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 [120]. Importantly, FN EDA enhances TLR4 response, which in turn has been reported to augment TGF-β signaling [121]. 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 [111]. Endothelial cells on developing and adult lymphatic vessels also express α9β1 [122]. Depending on the relative levels of different receptors, the effect of FN EDA 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 [123, 124]. In contrast, mice lacking both isoforms die at E9–E10, due to cardiovascular defects and leaky blood vessels [125]. 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 [122]. However, other data suggests that Elastin Microfibril Interfacer 1 (EMILIN1) might play a more prominent role than FN EDA as an α9β1 ligand during lymph vessel development, especially in mature lymph vessels [126]. 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 [43].

Function of Fibronectin EDA 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 [127]. In another study, EDA induced a pro-fibrotic effect in dermal fibroblasts via binding to α4β1-mediated adhesion without affecting myofibroblast differentiation [128]. Similarly, studies of wound healing in an 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 [124, 129]. 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 [129]. 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 [130].

Function of Fibronectin EDA Domain in Fibrosis

A limited number of studies of EDA fibronectin function have been performed in fibrosis models. An *in vitro* study suggests that integrin α4β7 on lung fibroblasts stimulates myofibroblast differentiation [119]. In a mouse model, fibronectin EDA

deficiency prevented bleomycin-induced lung fibrosis [131]. 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 [132]. Finally, recent data suggests that FN EDA associated with TLR4 may play a role in keloids to couple a fibrotic response in the skin with an inflammatory response [121].

Function of Fibronectin EDA Domain in Tumorigenesis

In the context of tumors, *in vitro* and *in vivo* experiments have suggested different roles for fibronectin EDA. In colon carcinoma, FN EDA sustained tumor cell proliferation and induced lymphangiogenesis through VEGF-C secretion in mouse xenograft models [133, 134]. FN EDA has also been shown to induce EMT in lung and colon carcinomas, thus promoting metastasis [135, 136]. In a radiotherapeutic aspect, the presence of FN EDA reduced radiation sensitivity in head and neck carcinoma by inhibiting apoptosis of tumor cells [137]. Despite these findings, 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 [110].

In summary, FN EDA is highly expressed in granulation tissue, in fibrotic lesions, and in the tumor stroma. Critical analysis in genetic models demonstrated a moderate effect of FN EDA in wound healing and variable effects on myofibroblast differentiation in fibrosis models. It is thus notable that genetic lack of EDA was without effect in the tested tumor models. FN EDA in some cell models appears to influence myofibroblast differentiation and not in others. This might be related to the source of cells (embryonic origin, cell type, tissue, receptor repertoire expressed by cells). In the limited number of tumor studies performed in mouse models lacking EDA, the splice variant does not seem to be involved in inducing myofibroblast differentiation in the tumor stroma. Conditional deletion of both EDA and EDB forms in the tumor stroma is needed in order to exclude functional redundancy. Finally, the data from wound healing studies suggest that EDA during wound healing is more involved in stimulating recruitment of tissue fibroblasts to the area of fibroblast activation rather than affecting myofibroblast differentiation per se.

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). PGs play important roles during different aspects of cancer progression (for review, see [138–140]). Heparan sulfate PGs (HSPGs) 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 [141]. Protein binding is generally mediated by their sulfated GAG chains but may in a few cases involve interaction with core proteins [142]. 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, pleiotrophin, and the serine protease inhibitor antithrombin [143]. 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 HSPG GAG chains at the cell surface and becomes available for receptor activation only following enzyme-catalyzed release of specific sulfate groups from the heparan sulfate chains [144].

In addition to the direct effect of PGs 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 [145]. 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 [146, 147]. 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) [148]. In this chapter, we focus on the stromal PGs, the most characterized in the tumor context being shed syndecans and small leucine-rich proteins/proteoglycans (SLRPs), and summarize how their presence in tumor stroma influences cancer progression.

Syndecans

Syndecans are transmembrane heparan sulfate PGs with four members in vertebrates, syndecan-1 to syndecan-4. They are involved in diverse biological processes, such as regulating cell adhesion, cell migration, and cell differentiation as well as participating in the organization of ECM and the cytoskeleton [149]. Syndecans can serve as co-receptors 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 [150].

