The Constituents and Potential Targets of the Extracellular Matrix: Implications for Carcinogenesis and Cancer Treatment

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Abstract

The dense extracellular matrix consists of a multitude of proteins with important implications in tumorogenesis that extend beyond the maintenance of tissue integrity. Several of the main macromolecular constituents- proteoglycans, collagens, integrins, and syndecans will be discussed in this review, with particular attention to their roles in tumor initiation, invasion, angiogenesis, and metastasis. In addition, a brief synopsis of the role of enzymes that remodel the extracellular matrix will be provided. Finally, specific examples of targeted molecular therapies: anti-integrin agents, MMP inhibitors, and hyaluronidase will be discussed.

Abbreviations: ECM- Extracellular Matrix; BM- Basement Membrane; PG- Proteoglycan; HSPG- Heparan Sulfate Proteoglycan; GAG- Glycosaminoglycan; MMP- Matrix Metalloproteinase; ADAM- A Disintegrin And Metalloproteinase; TIMP- Tissue Inhibitor of Metalloproteinases; VEGF- vascular endothelial growth factor; HFG- Hepatocyte Growth Factor; FGF2- Fibroblast Growth Factor 2; TGFβ- Transforming Growth Factor β; PDGF- Platelet-Derived Growth Factor; IGF- Insulin-Like Growth Factor; EMT- Epithelial-Mesenchymal Transition

Introduction

The biology of cancer is intricate and multifaceted. Much is known about genetic and epigenetic changes that transform normal cells into an aggressive malignant phenotype. However, cancer cells exist in a complex microenvironment, dependant on feedback and paracrine signaling from other tumor cells and from stromal cells such as fibroblasts, immune cells, and pericytes. In addition, the dynamic, reciprocal network of proteoglycans, polysaccharides, glycoproteins, growth factors, and other soluble molecules that constitute the Extracellular Matrix (ECM) also has a profound influence on carcinogenesis and metastasis. These main constituents of the ECM as well as their roles in tumorogenesis, invasion, angiogenesis, and metastasis will be discussed in this review. Specific ECM targeted molecular anti-cancer therapies currently under investigation will be highlighted.

Extracellular Matrix

Extracellular matrix (ECM) is the dense, often rigid, non-cellular environment that is in direct physical contact with cancer cells during all stages of tumorogenesis and metastasis. The components of ECM include: proteoglycans, glycoproteins, polysaccharides, water, and soluble factors [1,2]. According to *Hynes and Naba*, the "core matrisome" of proteins in the ECM is vast, numbering around 300 [3]. This milieu of macromolecules constantly undergoes post-translational modification, degradation, and remodeling by enzymes such as Matrix Metalloproteinases (MMPs). Typically, the term ECM is used to describe the interstitial matrix, or the space between cells. A more specialized version of ECM is located basolaterally to epithelial and endothelial cells, and is termed the Basement Membrane (BM) [4]. Besides forming the architectural scaffolding and imparting structural integrity to tissues, the ECM plays an important role in biochemical and mechanical signaling in the Tumor Microenvironment (TME). These cues modulate many aspects of carcinogenesis, from tumor formation to tumor migration and invasion to distant metastasis [1,2]. Communication between the ECM and cancer cells occurs directly through cell-ECM adhesion molecules such as integrins and syndecans and also indirectly through ECM bound growth factors and transmitted mechanical forces. The main macromolecules constituting the ECM as well as the adhesion molecules connecting cancer cells to the ECM will be described in the following sections.

