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Matrikine and matricellular regulators of EGF receptor signaling on cancer cell migration and invasion

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Cancer invasion is a complex process requiring, among other events, extensive remodeling of the extracellular matrix including deposition of pro-migratory and pro-proliferative moieties. In recent years, it has been described that while invading through matrices cancer cells can change shape and adapt their migration strategies depending on the microenvironmental context. Although intracellular signaling pathways governing the mesenchymal to amoeboid migration shift and vice versa have been mostly elucidated, the extracellular signals promoting these shifts are largely unknown. In this review, we summarize findings that point to matrikines that bind specifically to the EGF receptor as matricellular molecules that enable cancer cell migrational plasticity and promote invasion.
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The major part of cancer morbidity and mortality results from both metastatic dissemination and invasion from the primary tumor. The extracellular matrix (ECM) is the first obstacle that solid tumors encounter during this spreading. Matrix remodeling during tumor invasion does not only involve proteolytic degradation of the barrier but also a concomitant synthesis of bioactive matrix molecules, in a process resembling active matrix remodeling during wound healing. This new matrix environment in turn promotes and nurtures cancer cell spreading. Both tumor and stromal cells contribute to these changes, with the matrix components secreted by the tumor cells varying significantly in conjunction with their metastatic potential.[1] Many of these proteins have profound effects on cell morphology, inducing weaker states of cell adherence and thus promote migration. In this review, we will concentrate on the recent findings that point toward dysregulation of ECM components that promotes invasion of cancer cells from the primary site by enabling plasticity of migration strategies. In particular, this focused brief missive emphasizes ECM proteins that can bind to the epidermal growth factor receptor (EGFR), thus, subsuming established signaling networks that govern effective migration in various conditions. The wealth of other matricellular molecules that are implicated in carcinogenesis and metastasis, often by regulating adhesion and migration and thus invasion, have been elegantly reviewed elsewhere.[2,3]

The EGFR signaling axis is the growth factor system most often implicated in tumor progression via upregulation or activation of the receptor or of its numerous ligands.[4] Even though EGFR activation by its traditional soluble ligands leads to both mitogenesis and motogenesis, it is the motility that correlates to tumor progression.[5–7] More recently, EGFR activation by cryptic, ultralow affinity ligands, embedded within ECM molecules, has been recognized.[8,9] These matrikines limit EGFR signaling to the perimembrane area of the cytosol, a mode that is preferential for motility[10,11] and cell survival.[12,13] As these matrix components are upregulated during cancer progression, the role of EGFR in altering tumor behaviors is being re-examined in terms of such ECM-embedded signaling.[14–16] This perspective aims to provide the background for addressing such questions.

Cancer cell invasion programs

Cancer cells are known to use both protease-dependent and protease-independent invasion strategies. During the mesenchymal mode of invasion, in the presence of proteases that can degrade the surrounding ECM,[17,18] movement of cells is a multi-step process: (1) cell polarization and initial protrusion are followed by the attachment at the base of the leading edge to the ECM, (2) the cell surface-localized degradation of ECM generates space into which actomyosin contraction will move the advancing cell body deforming both the cell and the ECM;

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finally, (3) retraction of the cell rear and turnover of adhesions occur.[19,20] On the other hand, in the absence of significant proteolytic activity or an inability to degrade the surrounding substrate, cancer cells can still invade by using actin contractile force to generate a rounded morphology and amoeboid bleb-like protrusions that push and squeeze cells through spaces in the ECM.[17,18,21–23] This type of migration is possible if both the cell body deformability and the porosity of the matrix match so that the cells can fit through the spaces. Cytoskeletal organization and cell adhesion are modulated by the rigidity of the ECM, density and gap size, and orientation of fibers.[19] In 3D stiff matrices, mesenchymal migratory force generation is β1 integrin dependent,[18] whereas soft matrices do not reinforce focal adhesion (FA) formation and fail to support cell rounding.[24]

