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Review

Tumour–stromal interactions
Integrins and cell adhesions as modulators of mammary cell survival and transformation

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Abstract

Stromal–epithelial interactions modulate mammary epithelial cell (MEC) growth and apoptosis by influencing cell adhesion and tissue organization. Perturbations in the mammary stroma and cell adhesion characterize breast tumors and underlie the altered tissue organization, disrupted tissue homeostasis and enhanced survival phenotype of the disease. Apoptosis resistance likely arises during malignant transformation via genetic and epigenetic modification of cell adhesion pathways induced by a changing tissue microenvironment. Acquisition of adhesion-linked survival networks that enhance MEC viability in the absence of basement membrane interactions probably promote malignant transformation, and may render breast tumors sufficiently resistant to exogenous apoptotic stimuli to generate multidrug resistance.

Keywords: apoptosis, basement membrane, integrin, mammary epithelial cell, three-dimensional tissue organization

Introduction

Homeostasis in the mammary gland is achieved by a balance between cell proliferation and cell death (apoptosis), which is reflected by an organized tissue structure. Breast cancer, which is a loss of tissue homeostasis, is characterized by perturbations in mammary tissue architecture, that is linked to alterations in the extracellular matrix (ECM) and in the adhesion molecules expressed by the MECs [1]. Correcting the adhesion defects in the mammary tumor epithelia can restore tissue organization and normal behavior to some breast tumor cells [2]. Conversely, altering the stromal microenvironment of the breast promotes the expression of tumorigenic potential in MECs [3]. This suggests that aspects of the breast cancer phenotype arise from alterations in the dynamic interplay between the epithelial cells, the mammary stroma, and the structural organization of the breast. Gaining an understanding of how disturbances in this relationship relate to the pathogenesis of human breast cancer will depend on delineating the subtleties of this dialog. This will require the application of appropriate model systems that can reconstitute stromal–epithelial interactions in the context of a three-dimensional tissue structure.

Breast cancers typically exhibit low rates of cell proliferation (apoptosis deregulation), often recur after years of dormancy (apoptosis evasion), and once re-established frequently acquire resistance to treatment (apoptosis

BM = basement membrane; DCIS = ductal carcinoma *in situ*; ECM = extracellular matrix; EGFR = epidermal growth factor receptor; MEC = mammary epithelial cell; PI3-K = phosphinositide 3-kinase.

resistance). As such, alterations in apoptosis probably predominate in the pathogenesis of human breast cancer. Therefore, an understanding of how the stroma influences adhesion and tissue architecture to modulate MEC survival, and how these pathways become deregulated in mammary tumors, should help to define the critical events that regulate breast cancer pathogenesis. In the present brief review, we discuss how altered stromal–epithelial interactions and changes in cell adhesion and tissue architecture influence MEC survival to drive malignant transformation in the breast. We then present data that suggest that the interplay between the tissue microenvironment, cell adhesion, and tissue architecture may also underlie the origins of the multidrug resistant breast tumor phenotype.

Cell adhesion and survival in mammary epithelial cells

Normal cells require adhesion to grow and survive, and anchorage independence for growth and survival is considered a key feature of transformed cells [4]. Consistent with this concept, primary and immortalized nontransformed human and murine MECs require adhesion to an ECM basement membrane (BM) in order to maintain their survival in culture. For example, both primary and immortalized MECs will retain their viability *ex vivo* in the presence of growth factors such as insulin-like growth factor-I and epidermal growth factor, provided that they are in contact with a laminin-rich BM [5]. This response is specific, because primary MECs on fibronectin or in a collagen I ECM will undergo apoptosis [6–8].

