Toward an Integrative Analysis of the Tumor Microenvironment in Ovarian Epithelial Carcinoma

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Abstract Ovarian epithelial carcinomas are heterogeneous malignancies exhibiting great diversity in histological phenotypes as well as genetic and epigenetic aberrations. A general early event in tumorigenesis is regional dissemination into the peritoneal cavity. Initial spread to the peritoneum is made possible by cooperative signaling between a wide array of molecules constituting the tissue microenvironment in the coelomic epithelium. Changes in the activity of key microenvironmental components not constitutively expressed in normal tissue, including several disclosed adhesion molecules, growth factors, proteases, and G-protein coupled receptors (GPCRs), coordinate the transition. Remodeling of the extracellular matrix (ECM) and subsequent cell surface interactions enable transformation by promoting chromosomal instability (CIN) and stimulating several common signal transduction cascades to prepare the tissue for harboring and facilitating growth, angiogenesis and metastasis of the developing tumor.

Keywords Ovarian cancer · Tumor microenvironment · E-cadherin · Integrins · HB-EGF

Introduction

Ovarian cancer is consistently one of the leading causes of cancer-related mortality among women. Approximately 90% of ovarian cancers are ovarian epithelial carcinomas

(OECs), arising principally from the fimbriae of the fallopian tube, but sometimes originating from dysplastic inclusion cysts derived from modified ovarian surface epithelium (OSE) [1]. Regional dissemination from direct peritoneal contact is a frequent early event, occurring well before distant metastasis via hematogenous or lymphatic routes.

Ovarian cancers are classified by the type of Müllerian histology exhibited by malignant cells, as well as by invasive potential. The predominant histological subtype is serous adenocarcinoma, followed by endometrioid and the rarer mucinous, undifferentiated and clear cell carcinomas. Type I low grade, or low malignancy potential tumors, are characterized by a gradual accrual of morphological and genetic changes over time. These carcinomas progress slowly from borderline tumors and often display high mutation rates of *KRAS*, *BRAF*, and *ERBB2* [2, 3]. The remaining malignancies are type II high grade carcinomas. Tumors with this classification exhibit a highly invasive phenotype very rapidly with more widespread chromosomal instability (CIN) and pronounced epigenetic alterations of gene expression. Ras mutations are uncommon in high grade OEC. While *TP53* mutations are relatively rare in low grade carcinomas, they present in up to 97% of high grade serous tumors [4].

Because ovarian carcinomas are highly heterogeneous, the structural changes and molecular interactions that take place within the tumor microenvironment of the extracellular matrix (ECM) and on the cell surface may provide key insights into ovarian cancer progression, including tumor genesis, development and metastasis. In addition, delineating the changes in expression patterns of extracellular ligands that alter signaling cascades during transformation toward a mitogenic phenotype should have direct implications on the discovery of novel biomarkers for diagnosis and therapeutic targets for intervention.

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Despite the variegated nature of OEC, direct peritoneal dissemination is a hallmark of early disease. Malignancies showing only regional spread from the primary tumor site are far more curable than those carcinomas that have metastasized to distant tissues. For this reason, knowledge of the early events predisposing to tumorigenesis and peritoneal invasion is critical in elucidating common mechanisms in the pathogenesis of different forms of OEC. As patterns in alterations to the microenvironment that fosters and facilitates tumor genesis and growth are better recognized, identification of unifying factors across broad spectra of otherwise different diseases manifesting as ovarian carcinomas will present opportunities for clinicians to diagnose OEC more rapidly and possibly offer more successful treatment options as well. The following review briefly highlights areas of research that have contributed to our understanding of the elusive tumor microenvironment in OEC, with emphasis on the cross-talk and signaling between components that prepare the tissue for malignancy.

