Cell–Matrix Interactions in Mammary Gland Development and Breast Cancer

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The mammary gland is an organ that at once gives life to the young, but at the same time poses one of the greatest threats to the mother. Understanding how the tissue develops and functions is of pressing importance in determining how its control mechanisms break down in breast cancer. Here we argue that the interactions between mammary epithelial cells and their extracellular matrix (ECM) are crucial in the development and function of the tissue. Current strategies for treating breast cancer take advantage of our knowledge of the endocrine regulation of breast development, and the emerging role of stromal–epithelial interactions (Fig. 1). Focusing, in addition, on the microenvironmental influences that arise from cell–matrix interactions will open new opportunities for therapeutic intervention. We suggest that ultimately a three-pronged approach targeting endocrine, growth factor, and cell–matrix interactions will provide the best chance of curing the disease.

Cellular interactions with the ECM are one of the defining features of metazoans (Huxley-Jones et al. 2007). Matrix proteins are among the most abundant in the body, and are integral components of cell regulation and developmental programs operating in all tissues. They provide structure and support to tissues, and they interact with cells through diverse receptors to guide development, patterning, and cell fate decisions (Streuli 2009). Together with cytokines and growth factors, and cell–cell interactions, the ECM determines whether cells survive, proliferate, differentiate, or migrate, and it influences cell shape and polarity (Streuli

and Akhtar 2009). Cell–ECM interactions also are central in the assembly of the matrix itself, and in determining ECM organization and rigidity (Kadler et al. 2008; Kass et al. 2007). The cell–matrix interface is therefore pivotal in controlling both cell function and tissue structure, which together build organs into operational structures. Thus, elucidating precisely how the matrix directs cell phenotype is crucial for understanding mechanisms of development and disease.

Mammary gland tissue contains epithelium and stroma (Fig. 2). Mammary epithelial cells (MEC) form collecting ducts and, in pregnancy

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Figure 1. Mammary gland development. Whole mounts of (A) virgin and (B) mid-pregnant mouse mammary gland. The thin, branched epithelial ducts that are characteristic of nonpregnant gland undergo dramatic alterations in pregnancy, when new types of epithelial structures, the milk-producing alveoli, emerge. The huge amount of proliferation that accompanies this change occurs in a discrete and controlled fashion. The formation of ducts and alveoli is under three types of environmental control. The first is long-range endocrine hormones, which includes estrogen, progesterone, glucocorticoids, and prolactin. The second is locally acting growth factors, which arise from stromal–epithelial conversation, and includes amphiregulin, FGF, HGF, and IGF. Finally, microenvironmental adhesive signals from adjacent cells (e.g., via cadherins) and from the ECM (e.g., integrin) have an equally central role in all aspects of mammary development and function. Importantly, the proliferation that occurs in breast cancer is not well controlled, indicating not only defects in growth signaling, but also in cellular organization. Chronologically, breast cancer drugs were initially developed against endocrine regulators, e.g., estrogen, and more recently against the stromal/epithelial regulators, e.g., receptor tyrosine kinases. A complete control of the disease will only happen when therapies targeting the microenvironmental adhesion breast regulators, e.g., cell–matrix interactions, are formulated, and used in combination.

and lactation, milk-secreting alveoli (or lobules). The mammary epithelium is bilayered, with the inner luminal cells facing a central apical cavity and surrounded by the outer basal, myoepithelial cells. It also harbors stem and progenitor cells, which are the source of both luminal and myoepithelial cells (Visvader 2009). The epithelium is ensheathed by one of the main types of ECM, basement membrane (BM), which separates epithelium from stroma, and profoundly influences the development and biology of the gland (Streuli 2003). The stroma includes fibrous connective tissue ECM proteins, and a wide variety of cell types, including inter- and intralobular fibroblasts, adipocytes, endothelial cells, and innate immune cells (both macrophages and mast cells). The stroma is the support network for the

epithelium, providing both nutrients and blood supply, and immune defenses, as well as physical structure to the gland. Importantly, each of the different stromal cell types secrete instructive signals that are crucial for various aspects of the development and function of the epithelium (Sternlicht 2006).

BMs surround three cell types in the mammary gland: the epithelium, the endothelium of the vasculature, and adipocytes (Fig. 3). These ECMs are thin, ~100-nm thick sheets of glycoproteins and proteoglycans, which are constructed around an assembled polymer of laminins and a cross-linked network of collagen IV fibrils (Yurchenco and Patton 2009). Laminins form $\alpha\beta\gamma$ trimers, and in the breast at least four distinct isoforms are present: laminin-111, -322, and -511 and -521 (previously

Figure 2. Ducts and alveoli in early pregnancy. Transverse section of ducts surrounded by a thick layer of collagenous (stromal) connective tissue containing fibroblasts and the fat pad. Also visible are small alveoli, which fill the fat pad by the time the gland lactates, but note that they are not surrounded collagen. A capillary is evident, and macrophages and mast cells are also present, though they require specific staining to visualize. A basement membrane is present directly at the basal surface of both ductal and alveolar epithelium (see Fig. 3).

known as LM-1, 5, 10, and 11) (Aumailley et al. 2005; Prince et al. 2002). Similarly, BM proteoglycans are diverse and show complexity in their GAG chain modifications that vary with development of the mammary gland, though the major species is perlecan (Delehedde et al.