The mechanism by which adhesion to a laminin-rich BM mediates MEC survival is not completely known, but ligation and activation of the laminin receptor $\alpha_3\beta_1$ integrin is believed to be a part of the process [7,9]. Ligation of MEC β_1 integrins alters the activity of adhesion-associated kinases, such as focal adhesion kinase and integrin-linked kinase [4,6,10]. BM-mediated survival in MECs probably requires co-operative signaling with cytokine receptors, such as the insulin receptor [5] or the epidermal growth factor receptor (EGFR) [11]. Synergistic interactions between growth factor receptors and integrins in MECs presumably lead to the activation of downstream effectors such as phosphinositide 3-kinase (PI3-K), mitogen-activated protein kinase and/or nuclear factor-κB [6,12]. These enzymes in turn are functionally linked to pathways that can actively repress death by modulating the expression and/or activity of various apoptosis repressors, including members of the bcl-2 family [13]. In primary and immortalized murine MECs, for example, adhesion-dependent survival is associated with PI3-K induced repression of bax translocation to the mitochondria [14]. Moreover, integrin-linked kinase can stimulate Akt activity via PI3-K, and this in turn can influence murine MEC survival by altering the functional status of BAD [10,15].

Whether BM-directed integrin-linked pathways also operate to mediate MEC survival in the mammary gland has not been directly established. Nevertheless, there is good concordance between remodeling of the mammary gland *in vivo* and expression of genes associated with involution (apoptosis) [16]. Furthermore, loss of mammary gland function and apoptosis also correlate with increased expression of metalloproteinases, which are ECM-degrading enzymes. Indeed, parallel studies conducted *ex vivo*, using MECs ectopically expressing the metalloproteinase stromelysin-1 [17], directly demonstrated that acute exposure to metalloproteinases and rapid degradation of the BM would lead to apoptosis.

It should be noted that two waves of apoptosis occur during involution in the mammary gland. The first wave, or initiation stage, of apoptosis involves the death of a small population of differentiated MECs, and probably occurs as a consequence of changes in systemic hormones and/or mechanical forces. The second stage involves the death of the remaining acinar MECs, and is linked to the activation of metalloproteinases, ECM degradation, and the irreversible commitment to remodel the mammary gland [16]. The precise roles of cell adhesion and integrin signaling in either of these stages of involution have not been well defined. Nevertheless, it was reported [18] that chronic exposure of MECs to stromelysin-1 both *in vivo* and in culture resulted in malignant transformation, suggesting that MECs that circumvent BM-dependent survival are tumorigenic. Whether malignant transformation in the breast requires absolute independence from adhesion-linked survival cues is yet to be determined.

Anchorage independence for survival and malignant transformation of the breast

The perception that anchorage independence for survival is an essential feature of malignant breast tumors is consistent with reports that immortalized breast tumor cells are able to grow and survive in soft agar. More specifically, we and others have found that malignant human MECs no longer depend on ligation and activation of β_1 integrins for survival in culture [2,7,9]. Using a tumor progression model called HMT-3522, in which it is possible to study the early changes that occur during malignant transformation [1], we found that as the nontransformed cells in this series progress towards malignancy, they gradually lose their dependency upon β_1 integrin for survival [7]. This suggests that circumvention of β_1 integrin adhesion-dependent survival signaling may play a critical role in driving malignant transformation of the breast.

More recently, we determined that loss of β_1 integrin dependency for survival in this cell series is associated with a dramatic increase in the expression and activity of EGFR (Weaver *et al*, unpublished data). We also found

that inhibiting the activity of the EGFR was sufficient to revert the malignant phenotype of the tumor cells and repress their anchorage independence for growth and survival [19]. Alterations in β_1 integrin-dependent survival and EGFR activity occur concomitantly with a perturbed ability of the HMT-3522 cells to form breast tissue-like structures in response to a reconstituted BM (Weaver *et al*, unpublished data). This emphasizes the existence of an association between cell adhesion-directed tissue architecture, growth control, and apoptosis regulation in MECs. These findings also imply that deregulation of this relationship could lead to malignant transformation.

Is anchorage independence necessary for malignant transformation in mammary epithelial cells?

Clinical data support the idea that independence from BM-directed survival is linked to malignant transformation in the breast. Immunologic studies [20] have shown that invasive breast tumor cells exhibit a reduced level of apoptosis when compared with cells located in benign ductal carcinoma *in situ* (DCIS) lesions. Similarly, intense staining for focal adhesion kinase, a tyrosine kinase that can induce anchorage-independent survival in epithelial cells, was detected both in invasive tumor cells and in groups of premalignant cells within adjacent DCIS lesions [21]. Unfortunately, these data do not establish whether the enhanced survival in the transformed cells is due to genetic selection or is mediated via microenvironmental factors.