Cell-matrix Transitions Mediated by Cadherins

Specialized junctions, such as adherens and tight junctions, are responsible for holding epithelial cells together. Important mediators of cell-cell interactions in adherens junctions are members of transmembrane proteins belonging to the cadherin superfamily (Table 1). Normal OSE derived from the coelomic layer expresses N-cadherin [5]. E-cadherin is not found in physiologic OSE but is highly expressed in surface clefts and inclusion cysts [6]. Cells transplanted from contiguous tissue, such as those from the fallopian tube epithelium, are likely sources of increased E-cadherin. Epithelial-like characteristics of newly migrated cells appear to provoke a mesenchymal-to-epithelial (MTE) transition and subsequent switch to Müllerian duct histology within the ovary [7]. This sudden up-regulation of E-cadherin leads to a gain of function effect responsible for inducing an anomalous hyperdifferentiated state very different from dedifferentiation typically observed in cancers. Cells

within the ovary then resemble those confined to nearby tissue [8]. For example, cells destined for serous carcinoma take on a Fallopian tube-like histology, clear cell carcinoma cells present with a vaginal phenotype, mucinous carcinoma cells display an endocervical histology, and endometrioid carcinoma cells show an endometrial histology. The observed phenotypes appear to reflect the source of invasive cells from adjacent tissue, making ovarian cancer and amalgam of different primary cancers.

Following the MTE transition, ovarian epithelial cells are not yet in a state promoting excessive proliferation, survival and invasiveness that would favor a shift to a tumorigenic phenotype. High concentrations of E-cadherin are associated with β -catenin transcriptional inactivity, as they bind to one another on the cell surface to prevent nuclear translocation and subsequent transcription of the S-phase regulator cyclin D1 and oncogenes such as *c-Myc* [9]. In addition, E-cadherin interacts with epithelial growth factor receptor (EGFR) to weaken its ligand affinity [10]. A dampened EGFR ligand response in E-cadherin-positive cells, as well as pre-dysplastic N-cadherin expressing cells, is believed to be involved in growth suppression via contact inhibition due to increased p27 expression [11, 12].

Peritoneal dissemination is not capable of occurring until an epithelial-to-mesenchymal (EMT) reversal takes place following initial MTE transition. Epithelial-like adhesive qualities exhibited by newly implanted cells may be overridden by inhibitory signaling localized within the microenvironment of the transcoelomic mesothelium.

While epithelial cells exhibit apical/basolateral polarity on a basement membrane with strong adhesive characteristics, mesenchymal cells display a front/back polarity within an interstitial matrix, endowing them with the ability to invade the basal lamina, the major barrier to cell migration. The potential for EMT reversal is high in OSE cells, as these mesothelial-like cells exhibit reversible mesenchymal characteristics due to their regenerative capacity in post-ovulatory repair [13].

Because of the inhibitory effects on growth induced by E-cadherin when expressed in the ovary, its

Table 1 Patterns of cadherin expression in OEC compared to normal OSE

Cadherin type	OSE (+/-)	OEC expression	Function	References
E-cadherin	(-)	Expressed (MTE) Down-regulated (EMT)	Cell adhesion, morphogenesis, contact inhibition, p27 expression	[5–7, 11, 122, 123]
N-cadherin	(+)	Expressed (serous/endometrioid)	Cell adhesion, cell survival, epithelial integrity, p27 expression	[12, 123–126]
H-cadherin	(+)	Down-regulated	Cell recognition, contact inhibition, p21 expression	[127, 128]
P-cadherin	(-)	Expressed after regional metastasis	GnRH-dependent p120 activation, cell invasion	[5, 26, 123, 129]

repression is vital in the commencement toward EMT. A major transcriptional repressor of E-cadherin is Snail1 [14]. Several growth factors and related proteins up-regulate Snail1, including EGF, vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), p70 S6 kinase, nuclear factor-kappa beta (NF- κ B) and integrin-linked kinase (ILK), which are often highly expressed in OEC [15–17]. Snail1 is induced by hormonal triggers as well. Its activation has been demonstrated to be associated with estrogen binding to its ER- α receptor [18]. Zeb1/2 and Twist are other transcriptional repressors of E-cadherin, with Twist being upregulated by EGF via STAT3 activation [19–22]. Activated EGFR can suppress E-cadherin directly by disassembling the cadherin/catenin complex and triggering endocytosis of E-cadherin [23]. Silencing by promoter methylation is quotidian in many cases as well. Finally, the ectodomain of E-cadherin responsible for promoting cell-cell adhesion and directing binding specificity, is shed upon interaction with the gelatinase matrix metalloproteinase (MMP)-9 to further inhibit its activity [24].