Signaling integrators that control the adoption of either the mesenchymal or the rounded mode of migration are the Rac and Rho/ROCK signaling pathways.[17] In the mesenchymal mode of migration, formation of actin-rich lamellipodia is Rac1 dependent.[25–27] Cdc42 and Rac regulate WASP/WAVE proteins that promote the nucleation of actin filaments and the formation of the leading edge.[28,29] Superimposed upon these is the small GTPase Cdc42, which provides for directionality.[30,31] The protruding leading edge is then stabilized by integrin interactions with the ECM and the formation of FAs. Rho and its downstream effector ROCK have been shown to be dispensable for the mesenchymal mode of migration,[17] in a situation where Cdc42 can compensate the loss of Rho/ROCK signaled contractility.[32] In contrast, the rounded mode of motility is dependent on Rho and ROCK activity,[17] where ROCK-dependent myosin light chain (MLC) phosphorylation is crucial for the correct organization of MLC and force generation within the moving cell.[22] Phosphorylated MLC increases ATPase activity to promote actin–myosin interactions and contractile force generation. The intracellular pressure results in the rupture of the actomyosin cortex and the formation of membrane blebs.[33] After the formation of the bleb, the contractile cortex re-assembles.[34] One major difference between mesenchymal and amoeboid movement is therefore the driving force for the formation of protrusions, which are actin polymerization and cytoplasm inflow, respectively. The silencing of ROCK pathway induces an amoeboidal to mesenchymal shift[17,35] and the silencing of Rac induces cells to attain the opposite morphology.[35,36] This mesenchymal to amoeboid transition is summarized in Figure 1.

This plasticity of migrational modes allows great adaptation during invasion and although underlying cellular machinery has been extensively studied, only recently has attention been given to the ECM signals that might induce these shifts.

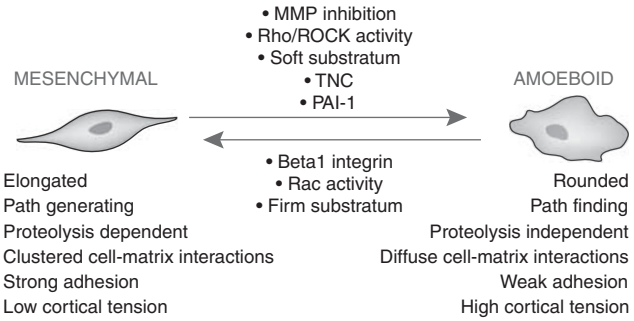

Figure 1 Mesenchymal and amoeboid cell phenotypes. The summary of phenotypic characteristics and known signaling controls that govern mesenchymal to amoeboidal shift.

Matricelular proteins as regulators of migration

The concept of matricellular proteins has been proposed in order to define protein domains from the ECM, which can signal to the surrounding cells.[37] It has become appreciated that some of these signals can be provided by cryptic sites within ECM molecules, which are revealed/accessible to cells only after structural or conformational alterations in the components.[38,39] In the course of tumor invasion, matrix alterations because of ECM denaturation, enzymatic breakdown, mechanical forces or protein multimerization and adsorption provide for a plethora of matrycriptic signals. Matricellular proteins can interact with multiple other matrix proteins and cellular receptors and therefore have complex biological functions. Some of the matricellular proteins and their peptide fragments that can signal through growth factor receptors have been denoted as matrikines to emphasize their direct cell signaling capacities.

Matricellular proteins that lessen adhesion, via integrin or growth factor signaling, have a profound influence on cell motility as the intermediate state of cell adhesion favors motility.[40,41] ECM proteins that promote this intermediate state of adhesion, such are tenasicin C, thrombospondin, laminins, and secreted protein-rich in cysteine (SPARC), are increased in expression at the exact sites of remodeling that require cell migration—during embryogenesis, wound healing, inflammation, and tumor invasion. This suggests a role for the matricellular proteins in promoting migration by enabling the intermediate adhesive state.

A mathematical model developed by DeMilla, Barbee, and Lauffenburger predicts that the maximal motility of cells is achieved when the ratio of the transcellular force, achieved through cytoskeletal contractility, and the adhesive strength, achieved through integrin–matrix interactions, is intermediate.[41] Weak cell adhesion does not generate sufficient force for cell movement, and excessive adhesion prevents the releasing of the cell from the ECM.[42–44] It must be noted that the assays that test effects of ECM proteins on cell motility impose certain requirements for cytoskeletal organization and adhesion for the motility to be considered successful. Mesenchymal moving cells that form strong adhesions perform better in 2D migrational assays (such as wound-healing assay or live cell tracking on 2D matrices), whereas amoeboid-moving, less adherent, cells move slowly on rigid

2D substrates, but more swiftly in 3D migration assays. Thus, seemingly discrepant findings of the role of a certain ECM protein in cell motility are partly due to different contexts used to ascertain migratory performance. Our recent findings that epidermal growth factor-like repeats (EGF-like; EGFL) of tenascin C (TNC) can promote mesenchymal to amoeboid shift in migration in melanoma cells,[15] with the cells expressing the EGFL domain moving slower on 2D substrates than the control melanoma cells, but nevertheless moving faster in 3D environments; and this is without an increase in MMP activities.

