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Microenvironmental regulation of E-cadherin-mediated adherens junctions

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1. ABSTRACT

The interaction between tumor cells and the microenvironment has substantial effects on tumor cell behavior by influencing cell-cell as well as cell-matrix contacts. The underlying molecular mechanisms are only partially unraveled. In this review we focus on the influence of the stromal microenvironment, especially collagen type I and type III on cellular adhesion and epithelial to mesenchymal transition (EMT). Extensive studies have emphasized that components of the microenvironment such as fibrillar collagen or growth factors like transforming growth factor beta are involved in induction of dedifferentiation of epithelial cells accompanied by disruption of the E-cadherin adhesion complex and reduced E-cadherin concentrations. On the molecular level many different proteins have been identified which are involved in the regulation of EMT, such as activation of integrins, intracellular kinases such as Src, focal adhesion kinase (FAK) or phosphatidylinositol-3 kinase (PI3-kinase) and alteration of catenin phosphorylation. The reduced cellular adhesion influences the tissue integrity and allows tumor cells to disseminate from the primary tumor representing an early step in cancer metastasis.

2. INTRODUCTION

The development of invasive and metastatic cancer is one of the most threatening visions. To develop strategies against cancer or for controlling metastatic events, the relationship between tumor cells and their microenvironment gains new attention (1, 2). A better understanding of the immediate microenvironment in developing tumors and its interaction with cancer cells should allow the development of new diagnostic and therapeutic strategies in cancer treatment (3). The tumor microenvironment is a complex system of extracellular matrix proteins, mainly composed of collagen type I and III, fibronectin, laminin, proteoglycans, as well as many cell types such as fibroblasts of various phenotypes, endothelial cells, and immune-competent cells (4, 5). Many types of carcinomas, especially breast, colon, liver and pancreatic cancer, are characterized by significant amounts of extracellular matrix proteins in the tumor environment. Therefore most authors agree that stromal cells and their associated matrix play an important role in carcinogenesis and tumor progression (1, 2). Although many signal transduction proteins have been identified in the last years, which are involved in the interaction between tumor cells and the surrounding microenvironment, the responsible

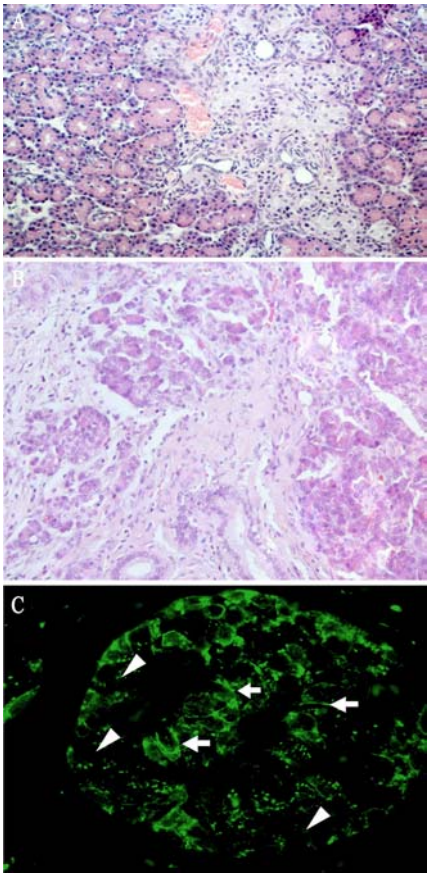

Figure 1. Histological analyses of pancreatic tissue samples. A) The pancreas of transgenic mice overexpressing TGF-beta 1 under the control of the insulin like growth factor 2 promoter develops a pronounced fibrosis. Sections of the pancreas of a 14 days old transgenic mouse contain significant amounts of extracellular matrix in this H/E staining. Magnification: 50x. B) Human pancreatic ductal adenocarcinomas are characterized by high amounts of extracellular matrix as demonstrated by H/E staining. Magnification: 45x. C) Immunohistochemical detection of E-cadherin in a section of a human pancreatic ductal adenocarcinoma. Central areas of the tumor show E-cadherin signals which are located at the plasma membranes and enriched in areas of cell-cell contacts. In the border area of the tumor, where tumor cells have contact with the stroma of the microenvironment, E-cadherin staining is reduced to a punctated staining. Magnification: 80x.

molecular signal mechanisms are only marginally established.