Confluence of the EMT-transformed OSE with the peritoneum helps enable the ovarian cells with newly acquired invasive mesenchymal characteristics to anchor into the peritoneal mesothelium and spread into the fluid in the peritoneal cavity. This environment is rich in growth factors, inflammatory mediators, bioactive lipids and ECM components. Matrix elements such as the hyaluran/CD44/versican complex can form a protective pericellular matrix to coat aggregated cells and thus likely aids in immune system evasion [25]. In the peritoneum, a second cadherin switch may occur, as the down-regulation of E-cadherin in newly implanted ovarian cells is accompanied by a surge in native P-cadherin upon peritoneal migration [26]. P-cadherin expression in breast and endometrial cancer has been associated with platelet interactions, angiogenesis and metastasis [27, 28].

Integrin Involvement in Adhesion and Signal Transduction

Integrins constitute a family of transmembrane receptors that mediate a variety of cellular functions, including cell adhesion, cell cycle regulation and signal transduction [29, 30]. They are heterodimeric proteins composed of different combinations of α and β subunits. Strength of ligand binding is mediated by cationic interactions with α subunits in the extracellular domain, while signal transduction is mediated by interactions between intracellular β subunits and various signaling proteins [31]. Types that have frequently been implicated in OEC are α_v , α_5 , and β_1 subunits [32–34].

Integrin function is critical to both early peritoneal dissemination as well as sustenance of tumor cells in ascitic fluid, which gradually accumulates and commonly reaches volumes greater than 500 ml by advanced stages [35, 36]. Increased α_v subunit expression in ascites has been associated with carcinoma cells while α_5 expression is coincident with spheroid aggregation [33, 34, 37].

A major pathologic mechanism of α_v integrin lies in its ability to bind osteopontin (OPN). This secreted glycoprotein mediates cell signaling events favoring survival and motility when bound to $\alpha_v\beta_3$ integrin [38]. Experiments with lung cancer cells have revealed multifarious pathway involvement resulting from $\alpha_v\beta_3$ -mediated OPN signaling, including PI3K/Akt, ERK1/2, FAK, and NF- κ B pathways [39]. Expression of CD44 and up-regulation of (MMP)-2 and MMP-9 have been associated with $\alpha_v\beta_3$ integrin bound to its ligand [40, 41]. Caution should be exercised in extrapolating results from other types of solid tumors to ovarian cancer, however.

Up-regulation of β_1 integrin is a frequent finding in ascitic fluid. Increased expression of β_1 integrin in OEC is strongly associated with increased heparan-binding (HB)-EGF, elevated lysophosphatidic acid (LPA), and decreased E-cadherin [42, 43]. Multicellular aggregation into the spheroid topology mediated by $\alpha_5\beta_1$ integrin has been linked to two splice variants of the serine protease kallikrein-7 [44]. Proteases in the large kallikrein family are associated with ECM degradation and tissue remodeling, and in malignancies they are often implicated in invasion and metastasis. Several kallikreins are dysregulated in OEC, including kallikrein-7 [45]. Many details regarding the mechanisms of these steroid-dependent proteases have not yet been elucidated, although their functions in establishing and maintaining the tumor microenvironment are gradually being uncovered (Table 2). An inverse relationship between E-cadherin loss and $\alpha_5\beta_1$ integrin has additionally been observed, consistent with remodeling associated with EMT transition to an invasive phenotype [46]. The membrane trafficking protein responsible for displaying $\alpha_5\beta_1$ integrin at the cell surface is Rab25, and tumor development has been shown to be facilitated by enhanced Rab25 activity [47].

Key downstream signaling pathways involved in cell growth, survival and migration become activated through the formation of a submembrane signalosome complex consisting of the cytoplasmic domains of integrins as well as associated proteins. These include the MEK/ERK and PI3K/Akt pathways [48–50]. Activation of the PI3K pathway has been linked to resistance to apoptosis in lung and ovarian cancer [50, 51]. Proteins acting as mediators for signal transduction upon binding to β integrin subunits in signalosome formation include ILK and focal adhesion kinase (FAK). ILK functions in both structural arrangement