This finding impels us to examine other matricellular proteins that have anti-adhesive properties. What is common for these proteins is that they bind multiple cellular receptors and they can induce multiple, sometimes even opposing cellular responses. TNC, Thrombospondin 1 (TSP1), Laminin 5, and SPRC are all anti-adhesive, all can bind multiple different integrins, and all possess EGFL domains or modules. In this review, we posit that these matricellular proteins can dictate the mode of migration, mesenchymal or amoeboid, by signaling cytoskeletal adaptation to the cell surroundings, and generally promoting tumor invasion. This view imposes a demand for targeted therapies that account for the plasticity that enables cells to switch between both modes of motility during invasion, for any hope of success in limiting cancer dissemination.

Stimulatory molecules

There are a large number of molecules that can be considered 'onco-fetal-wound' markers, in that they also appear or are upregulated in the matrix during tumor invasion. These molecules are present during development and re-appear during the regenerative phase of wound repair. They not only mark a period of rapid ingrowth of cells of all lineages, but also promote this exuberant expansion both by altering the density of the matrix and directing the cells to migrate. Thus, during tumor invasion, the malignant turn hijacks this physiological processes to promote dissemination. The following will discuss a number of the best-characterized and most strongly mechanistically correlated proteins.

TNC

The physiologic role of TNC lies in establishing interactions between the epithelium and the mesenchyme during embryonic development, tissue differentiation, and wound repair. Therefore, expression of TNC is transient during these periods being strictly regulated.[45] Persistent high levels of TNC are present in various tumor tissues, including brain, bone, prostate, intestine, lung, skin, and breast.[46]

TNC is a hexameric glycoprotein composed of 180–320 kDa monomers (it is actually a homodimer of homotrimers), which are disulfide-linked at their N-termini. The different molecular weights of TNC monomers are the consequence of glycosylation and alternative splicing. Each subunit contains: the N-terminal assembly domain, a domain composed of 14.5 EGFL, a domain composed of a varied number of fibronectin type III-like (FNIII) repeats, and a fibrinogen-like sequence on the C terminus.[47–52]

Cells can interact with the FNIII-like domain of TNC via integrins $\alpha2\beta1$, $\alpha7\beta1$, $\alpha9\beta1$, $\alpha V\beta1$, $\alpha V\beta3$, and $\alpha V\beta6$, thus allowing for cell attachment, and via syndecans-1 and -4, and annexin II to signal de-adhesion (reviewed in Erickson and Bourdon[51] and Prieto *et al*[53]). Therefore, the response to TNC differs depending on the receptor repertoire present on the cell surface. The EGF-like repeats of TNC also have counter-adhesive properties[54,55] and have been shown to bind and signal through the EGFR.[8,10] Interestingly, the binding of TNC EGFL to EGFR preferentially promotes cell migration by limiting receptor signaling to the perimembrane space.[11]

TNC has been shown to promote cancer invasion by both MMP-dependent and -independent mechanisms.[56] TNC induces expression of MMPs 1, 3, 9, and 13[56,57] and the activation of MMP2, and thus there is a positive feedback loop between the induction of MMPs by TNC and its cleavage by these MMPs.[58] Interestingly, cleavage sites for all MMPs tested to-date are outside the EGFL repeats domain in the TNC molecule,[59] leaving the EGFL intact in the face of increased ECM remodeling. Therefore, TNC is equipped not only to modulate ECM architecture but also to dramatically influence the behavior of cells by exposing its active EGFL matrikine domain.