3. THE TUMOR ENVIRONMENT

During the last years it has been shown that an intensive communication between microenvironment and tumor cell supports invasive growth and metastasis and regulates survival, differentiation, and migration of many tumor cells (1, 6). This observation culminates in the question what comes first: the dysfunction of epithelial cells or changes in their microenvironment (7). Several reports underline the ability of mesenchymal cells to control epithelial differentiation as for instance during development (8). Rather than being a passive actor in tumorgenesis, the microenvironment might represent a primary active factor in determining whether dysfunctional epithelial cells might develop to invasive tumor cells or will be eliminated (7).

Although some tumor cells contribute directly to stromal tissue, the main fraction of ECM proteins is secreted by mesenchymal cells such as stromal fibroblast, activated myofibroblast or activated stellate cells (9). The latter have been described in liver and recently in pancreas and are classified as mesenchymal cells by expression of glial-fibrillary-acidic protein and storage of vitamin A in their cytoplasm (10). Stellate cells can be activated by damage or stress as well as cytokines and growth factors released from inflammatory or neoplastic cells. Upon activation stellate cells synthesize and release excessive amounts of ECM proteins (Figure 1) (10, 11). The resulting stroma contains mainly fibrillar type I and III collagens, fibronectin and laminin which stimulates tumor cell growth, migration, angiogenesis and promotes resistance to chemotherapeutic agents (5, 12). The most potent fibrogenic stimulants were identified as TGF-beta 1, fibroblast growth factor 2 (FGF-2), and platelet-derived growth factor (PDGF), which are secreted by immune cells, and later on by tumor cells and activated fibroblasts themselves (13). Especially TGF-beta 1 induces tumor progression by influencing multiple processes, such as angiogenesis, fibrotic changes and adherens junction stability (14).

3.1. Epithelial-mesenchymal transition in tumor progression

A first step leading to metastasis is the acquisition of local invasiveness by tumor cells, which involves major changes in the phenotype of cells within the primary tumor (1, 15) characterized as epithelial to mesenchymal transition (EMT). During this process cells partially lose their epithelial markers and gain mesenchymal characteristics (16, 17). One typical feature of EMT is the reduction of strong epithelial cell-cell adhesion by dissociation of adherens junctions which correlates with reduced E-cadherin concentration and with upregulation of the mesenchymal markers vimentin and N-cadherin. On cellular level EMT is characterized by epithelial dedifferentiation, phenotypic alterations, and increased migration (16-18).

Loss of E-cadherin is not uniformly found in carcinomas. Some carcinomas are characterized by complete deficiency of E-cadherin, whereas in others the membranous localization of E-cadherin is lost only in the dedifferentiated areas of the tumor particularly near the invasive front (Figure 1C) as shown for colon and less impressive for pancreatic tumors (19, 20). One possible explanation may be the presence of specific ECM proteins in the environment of tumor cells near the invasive front compared to central tumor areas or compared to metastases where less stroma is often present.

3.2. The microenvironment activates integrin signaling

The influence of individual ECM proteins on invasiveness and especially on EMT is still a matter of discussion (15). On the molecular level, ECM proteins are ligands for integrins, which are transmembrane proteins binding extracellular proteins such as collagen type I or type III, fibronectin or laminin. Ligation of ECM proteins to integrin heterodimers leads to integrin activation and initiation of many intracellular signal transduction processes. Because integrins themselves have no intrinsic catalytic activity, ECM-integrin signals are transduced into the cell through activation of integrin-associated proteins, which are assembled in focal adhesions (FA). One current model of integrin signal transduction highlights the binding of talin with its FERM domain to the short cytoplasmic tail of activated integrins. Talin has been suggested as a scaffold protein, which contains multiple interaction domains for adaptor proteins, kinases and phosphatases (21). Furthermore, Talin has been involved in the linkage of filamentous actin with integrin-containing focal adhesions. The link between activated integrins and signaling pathways of Src-family kinases, the Ras-MAPK- or the Rho-GTPase-cascade is mediated by various adaptor proteins localized in Fas.

