Published in final edited form as:

Semin Cancer Biol. 2002 April ; 12(2): 97–104. doi:10.1006/scbi.2001.0417.

The dominance of the microenvironment in breast and ovarian cancer

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Abstract

That cancer development is a multistep process, driven in large part by genetic change, is well established. However, it is becoming increasingly clear that, prior to its emergence, the tumorigenic phenotype must overcome the suppressive effects of the surrounding microenvironment. Because the microenvironment is tissue-specific, cancer in each organ must develop unique strategies to overcome these normal epigenetic suppressors. Surprisingly, the induction of glandularity during the earliest stages of ovarian carcinoma development produces a microenvironment that has much in common with the normal mammary gland. This phenotypic convergence may explain why similar genetic and epigenetic changes appear to play a role in breast and ovarian tumor progression.

Keywords

basement membrane; E-cadherin; epigenetics; integrins; morphogenesis

Introduction

In the breast, the target cell for carcinoma formation is located in the bilayered, polarized epithelium of the mammary gland.¹ The basal myoepithelial cells directly contact the basement membrane (BM) while the luminal epithelial cells form the apical layer that contacts the central lumen. Because of microscopic gaps in the myoepithelium, luminal cells also contact the BM, although this interaction is more prevalent in the lobules of the gland than it is in the ducts. While breast carcinoma cells often retain some characteristics of luminal cells, evidence is emerging that the target cell is bipotent.²

In the ovary, the major target cell for carcinoma formation is located in the modified mesothelium that covers the organ.³ This single-layered ovarian surface epithelium (OSE) is not glandular; all of the cells directly contact the BM basally and face the peritoneal fluid of the abdominopelvic cavity apically. Thus, there are fundamental microenvironmental differences in the normal breast and ovarian target tissues. However, the fact that many of the same risk factors (e.g. hormonal) and genes (e.g. BRCA1, erbB2) have been implicated in the development of breast and ovarian carcinomas suggests that a phenotypic convergence may play a role in the ultimate clinical outcome of the two diseases. Indeed, the acquisition of a polarized, glandular phenotype is a hallmark of early pre-neoplastic change

in the OSE. In this review we will first address this issue of convergence. We will then discuss how the progression to the metastatic state in both the breast and ovary is critically influenced by the co-opting of common epigenetic effectors present within the glandular microenvironment.

Glandular phenotypic convergence in the ovary is regulated epigenetically by E-cadherin

As tissue-specific promoters for the OSE are just now being identified, there is not yet a definitive mouse transgenic model of ovarian carcinoma formation.⁴ Thus, there is still some doubt about the linearity of tumor development and progression.^{5,6} However, histopathological studies of clinical specimens indicate that the early stages of ovarian carcinoma development are unusual in that they are not associated with a decrease in tissue organization; both pre-malignant structures and tumors of low malignant potential are very often polarized, differentiated and glandular. The glandular architecture of these differentiated ovarian lesions is so striking that many are classified on the basis of their similarity to normal oviductal (serous tumors), endocervical (mucinous tumors) or uterine (endometrioid tumors) epithelia.⁷ All of these lesions arise from the OSE,⁸ although there is evidence that ectopic endometriotic deposits can also be transported to the ovary by 'retrograde' menstruation.⁹ Regardless, this early acquisition of ovarian glandularity signals a phenotypic convergence with the normal mammary gland, in terms of both architecture and microenvironment (Figure 1).

The OSE is a simple cuboidal epithelium that develops as an embryonic specialization of the coelomic mesothelium located on the posterior abdominal wall. Despite the fact that they form a lining epithelium, OSE cells exhibit a number of mesenchymal characteristics, which likely reflects the fact that the mesothelium is mesodermally-derived.¹⁰ One of these mesenchymal characteristics is the absence of the epithelial cell-cell adhesion molecule E-cadherin.^{11,12} We have speculated that this lack of E-cadherin facilitates rapid OSE cell migration into post-ovulatory wounds after follicular rupture, a hypothesis that is supported by studies in tissue culture. When OSE cells are maintained in monolayer culture under conditions that mimic wounding, they become migratory and rapidly undergo an epithelial to mesenchymal transformation (EMT).¹³ When these mesenchymal OSE cells are placed on reconstituted BM gels they do not form organized glandular structures as is the case with mammary epithelial cells (see below). Instead, the OSE cells migrate into the BM matrix.¹⁴ This wandering, mesenchymal behavior also occurs in three-dimensional (3D) collagenous sponge cultures where OSE cells migrate into the spaces between the sponge spicules by depositing their own stromal extracellular matrix (ECM).¹⁵