We found that TNC EGFL induce melanoma cell rounding and decreased adhesiveness through activation of ROCK[15] and that this allows transition from the mesenchymal to amoeboid mode of invasion through the dermis. On the other hand, FNIII repeats of TNC have been shown to suppresses Rho A activation while maintaining the level of active Cdc42 thus preventing stress fiber formation.[60] As TNC is being deposited at the front of invading cells,[15] and amoeboid cell morphology is observed at the fronts of invasion in tumors,[61] it is possible that TNC can induce cytoskeletal changes in cancer cells that lead to a shift toward amoeboid movement and allow greater plasticity of invading cells. We speculate that invadopodia that can localize MMPs in the front of migrating cells[62] could cleave TNC to expose the EGFL as a mechanism to promote this invasion. It is likely that after proteolytic cleavage, TNC fragments may have distinct signaling activity compared with the full-length TNC protein.

There are other members of the tenascin family, generated by alternative splicing; some of which present multiple EGFL.[63] However, these have not been examined for ability to bind and activate EGFR.

Laminin-332 (formerly laminin 5)

Another matrikine protein that can signal via its EGFL-activating EGFR is laminin-332 (Ln-332),[9] a widespread constituent of the basement membrane. It is composed of α-3, β-3, and γ-2 chains, coiled together and stabilized by

disulfide bonds forming the long arm from which portions of all three chains protrude forming short arms.[64] In normal physiological conditions, epithelial cells adhere to Ln-332 through $\alpha3\beta1$ and $\alpha6\beta4$ integrins and form focal contacts and hemidesmosomes (reviewed in Koshikawa *et al*[65]). But, in cases of active remodeling, during wound healing or tumorigenesis, MT1-MMP[66] and MMP-2[67] can cleave Ln-332 and reveal cryptic pro-migratory sites. These cryptic sites were shown to be EGF-like repeats within γ-2 chain that stimulate cancer cell migration in an EGFR-dependent manner.[9] On the other hand, the laminin α-3 chain interacts with $\alpha3\beta1$ integrin and can stimulate cell adhesion, spreading, and migration,[68] which involves Src/FA kinase activation and subsequent Rac1-induced lamellipod extensions.[69] β-3 Chain and its cleaved products have also been shown to promote cell migration in multiple cancers (reviewed in Pyke *et al*[70]).

Invading cancer cells preferentially express increased amounts of γ-2 chain of Ln-332[71] and this is the only chain that can be secreted in the monomeric form.[72] Ln-332 γ-2 chain can also be processed by uPA and MMP9,[73] where uPAR signaling seems to be essential for production and secretion of Ln-332 itself.[74] In cancers of epithelial origin, Ln-332, uPAR and plasminogen activator-inhibitor-type 1 (PAI-1) are upregulated at the invasive fronts,[75,76] with the uPAR and PAI-1 upregulation being promoted in a feed forward manner by EGFR signaling.[77] This further supports our model that not only matrix degradation, but also cryptic domains released from matricellular proteins promote migration and possibly motility shift toward amoeboid migration at the leading edges of invasive tumors. In colon adenocarcinoma, addition of Ln-233 activates $\alpha3\beta1$ to decrease RhoA activity, which causes $\alpha2\beta1$ to $\alpha3\beta1$ switch in adhesion and increases attachment to Collagen IV and differentiation of cells into enterocytes.[78] A similar observation was made in squamous cell carcinoma cells, where attachment to Ln-233 through $\alpha3$ integrin caused decrease in RhoA activity, whereas the attachment on collagen I through $\alpha2$ integrin strongly activated RhoA.[79] In this case, $\alpha2\beta1$ attachment to collagen decreased, whereas Ln-322 $\alpha3\beta1$ integrin activation induced FA disassembly and stimulated migration on 2D-coated surfaces through decrease in RhoA and increase in Cdc42 activity. On the other hand, it has been shown that Ln-233 activates RhoA in keratinocytes on matrices through activation of both $\alpha31$ and $\alpha6\beta4$ and this enables subsequent spreading on collagen via $\alpha2\beta1$.[80] Depending on the cell type, different integrins can elicit various RhoA levels of activation in response to Ln-332 and this also depends on the other ECM proteins present.