Although there are a rapidly increasing number of reports about the molecular mechanisms of integrin-induced signaling, the molecular details are presently mainly unknown. Understanding is further complicated by the high number of different integrin subunits present in cells, which seem to vary between different cell types and with the changing microenvironment.

In the following parts, we focus on recent data dealing with the influence of the microenvironment on E-cadherin-mediated cell-cell adhesion of epithelial tumor cells.

3.3. E-cadherin-mediated adherens junctions

The acquisition of a motile behavior early in metastasis depends on the EMT process, which is mainly driven by inactivation of epithelial cell-cell contacts particularly E-cadherin-mediated adherens junctions (22-24). E-cadherin is a calcium-dependent transmembrane glycoprotein present in most epithelial cells. E-cadherin binds intracellular directly to several cytoplasmic proteins including beta-catenin, alpha-catenin and $p120^{ctn}$, which mediate the association of E-cadherin with the actin cytoskeleton (25, 26). The interaction is induced by Rho and Rac GTPases and subsequent actin filament polymerization as well as by binding to further, not so well characterized actin-binding proteins (27). Not all E-cadherin molecules present at the cell membrane are involved in E-cadherin-mediated cell-cell adhesion. Only E-cadherin which is linked via beta-catenin to the actin cytoskeleton contributes to strong cellular adhesion (28). Disturbance and loss of E-cadherin-mediated adherens junctions as well as reduction of E-cadherin concentration strongly correlates with epithelial dedifferentiation and phenotypic alterations of epithelial cells (29, 16, 17). The assembly and maintenance of adherens junctions is under tight transcriptional and posttranscriptional control.

4. REGULATION OF E-CADHERIN GENE EXPRESSION

E-cadherin is considered as a tumor suppressor molecule due to two main reasons: transcription of E-cadherin is silenced in various carcinomas and reexpression of E-cadherin in carcinomas *in vitro* and animal models is sufficient to reduce the aggressiveness of tumor cells (24, 30).

In some human carcinomas the E-cadherin loss is due to transcriptional silencing of the E-cadherin promoter by hypermethylation or histone deacetylation (31). Moreover, different transcription factors have been described which bind to the E-cadherin promoter especially to the palindromic E-boxes resulting in inhibited promoter activity, which will be discussed later in the context of TGFβ-induced regulation of transcription factors. The expression of Ets-1 has been shown to repress E-cadherin expression in breast carcinoma and keratinocyte cell lines by binding to Ets binding sites in the promoter region (32, 33).

In addition to the loss of E-cadherin by modification of gene expression, the balance between assembly and disassembly of the E-cadherin adhesion complex as well as its stability and association with the actin cytoskeleton is tightly controlled. An increasing number of proteins have been identified to be involved in regulating the E-cadherin complex assembly. The underlying mechanisms are highly diverse starting with altered expression of genes coding for adhesion proteins, continuing with altered interactions between adhesion proteins such as N-cadherin and E-cadherin (34, 35) and ending with the presence of regulatory proteins such as $p120^{ctn}$ (36, 37).