Macromolecules of the ECM

Proteoglycans

Proteoglycans (PGs) are composed of a core protein covalently attached via serine residues to long unbranched polysaccharides made up of repeating disaccharide units- glycosaminoglycan chains (GAGs). These GAGs are highly sulfated imparting a net negative charge that attracts water as well as cations and creates a hydrated gel-like environment that permits resistance to compressive forces and sequesters soluble growth factors. There are five known GAGs: heparan sulfate, chondroitin sulfate, dermatan sulfate, keratin sulfate, and hyaluronan. All exist bound to a protein core except hyaluronan, also known as hyaluronic acid (HA), which is released into the ECM independent of the Golgi apparatus and exists bound to the matrix and to the cell surface via CD44 receptors. PGs can be divided into four main families: glypicans (six heparan sulfate PGs covalently anchored to the cell surface via GPI-glycosylphosphatidylinositol), syndecans (four primarily heparan sulfate or chondroitin/dermatan sulfate transmembrane PGs), lecticans (four chondroitin or keratin sulfate soluble PGs located extracellularly), and SLRPs- small leucine-

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rich repeat proteoglycans (six chondroitin/dermatan sulfate or keratin sulfate PGs also located extracellularly). There are many PGs that are not members of a specific family, for example, CD44, thrombomodulin, perlecan, endocan, and type IX collagen among others [5,6].

In tumorogenesis, much attention has been paid to the ubiquitous heparan sulfate proteoglycans (HSPGs) [7]. The repeating disaccharide units in HSPGs are composed of glucuronic acid and N-acetylglucosamine. Complexity is conferred to these molecules by epimerization, sulfation, and de-acetylation [6]. The main types of interstitial matrix HSPGs are glypicans and syndecans, while examples of BM HSPGs are collagen type XVIII, perlecan, and agrin [1,5]. In addition to contributing to tissue integrity via their interactions with collagens and other glycoproteins such as fibronectin and laminin in the ECM, HSPGs also mediate cellular uptake of growth factors and other soluble ligands and function as co-receptors for multiple ligands. They mediate important cell signaling pathways involved in cellular growth, proliferation, and migration [7]. Multiple studies also implicate HSPGs as extracellular reservoirs for growth factors, chemokines, morphogens, and cytokines. In this bound form, these soluble factors are inactive and unable to influence their target cells. However, cleavage of HS by heparanase (an endoglucuronidase) results in the release of HS bound factors such as Vascular Endothelial Growth Factor (VEGF), Hepatocyte Growth Factor (HGF), Fibroblast Growth Factor 2 (FGF2), Transforming Growth Factor β (TGFβ), and Platelet-Derived Growth Factor (PDGF), which can potentiate tumor cell growth and invasion as well as angiogenesis and metastasis [6,8]. Studies have shown that in melanoma, for example, the release of HS by heparanase promotes melanoma tumorogenesis and local angiogenesis [8]. Elevated heparanase levels have been detected in many other cancers, and have been correlated with invasion and metastasis [9]. However, non-enzymatic functions of heparanases in carcinogenesis have been described [6].

Another class of enzymes that act on HSPGs, sulfatases (Sulfs), is also frequently dysregulated in cancer. These enzymes modify the sulfation status of HSPGs by selectively removing a sulfate group from the 6-O position, resulting in altered binding of ligands. While classic theory holds that Sulf-1 is a tumor suppressor and Sulf-2 is pro-tumor, Sulf-1 and Sulf-2 levels have been shown to be both up-regulated and down-regulated in human cancers [10,11]. Thus, the role of HSPGs in carcinogenesis is highly complex.

Collagens

Collagens are a group of 28 fibrous proteins that exist as supramolecular complexes of triple-stranded α-helices. Post-translationally, hydroxylation occurs at proline and lysine residues, while glycosylation occurs at hydroxylysine residues [12]. These fibrils and networks are further organized into sheets and cables by fibroblasts and provides tensile strength to the ECM and BM [1]. The main collagen components of the interstitial matrix are types I and III, whereas collagen type IV is abundant in the BM [4]. Collagens regulate cell adhesion to the ECM by binding to cell surface adhesion molecules including integrins, syndecans, and other PGs. The ECM of cancer is characterized by a dense, rigid, parallel orientation of collagen fibers that promotes epithelial tumor cell migration [12]. Stiffness of the microenvironment is created in part by covalent crosslinking of collagen fibers, facilitated by lysyl oxidase enzymes (LOX and LOXL1-4). These copper dependent enzymes are upregulated in many cancers, especially under hypoxic conditions, and their overexpression results in a rigid ECM that is tumorogenic and supportive of invasion and metastasis. The LOX

dependent collagen crosslinking up-regulates the formation of integrin focal adhesion complexes stimulating downstream signaling, further demonstrating the complex dynamic interactions that occur during tumor development and progression [13]. Studies have shown that up-regulation of LOX occurs in more invasive and aggressive cancers, and correlates with poor prognosis [12,14,15].