Table 2 Kallikrein-related peptidases that are frequently overexpressed in OEC

Kallikrein-related peptidase	Prognosis	Known targets/functions
KLK4	unfavorable	Pro-uPA activation, meprin metalloprotease β activation, pro-KLK7 activation, morphological changes [130, 131]
KLK5	unfavorable	Meprin metalloprotease α , β activation, cell proliferation, morphological changes [131]
KLK6	unfavorable	PAR1 activation, lymphocyte survival [132]
KLK7	unfavorable	$\alpha_5\beta_1$ integrin-dependent spheroid aggregation in ascitic fluid [44]
KLK8	favorable	Meprin β metalloprotease activation, pro-KLK7 activation, morphological changes [131]
KLK9	favorable	unknown
KLK10	unfavorable	Cell proliferation (unknown mechanism) [133]
KLK11	favorable	IGF activation [134]
KLK14	favorable	Regulation of activation of proKLKs [135]
KLK15	unfavorable	Regulation of activation of proKLKs [136]

of molecules and cell signaling. This focal adhesion plaque component is involved in integrin and growth factor binding to cytoskeletal actin and causes nuclear translocation of β -catenin via inhibition of GSK-3 β [52, 53]. β -catenin is a binding partner of E-cadherin that induces transcriptional activity of Snail-1 and cyclin D1 when present in the nucleus [54]. The non-receptor kinase, FAK, is activated by autophosphorylation following galectin, talin or paxillin interaction with β_1 integrin, and becomes phosphorylated at additional sites in response to growth factors to regulate different signaling pathways [55, 56]. Activation of FAK plays a part in the development of anchorage-independent tumor aggregates in ascites to promote metastatic potential by overriding anoikis, which causes cell death upon disassociation from the ECM under normal circumstances [57, 58].

Integrin expression has been correlated with angiogenesis. Activation of both VEGF and FGF is associated with increased α_v expression [59]. Endothelial cell proliferation is associated with $\alpha_5\beta_1$ binding to fibronectin in the ECM [60]. In addition, pericyte adhesion and differentiation is regulated in part by β_1 subunit-mediated signaling [61]. Taken together, these data show a critical role for integrins in not only tumor initiation and invasion, but in neovascularization necessary for tumor growth as well.

Metastasis and angiogenesis related with altered β_1 integrin signaling is due in part to alterations in carbohydrate binding characteristics. The sialyltransferase ST6Gal-I is frequently overexpressed in ovarian cancer, and this enzyme has been shown to increase branched $\alpha_2,6$ -sialylation of the β_1 subunit, altering its adhesion characteristics to favor an invasive phenotype [62]. In addition, hypoglycosylated variants of mucin-1 (MUC1) are common in OEC [63]. Mucins are large proteins heavily O-glycosylated on proline-threonine-serine tandem repeat

sites that form a protective barrier on the surface of epithelial cells. Alternate splice variants lacking these domains instead feature short oligosaccharides, including Thomsen-Friedenreich (TF) antigen [64]. Without the protective barrier of O-glycosylated MUC1 on the cell surface, the TF antigen in its asialylated form enhances galectin-3 binding to β_1 integrin, facilitating both cell motility and angiogenesis [65, 66].

MMPs and Growth Factors in Matrix Remodeling

The transformation of a microenvironment conducive to creation of large scale ostensible alterations in cell polarity and cytoskeletal binding characteristics involves the actions of a number of proteases for degradation and growth factors for remodeling. Signaling mediated by EGF and HB-EGF with the EGFR receptor helps to promote a shift toward a pro-survival and pro-proliferative state, while MMP activation is critical in breaking down the substratum to favor migration and invasion. Although overexpression of several MMPs is subsumed in the malignant phenotype, MMP-2, MMP-9 and membrane type-1 (MT1)-MMP have particularly important known functions in tumorigenesis and metastasis [67].

MMPs constituting the gelatinase subfamily (MMP-2, MMP-9) are necessary for non-specific degradation of a number of ECM components during the large scale remodeling needed for EMT transition. MMP expression is hormonally regulated in healthy ovaries by LH in response to the need for tissue remodeling during ovulation to support follicular growth. These proteases additionally maintain the corpus luteum and dissolve the follicular wall and corpus luteum in post-ovulatory repair [68]. Gelatinase concentrations are especially high, as they localize around developing follicles to support their growth. Larger follicles

over 25 mm diameter are associated with increased amounts of MMP-9 [69].