4.1. $p120^{ctn}$ stabilizes E-cadherin-mediated adhesion

The interaction between cadherins and $p120^{ctn}$ is believed to play a pivotal role in the regulation of different aspects of E-cadherin complex assembly. $p120^{ctn}$ promotes cell surface trafficking of E-cadherin through its ability to bind kinesin and to transport E-cadherin along microtubuli (38). Another core function of $p120^{ctn}$ in epithelial cells is to control the availability of E-cadherin by regulating the recycling of endocytosed molecules (reviewed in Reynolds and Roczniak-Ferguson, 2004). While E-cadherin is rapidly degraded in cells with low $p120^{ctn}$ content, forced expression of $p120^{ctn}$ prevents the lysosomal degradation of E-cadherin (40). The complex role of $p120^{ctn}$ in epithelial cells is underscored by the observations that $p120^{ctn}$ increases lateral clustering of E-cadherin molecules to build zipper like adherens junction thereby strengthening cell-cell adhesion (41). Besides directly influencing E-cadherin protein content and cell-cell adhesion, $p120^{ctn}$ controls gene expression by regulating the transcription factor Kaiso (42, 43).

4.2. IQGAP influences the association of the E-cadherin complex with actin filaments

IQGAP is a scaffolding protein that binds to a set of signaling and structural molecules and participates in the

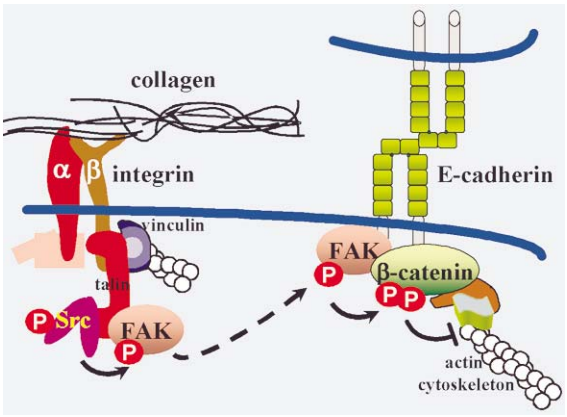

Figure 2. Crosstalk between extracellular matrix and adherens junctions. A hypothetical model summarizes data about the molecular mechanisms how ECM-activated integrins lead to destabilization of E-cadherin-mediated adhesion complexes.

regulation of multiple cellular functions such as organization of cell migration, cell-cell adhesion, and the cytoskeleton (44). IQGAP colocalizes with actin in lamellipodia and enhances actin polymerization (45, 46). Detailed analyses document that IQGAP modulates the cytoskeleton indirectly through Rho GTPases, mainly by stimulating Rac and Cdc42 activity (44). Furthermore, the translocation of IQGAP from the cytoplasm to the cell membrane (47) and its association with E-cadherin and beta-catenin reduces the interaction between the cadherin complex and the actin cytoskeleton thereby weakening cell-cell adhesion (48). These findings correlate with E-cadherin dysfunction and tumor dedifferentiation in gastric carcinoma (49). Furthermore, enhanced IQGAP expression was detected in human colorectal carcinomas (50), particularly in cells near the invasion front. With regard to the molecular mechanisms, the available data imply that E-cadherin/beta-catenin-associated IQGAP does no longer activate Rac and Cdc42, resulting in decreased actin polymerization and reduced attachment of the E-cadherin complex with cortical actin filaments which then promotes tumor cell invasion.

5. EXTRACELLULAR MATRIX PROTEINS REDUCE THE STABILITY OF ADHERENS JUNCTIONS

Another interesting crosstalk occurs between integrins and cadherins (51). Recent data illustrate the influence of defined ECM proteins such as fibrillar collagens or laminin on the assembly of E-cadherin-mediated adherens junctions (24, 19, 52). Carcinoma cell lines of human pancreatic or colon origin which were cultured on collagen type I, type III, or on laminin showed reduced concentration of E-cadherin and beta-catenin. A dissociation of the E-cadherin/catenin adhesion complex and its detachment from the cytoskeleton was detected a few hours after collagen-treatment of pancreatic cancer cells (52, 53). The observed collagen-stimulated reduction of cytoskeleton-associated E-cadherin correlated with an inhibition of the aggregation capacity of pancreatic carcinoma cells. Most interestingly, carcinoma cells expressing a chimeric protein of E-cadherin and alpha-catenin, which is insensitive to regulation by catenin phosphorylation, did not respond to collagen stimulation with reduced cellular aggregation (54, 52). These data imply that collagen-induced inhibition of epithelial cellular aggregation is induced by a phosphorylation-dependent detachment of the E-cadherin complex from the actin cytoskeleton.