Desmoplasia, or fibrotic reaction, driven primarily by stromal fibroblasts, either resident or recruited, represents an important step in tumorogenesis. ECM proteins, including collagens, are laid down in an extensive, dense network and facilitate directional migration of tumor cells through this collagen "highway" [12,13]. Using a pancreatic cancer model, one study reported that stromal fibroblasts expressing the protease- Fibroblast Activation Protein (FAP) promote the formation of an organized and parallel network of collagen that enhances velocity and directionality of invading tumor cells in a β1-integrin/FAK dependent fashion [16]. In addition, the mechanical force of this stiff matrix is sensed and transmitted via integrin receptors, resulting in increased epithelial tumor cell proliferation [12,13].

Collagen fibril formation is also greatly influenced by the glycoprotein fibronectin, which can bind directly to collagen fibers [12,17]. In many cancers, fibronectin dimer production by stromal fibroblasts is upregulated. These globular dimers associate with α5β1 integrins on the cell surface and the protein is stretched and elongated into a more linear form, revealing cryptic binding sites on fibronectin that when exposed may further contribute to fibril strength and rigidity [18]. Thus, the relationship between collagen fibril formation and fibronectin fibrillogenesis is reciprocal in nature and involves integrin receptors [17].

ECM-Cell Adhesion Molecules

Integrins

Integrins are a class of 24 distinct heterodimeric cell-surface glycoproteins composed of α and β subunits. Multiple classifications exist for integrins, but generally revolve around binding specificities. They are characterized by the presence of multiple binding and activation sites and are known to interact with a multitude of cell adhesion molecules, other proteins of the ECM, and growth factors [19-21]. This complex integrin network, or "integrin adhesome" consists of 156 linked proteins and lipids characterized by 690 interactions (379 binding, 213 activation, and 98 inhibitory) [22].

Integrins do not possess intrinsic enzymatic activity, but they facilitate cell adhesion to the ECM and mediate "inside-out" signal transduction between the ECM and cancer cells through non-enzymatic mechanisms. When bound to other proteins or growth factors in the ECM, integrins undergo receptor clustering and conformational changes that expose effector-binding sites. The result is the activation of cytoplasmic kinases, such as the phosphorylation of Focal Adhesion Kinase (FAK), which results in the transmission of signals to the cell's nucleus. These transmitted signals modulate cell migration, proliferation, and metastasis [19-21]. In addition, integrin signaling pathways regulate polymerization and de-polymerization of the actin cytoskeleton at the leading edge of cells, necessary for cell spreading on the ECM [20].

A major class of integrins up-regulated in the ECM of many tumors is arginine-glycine-aspartate (RGD) binding integrins. Examples of this family implicated in cancer and angiogenesis include: αvβ3, αvβ1, αvβ5, and α5β1. Tumor cells deposit glycoproteins containing the RGD sequence (ex. fibronectin and vitronectin) into the ECM during

the initiation of tumorogenesis. These protein-protein interactions promote tumor formation and migration [23,24].

Syndecans

Syndecans are a class of transmembrane HSPGs that are crucial for cell-ECM adhesion and paradoxically, also cell migration. Four syndecans (syndecan-1, -2, -3, and -4) have been characterized, but syndecan-1 is currently the most described in cancer literature [25]. It has been shown that basolaterally expressed syndecan-1 functions to anchor epithelial cells by connecting the ECM to the actin cytoskeleton via its associations with collagens, fibronectin, and thrombospondin. Investigators have also demonstrated that syndecan-1 associates with integrins and stabilizes focal adhesion complexes. The expression of cell surface syndecan-1 on epithelial cells promotes cell adhesion and prevents cell locomotion and migration [26]. Somewhat paradoxically, the expression of syndecan-1 on stromal fibroblasts actually promotes tumor cell migration. In models of breast cancer, it has been shown that syndecan-1 positive stromal fibroblasts orchestrate the formation of an organized ECM with collagen fibers in a parallel orientation, which promotes directional migration and invasion of cancer cells [25].

