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Regulation of the basement membrane by epithelia generated forces

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Abstract

Tumor metastasis involves a progressive loss of tissue architecture and dissolution of structural boundaries between the epithelium and connective tissue. The basement membrane (BM), a specialized network of extracellular matrix proteins forms a barrier that physically restricts pre-invasive lesions such that they remain as local insults. The BM is not a static structure, but one that is constantly regenerated and remodeled in the adult organism. Matrix organization also regulates cell function. Thus alterations in the balance of synthesis, remodeling and proteolytic degradation of the extracellular matrix proteins may contribute to a loss of structural integrity. However, the de novo assembly and maintenance of the complex structural properties of in vivo basement membranes remain elusive. Here, this paper highlights the current understanding on the structural properties and the establishment of the BM, and discusses the potential role of self-generated forces in adult tissue remodeling and the maintenance of the BM as a malignancy suppressor.

Introduction

Loss of tissue architecture is an early pathological signature associated with the malignant transformation[1]. Advanced stages of malignancy are generally characterized by the disruption of structural boundaries that lie between the epithelial and stromal tissues [2]. In humans, pre-invasive mammary lesions, such as ductal carcinoma in situ (DCIS) for the breast and more generally carcinoma in situ (CIS) for epithelial organs including the prostate, lung and colon [2, 3] accompany the first clinical presentations of the malignant transformation. Earlier works determined that these pre-invasive lesions are present and undetected in a percentage of the population as much as ~34–39% for human prostate and breast lesions respectively [4–6]. These lesions remain quiescent for several decades and in some cases never progress to its fully invasive potential. The CIS remains a local aberration as long as the basement membrane (BM) remains intact (Figure 1) [7, 8]. In a review, Bissell et al suggest that “We get more cancer” when the normal tissue architecture can no longer suppress the local malignancy[9]. So why are some lesions quiescent while others progress rapidly to metastasis? Do tumor cells force their way through or do the structural properties of the BM simply degrade during aging?

Focus has been primarily on mechanisms used by tumor cells to penetrate the basement membrane. Tumor cells exploit different migration strategies to metastasize to distant anatomical sites. They possess the ability to switch between mesenchymal amoeboid movements [10–18]. Possible underlying mechanisms involve localized proteolytic degradation and navigation through preformed gaps in the BM [19–25]. Metastatic tumor cells show an overexpression of matrix degrading proteases including matrix metalloproteinase (MMPs) [26]. Initial clinical trials where patients with advanced stages of cancer were treated with MMP inhibitors did not improve survival in part due to the fact that the drugs were highly non-selective for specific MMPs and that these drugs may be more

effective for patients at early stages of cancer [27, 28]. Future efforts involving modified drug design and clinical protocols may prove to be more successful. But what about those patients who clinically present with pre-invasive lesions? Can we determine who should receive immediate treatment and who should simply be monitored? Can we provide therapy that will stabilize the BM so that the lesion remains quiescent? We must first understand the regulation of secretion of ECM proteins and de novo assembly and remodeling of the BM in the adult organism. Far from being impenetrable, some cells such as immune cells are able to traverse freely, locally remodeling the BM as they hunt for potential pathogens and during normal development [21, 29, 30]. Far from being static, the assembly/disassembly of the BM permits sprouting of neovasculature [31–33] where changes in the composition and/or reduced basement synthesis may explain localized gaps in the BM [8, 34, 35]. The remodeling of the fibroblasts and macrophages in response to wounding has been extensively studied [36, 37]. But what are the normal epithelial cells doing to maintain this protective cocoon? Here I present a framework to address the mechanisms by which epithelial cells establish and maintain tissue architecture via regulation of the BM and how these are corrupted in the malignant transformation.

What is the Basement Membrane? - Structural and chemical composition

First expressed in early embryogenesis, the BM is basally cell-adherent in every epithelium [38–41]. This specialized network of ECM proteins also surrounds muscle fibers, adipocytes, endothelial cells and Schwann cells of peripheral nerves [38, 42]. Throughout the body, it provides the tissue-specific structural support and orientation cues that are maintained for decades despite chemical, environmental and physical insults [43]. The process by which this template is established and maintained in the adult organism remains elusive. In the intact, injury free organs, BMs are not static structures but there is a continual turnover in quiescent tissue fueled by de novo synthesis, integration and degradation of ECM proteins [44–53]. Analysis revealed lifetimes ranging from a few weeks to a few years for the gut and kidney respectively [54–56].