SPARC

SPARC is an ECM protein with highest expression in bone tissue, but distributed throughout other tissues at the sites of remodeling, angiogenesis, and in pathological conditions such as tumorigenesis. SPARC is a 32-kDa protein with an acidic domain, a follistatin-like domain, and an extracellular calcium-binding domain.[81,82] The follistatin-like domain contains three EGFL modules, which are twisted by disulfide bonds,[83] and a copper-binding region that interacts with $\beta1$ integrin.[84] SPARC signaling through $\alpha v\beta3$ and $\alpha v\beta5$ integrins has also been demonstrated.[85,86] Like TNCs, effects of SPARC are context and cell type dependent with seemingly contradictory roles in tumor progression (reviewed in Chlenski and Cohn[87]). In ovarian, prostate, and colorectal cancers, SPARC expression is downregulated by methylation of the promoter, compared with normal tissues (reviewed in Sage *et al*[88]). In these cancers, overexpression of SPARC suppresses growth and survival of cancer cells. On the other hand, in glioma and breast cancer, SPARC expression is increased and it promotes invasion. These different effects on tumor progression can be explained by different requirements of tumor cell–matrix interactions for progression of neoplasms.

SPARC has anti-adhesive properties, inducing cell rounding and FA disassembly, which is induced by the EGFL module of SPARC;[89,90] suggesting that these might signal via the EGFR similar to TNC and laminin 322, although they have not been experimentally tested for such activity. The acidic domain of the molecule also has anti-adhesive properties.[91]

SPARC can bind integrin-linked kinase and augments fibronectin-induced integrin-linked kinase activation, formation of stress fibers and cell contractility.[92] SPARC is also a regulator of the ECM remodeling, it interacts with collagens I, II, III, IV, V, vitronectin, and thrombospondin (reviewed in Sage *et al*[88]) and induces MMP-1, -2, -3, and -9 secretion.[93,94] SPARC also directly binds VEGF and PDGF, and interferes with their signaling.[95,96] Therefore, this plethora of effects that SPARC can impose on cancer cells has different effects on progression in different cancer types. In glioma, overexpression of SPARC promotes tumor invasion by increasing MMP production, but also by the activation of RhoA and uPA-uPAR signaling.[94]

As expected for anti-adhesive domains, deletion of the EGFL module decreases SPARC-induced directional 2D migration on fibronectin, which is p38 mitogen-activated kinase dependent.[91] On the other hand, SPARC overexpression in medulloblastoma suppresses activity of Rho, Rac, and Cdc42 and inhibits invasion.[97] In ovarian cancer, SPARC abrogates cancer cell adhesion to the peritoneal mesothelial cells and ECM, thus inhibiting implantation and cancer progression.[98] These seemingly opposite effects are a consequence of a requirement for a certain level of adhesion and the adequate mode of migration that is needed for invasion through different matrices. Thus, based on environment, SPARC-induced increased de-adhesiveness could promote or ameliorate invasion. Interestingly, SPARC treatment of endothelial cells decreases production of TSP1, but induces production of plasminogen activator inhibitor 1,[99] with these

two ECM proteins driving mesenchymal to amoeboid migration transition by these changes in levels.

Thrombospondins

TSP1 is a 420-kDa trimer composed of three identical 145 kDa peptides linked by disulfide bonds. Like SPARC and TNC, TSP1 is expressed at the sites of tissue remodeling, associated with wound healing and tumorigenesis (reviewed in Liu *et al*[100] and Murphy-Ullrich and Poczatek[101]). It contains N-terminal globular domain, inter-chain disulfide knot, segment homologous to pro-collagen I, three properdin repeats, three EGFL, seven calcium-binding repeats, and carboxy-terminal L-lectin-like domain.[100] Its role in tumor progression has been controversial, as findings that support both promotion and suppression exist. This is, again, a consequence of multiple binding partners of TSP1 and especially its ability to bind and activate latent complexes of TGFβ.[102] TSP 1 binds syndecans-1[103] and -4[104] and multiple integrins-$\alpha6\beta1$, $\alpha4\beta1$, $\alpha9\beta1$, $\alpha v\beta3$, and $\alpha3\beta1$.[105,106] It can also indirectly modulate integrin signaling. For example, the C-terminal domain of TSP1 binds to integrin-associated protein and modulates $\alpha v\beta3$ signaling.[107,108] TSP1 is also an endogenous angiogenesis inhibitor[109] (reviewed in Fontana *et al*[110]), but some cancer cells can override this inhibitory effect.[111,112] Overexpression of TSP1, thus, in some cancers promotes[113,114] while in others, inhibits invasion and metastasis (reviewed in Bein and Simons[115]). TSP1 binds MMP2 and is believed to inhibit its activity,[116] whereas it upregulates MMP-9 expression.[117] The N-terminal domain of TSP1 induces disassembly of FAs,[118,119] which is stimulated by RhoA inactivation through FA kinase and activation of ERK and PI-3-kinase.[120] TSP1-induced FA disassembly is signaled through calreticulin and can promote migration of endothelial cells and fibroblasts.[121] EGFL repeats of TSP1 were found to activate EGFR and increase motility, but direct binding was not demonstrated, and MMP9 activity was required.[122] Interestingly, TSP1 increases secretion of matrix-bound PAI-1 in breast and lung cancers,[123,124] mediated through TGFβ activation.[124] PAI-1 has recently been found to promote mesenchymal to amoeboid migration transition by RhoA-ROCK-MLC pathway.[125] TSP1 also increases expression of other members of plasminogen system, uPA and uPAR[126] and thus increases invasion.[127]