5.1. Integrins mediate collagen-induced E-cadherin complex disassembly

Activation of alpha 1/beta 1 or alpha 2/beta 1 integrins by collagen type I or type III represents the first step in collagen-induced E-cadherin down-regulation (24, 52). Integrin ligation is known to activate multiple downstream signaling molecules including the kinases Src and focal adhesion kinase (FAK), as well as structural proteins such as talin, paxillin, or vinculin (55-58). As shown in many cell types collagen-activated integrins induce phosphorylation and activation of FAK and Src within minutes which is accompanied by the formation of focal adhesion complexes (24, 59, 60). The non-receptor tyrosine kinase FAK represents a key player in cell-matrix induced cellular signaling by interacting with a plethora of intracellular proteins such as the p130/Crk/DOCK1 cascade (58) and members of the Raf-MEK-MAPK pathway (61) (Figure 2).

5.2. Activated focal adhesion kinase associates with the E-cadherin complex

In collagen type I-stimulated pancreatic carcinoma cells a fraction of activated FAK can be detected in the apical part of differentiated epithelial cells associated with E-cadherin complexes, while the main fraction of FAK is localized in focal contacts. The increased amount of activated FAK associated with the E-cadherin complex correlated with increased tyrosine phosphorylation of beta-catenin, disassembly of the E-cadherin adhesion complex, detachment of E-cadherin from the actin cytoskeleton and reduced cell aggregation (52). This modification of beta-catenin and repression of cellular aggregation by collagen-activated FAK was suppressed by addition of neutralizing antibodies against beta 1 integrins, pharmacological Src inhibitors or the expression of the dominant-negative FAK mutant FRNK (52). Data obtained from colon cancer support the role of FAK in the assembly of the E-cadherin complex. Avizienyte and colleagues reported that the expression of FAK mutants, which could not be phosphorylated by Src kinase, resulted in increased E-cadherin-mediated cell-cell adhesion (57). Furthermore, the pharmacological inhibition of Src or expression of dominant-negative FAK in Src-overexpressing epithelial cells restored E-cadherin-mediated cellular adhesion (62). Taken together, these findings point towards a new role of FAK in the induction of EMT by modulating E-cadherin-dependent cell-cell adhesion. This leads to the hypothesis that components of the extracellular matrix, which are produced by many tumors in large quantities, support tumor invasion by activation of integrins, Src and FAK (Figure 2). This results among others in dissociation of the

E-cadherin-adhesion complex by phosphorylation of beta-catenin (52, 63-65).

5.3. Catenin phosphorylation in the E-cadherin adhesion complex assembly

Phosphorylation of beta-catenin has been suggested to be an integral part of ECM- or growth factor-induced inhibition of the E-cadherin adhesion complex (65-67). Recently, the phosphorylation of tyrosine residues Y142, Y489 and Y654 of human beta-catenin has been described to be involved in the regulation of beta-catenin-E-cadherin or beta-catenin-alpha-catenin binding (reviewed by Lilien and Balsamo, 2005 (65)). The increased phosphorylation of beta-catenin is also associated with the activation of different kinases such as the epidermal growth factor (EGF) receptor, Src or Fer (68-70) and the inhibition of phosphatases such as SH2 domain-containing inositol-5'-phosphatase 2 (SHP2), leucocyte antigen related (LAR) protein tyrosine phosphatase, or phosphatase and tensin homolog (PTEN) (63, 71-73). A large body of evidence points towards a role of Src kinases in the regulation of cell-cell adhesion (Figure 3). Years ago two reports described the effect of activated Src kinase on E-cadherin-mediated cellular adhesion. Both studies suggested a direct influence of activated Src on catenin phosphorylation followed by inhibition of cell aggregation (74, 75). Moreover active Src regulates the cellular amount of E-cadherin. Upon activation Src phosphorylates E-cadherin at tyrosine residues which results in ubiquitylation by Hakai, a Cbl-like E3-ubiquitin ligase, and subsequent endocytosis and lysosomal degradation of E-cadherin (76). The described data outline signaling pathways which allow the tumor stroma to decrease cell-cell interactions, eventually leading to tumor cell dissemination (Figures 2,3).