BM form dense interconnected sheets of extracellular matrix proteins, 100–300nm thick as determined by electron microscopy [57]. This meshwork of more than 50 distinct macromolecules provides support the mechanical support for adherent cells while allowing small molecules to passively diffuse through pores of size on the order of 50nm [58]. This scaffold is largely defined by four ECM proteins, laminin, collagen type IV, Nidogen and perlecan where the relative amounts are tissue specific [59–61]. These macromolecules are approximately 75–400nm long and weigh 100–500 kDa [62]. There are 16 Laminin isoforms that are comprised of three chains, an α , β and γ chains that form a trimer (cross-like) stabilized by disulfide bonds. These trimers self-assemble into suprastructures at the cells' plasma membrane that provide the initial template for BM assembly [63]. Collagen type IV is comprised of six individual chains that also assembles into highly ordered macrostructures at the cell's surface stabilized via covalent crosslinks [64]. Targeted inactivation of one of the major isoforms of Collagen type IV in mice resulted in embryonic lethality due to impaired mechanical stability of the basement membrane [65]. Point mutations involved in the reduction of synthesis of Collagen type IV compromised the microvasculature concomitant with the development of porencephaly and hemorrhagic stroke. These data highlight the role of collagen type IV in conferring the mechanical stability to the BM. Finally, Nidogen/entactin and perlecan serve as a bridge between the laminin and type IV collagen networks further increasing the stability and structural integrity of BM [66].

The mammary gland: a model to understand BM synthesis and remodeling

At the onset of puberty, the rudimentary anlage rapidly transformation to an elaborately branched epithelial structure enclosed by the BM within stromal connective tissue. This arboreal structure consists of inter-connected duct network (tubes) terminated by basic functional units, acini (spherical-like structures). During pregnancy, there is an increased proliferation of epithelial cells to make the breast tissue ready for lactation [67, 68]. Once lactation ceases involution occurs through a process of controlled apoptosis and tissue remodeling, returning the gland to the architecture of its virgin state. This cyclic event is accompanied by active tissue remodeling specifically involving the remodeling of the stroma and re-assembly of the BM [69–72]. The mammary gland is unique organ as it undergoes structural changes during the estrus cycle as well as during pregnancy with a dramatic reversion to nulliparous architecture (virgin gland). Three-dimensional (3D) culture models have been developed that allow robust recreation of the acinus, the terminal functional unit of the mammary gland. The cancer progression series, HMT3522[73], developed in the Petersen/Bissell labs, is an isogenic transformation model consisting of cells from a reduction mammoplasty grown comprised of the non-malignant, S1, to the malignant, T4-2 derived without exogenous oncogenic manipulations (Figure 2) [74]. Specifically, in 3D cultures, when non-malignant HMECs are placed in a surrogate basement membrane gel, IrECM, they undergo multiple mitoses to achieve the multicellular spherical structure [73]. These cells establish polarity whereas malignant cells show a differential response where they form non polarized aggregates reminiscent of in vivo tumors. Intermediate stages of this series, S2 and S3s also recapitulate the progressive loss of architecture observed for in vivo pre invasive lesions [73]. T4-2 cells can be ‘phenotypically reverted’ in 3D IrECM by modulating intracellular and extracellular signaling malignant cells could form growth arrested, polarized acinar-like structures despite maintaining a malignant genome (Figure 2) [75–79]. Thus, the HMT3522 series and the mammary gland provide appropriate platforms to understand the de novo synthesis and remodeling of the BM and how it is compromised in the malignant transformation.