One interesting feature of syndecans is the shedding of the extracellular domain that enables syndecans to act as soluble factors [151], which plays an important role in tumorigenesis. The shedding occurs next to the plasma membrane and is processed by different MMPs: MMP-7 is involved in syndecan-1 and syndecan-2 shedding, and MMP-2 and MMP-9 can cleave syndecan-1, syndecan-2, and syndecan-4, whereas MMP-14 can cleave syndecan-1 and syndecan-4 [152–154]. The shedding is regulated by different growth factors and cytokines present in the tumor microenvironment, such as FGF-2 and TNF-α [155, 156]. In addition, heparanase, an enzyme that cleaves the heparan sulfate chains, regulates syndecan-1 expression and promotes syndecan-1 shedding, resulting in increased myeloma tumor growth [157].

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 [158]. This correlation was also observed in myeloma and bladder carcinoma [159, 160]. 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 [161, 162]. However, another study demonstrated an inhibitory effect of shed syndecan-1 on breast adenocarcinoma cell proliferation [163]. The study interestingly suggested the duality of membrane-bound and membrane-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 [163]. More recently, shed syndecan-2 has been shown to contribute to colorectal tumor growth and metastasis by upregulating MMP-7, suggesting a positive regulatory loop between these two proteins [164].

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 downregulates histone acetylation, leading to increased gene transcription [165]. This mechanism is suggested 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 [166]. Additionally, shed syndecan-1 contributes to chemotherapy resistance in colon cancer [167]. 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

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 [168]. 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) [148]. In this section, we will focus on the role of four SLRPs from classes I and II in tumor progression.

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 [169], decorin is often described as having antitumor properties, as listed below. Decorin expression is downregulated in bladder cancer [170], prostate cancer [171], lung cancer [172], and breast cancer

[173, 174] where a reduced expression is associated with poor survival [175]. Consistent with these observations, liver carcinogenesis was promoted in decorin-null mice [176]. Moreover, overexpression of decorin was shown to inhibit metastasis of prostate cancer [177], inhibit proliferation of bladder tumor cells [170], and inhibit colorectal carcinoma cell growth and migration [178, 179]. 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 downregulated [180].

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 [181]. Decorin can also antagonize Met, a receptor for hepatocyte growth factor, via degradation of β-catenin leading to reduced cell migration and invasion [182]. The decorin/Met axis appears to be required for the induction of an oncostatic mitochondrial protein, mitostatin [183]. In addition, decorin has been shown to bind and antagonize VEGFR2, inhibiting angiogenesis through endothelial cell autophagy [184, 185], and to bind IGF-IR to inhibit tumor cell migration and invasion [186].

Based on these observations, decorin is considered as a promising therapeutic protein in cancer progression treatment [177]. However, similar to syndecan-1, decorin has also been reported to induce resistance to some chemotherapeutics [187, 188].

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 [189, 190]. Moreover, biglycan was shown to promote migration and invasion of gastric carcinoma through FAK signaling activation [191]. However, biglycan also displays antitumor activity, inhibiting bladder carcinoma and pancreatic carcinoma cell proliferation [192, 193].

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 [194]. In tumor tissues, lumican is often overexpressed by stromal cells and/or tumor cells, and the correlation of its expression to malignancy is complex [195, 196]. In advanced colorectal cancer, Seya et al. have shown that lumican expression in tumor cells is associated with poor survival [197], whereas de Wit et al. have described a correlation with good survival in stage II patients [198]. In breast cancer, lumican expression was found to decrease with the progression of disease [199]. Consistent with this observation, high expression of lumican is associated with good survival in invasive stages of breast cancer [175]. 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 [200]. However, a recent study showed that lumican expression in pancreatic stroma was only correlated with good survival

after surgery [201]. 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 [202]. We suggest that these differences could be related to the secretion of different glycosylated forms of lumican in different cellular contexts.