The mesenchymal characteristics of the OSE are not apparent in pre-malignant ovarian inclusion cysts.³ In this condition OSE cells become trapped within the ovarian stroma, remain stationary and they form cystic epithelial linings that are often columnar, polarized and secretory. Therefore, cystic OSE cells undergo a metaplastic change in which they become epithelially restricted and somewhat glandular. Not surprisingly E-cadherin expression is upregulated in these metaplastic cells.¹¹ To determine if E-cadherin is a cause or consequence of cystic metaplasia, we force-expressed the cell adhesion molecule in normal OSE cells (designated OSE-EC). This reversed the ovarian EMT normally observed in two-dimensional (2D) monolayers and it caused OSE-EC cells to form cyst-like linings in 3D collagenous sponge cultures.¹⁶ Cystic OSE-EC cells form functional adherens junctions and the expression of tight junction proteins is upregulated. In addition, OSE-EC cells are polarized and secretory; they express the high molecular weight glycoprotein CA125 which is secreted apically in a vectorial fashion.

Like pre-malignant inclusion cysts, glandular ovarian carcinomas express E-cadherin and polarizing tight junction proteins.^{11,12,17,18} They also produce and secrete CA125. The latter is diagnostically significant as a severely elevated serum CA125 level is an important non-surgical indicator of ovarian carcinoma.¹⁹ CA125 is also a normal differentiation antigen of Mullerian duct-derived epithelia which line the oviduct, endocervix and the endometrium. As described above, ovarian carcinomas are classified on the basis of their morphological similarities to these glandular epithelia. Embryologically, the Mullerian ducts arise when the same coelomic mesothelium that gives rise to the developing OSE migrates into the underlying stroma. Thus, while alternative hypotheses exist,²⁰ it has been proposed that cystic OSE trapped within the ovarian stroma can undergo a subtle Mullerian transdifferentiation and directly give rise to ovarian carcinomas.^{8,21,22} Our finding that CA125 is produced by OSE-EC cells suggests that ectopic E-cadherin expression is one of the initiators of this transdifferentiation. This begs the question, what upregulates E-cadherin during the early stages of glandular ovarian carcinoma development? While the specific microenvironmental effectors that mediate this ectopic expression have not yet been identified, the E-cadherin promoter is transactivated both in metaplastic OSE and in differentiated ovarian carcinoma cells.²³ Therefore, the upregulation is clearly epigenetic.

At first glance it appears somewhat paradoxical that E-cadherin could facilitate early ovarian carcinoma progression as this cell adhesion molecule is clearly a late stage tumor suppressor.²⁴ However, we would like to suggest that E-cadherin confers an early selective advantage in the ovary precisely because it contributes to transdifferentiative glandular morphogenesis. The rationale for this tentative hypothesis is the unusual route by which ovarian carcinomas can be initially disseminated. Instead of migrating through the ovarian stroma, glandular tumor nodules are often exfoliated from the surface of the ovary where they float free in the peritoneal fluid of the abdominopelvic cavity. These nodules are often so prevalent that surgical ‘debulking’ procedures have been developed in an effort to remove them.²⁵ Normally, epithelial cells apoptose when they are placed in suspension, a process that has been described as anoikis.²⁶ Interestingly, anoikis can be inhibited by E-cadherin-mediated cell-cell adhesion, at least in part because this adhesion leads to the maintenance of anti-apoptotic PI-3-kinase signaling.^{27,28} As ovarian carcinoma cells are particularly susceptible to pharmacologic PI-3 kinase inhibition,²⁹ it has been suggested that E-cadherin may facilitate cell survival when glandular tumor nodules leave the ovary and begin to metastasize into the abdominal cavity.³⁰