Syndecan-1 is also implicated in cancer progression via another mechanism. The syndecan proteins have an extracellular domain (ectodomain) that can be cleaved or shed by sheddases, which are membrane-bound enzymes of the MMP or A Disintegrin And Metalloproteinase (ADAM) families [26,27]. For example, one study reported that in vitro, membrane type matrix metalloproteinase-1 (MT1-MMP also termed MMP-14), cleaves a Gly245-Leu246 peptide bond that results in the shedding of syndecan-1 and the stimulation of cell migration [28]. When this ectodomain is shed, this soluble protein promotes migration and angiogenesis. In fact, high levels of shed syndecan-1 in patients' serum of many cancers have been correlated with a poor prognosis. For example, in multiple myeloma, heparanase expression in the bone marrow, which results in the shedding of syndecan-1, is a bad prognostic factor [29]. Another study suggested that levels of shed syndecan-1 in the serum might even be an independent prognostic marker in multiple myeloma [30]. One proposed mechanism of how shed syndecan-1 participates in carcinogenesis is through its binding to angiogenic growth factors, VEGF and FGF-2. Receptors such as VEGFR and integrins on endothelial cells can recognize these growth factor-syndecan-1 complexes and initiate a process of endothelial budding and invasion eventually leading to neovasculature formation [26].

ECM Remodeling and Degradation

MMPs/TIMPs

The ECM of tumors is continually undergoing degradation and remodeling by proteases. The primary proteases involved are members of the MMP family, zinc dependent endopeptidases. Currently over 20 members have been identified and are divided into four main groups: collagenases, gelatinases, membrane type, and stromelysins. Other MMPs such as matrilysin (MMP-7) do not readily fit into any of the above groups. Both tumor and stromal cells secrete mMPs as inactive zymogens in the form of pro-MMPs and are activated in the ECM by other proteases or by already active MMPs. The mechanisms and functions of MMPs in tumorogenesis, angiogenesis, and metastasis are diverse [31-33].

Normal tissue homeostasis is maintained by tight control of MMP activity, however in tumorogenesis, cancer cells exploit the function of these enzymes to promote an invasive and metastatic phenotype

[31-33]. In addition, when normal epithelial cells lose their connection to the ECM they undergo anoikis (programmed cell death), which is triggered by detachment. Cancer cells, on the other hand, have evolved mechanisms to avoid anoikis, allowing them to survive even after MMPs disrupt their attachments to the ECM and to each other [34].

Two tumorogenic MMPs that have been extensively studied in cancer are the gelatinases: MMP-2 and MMP-9. These MMPs and others cleave ECM protein-growth factor complexes and release bound factors, such as VEGF, TGFβ, and IGFs, which can then bind to growth factor receptors and promote tumor proliferation and angiogenesis [35,36]. MMP-9 has also been shown to interact with the αvβ3 integrin receptor in breast cancer [31]. In addition, MMPs are involved in the disruption of epithelial and endothelial cell basement membranes, which promote cell migration and angiogenesis [31-33]. For example, cleavage of type IV collagen and laminin in the BM uncovers cryptic binding sites for growth factors and other proteins that promote tumor cell growth, migration, and angiogenesis. [37] On the other hand, cleaved products from type IV collagen are also known to be anti-angiogenic [38].

Another important step of tumorogenesis promoted by MMPs as well as ADAMs is the degradation of cell-cell adhesion molecules such as cadherins, a family of calcium binding transmembrane glycoproteins that form adherens junctions between epithelial cells. The down-regulation of E-cadherin, a process regulated by many factors in addition to MMPs and ADAMs, results in the translocation of β-catenins to the nucleus and the polymerization of the actin cytoskeleton into stress fibers. A more motile mesenchymal-like cell now characterizes the invasive and metastatic phenotype [31]. This phenomenon termed the epithelial-mesenchymal transition (EMT) is mediated largely by TGFβ. Other proteins such as N-cadherin, vimentin, tenascin-C, and fibronectin are up-regulated in the mesenchymal phenotype. Further details of EMT are beyond the scope of the discussion in this review [39-41].