The serine protease responsible for activation of most MMPs, including the transmembrane type MT1-MMP and MMP-9, is urokinase plasminogen activator (uPA) bound to its uPAR receptor [70, 71]. Activation of the uPA/uPAR complex is mediated by several microenvironmental constituents, including lysophosphatidic acid (LPA) and VEGF [72, 73]. MT1-MMP is a key regulatory MMP in modulating the pericellular microenvironment, and its expression stimulates cooperative communication with integrins during ECM remodeling as well as activation of MMP-2, which is otherwise not directly expressed by uPA [68, 74]. Tight junction proteins in the claudin family are thought to play a role in MMP expression as well. Claudin-3 and claudin-4 are frequently expressed in OEC, as they aid in altering membrane permeability to facilitate invasion. Both of these proteins have been implicated in gelatinase activation [75, 76].

Growth Factors in EMT and Angiogenesis

When the inhibitory effects on proliferation and invasion mediated by E-cadherin are abrogated through its inactivation, EGFR is then freed by the changing microenvironment to enhance its ligand binding affinity to EGF and HB-EGF. MMP-9 activity is strongly correlated with increased EGFR activity [43, 77]. EGFR activation in the OSE facilitates a number of EMT inducing events, including suppression of the pro-apoptotic cyclin inhibitor protein p27, increased β_1 and particularly $\alpha_5\beta_1$ signaling, activation of VEGF, ERK, and ILK, decreased caveolin-1 expression, and increased gelatinase expression [43, 46, 78].

A major EGFR ligand observed in OEC cells is HB-EGF [43]. This growth factor is highly expressed proportionately as disease pathology progresses, yielding high concentrations in advanced stages and ascites in particular [79]. HB-EGF contains a heparan sulfate binding motif that enables its binding to heparan sulfate proteoglycans (HSPG) in the coelomic epithelium. An analysis of HSPG expression in normal OSE compared to that derived from tumors evinced normal patterns for all cell surface PGs except syndecan-1 (SDC1), which was highly expressed in tumor cells but absent from normal epithelium [80]. In addition, only SDC1 and glypican-1 were associated with poor prognoses in univariate analysis. It can thus be inferred that induction of SDC1 is a critical factor in EGFR activation upon heparan sulfate binding to HB-EGF to enable cell surface accumulation of this ligand. Hypersulfation of HSPGs secondary to inactivation of the arylsulfatase HSulf-1 is a common event in OEC, and may contribute to neo-

vascularization by increasing binding capacity of endothelial cells to the heparin-binding domains of VEGF_A and FGF [81].

The cross-talk between MMPs and growth factors is apparent during angiogenesis. Both MMP-2 and MMP-9 induce expression of VEGF, and VEGF, in turn, can induce expression of MMP-9 [82, 83]. VEGF plays an important role in stromal invasion and neovascularization upon binding to endothelial cells, and is one of the most predominant angiogenic agents in tumor formation. Nerve growth factor (NGF), through its tyrosine kinase A (TrkA) receptor, is another potent angiogenic factor. Downstream signaling via the PI3K/Akt pathway induces MMP-2 [84]. This effect is likely due to increased expression of its activator, MT1-MMP [85]. In breast cancer cells, TrkA receptor-mediated signaling by NGF through both the PI3K/Akt and MAPK/ERK2 pathways were shown to activate MMP-2 and VEGF, as well as endothelial nitric oxide synthase (eNOS) [86]. This produces a highly invasive phenotype with high vascular permeability to strongly favor angiogenesis. Hormonal effects of NGF/TrkA interactions include an anti-luteinization effect via progesterone inhibition coupled with follicle stimulating hormone receptor (FSHR) and estrogen sensitization [87]. Overexpression of gonadotropins, such as FSH and LH, is associated with OEC progression, and progesterone is believed to have an inhibitory effect, as both progestin-only contraceptives and pregnancy, which is characterized by elevated progesterone levels, are associated with risk reduction. Because of the ubiquitous nature of NGF in establishing the tumor microenvironment and facilitating angiogenesis, phosphorylated TrkA has recently been proposed as a potential biomarker for EOC [88].