Although genetics undoubtedly plays a critical role in driving malignant transformation and apoptosis resistance in the breast, evidence is slowly accumulating that microenvironmental factors must also play a role in these processes. For example, angiogenesis can enhance mammary tumor viability, irrespective of genetic selection [22], whereas nonmalignant MECs exposed to a reactive stromal ECM can be induced to develop a tumor-like behavior in the absence of genetic events [3]. Indeed, stromal fibroblasts associated with mammary tumors have been shown to display a 'fetal-like' behavior, and this altered phenotype has been suggested to modify significantly the kinetics of tumor progression [23]. Interestingly, data show that primary human breast tumors frequently exhibit a decrease in the expression of the 'differentiation-associated' laminin/collagen integrin receptors α_2, α_3, and β_1, but they often express the 'invasion and growth-linked' tenascin and fibronectin receptors α_v integrin and α_5 integrin [1]. Some aggressive breast tumors even retain expression of the laminin integrins α_6 and β_4, and secrete BM proteins [24].

Because MECs within DCIS lesions seldom show changes in their integrin expression, this indicates that the dramatic shifts in integrin expression may be necessary to support tumor cell survival and drive malignant transformation. Although one could argue that the changes in integrin expression are solely due to selection of a genetically variant population of cells, it is also possible that the altered integrin expression observed in breast tumors reflects a dynamic adaptive survival response by the tumor cells to the interstitial stromal ECM. This would depend on the ability of the tissue microenvironment to modulate integrin expression and apoptosis resistance epigenetically in MECs.

In support of this concept, significant and rapid changes in integrin expression have been documented in primary tumor cells before (in tumors *in situ* that are in contact with a reactive stromal ECM) and after growth within a reconstituted BM *ex vivo* [25]. We and others [19,26,27] have also observed that the ECM microenvironment can dynamically modulate integrin expression in both primary and immortalized MECs. Most recently, we found that malignant transformation and β_1 integrin independence in the HMT-3522 tumors occurs in conjunction with, and is dependent on, ligation of $\alpha_6\beta_4$ integrin and secretion of BM protein (Zahir *et al*, unpublished data). These results are consistent with reports that high levels of expression of α_6 and β_4 integrins, and BM proteins in human breast carcinomas correlate with reduced patient survival, and functional deletion of α_6 integrin in metastatic breast tumor cells results in a significantly higher rate of apoptosis [24,28,29]. Therefore, a more realistic interpretation regarding the evolution of apoptosis resistance and malignant transformation in the breast is that it arises by a combination of genetic 'mutation/selection' pressures and epigenetic 'adaptation' responses induced by the tissue microenvironment.

Studies conducted with isolated primary human breast tumor cells support the idea that stromal–epithelial interactions are primarily responsible for promoting survival in primary breast tumors *in vivo,* as opposed to cell autonomous 'selection' events. 'Tumorigenic' breast cell lines isolated from primary breast tumors that represent earlier, less aggressive breast cancer phenotypes do not always exhibit true anchorage independence for growth and survival. Indeed, the majority of immortalized tumor cells used to study apoptosis regulation and anchorage independence in human breast cancer have been generated from late-stage disease cells isolated from metastatic pleural effusions, in which stromal interactions are minimal and cell–cell interactions are predominant [30]. Along this vein, Giovanella *et al* [31] reported that a mere 6.1% (16/262) of primary infiltrating duct-cell human breast carcinomas survived and grew following injection into nude mice. Only cells from those tumors that were highly cellular (enhanced cell–cell interactions) and lacked detectable desmoplastic hyperplasia (minimal stromal involvement) could be grown and serially transplanted into nude mice.