The proteolytic degradation and remodeling of MMPs is inhibited by the activity of four tissue inhibitors of metalloproteinases (TIMP1-4). The N-terminus of TIMPs binds to the catalytic domain of MMPs and inhibits their activity. In tumorogenesis, the balance of MMPs and TIMPs is altered, and decreased expression or down-regulation of TIMPs promotes tumor metastasis as well as angiogenesis [31].

As mentioned above, one class of MMPs is the membrane type MMPs that are expressed on the cell surface. MMP-14 or MT1-MMP is a well-studied member of this class. This isoform functions to degrade collagen types I, II, and III as well as fibronectin, laminins, vitronectin, and aggrecan. It has been shown to be present on the motile edge of tumors and serves to cleave the hyaluronan receptor CD44, promoting cell migration [42]. In fact, elevated levels of soluble CD44 have been detected in the plasma of patients with metastatic cancer [43]. In addition, MT1-MMP, in conjunction with TIMP-2 recruits and activates pro-MMP-2. This paradoxical pro-tumor effect of TIMP-2 is attributed to its C-terminal domain [31,33,44,45].

ECM-Targeted Molecular Anti-Cancer Agents

Anti-Integrin Drugs

Goodman and Picard, in their review on anti-integrin agents, highlighted the properties of integrins, specifically their cell-surface location and their sensitivity to blockade that make them attractive therapeutic targets. Approximately 15 anti-integrin agents were currently in active clinical trials as of 2012 [23]. Cilengitide, the only

anti-integrin drug in a Phase III trial- CENTRIC (clinicaltrials.gov, NCT00689221), is a small molecule drug with an RGD sequence that inhibits both αvβ3 and vβ5 integrin receptors. This drug has shown favorable safety profiles in earlier trials, however, in February of 2013, Merck reported that the study failed to meet its primary endpoint of a survival advantage in the group receiving cilengitide in conjunction with standard chemoradiotherapy compared to the group receiving only standard chemoradiotherapy in patients with glioblastoma multiforme [46]. The full results of the study are not yet available, but will reportedly be available in June 2013. Currently, other Phase I and II trials using cilengitide as a therapeutic for multiple other cancers are in various stages of development [47-50]. (See also: NCT01276496, NCT01118676, NCT00705016, NCT00077155)

Other integrin inhibitors demonstrating potentially promising results in Phase I and II clinical trials include: 1. Etaracizumab (MedImmune), a monoclonal antibody to αvβ3 integrin; 2. Volociximab (PDL BioPharma/ Biogen Idec), a monoclonal antibody to α5β1; 3. ATN-161 (Tactic Pharma), an oligopeptide that inhibits αvβ3, α5β1, and αvβ5 in an RGD-independent manner; 4. Intetumumab (Janssen Biotech), a monoclonal antibody to αv integrin; and 5. DI17E6 (Merck KGaA/EMD Serono), also a monoclonal antibody to αv integrin.

Etaracizumab (MEDI-522) is currently under investigation in melanoma (NCT00066196) and advanced prostate (NCT00072930) and colorectal cancer (NCT00027729). A randomized phase II open-label trial comparing the combination of etaracizumab and dacarbazine versus dacarbazine alone in patients with metastatic melanoma, however, showed no benefit in time to progression or progression free survival with the addition of etaracizumab [51]. Volociximab is in various stages of safety and efficacy trials in melanoma (NCT00099970), non-small cell lung (NCT00654758), renal (NCT00100685), ovarian (NCT00516841), and pancreatic cancer (NCT00401570). However, a phase II study showed progression of disease in 13 of 14 patients receiving monotherapy volociximab for platinum-resistant ovarian or peritoneal cancer [52].

