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Review Article

The extracellular matrix in breast cancer predicts prognosis through composition, splicing, and crosslinking

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ABSTRACT

The extracellular matrix in the healthy breast has an important tumor suppressive role, whereas the abnormal ECM in tumors can promote aggressiveness, and has been linked to breast cancer relapse, survival and resistance to chemotherapy. This review article gives an overview of the elements of the ECM which have been linked to prognosis of breast cancers, including changes in ECM protein composition, splicing, and microstructure.

Contents

1. Introduction

When metastatic breast cancer cells are mixed with murine mammary epithelial cells and injected into the mammary fat pad, one would expect to observe frank tumors [1]. However, instead of tumors, these cancerous cells incorporate into histologically normal ductal structures, respond appropriately to hormones, and even secrete milk proteins [2]. Furthermore, breast epithelial cells with surprisingly abnormal genomes can be found in histologically normal human breast ducts [3–5].

These studies, and many others, show that the correct context can induce non-malignant behavior, whereas, the abnormal environment in tumors can induce progressive genomic instability and tumorigenesis even in non-malignant cells, both in vitro and in animal models [6–8]. Recent work has linked the ECM in tumors to dormancy [9], resistance to chemotherapy or radiation [10–12], metastasis and metastasis tropism [13] again demonstrating the importance of understanding cell–ECM interactions. It has become apparent from both in vitro and clinical work that the ECM signals to cells through both biochemical and physical means with complex interactions between ECM composition, splicing, microstructure, and biomechanics.

This work gives a survey of the alterations to ECM observed in the progression from healthy breast to breast cancer with special attention to biomechanics. We will focus on data from the breast

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and breast cancer, as cell–matrix interactions have been studied extensively for this organ system and cell culture models of breast development and breast cancer show clear clinical relevance [14,15].

2. ECM in the healthy breast suppresses tumorigenesis

2.1. The basement membrane in the normal breast is a tumor suppressor

The epithelial structures in the breast originate at the nipple, form a branching set of ducts, and end in terminal ductal lobular units, where milk is synthesized. Breast ducts and lobules are bi-layered structures: the inner ring of luminal epithelial cells, which secrete milk during lactation, is surrounded by a ring of myoepithelial cells, which are contractile cells with the ability to secrete and organize ECM proteins. Subtending both these layers of cells is a highly specialized layer of extracellular matrix proteins termed the basement membrane (BM). Myoepithelial cells are lost with malignant progression [16–18] and are believed to play an important tumor suppressive role in the healthy breast due to their ability to secrete the specialized extracellular matrix proteins of the BM [16,19]. Myoepithelial cells surrounding tumors show a shift in ECM protein secretion, losing expression of tumor-suppressive laminins and increasing expression of collagens [16,20].

The basement membrane (BM), a complex, crosslinked layered structure of multiple laminins, collagen IV and other collagens, proteoglycans including perlecan/heparin sulfate proteoglycan nidogen/entactin, and others. Loss of an intact basement membrane is a key stage in malignant progression with high predictive value for prognosis [21], and animal models show that destruction of the BM results in genetic instability and tumorigenesis [7,8].The innermost layer of the basement membrane, at the epithelial cell surface, is a network of laminins [22,23]. In the presence of cell surface ECM receptors, such as dystroglycan, laminin-111 can polymerize into a soft, cohesive network [23,24], which then induces formation of a more structurally stable collagen IV network subtending the Ln-111 network [25], which epithelial cells do not typically contact. These independent networks are then linked by proteins such as fibronectin and nidogens [26], permitting formation of a cohesive mat of proteins.