6. REGULATION OF ADHERENS JUNCTIONS BY TGF-BETA

The growth factor transforming growth factor beta (TGF-beta) has been mentioned before as a potent profibrotic factor which activates mesenchymal cells, fibroblasts or stellate cells, to produce ECM proteins and tumor stroma. In the last years several publications underscore the existence of a more direct influence of TGF-beta on the regulation of cell-cell adhesion in epithelial cells, especially during tumorgenesis (14, 77-79). The mammalian TGF-beta family consists of three proteins TGF-beta 1, 2, and 3 and is known to be involved in many cell types in the regulation of a number of biological processes, such as proliferation, differentiation, migration, and adhesion (14, 77-79). TGF-beta signals via two discrete serine/threonine kinases: the type I TGF-beta receptors (TbRI) and the type II TGF-beta receptors (TbRII). Upon ligand binding, the activated type II receptor transphosphorylates TbRI which is recruited to the TGF-beta-receptor-ligand complex. This transactivation is followed by the phosphorylation of downstream mediators belonging to the Smad family (Smad2 and/or Smad3). Phosphorylated Smad2 or -3 oligomerizes with Smad4 and translocates into the nucleus to act in concert with various binding partners as a regulator of gene expression (80, 81, 82). Besides Smad-dependent regulation of gene expression, a number of non-Smad-mediated signaling mechanisms and effects have been described, which include activation of the mitogen-activated protein kinases ERK, JNK and p38, the PI3-kinase, Ras and Rho GTPases as well as PTEN and other proteins (80, 83-86). A possible link between TGF-beta and tyrosine kinase signaling has recently been discovered by Lee *et al.*, (2007). Activation of an intrinsic tyrosine kinase domain of TbRI by TGF-beta results in direct tyrosine phosphorylation of the adaptor protein ShcA, which associates with Grb2 and Sos, well known mediators of tyrosine kinase signaling, such as the ERK MAP kinase pathway (87).

In epithelial cells, TGF-beta induces the production of proteins which inhibits cell cycle progression (88) and promote differentiation of epithelial cells (89, 90). Given that many malignant tumors express high amounts of TGF-beta an additional role for TGF-beta in cancer progression, especially in late tumor stages has been suggested after silencing of Smad-signaling. Here, tumor-cell-autonomous signaling and host-tumor cell interactions, such as stromal-epithelial interaction, inflammation or angiogenesis are equally important (recently reviewed in Bierie and Moses, 2006). With regard to epithelial tumor cells it has been documented that TGF-beta induces morphological alterations as well as biochemical and transcriptional changes towards a mesenchymal phenotype (91-93). This EMT is accompanied by the dissociation of the E-cadherin adhesion complex and increasing evidence suggests a direct role for TGF-beta 1 in the regulation of this process. A central step in determination of the adhesion complex turn over is the modulation of beta-catenin by phosphorylation of various tyrosine residues (65), as already mentioned above. It has recently been shown that TGF-beta 1 induces the disassembly of the E-cadherin adhesion complex by enhanced phosphorylation of beta-catenin in pancreatic carcinoma cells (73). In these cells TGF-beta 1-induced phosphorylation of catenins is accompanied by activation of E-cadherin complex-associated PI3-kinase and relocalization of the phosphatase PTEN from the E-cadherin-adhesion complex. Phosphorylation of beta-catenin, which is probably mediated by activated Src-family tyrosine kinases and supported by reduced dephosphorylation by PTEN, results in destruction of the adherens junctions and concomitant decrease in cell aggregation and increase in cell migration (73). In addition to destabilization of the adherens complex via catenin phosphorylation, E-cadherin levels can be downregulated post-translationally through enhanced clathrin-mediated endocytosis and lysosomal degradation as shown in mammary epithelial cells (94). This mechanism is regulated by the cooperative activity of TGF-beta- and Raf-dependent signal transduction pathways and might by especially important for the early stages of EMT (94), whereas the transcriptional downregulation of E-cadherin seems to be a late event in EMT (Figure 3).