Epigenetic effectors of morphogenetic change during breast and ovarian carcinoma progression: the microenvironment is dominant

Transgenic mice have been used extensively to identify genes that contribute to breast carcinoma formation. Unlike the situation in the ovary, the development of these experimental models has been greatly facilitated by the availability of regulatory elements that are specifically transactivated in the mammary epithelium. However, while the transgenes are expressed throughout the gland, true malignant tumor formation is a rare event. Although screens are being used to identify secondary genetic changes that help drive tumorigenesis,³¹ it is clear that a common epigenetic theme in the emergence of these experimental tumors is the progressive architectural disruption of the glandular microenvironment.³² Successive and cumulative architectural changes in the transgenic glands, which often mimic those that occur during true clinical breast tumor progression, include: a relatively common anti-apoptotic, proliferative multilayering of the epithelium in pre-malignant hyperplastic lesions; a rarer intraepithelial cellular disorganization that often leads to obliteration of the central glandular lumen in non-malignant carcinomas *in situ*; and, a still rarer migration of epithelial-derived tumor cells into the surrounding stromal compartment in invasive carcinomas. The increasing rarity of each architectural change

suggests that microenvironmental suppression becomes more stringent as tumor progression proceeds. A major epigenetic effector of this suppression is the ECM.³³ The mammary epithelium begins as a surface ectodermal thickening that migrates into the underlying stroma to form cohesive cords during embryonic development. These cords canalize and undergo a restricted branching to form ductal structures that increase in length proportionally with increased body size from birth to puberty. Ductal elongation and branching then accelerate and the terminal ends of the ducts expand. These terminal endbuds give rise to the lobular portions of the gland that expand and differentiate into milk-producing alveoli during adult cycles of pregnancy and lactation.³⁴ A critical microenvironmental effector of these architectural and differentiative changes is the specialized BM ECM that forms at the interface between the basal surface of the glandular epithelium and the stromal compartment.³⁵ Thus, when mammary epithelial cells are separated from the BM by enzymatic digestion and maintained in 2D monolayer culture they remain epithelial but glandular architecture and differentiated function are rapidly lost. If these cells are instead placed on flexible, floating gels of stromal collagen, they now deposit an endogenous BM and achieve a modicum of glandular morphogenesis and differentiated function.^{36,37} More dramatically, when these cells are maintained directly in 3D BM gel culture they undergo a complete glandular, alveolus-like morphogenesis and they fully differentiate.^{38,39} The BM glycoprotein laminin-1, particularly in its cross-linked form, is a crucial regulator of this inductive process.^{40,41} Acting first in an integrin-independent manner, laminin-1 causes the cells to round up and to aggregate into solid, disorganized spheroids. This morphologic action of laminin inhibits proliferation and it is a pre-requisite for differentiation.⁴² Laminin-mediated integrin signaling then initiates apical/basal polarization within the cellular aggregates, cavitation of a central lumen, and differentiative milk protein gene expression.^{43,44} 3D BM gel culture also induces normal human mammary epithelial cells to undergo a non-proliferative, polarized, alveolus-like morphogenesis.⁴⁵

Pre-malignant change in the breast is associated with a proliferative multilayering of the glandular epithelium that causes many of the cells to move away from the BM towards the central lumen. Normally, mammary epithelial cells that are no longer in contact with the BM undergo apoptosis. This phenomenon is physiologically relevant at the end of lactation when glandular involution is partially driven by the transient destruction of the BM by metalloproteinases.⁴⁶ This apoptosis is suppressed by BM-dependent $\alpha 1$ integrin signaling.⁴⁷ Thus the treatment of mammary epithelial cells in contact with the BM⁴⁷ or purified laminin⁴⁸ with $\alpha 1$ integrin blocking antibodies initiates apoptosis while anchorage-independent stimulation of $\alpha 1$ integrin signaling prevents it.⁴⁹