Thrombospondin 2 is encoded by a different gene and has different temporal and spatial distribution compared with TSP1 (reviewed in Angelucci *et al*[128]). This isoform has been shown to be a matricellular protein that modulates both MMPs and growth factor signaling (VEGF in particular)[129] to inhibit angiogenesis in wound repair and tumor progression. However, this isoform has not been shown to interact with EGFR, the subject of this review.

Osteopontin (OPN)

OPN, a matricellular protein initially found in bone and thus also named bone sialoprotein-1, binds integrin $\beta1$ and via that colocalizes with EGFR. OPN permissive and even enhancing effects on tumor cells appear to need EGFR signaling, but this is secondary to co-clustering and increased EGFR and TGFα levels.[130,131]

Fibulins

Fibulins are a family of secreted glycoproteins with modular structure[132] that contain calcium-binding epidermal growth factor-like (EGFL) modules[133] and can also modulate cell adhesion through integrin signaling (reviewed in Obaya *et al*[134] and Camaj *et al*[135]). As in the case of many modular matricellular proteins that can interact with various other ECM components, fibulins have been described to have both pro- and anti-tumor progression activities (reviewed in Yates *et al*[136]).

Although it has not been conclusively explored whether fibulins can bind EGFR, a related molecule EFEMP1 appears to bind and activate EGFR.[137] Whether this family of proteins has an impact on cancer cell migration or invasion, given that its structure implies possible integration of signals similar to the above-discussed molecules, awaits further exploration.

Suppresive molecules

The ECM of quiescent, mature tissue contains numerous molecules that suppress the proliferative and migratory properties of the resident cells, and steer them toward a differentiated state. These molecules appear late in development after the formative burst, and during the transition from regenerative to resolving phase of wound repair.[138] A similar but inverted transition occurs in tumor progression.[139] At this emergence of invasiveness, these suppressive ECM components are decreased. Key among these are the structural collagens, which have been reviewed extensively elsewhere,[140–142] and a family of small leucine-rich proteoglycans.[143] The best characterized of the latter is decorin (DCN), the only molecule we will explore in detail due to its description as an EGFR-binding molecule.

DCN

DCN is the small (40 kDa) leucine-rich proteoglycan synthesized chiefly by stromal fibroblasts, endothelial cells under stress, and smooth muscle cells.[16] Unlike above-mentioned matricellular proteins, DCN has strictly anti-tumor activities. DCN consists of a protein core and a single chondroitin/dermatan sulfate glycosaminoglycan chain attached to a serine near the N terminus.[144] It is mostly found in collagen-rich connective tissues,[145] where it interacts with high affinity with collagen fibers ('decorates') and is involved in collagen fibrilogenesis.[146–148]

DCN can bind to and inhibit the activation of a number of growth factor receptors including EGFR,[149] Met receptor,[150] PDGF receptor,[151] and IGF-1R.[152] It can also sequester TGFβ family members into the ECM, as there are two binding sites for TGFβ in the DCN core.[153] DCN can be considered an