Among BM proteins, laminin-111 is absolutely necessary for epithelial specific functions in 3D culture assays, including formation of polarity in human breast epithelial cells [16], and induction of milk protein expression (including beta-casein) in murine mammary gland epithelial cells [27]. Furthermore, tumor reversion, or induction of a quiescent phenotype in malignant cells requires laminin and induction of normal cell–ECM signaling [16,28]. Laminin-111/Ln-1 has three head domains which can crosslink into a soft cohesive 3D network, whereas other laminin isoforms with truncated head domains, such as laminin-332/Ln-5, laminin-511/Ln-10 or laminin-521/Ln-11, cannot form a network [22,29], and do not support normal epithelial cell function in vitro, despite the fact that all these isoforms present similar tail domains to cells [16]. Furthermore, some evidence suggests that laminin-332, or collagen IV may support tumor invasion or aggressiveness [30–32].

Both the biomechanics and composition of this laminin network regulate epithelial function: artificially stiffening the laminin network induces epithelial cells to enter an invasive phenotype due to disrupted clustering of β-4 integrin into hemidesmosomes [33] and increased β1 integrin signaling [34,35]. Increasing the density of laminin sites can overcome increased matrix stiffness [33], showing that cells integrate multiple aspects of ECM.

Given the small dimensions of the breast BM (30–50 nm in the human breast [36]), the biomechanical properties of the mammary gland basement membrane itself have never been experimentally determined (though breast stromal tissue has a modulus of 200–400 Pa [35,37]). The BM subtending the retina (which has a similar laminin-rich composition) has a modulus between 1 and 4 MPa, with a difference in matrix biomechanics between its two faces [38], suggesting that despite the thinness of this structure it specializes into sides.

2.2. Stroma

Surrounding the ducts and lobules of the glandular epithelium is the breast stroma, comprised of adipocytes, fibroblasts, and capillaries embedded in a different mix of ECM [39]. The stroma contains blood vessels, adipocytes and fibroblasts embedded in abundant collagen I, chondroitin sulfate and fibronectin [40] (note that the blood vessels have their own laminin-rich BM [41]). Despite their separation by the BM, stroma communicates with epithelia, and stromal changes are observed even in the early stages of malignancy [42]. Stromal ECM plays a major role in tumorigenesis: genetic work from both animal models [8,43] and the clinic [44,45] show that stromal gene expression can alter probablility of developing breast cancer. Importantly, gene expression patterns of normal stroma adjacent to breast cancers shows a different gene expression pattern from normal tissue from unaffected patients [46]. Changes in stromal ECM are observed even in ductal carcinoma in situ (DCIS), where carcinoma cells are confined within an intact basement membrane, including increased deposition of versican [47], loss of decorin [48], and altered expression of Col11A1 [46,49].

Direct contact between stroma and non-malignant epithelia is not observed except during involution [50]. Breast cancers arising during pregnancy and involution tend to be highly aggressive and metastatic [51], suggesting that the collagen-1 rich stromal ECM, along with inflammatory environment observed in lactation and involution, could be pro-tumorigenic [52]. Supporting this, mouse models of BM destruction or stromal collagen I overexpression, which would tend to increase exposure of epithelia to stromal ECM, show increased tumorigenesis [8,53]. Furthermore, non-malignant epithelial cells exposed to increased density of stromal-like collagen I and associated increases in ECM biomechanics undergo transition between formation of normal structures and loss of cell structure and increased growth [35]. The microarchitecture of the fibrilar collagen network (typically collagen I) in the stroma is believed to play a major role in specifying both risk of BC and the stiffness of the stromal ECM [37,50,54,55], suggesting that stiff stroma could encourage tumor initiation or progression. Depending on species, age and testing method, breast interstitial ECM has been measured to have a modulus of 167 ± 31 Pa [35], 0.4 kPa [37] and 1.13 ± 0.78 kPa [55], and the risk of developing BC has been linked to increases in total breast stiffness both clinically [54].

3. ECM in tumors

3.1. Altered ECM and altered cell response

In breast cancers, high levels of fibronectin and its splice variants, crosslinked collagen I, and tenascin-C are associated with poorer survival or time to progression for breast cancer patients, whereas high levels of laminins, high molecular weight hyaluronic acid, heparins, versican, lumican or decorin correlate with better outcomes (summarized in Table 1). While biological mechanisms for some of these links between ECM signatures and prognosis, many open questions remain.

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