Numerous changes in integrin expression have been noted in breast carcinomas. However, the data are often conflicting with respect to upregulation, downregulation and the integrin subunits affected.⁵⁰ These data suggest that changes in functional integrin signaling rather than expression may be critical for breast tumor development. We have shown this to be the case using a human breast tumor progression series. As these cells are passaged in culture they acquire numerous genetic abnormalities, proliferation is dysregulated, the ability to form 3D structure is lost, and the cells no longer respond to architectural cues in the BM. These changes are accompanied by a chronic activation of $\alpha 1$ integrin signaling at the expense of $\alpha 6 \alpha 4$ integrin signaling which normally acts to initiate apical/basal polarity⁵¹ in response to contact with laminin in the BM. When this integrin ‘switching’ is reversed, proliferation is controlled, morphogenesis is restored and tumorigenesis is dramatically reduced *in vivo*, despite the fact that the genetic abnormalities acquired during progression are unaffected.⁵² Therefore, appropriate integrin signaling is a microenvironmental epigenetic effector that can act dominantly to suppress the emergence of the breast cancer phenotype.

The $\alpha 6 \beta 4$ integrin is present in cyst-like OSE-EC cells (Wu and Roskelley unpublished obs) and in differentiated glandular tumors where it interacts with basally deposited laminin.⁵³ In contrast, when ovarian carcinoma nodules are exfoliated into the abdominal cavity, the interaction between $\alpha 6 \beta 4$ integrins and laminin is disrupted. $\beta 1$ integrin signaling then contributes to the attachment of ovarian carcinoma cells to the peritoneal lining,⁵⁴ which is required for the development of secondary extraovarian tumor deposits. Once this secondary attachment has occurred the nodule can expand, glandular architecture can become increasingly disrupted, and local invasion into sub-peritoneal organs can follow. Therefore, ovarian carcinomas may progress by co-opting the same epigenetic effectors that normally suppress breast carcinoma progression. Ironically, in addition to integrin switching, another epigenetic suppressor of late stage tumor progression in both the breast and ovary is E-cadherin.

E-cadherin as a late stage tumor suppressor in the breast and ovary

As described above, forced expression of E-cadherin causes normal mesenchyme-like OSE cells to become epithelialized. Conversely, a loss of E-cadherin causes many epithelial cell types to undergo an EMT.⁵⁵ This process is required for tissue rearrangements during development where it is tightly regulated by morphogenetic signaling molecules such as the Wnts.⁵⁶ However, when it is recapitulated inappropriately during tumor progression, an EMT can contribute to invasion and metastasis.²⁴ While mutational losses of E-cadherin have been noted in late stage breast⁵⁷ and ovarian tumors,⁵⁸ expression of this cell adhesion molecule is often downregulated epigenetically. Regulators of the latter downregulation include inappropriate Wnt-like signaling, hypermethylation of the E-cadherin promoter and the upregulation of the transcriptional repressor Snail.⁵⁹⁻⁶²

Mesenchymal cells are inherently migratory. Thus, this characteristic is often cited as the major reason why EMT contributes to the metastatic phenotype. However, mesenchymally-transformed cells also respond inappropriately to soluble factors in the microenvironment. For example, stromally-produced neuregulin acts in a paracrine manner to promote glandular morphogenesis and differentiation of the normal mammary epithelium during pregnancy.⁶³ In contrast, neuregulin, which acts, at least in part, by activating the erbB2 (Her-2/Neu) tyrosine kinase, increases the invasiveness of mesenchymal breast tumor cells in 3D culture.⁶⁴ When E-cadherin is force-expressed in the latter cells, neuregulin once again promotes glandular morphogenesis.⁶⁴ Thus, an appropriate 3D tissue architecture suppresses the oncogenic action of erbB2 stimulation, although direct activation of the kinase can disrupt apical-basal polarity and block proliferation suppression.^{64a} Similarly, signaling outputs from the EGF receptor (erbB1) are also regulated in an architecture-dependent fashion.⁶⁵ These microenvironmental effects may have clinical relevance as erbB blocking antibody strategies have shown significant efficacy in the treatment of late stage breast and ovarian tumors.⁶⁶