The antitumor properties of lumican have mainly been reported in melanoma, where lumican is expressed in the peritumoral stroma [203] 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 [204–206]. More recently, lumican was defined as a new inhibitor of MT1-MMP in melanoma cells, thus inhibiting tumor environment proteolysis and invasiveness [207]. Antitumorigenic activities of lumican were also found in prostate cancer [208], in colon cancer by affecting tumor cell migration through upregulation of gelsolin [209], and in pancreatic cancer, in which lumican reduced EGF receptor expression resulting in reduced Akt signaling and tumor cell growth inhibition [201].

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 [210]. 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 antitumorigenic properties.

SLRPs also function to sequester TGF-β [211], 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 Nonobese diabetic/severe combined immunodeficiency (NOD-SCID) mice [212]. Interestingly, asporin expression is induced by TGF-β, thus asporin and TGF-β appear to regulate each other in an intricate feedback loop.

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 [213]. In cancer, matricellular proteins are involved in different steps of tumorigenesis due to their ability to bind different cell receptors [214]. 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.

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 [215]. Although all tenascin isoforms are expressed in different cancer forms, tenascin-C has been studied the most.

Tenascin-C is absent or lowly expressed in adult tissues, in contrast to the strong expression observed in cancer. Tenascin-C expression is induced in several solid tumors and is often associated with poor prognosis (for review, see [216]). It is clear that tenascin-C promotes tumorigenesis, acting at different steps of this process. On one hand, tenascin-C stimulates tumor growth by abolishing the cell proliferation-suppressing effect of fibronectin [217, 218]. Tenascin-C has also been demonstrated to compete with fibronectin for syndecan-4 binding, thus weakening breast carcinoma cell adhesion and spreading on fibronectin [219]; this cell adhesion inhibition leads to cell rounding that enhances tumor cell proliferation. On the other hand, tenascin-C can reduce apoptosis of pancreatic cancer cells, by activating the antiapoptotic Bcl-2 and Bcl-xL and inhibiting cleavage of caspase-3 [220].

Tenascin-C also stimulates EMT of breast cancer cells, in an αvβ1- and αvβ6-dependent manner [221, 222]. The Wnt/β-catenin signaling pathway, which is known to induce EMT [223], is enhanced in the presence of tenascin-C via the downregulation of the Wnt inhibitor Dickkopf 1, which stabilizes β-catenin [218, 224]. It is interesting to note that the tenascin-C 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 [225]. Furthermore, tenascin-C plays a role in tumor cell migration and invasion [226, 227]. 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 [228]. Interestingly, tenascin-C can also upregulate MMP-9 and MMP-13 expression in breast cancer, thus promoting cancer cell invasion [229, 230]. 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 [231]. A more recent publication demonstrated that in lung metastatic sites, tenascin-C is overexpressed by $S100A4^{+}$ stromal cells, most likely fibroblasts, supporting metastatic colonization [232]. In the same study, tenascin-C-null mice injected with 4T1 murine breast cancer cells displayed fewer and smaller metastatic lung nodules [232]. Another interesting study initiated by Oskarsson et al. showed that tenascin-C secretion by breast cancer cells is required to form a metastatic niche for the establishment of lung metastases [73].

Tenascin-W has been the last tenascin member to be described, and relatively little is known about this tenascin family member. Expression of tenascin-W has been shown to be regulated by TGF-β [233] and was initially observed to be strongly upregulated in the tumor stroma of breast and colon cancer patients [234, 235]. In the context of breast cancer, tenascin-W has been shown to promote

migration of breast tumor cells through interaction with α8β1 integrin [236]. 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 [237].

Tenascin-X is expressed in several tissues, with high expression in skin and skeletal muscle [238]. Deficiency or mutation in tenascin-X gene leads to a form of Ehlers-Danlos syndrome, characterized by skin and joint hyperextensibility [239]. In contrast with other tenascins, tenascin-X was first predicted to be antitumorigenic: its expression was strongly decreased in malignant melanoma [240], and mice deficient in tenascin-X displayed increased melanoma invasion and metastasis [241]. 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 [242]. Alcaraz et al. have suggested a different role of tenascin-X in breast cancer progression. In their study, tenascin-X was suggested to contribute to TGF-β activation via its interaction with α11β1 integrin, thus promoting EMT [243]. It will be interesting to determine if the binding of tenascin to α11β1 is direct, and if so, which part of integrin α11β11 binds to tenascin-X.