Preliminary data from a phase I trial of ATN-161 in 26 patients with solid tumors showed the drug was well tolerated [53], and a current trial is underway in malignant gliomas (NCT00352313). Also, according to Tactic Pharma, phase II trials in head and neck cancer as well as glioblastoma multiforme are in the planning stages. A randomized phase II trial (NCT00246012) in metastatic melanoma patients of intetumumab administered alone or in combination with dacarbazine showed no significant difference in progression free survival [54]. Another study, also phase II (NCT00537381), however, showed inferior progression free survival in patients who received intetumumab in combination with docetaxel and prednisone compared to docetaxel and prednisone alone in patients with metastatic castration-resistant prostate cancer [55]. Finally, as of April 2012, a phase II study (PERSEUS, NCT01360840) of DI17E6 (also referred to as EMD 525797) had enrolled 106 patients with metastatic castrate-resistant prostate cancer. Results of that study are not yet available [56].

Anti-integrin therapy is still in its infancy, and there are still many unanswered questions regarding the therapeutic potential for drugs like Cilengitide. The reasons for failure of many of the above-mentioned studies are likely not a simple answer. There is concern about target specificity, as normal cells express integrin receptors as well. In addition, preclinical animal data has raised concerns that anti-integrin agents might actually increase tumor growth and angiogenesis [57]. Hersey at al, also point out that determining the optimal dose of anti-integrins is crucial, and hypothesize that higher doses of etaracizumab (>10 mg/kg) might have produced a survival difference in their study [51]. Finally, the majority of these trials have been conducted in advanced stage cancers. Perhaps anti-integrin therapy might prove more beneficial if given early on in carcinogenesis. Future studies using these targeted molecular therapies will hopefully answer these questions.

MMP Inhibitors

Unfortunately, despite the fact that MMPs are up-regulated in almost all human tumors and play a vital role in tumor progression via matrix remodeling and digestion of cell adhesion molecules, the results from clinical trials looking at MMP inhibitors have been largely disappointing. Early studies showed that with prolonged usage, MMP inhibitors resulted in significant musculoskeletal pain and inflammation. This toxicity was reduced by the development of drugs with diminished activity against sheddases. However, Phase III clinical trials have failed to show therapeutic benefit or improved survival [32,58-60]. A phase III trial (NCT00004199) comparing prinomastat in combination with gemcitabine-cisplatin in patients with non-small cell lung cancer was closed early due to lack of efficacy [58]. A randomized phase III trial of tanomastat was conducted after administration of chemotherapy to patients with ovarian cancer, but failed to demonstrate any prolongation in progression free survival [59]. Marimastat, administered after chemotherapy in a phase III trial (NCT00003010) to patients with stable metastatic breast cancer, also failed to prolong progression free survival [60].

These results were especially discouraging as preliminary animal research using MMP inhibitors showed promising results. *Coussens et al.* points out, however, that when MMP inhibitors were used in animal models of cancer they were generally given early on in tumor formation, whereas in human studies the majority of patients had advanced stage or even metastatic disease [32]. Thus, MMP inhibitors may actually be useful in treating human cancer if given earlier on in disease progression, but this remains to be proven.

Enhancing drug delivery through the ECM

The desmoplastic reaction that results in a dense and stiff ECM not only promotes tumor progression by creating cell-ECM interactions but also provides chemoresistance to the developing tumor. Intratumoral vasculature is leaky and the lack of functional lymphatics contributes to this chemoresistance by raising the interstitial fluid pressure [12]. The presence of elevated amounts of HA in most cancers, a polysaccharide that attracts water, expands the matrix volume, and increases viscosity, further increases interstitial fluid pressure, creating issues with hematogenous chemotherapeutic drug delivery. It is also thought that the anionic nature of HA prevents adequate tumor penetration by chemotherapeutic agents [61]. Finally, it is likely that collagen fibers bind to and sequester drugs, further preventing their action in the tumor microenvironment [12].

Several studies carried out in animals have shown that there is a reduction in tumor volume with inhibition or depletion of stromal components, such as fibroblasts, that are primarily responsible for the fibrotic reaction in the ECM [62,63]. Others have demonstrated that inhibition of signaling pathways that potentiate stromal desmoplasia transiently increases the delivery of chemotherapeutic agents [64]. A temporary reduction in interstitial fluid pressure and an associated increase in delivery of antibodies were seen with collagenase and hyaluronidase in an osteosarcoma xenograft model [65-67].