Several microenvironmental effectors have been identified that have the potential to induce a mesenchymal transformation in breast and ovarian cancers. As described above, inappropriate $\beta 1$ integrin signaling disrupts BM-dependent mammary morphogenesis in 3D culture. When $\beta 1$ integrin signaling is chronically activated by forced expression of the integrin-linked kinase or the activation of one of its distal regulators, the c-fos transcription factor, E-cadherin expression is downregulated, and an invasive EMT occurs.^{67,68} Such a downregulation of E-cadherin expression is prevalent in late stage invasive lobular breast carcinoma.⁵⁷ Mammary EMT can also be achieved by disrupting the BM itself via the forced expression of the metalloprotease stromelysin-1 (MMP-3).⁶⁹ In this case, E-cadherin is partially cleaved which initially perturbs cell-cell adhesion and apical/basal polarity in 3D culture. Such perturbations in polarity are frequently observed in invasive ductal breast

carcinoma.⁷⁰ In patients with significant ovarian tumor burdens, ascites often builds up within the abdominopelvic cavity. A prominent constituent of ascites is the bioactive phospholipid lysophosphatidic acid (LPA) which increases the invasiveness of ovarian carcinoma cells.^{71,72} While it is not clear that LPA initiates a true EMT, it does disrupt E-cadherin-mediated cell-cell adhesion.⁷³ In addition, E-cadherin expression is often downregulated in late stage ovarian carcinomas.¹⁷ Therefore, as is the case in the breast, E-cadherin appears to act as a late stage microenvironmental tumor suppressor in the ovary.

Lost suppression—disrupting the microenvironment can induce tumor formation

If morphogenesis is truly a dominant epigenetic tumor suppressor, it follows that a prolonged disruption of the glandular microenvironment alone should, in and of itself, induce tumor formation. To test this overarching hypothesis we generated transgenic mice that express an autoactivated form of stromelysin-1 in the mammary epithelium. As described above, stromelysin-1 degrades the BM and it initiates an EMT in culture. *In vivo*, the stromelysin-1 mice first exhibit more subtle morphogenic defects in ductal and lobular branching.^{74,75} Later, after multiple rounds of pregnancy, the morphogenic alterations become more severe and true tumors emerge.^{76,77} Will such a morphogenic disruption of the microenvironment initiate tumor formation in the ovary? This question awaits the development of an ovarian-specific transgenic model. However, we predict that such a model will have to incorporate an initial glandular morphogenesis step (i.e. phenotypic convergence) if it is to generate information that sheds light on the true epigenetic suppressors of ovarian carcinoma progression.

In conclusion, we have briefly discussed differences and similarities between normal and cancerous tissues in the ovary and the breast. The data discussed reinforce the fact that cancer is a tissue-specific disease controlled by both genetic change and epigenetic effectors, many of which are regulated by tissue structure. As such, a thorough understanding of disease progression and the efficient development of rational treatment strategies await a more complete elucidation of each organ's microenvironmental milieu.

Acknowledgments

Work in the authors' laboratories is supported by grants from the Canadian Breast Cancer Research Initiative (to C.D.R.), the Canadian Institutes of Health Research (to C.D.R.), the US National Institutes of Health (grants CA-64786-02 and CA57621 to M.J.B.) and the US Department of Energy Office of Biological and Environmental Research (contract DE-AC03-76SF00098 to M.J.B.).

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Figure 1.

Phenotypic convergence during breast and ovarian carcinoma development. The normal OSE is not glandular. OSE cells are cohesive and rest on a BM. However, after follicular rupture the cells migrate into the post-ovulatory wound and lay down their own stromal ECM. During the early stages of ovarian carcinoma formation OSE cells acquire a glandular phenotype such that they come to resemble the normal mammary epithelium architecturally. We have termed this phenotypic convergence. In both the breast and ovary, architectural cues in the glandular microenvironment suppress the emergence of the tumorigenic phenotype. These epigenetic effectors include the BM (black deposit around glandular structure) and cell-cell junctions (small black circles between glandular cells).