Periostin

Periostin is a matricellular protein, which is highly expressed in mesenchymal tissues during development [244]. Genetic deletion of periostin leads to tooth defects and a periodontal-like disease, which result in dwarfism [244]. Wound healing studies suggest a promoting effect of periostin in dermal myofibroblast differentiation and collagen gel contraction [245]. A pro-fibrogenic role for periostin in cardiac and skeletal muscle fibrosis has also been reported [246, 247]. Interestingly, periostin has been observed to interact with fibrillar collagen, and in the absence of periostin the collagen fibrillar diameter increases [248, 249].

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 [250]. Later studies have focused on the presence of periostin in the tumor stroma of gastric cancer, melanoma, and glioblastoma and in metastatic niches [251–253]. 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 [74, 254].

In summary, proteoglycans and matricellular proteins show different effects on tumorigenesis, sometimes with opposite effects in different tumor types. Figure 4.3 and Table 4.1 summarizes the role of stromal proteins in tumorigenesis and the experiments we have mentioned in the text, respectively.

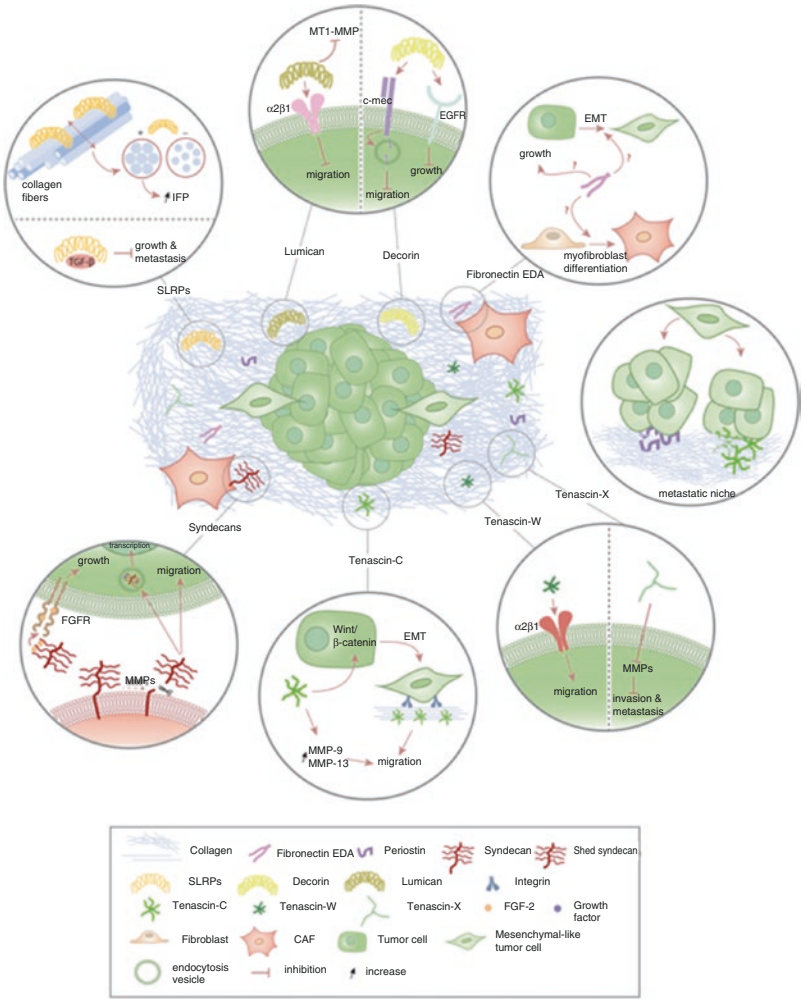

Fig. 4.3 Role of stromal ECM proteins in tumorigenesis. (**a**) SLRPs have a major function to regulate collagen fibrillogenesis. As shown for fibromodulin, SLRPs could increase thickness of collagen fibers resulting in increased interstitial fluid pressure (IFP). Some SLRPs such as asporin have the ability to bind and sequester TGF-β resulting in cancer growth and metastasis inhibition. (**b**) 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 c-met leads to internalization of the receptor and inhibition of cancer cell migration. Binding to EGF receptor (EGFR) leads to cell cycle inhibition. (**c**) The role of EDA fibronectin is uncertain; it has been shown to mediate EMT and to stimulate cancer growth; however, absence of EDA does not seem to affect tumorigenesis. (**d**) Syndecans at the cell surface of CAFs can be shed by MMPs to induce its effect on cancer cells. Syndecans as a cofactor 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. (**e**) Tenascin-C has been shown to contribute to EMT by stabilizing β-catenin. Tenascin-C also mediates cancer cell migration through upregulation of MMPs and by forming tubular structures with collagen and fibronectin. (**f**) Tenascin-W also mediates cancer cell migration but via interaction with integrins. In contrast, tenascin-X reduces invasion and metastasis by inhibiting MMPs. (**g**) Periostin and tenascin-C are needed in metastatic niches to support the metastatic colonization

Table 4.1 Role of some stromal ECM proteins for tumorigenesis based on experimental models