Negative Regulation of Growth Factors and MMPs

With the large degree of matrix-associated remodeling necessary for tumorigenesis, it is reasonable to asseverate that there will be a paucity of negative regulators of growth factors and MMPs as cells become more invasive and resistant to death with disease progression. Indeed, this is what is observed. Growth factor regulation is often attributed to solubility. As a general rule, soluble growth factor receptors have inhibitory functions and intact receptors promote survival, growth and proliferation. One potential biomarker for OEC is the lack of soluble (s)EGFR. This receptor is a secretory splice variant that inhibits tyrosine kinase activity when bound to its ligand. The amount of sEGFR progressively decreases with age and with advancing stages of OEC [89, 90]. Its reduction is additionally correlated with increased gonadotropin con-

centrations [89]. Other potential risk and disease markers are TIMP family inhibitors of MMPs. TIMP1 is specific for MMP-9 inactivation, while TIMP2 inhibits MMP-2 and TIMP3 suppresses both MT1-MMP and MMP-9. Decreased levels of these three TIMPs have been associated with OEC through LPA-mediated interactions [91].

Not much information is available regarding negative regulation of angiogenic growth factors. Two candidate markers that may impact future ovarian cancer research are G-protein receptor kinase 5 (GRK5) and neurotrophin receptor-interacting MAGE homolog (NRAGE). In a study by Zhou et al., expression of GRK5 in human coronary artery endothelial cells reduced VEGF signaling to abrogate cell proliferation and migration [92]. Activation of TrkA-mediated signaling upon interaction with NGF is inhibited by NRAGE [93]. This has been demonstrated to cause a significant reduction in MMP-2 expression and diminished motility and invasion in melanoma and pancreatic cancer cells both in vitro and in vivo [94]. NRAGE is a binding partner of BRCA2 that negatively regulates cell proliferation in a p53-independent manner, and is down-regulated in 37.5% of breast cancer cell lines studied [95]. Despite the strong association between ovarian cancer and *BRCA2* gene inactivation through either mutation or loss of heterozygosity, the possibility of altered NRAGE expression in OEC has yet to be examined.

GPCRs in the Regulation of Tumor Development

A major convergence point of several mechanisms that lead to peritoneal dissemination and spreading of EMT-converted cells derived from the ovary is the activation of GPCRs. These receptors regulate many aspects of tumor growth and development, such as cell growth, cell survival, motility, cytoskeletal remodeling, angiogenesis, cell cycle progression and chemotaxis [96]. Two principal ligands involved in GPCR-mediated processes are LPA and ET-1, and their tumorigenic activities are mainly exerted through LPA₂, LPA₃, and ET_AR.

LPA

LPA is a phospholipid that exerts its effects upon binding to one of five GPCRs, designated LPA₁₋₅. Of these receptors, LPA₁₋₃ are extracellular receptors and LPA₁ has an inhibitory role in inflammation and growth regulation. LPA is produced by enzymatic activity of either ATX or PLA₂. ATX expression is growth factor dependent, and it has been shown to be up-regulated by bFGF, EGF and VEGF [97, 98]. The peritoneal mesothelium constitutively produces PLA₂, so

translocated ovarian cells are constantly exposed to its presence [99]. Of particular concern in OEC is the secreted form of PLA₂ (sPLA₂). This isoform displays little activity in intact membranes but has high affinity for microvesicles similar to those found in ascites [100]. Expression of sPLA₂ has been shown to stimulate cell invasion [101].

Receptor binding to LPA₂, and to some extent LPA₃, activates a number of signaling pathways important in tumor development and metastasis. GPCR-mediated LPA-induced events include FAK phosphorylation, NF- κ B up-regulation and activation of the PI3K and MAPK/ERK pathways [42]. Phosphorylated FAK may be required to override anoikis in ascitic fluid, and NF- κ B affects transcription of several factors involved in cell growth, endothelial permeabilization and cell migration, such as Snail1, IL-6 and IL-8 [57, 102, 103]. ERK activation associated with LPA-mediated GPCR binding induces expression of sphingosine kinase 1 (SphK1), which phosphorylates sphingosine to generate S1P [104]. When phosphorylated by SphK1, but not the inhibitory SphK2, S1P stimulates endothelial cell proliferation and migration to trigger neovascularization [105]. High concentrations of sphingosine-1-phosphate (S1P) are observed in ovarian cancer cells [106].