6.1. Inhibition of E-cadherin gene expression by TGF-beta

Besides epigenetic silencing of the E-cadherin promoter, E-cadherin gene expression is repressed by several transcription factors. These transcriptional

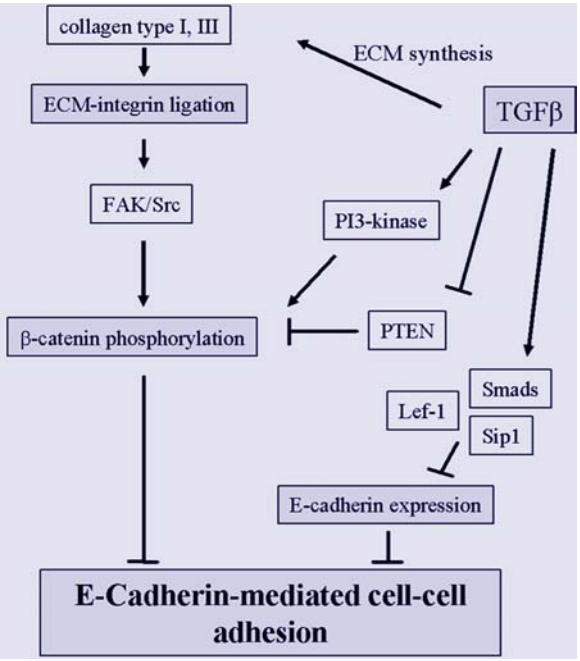

Figure 3. Summary of the influence of TGF-beta on E-cadherin-mediated cell-cell junctions during epithelial-mesenchymal transition.

repressors include the basic helix-loop-helix (bHLH) factors E12/E47 and Twist, the two-handed zinc-finger homeodomain proteins of the deltaEF1 family (deltaEF1/ZEB1 and SIP1/ZEB2) as well as the zinc finger proteins Snail and Slug (95, 96). The transcription factors directly interact with the E-boxes of the E-cadherin gene promoter and inhibit E-cadherin expression when overexpressed for example in MDCK cells (96). Moreover, downregulation of these transcription factors by siRNAs increases E-cadherin amounts and inhibits metastatic properties of many cancer cell (97). TGF-beta has been reported to stimulate the expression of Smad interacting protein 1 (SIP1) and Snail mRNAs in various epithelial cells (97-99). Upregulation of SIP1 has been shown for different primary tumor cells, such as gastric, ovary, esophageal, or oral squamous cell carcinomas and it has been correlated with reduced concentration of E-cadherin (100-104). SIP1 was identified as a mediator in ECM-, especially collagen type I-induced downregulation of E-cadherin. Pancreatic tumor cells exhibit a Src-dependent upregulation of SIP1 when grown on collagen type I, which correlates with reduced E-cadherin promoter activity (105). These data are supported by findings in SIP1-knockout mouse embryos, which show upregulation of E-cadherin in tissues where SIP1 is still expressed, such as the neuroepithelium and the neural tube (106).

TGF-beta-induced gene expression of Snail is induced via Smad3 in renal tubular epithelial cells (107) or via activation of the PI3-Kinase and the ERK pathway in MDCK cells (99). Recently, TGFβ has also been linked with traditional Wnt/Wg signal transduction (108), especially beta-catenin/ lymphoid enhancer-binding factor 1 (LEF-1) signaling, which is also associated with inhibition of E-cadherin gene expression (109). In MDCK cells expression of LEF-1 and Snail is induced in a Smad- and GSK3β-dependent manner and both transcription factors are necessary to fully repress E-cadherin expression and to complete EMT (110). Phosphorylated Smad2, Smad4 and LEF-1 form a complex that functions as a transcriptional repressor of the E-cadherin gene independent of beta-catenin in palate cells (111) (Figure 3).