ECM protein	Knockout phenotype mice	Potential ECM receptor in tumor stroma	Localization in tumors	Effects in tumor context	Type of model
Fibrillar collagens					
Collagen I	Embryonic lethal, severe structural defects in connective tissues [255–257]	α2β1, α11β1	Stroma	Barrier Highway for metastasis	MMTV-PyMT crossed with Col1a1^{tmJae} mice [259] mammary tumors [55] Prolyly-4-hydroxylase alpha subunit-2 knockdown xenograft
Collagen III	Perinatal, lethal [258]	α2β1, α11β1	Stroma	Restrict tumor growth [51]	Mammary tumors [56] 4T1 xenografts in Col III$^{+/-}$ mice [51]
COLINBRIs:					
Fibronectin EDA	Normal Defective lymph vessels [122, 125]	α5β1 α4β1 α9β1 TLR-2/4	Biomarker for myofibroblasts in stroma [125]	No effect in Rip1-Tag2 model	Rip1-Tag2 model pancreas cancer, B16 melanoma xenograft FN EDA$^{-/-}$ mice [125]
Periostin	Tooth eruption defect [260]	αv-integrins (αvβ3, αvβ5)	Stroma	Reduced glioblastoma growth, reduced breast cancer metastasis to lungs, retain decorin, concentrate Wnt in stem cell niches [74, 253, 261, 262]	Xenograft glioblastoma stem cells with knockdown of periostin [253], PyMT breast cancer model with periostin$^{-/-}$ [254]

(continued)

Table 4.1 (continued)

ECM protein	Knockout phenotype mice	Potential ECM receptor in tumor stroma	Localization in tumors	Effects in tumor context	Type of model
Proteoglycans:					
SLRPs:	Collagen organization				
Decorin	Skin fragility [263]	Tyrosine kinase receptor [266]	Stroma	Promotes tumor suppressor genes and cellular adhesion molecules	Systemic injection in MDA-231 breast carcinoma xenograft [180]
Lumican	Skin fragility, cornea opacity [264]	α2β1 [205]	Stroma and tumor cells	Inhibited melanoma growth and invasion	Lumican-transfected B16 melanoma xenograft [271]
Syndecan-1	Normal [265]	Cooperate with integrins [267–269]	Stroma and tumor cells	-Shedding [162], increased angiogenesis [162, 270], reduced myeloma growth	Xenograft model CAG myeloma knockdown syndecan-1 [272]
Matricellular proteins:					
Tenascin-C	Viable, subtle defects hair follicles [273, 274]	Integrin ligand [276] and steric hindrance of integrin-mediated adhesion	Stroma	Important for metastatic niche function [74]	Xenografts of mammary carcinoma MDA231 and CN34 with knockdown tenascin-C [74]
Tenascin-W	ND	αvβ1, α4β1 [234]	Biomarker-activated perivascular stroma in solid tumors [237]	ND	ND
Tenascin-X	A form of Ehlers-Danlos syndrome [275]	αvβ3	Stroma	Restrict melanoma invasion and metastasis	Xenograft B16 melanoma knockdown tenascin-X [241]

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 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 review, 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, inter-tumoral and intratumoral heterogeneity is necessary in order to fully address the central questions: Tumor stroma, friend or foe? Barrier or support?

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