Despite many promising animal models, enzymatic modification

Constituent	Classes/Families	Example Members	Proposed roles in cancer
Proteoglycans	Glypicans	Glypican 1-6	cell-ECM adhesion, proliferation
	Syndecans	Syndecan 1-4	*Cell surface*- cell-ECM adhesion, sequestration of growth factors *Shed ectodomain*- invasion, migration, angiogenesis
	Lecticans	Aggrecan, Versican, Brevican, Neurocan	structural integrity, aggregation
	SLRPs	Decorin, Biglycan, Fibromodulin, Podocan, Keratocan, and others	cell-ECM adhesion, fibrillogenesis of ECM proteins, growth factor sequestration and signaling, cell proliferation, migration, metastasis
	Others	CD44, Perlecan, Type IX collagen, Endocan, and others	cell surface co-receptors for growth factor signaling, migration, structural integrity, angiogenesis
Collagens		Type I, III	interstitial matrix, cell-ECM adhesion, facilitate directional migration, role in interstitial pressure
		Type IV, XVIII	basement membrane matrix
Integrins	RGD binding	α5β1, αvβ3, αvβ5, αvβ1, αvβ6, αvβ8	cell-ECM attachment and "inside-out" signaling, migration, invasion, metastasis, EMT
	α4 family	α9β1	cel-ECM adhesion, tumor growth, migration, invasion, angiogenesis, EMT
	I domain: collagen binding	α1β1, α2β1, α11β1	collagen fibril formation and polymerization
	LN binding	α6β4	migration, invasion, metastasis

Table 1: Specific examples of these ECM constituents and their reported roles in cancer.

of the ECM to improve drug delivery has been plagued with issues in human studies. For example, early studies using bovine hyaluronidase resulted in a high number of allergic and anaphylactic reactions. In addition, use of collagenases has been limited by its lack of specificity [61]. Currently, though, recombinant human hyaluronidase, Hyelenx®, is FDA approved for subcutaneous hydration and to enhance delivery of injectable drugs. In cancer research, Halozyme Therapeutics has developed a subcutaneous form that is under investigation, as Chemophase in superficial bladder cancer in early phase studies (NCT00782587, NCT00318643) in conjunction with Mitomycin C and as PEGPH20 in combination with gemcitabine for stage IV pancreatic cancer (NCT01453153). It has been shown that the addition of PEGPH20 to gemcitabine depletes HA, normalizes interstitial fluid pressure, increases tumor vasculature, decreases tumor size, decreases metastatic burden, and prolongs survival in a murine spontaneous pancreatic cancer model [68]. The hope is that the addition of PEGPH20 to chemotherapeutics will have the same positive results in human trials.

Additionally, Hoffman LaRoche has been granted a license by Halozyme to combine the recombinant human hyaluronidase with trastuzumab in the form of a subcutaneous injectable Herceptin®. A recently resulted phase III trial (NCT00950300), the HannaH study, demonstrated non-inferiority of the subcutaneous Herceptin® compared to the traditional IV Herceptin® in female patients with early stage HER2 positive breast cancer [69-70]. Two more trials, PrefHer (NCT01401166) and SafeHer (NCT01566721) are actively recruiting participants to analyze patient preference as well as drug delivery devices for subcutaneous Herceptin®.

Conclusion

The ECM, or the dense non-cellular component of tumors, is an important contributor to tumorogenesis and metastasis. Macromolecules such as proteoglycans, collagens, integrins, and syndecans, originally thought to function primarily in structural support have been found to participate in numerous diverse ECM-cell signaling pathways that promote tumor migration, invasion, and metastasis as well as the formation of neovasculature. The accompanying table (Table 1) provides specific examples of these ECM constituents and their reported roles in cancer. This table is by no means an exhaustive list of ECM members or function; interested readers should see cited reviews and other excellent reviews not cited due to space limitations.

Future studies should focus on improving current methods as well as developing new approaches to target these molecular components of the tumor microenvironment.

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