Although the zinc finger proteins Snail and Slug have been shown to repress E-cadherin gene expression (97, 112), neither Snail, Slug nor Twist exhibit significant effects on E-cadherin promoter activity in mouse mammary NMuMG cells. In these cells SIP1 and deltaEF1 are essentially required for inhibition of E-cadherin expression through interaction with E-box2 and E-box1 elements of the mouse E-cadherin promoter (113). Interestingly, SIP1 and deltaEF1 expression is controlled by Ets1, another TGF-beta target gene (114), which in turn is most likely controlled by Id2 (113). As mentioned earlier, the expression of Ets1 has been shown to repress E-cadherin expression (32, 33). Id proteins are targets of TGF-beta and act through repression of E12/E47 transcription factors. Thus downregulation of Id2 by TGF-beta relieves the inhibitory action of E12/E47 leading to E-cadherin repression and EMT of epithelial cells (115, 116).

All these findings points towards a complex regulation of E-cadherin expression orchestrate by TGF-beta (Figure 3), leaving the question open, whether these mechanisms are tissue and/or cell-type specific and whether these mechanisms act independently from each other or in concert (see also review by Moustakas and Heldin, 2007 (14)). Two recently discovered regulators of the described E-cadherin transcriptional repressors Snail, Slug, SIP1 and Twist might add to the question. Inhibition of TbRIII expression in mouse mammary epithelial cells results in increased activity of nuclear factor (NF) kappa B which in turn results in increased expression of the E-cadherin transcriptional repressors, especially of Snail. This finding classifies NFkappaB as a mediator of TbRIII induced repression of E-cadherin (117). The second TGF-beta-target implicated in regulating the above mentioned transcription factors is high mobility group factor A2 (HMGA2) (118). HMG2A and -1 constitute a family of nuclear factors that bind to AT-rich DNA sequences (119). Expression of HMGA2 is upregulated by TGF-beta-induced binding of Smad4 to the HMGA2 promoter region in NMuMG mouse mammary cells (118). Forced expression of HMGA2 leads to upregulation of Snail, Slug and Twist expression and downregulation of Id2 expression, which consequently results in marked inhibition of E-cadherin expression (118) introducing another TGF-beta signaling pathway resulting in repression of E-cadherin-mediated cellular adhesion.

7. FUTURE PERSPECTIVE

Although much progress has been made in the understanding of processes resulting in tumor invasion and metastasis, we are far away from successful strategies to

develop diagnostic or therapeutic tools to cure metastatic neoplasm. The disassembly of adherens junctions represents a hallmark in epithelial-mesenchymal transition. It inhibits cell-cell adhesion and enables tumor cells to disseminate from the primary tumor in the course of invasion and metastasis. To gain better insights into the regulatory processes which determine E-cadherin-mediated adherens junctions, regulation of tight junction and intercellular adhesive structures is a necessary requirement to develop therapeutic strategies against metastatic events in the future.

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Abbreviations: ECM: extracellular matrix; EGF: epidermal growth factor; ERK: extracellular signal-regulated kinases; EMT: epithelial-mesenchymal transition; FA: focal adhesion; FAK: focal adhesion kinase; FRNK:

FAK-related non-kinase; HMGA2: high mobility group factor A2; LAR: leucocyte antigen-related protein tyrosine phosphatase; LEF-1: lymphoid enhancer-binding factor 1; MAPK kinases: mitogen-activated protein kinases; NFkappaB: nuclear factor kappa B; PDGF: platelet-derived growth factor ; PI3-kinase: phosphatidylinositol-3 kinase; PTEN: phosphatase and tensin homolog; SHP2: SH2 domain-containing inositol-5'-phosphatase 2; SIP1: Smad-interacting protein 1; TGF-beta: transforming growth factor beta; TbR: transforming growth factor beta receptor; Wnt/Wg: wingless + Int/wingless transcription factor

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