The role of self generated epithelial forces in remodeling of the surrogate basement membrane

Recently, Tanner, Bissell and colleagues demonstrated that non-malignant human mammary epithelial cells undergo multiple rotations with centripetal velocity of ~ 1 rev/hr ($\sim 0.2\mu\text{m/hr}$) to establish acini while tumor cells are randomly motile during tumor formation [80]. Little translocation is observed and the motion is distinct from motility due to an Epithelial-Mesenchymal Transition[81] (Supplemental Movie 1). [82]. Actin asymmetrically basally localized (crescent shaped front) at the cell-ECM surface and precluded the direction of rotation for single cells [80, 83]. Pharmacological inhibition of effectors of actomyosin in non malignant cells disrupted rotation and cells failed to assemble into spherical acini but formed aggregates mimicking the disorganized geometries observed for 3D IrECM culture of tumor cells (Figure 3). Conversely, malignant cells were randomly motile within the surrogate basement gel and generated translational forces to form non-polarized aggregates. Upon ‘phenotypic reversion’ [78], the malignant cells regain the ability to generate centripetal forces. This coherent angular motion (CAMo) was restricted to the establishment of polarized acini starting from a single cell. In addition, loss of cell adhesion via silencing of PARD3 and functionally inactivating E-Cadherin disrupted spherical architecture concomitant with the loss of CAMo. Analysis of 3D in vitro culture of Madin-Darby canine kidney (MDCK) epithelial cells revealed that these cysts also rotate within surrogate ECM hydrogels [84, 85]. Similarly, live cell imaging of 3D organotypic culture of mammary tissue revealed that self propelled collective epithelial migration fueled ductal elongation

[86]. These data suggest that epithelial cells generate forces that directly remodel the surrogate ECM cocoon.

Integrating cues from the Extracellular matrix- Why Rotation?

Molecular studies have revealed key proteins that mediate and maintain cell- and tissue-polarity [87–91]. These proteins regulate a series of dynamic and interdependent processes, including reorganization of the cytoskeleton, assembly of intercellular junctional complexes and the specification of membrane domains coupled with bi-directional feedback with the surrounding ECM [92]. These observations suggest that cells rotate to establish radial symmetry where the single cell creates its own template to establish apical polarity by sequestering and/or releasing proteins/enzymes or creating localized gradients of soluble factors in the surrogate basement hydrogel to create a mechano-chemical feedback loop. The centripetal force may help orient the cell with respect to its progeny. The fact that actin is asymmetrically localized implies that this circular polarization of the cell orients the mitotic spindle to ensure that the daughter cell divided along the correct axis to maintain sphericity throughout division. This may explain why cells show staggered division to maintain a spherical geometry throughout acinar morphogenesis [80]. The chirality of the single cell rotation showed no bias probably because in this simple 3D culture model, presumably the external contributions from the stroma and connective tissue *in vivo* are missing. Recently, Guo et al showed that mature acini generated tractional forces that propagated as much as ~600µm away. The long range mechanical cross talk between neighboring acini was sufficient to break symmetry to induce interconnected tubule formation [93]. It precludes that one can exquisitely tune the assembly of a myriad of multicellular structures in culture conditions by temporally modulating an applied external force.

Is CAMo similar to existing embryonic morphogenetic programs to establish/remodel the BM?

This rotation observed in vitro 3D culture of adult mammary epithelial cells is similar to the morphogenetic program employed during organogenesis [94, 95]. Although, these adult cells have long been removed from the instructive structural cues of the *in vivo* environment, they are able to re-enter a morphogenetic program usually employed during embryonic development. Global rotation of epithelial tissue was recently described for follicle elongation during *Drosophila* oogenesis [96]. Individual follicles completed multiple rotations ~ 0.5µm/minute within its basement membrane. Local remodeling of the basement membrane was observed where collagen IV initially disorganized became increasingly aligned. However, one unanswered question for the *in vitro* culture is how do cells rotate/crawl in the ECM? Traction is required for cellular motion [97]. Moreover, cells secrete their own endogenously produced ECM proteins when placed in 3D culture [98, 99]. The question arises is this global rotation required for tissue remodeling of the surrogate basement membrane or the establishment of a de novo basement membrane, or both? Examination of malignant cells shows that key components of the BM are compromised and deposited within cell-cell boundaries during tumor formation (Figure 2) [98]. For the reverted malignant cells, the spatial deposition of these proteins is similar to what is observed for the non malignant structures. These data suggest that when CAMo is compromised, the de novo assembly and that the composition of the endogenous BM is altered further serving as misinformation for structural assembly. In both model systems of the human mammary epithelial cells and *Drosophila* follicles, the epithelia tissue generate centripetal forces that remodels the microenvironment but it remains to be seen if CAMo directly establishes the de novo endogenous BM [80, 96].