Interestingly, primary breast tumor cells that are first embedded within purified extracellular collagen I or a reconstituted BM can be successfully grown and propagated in nude mice [32]. It has also been observed that primary human breast tumor cells can be maintained and effectively studied *ex vivo* if the cells are maintained in the presence of either a reconstituted BM or purified collagen I matrix [33,34]. Therefore, a more prudent conclusion regarding malignant transformation and apoptosis resistance in breast cancer is that, rather than acquiring absolute anchorage independence for survival, most primary human mammary tumors probably depend on altered stromal factors and/or adhesive interactions to maintain their viability *in vivo*.

Cell adhesion, tissue architecture and apoptosis resistance in breast cancer

Breast tumors characteristically lack tight junctions [35], and exhibit disrupted E-cadherin organization [1]. These observations are consistent with the idea that loss of cell–cell adhesion is essential for tumor invasion [36]. However, aggressive breast tumors with a poor prognosis often upregulate different cell adhesion molecules such as P-cadherin, CD44, and Ep-Cam [37–39], and readily aggregate. Enhanced cell–cell interactions can repress apoptosis [40], and the success of culturing primary breast tumor cells *ex vivo* in three dimensions may be explained in part by the augmentation of nonclassical intercellular communication in the spheroid cultures [41]. A relationship between multicellular-mediated drug resistance, the metastatic phenotype, and cell adhesion has also been established [42]. These findings suggest that altered intercellular communication in breast tumors may be another mechanism whereby BM independence and apoptosis resistance can be generated in breast tumors.

It is not known how intercellular interactions support cell survival. However, Carmeliet *et al* [43] showed that VE-cadherin functionally interacts with the vascular endothelial growth factor receptor, PI3-K, and active Akt in a physical complex to mediate endothelial cell survival *in vivo*. Green fluorescent protein tagged protein kinase B/Akt, which facilitates adhesion directed survival in MECs, also localizes to both cell–ECM and cell–cell junctions [44]. Furthermore, the retinoblastoma protein, which is implicated in ECM-directed survival, mediates adherens-junction-dependent survival in MECs [45]. Thus cell–cell adhesion probably supports MEC survival by actively repressing apoptosis via cross-talk with growth factor and ECM-linked survival pathways.

We and others have shown that long-term survival and apoptosis resistance in MECs in a reconstituted BM is dependent on the formation of a multicellular tissue-like structure and the assembly of adherens junctions (Weaver *et al*, unpublished data) [46]. Studies have shown that acquisition of the multidrug-resistant phenotype can be significantly accelerated if the tumor cells being studied are grown as three dimensional spheroids [42]. Tumor cells grown as three dimensional spheroids exhibit enhanced cell–cell communication, change their integrin expression, and secrete ECM proteins [41,47]. This indicates not only that cell–cell interactions could modulate ECM-directed survival via integrin and growth factor receptor mediated events, but also that such cross-talk may additionally confer apoptosis resistance on MECs.

Conclusions

The molecular basis for the cross-modulation of survival networks by cell–cell and cell–ECM interactions remains ill defined. Cells dramatically reorganize their cytoarchitecture when cultured as three dimensional apoptosis resistant spheroids, however, and perturbing cytoskeletal organization restores their apoptosis sensitivity (Weaver *et al*, unpublished data). Because actin modifiers can regulate cell survival [48], and are themselves altered during apoptosis [49], this indicates that the regulation of apoptosis by cell adhesion cross-talk probably depends on the cytoskeleton and its molecular regulators. Nevertheless, the existence of dynamic and reciprocal cross-modulation of apoptosis by cell–cell and cell–ECM implies that any significant change in the breast stromal ECM, or in the adhesion molecules expressed by the breast epithelium, will have some impact on MEC survival and/or apoptosis sensitivity. Should alterations in any one or more of the adhesion-linked apoptosis regulatory network components confer enough of a survival advantage to the cells, then this could favor MEC viability in the absence of a BM, and thereby promote malignant transformation. If the changes were of sufficient magnitude, then they could even render some breast 'tumor' cells resistant to exogenous apoptotic stimuli, and hence give rise to the multidrug-resistant breast cancer phenotype. Indeed, this paradigm can explain the profound effects exerted by a reactive stroma on cancer progression, and why the site of tumor metastasis can exert such a strong influence on the efficacy of cancer treatment.

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