Hypoxia caused by ascites is a contributing factor toward mitogenic activity. A major angiogenic effect of LPA is its ability to activate VEGF under the hypoxic conditions within peritoneal fluid. Transcription of VEGF is up-regulated through LPA-mediated activation of HIF-1 α [107]. A cooperative effect is possibly exerted by α_v integrin-bound OPN. Induction of HIF-1 α has been observed in ovarian cancer cells under normoxic conditions from overexpression of OPN via PI3K/Akt activation [108]. Not surprisingly, VEGF has been demonstrated to be up-regulated in endothelial cells, leading to neovascularization, and these effects have been inhibited by administration of anti-OPN and anti- $\alpha_v\beta_3$ antibodies [109]. Cross-talk is possible as LPA-mediated signaling was shown to induce OPN expression via Akt activation in a hepatic cancer cell line [110].

ET-1

ET-1 is an endothelial vasoconstricting peptide that normally functions in smooth muscle cell contractions. Tumorigenic effects mediated through binding to ET_AR. Like LPA, this ligand is found in high concentrations in ascitic fluid and exhibits redundancy in stimulating many of the same pathways [111]. A major effect of ET_AR activation is induction of the ILK pathway, leading to Snail1 and β -catenin nuclear localization and activation [112]. Phosphorylation of FAK and activation of the MAPK and PI3K/Akt transduction cascades are also mediated by

ET-1/ET_AR binding [113, 114]. Increased expression of β 1 integrins and disrupted gap junction communication has been linked to ET-1 as well [115, 116]. The latter phenomenon has been attributed to ET-1-mediated phosphorylation of connexin 43 [116].

EGFR Transactivation by GPCRs

Both LPA and ET-1 can promote EGFR transactivation through their GPCRs. Although several molecules have been associated with EGFR transactivation in different cancer types, OEC cells have shown a close association between GPCR signaling mediated by LPA and ET-1 and activation of the HB-EGF ligand [117, 118]. Transactivation of EGFR is often the result of GPCR-mediated activation of members of the ADAM family of metalloproteases [119]. The enzyme displaying the greatest correlation to HB-EGF activity is ADAM-17, but ADAM-9 and -12 have also been implicated [120]. The major mechanism for EGFR transactivation by GPCR signaling appears to be proteolytic cleavage of HB-EGF from its transmembrane precursor to its active soluble form by the sheddase activity of ADAM-17. Once in soluble form, HB-EGF can then phosphorylate its receptor to initiate multiple mitogenic signals from at least five distinct pathways (PI3K/Akt, Ras/p44/42/MAPK, MEKK/p38 MAPK, PLC/PKC, Rho-GEF/RhoA), which increase the invasive and proliferative nature of cells [117, 121]. It is therefore apparent that the alterations in the microenvironment caused by GPCR-mediated signaling contribute to EMT transition in a number of different ways, making these receptors impor-

tant upstream regulators of tumor formation and sustenance (Fig. 1).

Conclusion

The preceding exposition elucidated several commonly occurring mechanisms factoring in the early stages of tumor formation and initial dissemination into the peritoneum, which is central to the transformation from local dysplasia to fully invasive malignancy. Discernment of the pathophysiology of ovarian cancer reveals several possible molecular markers while providing clues on where to hone in for finding possible drug targets, as large scale changes in tumor microenvironment as well as gene expression leave researchers with an arduous task on deciphering the most important developments that may be of therapeutic relevance. Because of the high propensity for outside-in signaling coordinated by the tissue matrix toward the activity of constituent cells, a thorough investigation of events unfolding in the tumor microenvironment will indubitably reveal signal modulators that regulate several downstream effectors and thus should serve as better therapeutic targets than any one of the redundant markers individually. Much further research is required before a more comprehensive depiction of the microenvironment that provides the necessary conditions required for development and metastasis of tumors can be achieved. Advancements in proteomics, transcriptomics and glycomics should reveal patterns that will help to uncover additional components to better understand the transformation of the tumor microenvironment in ovarian carcinoma.

Fig. 1 Proposed model of tumor microenvironment during EMT transition in high grade OEC. Repression of E-cadherin and subsequent increase in integrin signaling represent key early steps. GPCR activation by LPA and ET-1 controls several aspects of EMT cascade, including transactivation of EGFR and up-regulation of MMPs necessary for ECM remodeling. Induction of CIN reinforces the tumorigenic phenotype in a positive feedback mechanism by targeting key tumor suppressors, such as p53, for inactivation. The result is a transformed microenvironment that facilitates proliferation, survival, angiogenesis, and metastasis

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