ECM regulation of epithelial generated centripetal forces

Cells can modulate their force generation as a response to local extracellular stiffness [100, 101]. CAMo reinforces the notion that cells are not simply passive responders to external mechanical perturbations but that they can generate contractile forces to locally remodel their microenvironment. But how big are these centripetal forces and what is the effect of the mechanical properties of the surrounding matrix? Interrogation using AFM and rheology to determine the material properties of surrogate BM gels via yield similar results. The Young's modulus of ~450Pa obtained using AFM is comparable to the value of macroscopic shear storage modulus ~80 Pa obtained using rheology [102–104]. Moreover, BMs are thixotropic and largely viscous on long time scales[105]. Single cell analysis done with fibroblasts revealed that cells can generate forces of ~1–100nN[106]. It remains to be seen if the centripetal forces generated by epithelial cells are comparable. Data analysis techniques based on Fluctuation correlation spectroscopy provide both structure and dynamics of sub-cellular and cellular components [107–114]. Recently, Raub et al employed correlation spectroscopy to quantify the microstructure of collagen type I hydrogels [115]. Moreover, a link to the macroscopic mechanical properties obtained from rheology and cell dynamics was established using non invasive multiphoton microscopy [115, 116]. Thus, the combination of non invasive imaging modalities and correlation techniques is an attractive method to quantify the dynamic interplay between cell and ECM during structural reorganization.

Does CAMo translate to in vivo cancer progression?

The *in vivo* link remains an outstanding question. Hypothetically, CAMo or a modified version may be the mechanism by which architecture is reformed when there is a periodic reorganization of structure or turnover of tissue for both epithelia and mesenchymal tissue[117]. For example, gut epithelial cells have a lifetime of a few days[118]. In women, some aspects of tissue reorganization are linked to the reproductive state such as the periodic release of an ovum and the architectural changes during pregnancy[119]. If global CAMo were deregulated during tissue remodeling, this may be the mechanism that regulates pregnancy associated breast cancer. It may also describe why inflammation of a tissue increases the possibility of cancer progression of the gut conversely why there is little cancer of the endothelium as these cells have a lifetime of years. It may be that in tissue where there is a faster rate of turnover or more active remodeling of the basement membrane, there is a greater risk that the underlying process may go awry. But, revisiting the question of how do we decide who to treat at the DCIS level, are cells moving within these lesions? Using live cell imaging, Pearson and Hunter uncovered that cells were randomly motile for several hours within the confines of the BM in HMEC acini following inducible activation of ERK1/2[120]. These acini had an altered architecture reminiscent of DCIS. These data in conjunction with the fact that CAMo is observed for in vitro acinar morphogenesis suggest that, in principle, cells can become motile during the preinvasive stage of epithelial tumor growth [80, 120]. Imaging of tissue dynamics has refined our understanding of basic principles of cell migration. Continued development of non- invasive imaging modalities and biomarkers to obtain sub cellular resolution within thick tissues will undoubtedly uncover additional mechanisms that could assist in diagnosis and treatment of cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Tumor Progression

Figure 1. Schematic showing the malignant transformation of a spherical acinus where there is a progressive loss of normal architecture and loss of the basement membrane.

Figure 2.

3D IrECM culture of the HMT3522 cancer progression series. Non malignant and reverted malignant cells form growth arrested spheres when cultured in 3D while malignant cells continuously proliferate and form disorganized aggregates. Micrographs of the median cross- section are shown where immunofluorescence delineates the nuclei (white), endogenous pan-laminin (green), endogenous collagen IV (blue) and actin (red). White scale bar is 100 μ m.

Figure 3.

Left panel a) shows the realized geometry of 3D culture of non- malignant cells treated with a specific pharmacological inhibitor. b) Right panel shows indicates the signaling pathways regulating traction forces via actin polymerization.