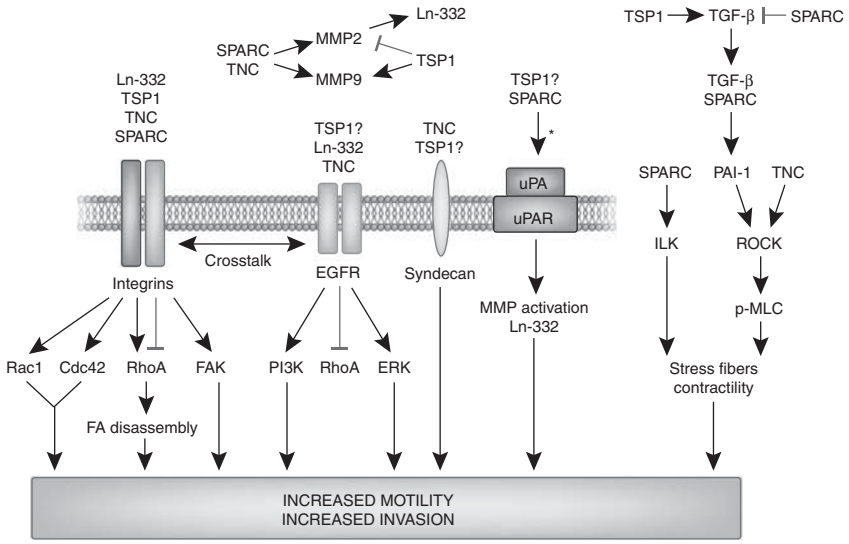

Figure 2 Signaling pathways activated by matricellular proteins promoting motility and invasion. Schematic of the extracellular molecules and their receptors that are linked to key signaling molecules.

endogenous matrix-centric pan-kinase inhibitor[16] and along with TGFβ sequestering function, which leads to tumor immunosupression and growth retardation, has been proposed to be 'a guardian from the matrix' to draw a comparison to 'guardian of the genome' p53.[16] Notably, the cooperation between DCN and p53 has already been established.[154] DCN inhibits cancer cell migration via inhibition of multiple growth factor receptors and by upregulation of E-cadherin.[155]

Quiescent fibroblasts are the main source of DCN, as proliferating fibroblasts produce significantly lower levels.[156] The disruption of DCN leads to abnormal collagen fibril morphology and tissue fragility.[147] In cancer, DCN is generally downregulated and can be found in peritumoral stroma, but not in tumor cells or dense tumor tissue (reviewed in ref. 154).

In recent years DCN has been explored as therapeutic agent, both alone and in combinational cancer therapy.[154] Our group has looked at effect of DCN on melanoma cell migration and found that it drastically inhibits it both in 2D and 3D migration assays (Grahovac and Wells, unpublished observations). This opens an avenue for further exploration of effects of DCN in presence of matricellular proteins that promote cancer cell migration and possibility of normalization of the cancer ECM by induction or administration of DCN.

Summary

Matricellular proteins are important regulators of tissue organization and cell activation status, and consequently their altered expression during tumorigenesis greatly impacts cancer progression. What is common for all of the above-discussed matricellular proteins that promote migration and invasion is that, in addition to regions binding to β1 integrin, they all have EGFL domains. TNC, TSP1, and Ln-332 have EGFL that have been shown to bind and activate EGFR, whereas SPARC and fibulins have EGFL that have not been examined for growth factor receptor binding. Furthermore, they all bind syndecans and induce or activate various MMPs that may in turn clip the molecules to expose these cryptic signaling moieties. TSP1 and SPARC also activate uPA/uPAR signaling and the generation of active HGF/Scatter Factor.[94,126] All of the described matricellular proteins can lessen the adhesiveness of the cells and enable signaling that can promote both mesenchymal or amoeboid cell movement depending on the ECM surroundings (Figure 2).

TNC, SPARC, TSP1, and Ln-322 are all expressed in the sites of active tissue remodeling, some distinctly present at the invasion borders. For example, Ln-322 γ-2 chains and TNC are co-deposited and form a physical complex at the invasion fronts and carcinoma-stroma borders.[155,156] We speculate that this localization is enabling shift toward amoeboid mode of migration as an adaptation to the previously un-encountered ECM.

The notion that the same matricellular protein can signal mesenchymal or amoeboid migration depending on the context imposes a requirement for inhibiting both modes of migration to limit invasion. Simultaneous inhibition of both MMPs and RhoA/ROCK signaling pathway could work toward that goal.

Another important signaling factor that sits at the crossroads of matricellular proteins is TGFβ. TGFβ induces production of SPARC and TNC, but decreases production of DCN. TSP1 binds and activates latent TGFβ complexes, whereas DCN can sequester TGFβ from the ECM, and SPARC indirectly diminishes TGFβ activity. This raises question whether TGFβ is the major regulator of the tumor stroma and whether normalization of the ECM through

TGFβ manipulation holds promise as a means for better cancer treatment.

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DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

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