2001). BM proteins interact with MEC via integrins and transmembrane proteoglycans dystroglycan and syndecan, which all couple to the cytoskeleton and assemble signaling platforms to control cell fate (Barresi and Campbell 2006; Morgan et al. 2007). The best-studied

Figure 3. Alveolar and ductal architecture of breast epithelia shown through fluorescence and histological images. (A) An alveolus from a lactating mammary gland, showing luminal epithelial cells with cell–cell adhesion junctions (green, E-cadherin) and cell–matrix interactions (red, laminin-111). The central lumen is where milk collects. (B) The duct of a nonpregnant gland is stained with an antibody to laminin (brown) and counterstained with hematoxylin. Note that the laminin-containing basement membrane surrounds the ductal epithelial cells, and outside this lie collagenous connective tissue and adipocytes. Figure B courtesy of Dr. Rama Khokha.

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MEC BM receptors are integrins, which are $\alpha\beta$ heterodimers: they include receptors for collagen ($\alpha1\beta1$ and $\alpha2\beta1$), LM-111, -511, -521 ($\alpha3\beta1$, $\alpha6\beta1$, and $\alpha6\beta4$), LM-322 ($\alpha3\beta1$ and $\alpha6\beta4$), and in some MECs fibronectin and vitronectin ($\alpha5\beta1$ and $\beta3$ integrins) (Naylor and Streuli 2006). BM proteoglycans have a further signaling role via their capacity to bind growth factors and cytokines: They act both as a reservoir and a delivery vehicle to GF receptors, thereby controlling the passage of GFs across the BM (Iozzo 2005). Because of these diverse roles, the BM is a dominant regulator of the mammary epithelial phenotype.

Apart from the endothelium and adipocytes, which contact BMs, the mammary stromal cells are mostly solitary and embedded within a fibrous ECM. Stromal matrix components include collagens type I and III, proteoglycans and hyaluronic acid, fibronectin and tenascins, and the composition varies with development and pregnancy (Schedin et al. 2004). Not a great deal is known about the specific interactions between breast stromal cells and their ECM, or how the matrix composition and density determines stromal cell function. However, it is becoming evident that the stromal matrix exerts a powerful influence on malignant breast epithelial cells, which invade the stroma and are further transformed by exposure to this distinct microenvironment (Kumar and Weaver 2009; Streuli 2006).

In this article we focus on cell–matrix interactions within mammary epithelium, and reveal known and possible mechanisms for its control on ductal development, alveolar function, and cancer progression.

HOW CELL–MATRIX INTERACTIONS CONTROL BRANCHING DUCTAL MORPHOGENESIS IN MAMMARY DEVELOPMENT

Mammary epithelium develops in the mouse embryo mid-way through gestation. An epithelial placode forms first, which invaginates from the ectoderm and then invades the presumptive mammary mesenchyme to form a naive ductal network (Hens and Wysolmerski 2005; Hinck

and Silberstein 2005). A simple structure is formed by iterative branching. The nascent gland maintains a continuous BM at the epithelial–mesenchymal interface as it emerges from the ectoderm, therefore the BM is omnipresent in mammary epithelial development. In humans, the BM of the mammary bud and early projections contains type IV and VII collagens, and laminin- $\alpha3$, and the epithelium displays $\beta1$, $\beta4$, and $\alpha6$ integrin expression, but little else is known about BM signaling in the embryonic gland (Jolicoeur et al. 2003). Embryonic mammary ducts remain dormant until the onset of puberty, when estrogen drives extensive growth and branching (Feng et al. 2007). The ducts grow into a pre-existing stromal mammary “fat pad,” forming long, thin tubes that are extensively branched. New ducts largely develop from their tips, which are enlarged multicellular structures called “endbuds” (seen in Figs. 1A and 4).

Cell–matrix interactions have a critical role throughout duct formation. Some mechanisms are known from transgenic studies and, as yet, limited culture analysis. Others are inferred from other tissues, e.g., lung and the salivary submandibular gland (SMG), but remain to be tested in mammary gland. Importantly, the bilayered ductal composition means that genetic studies to reveal how cell–matrix interactions are involved with duct formation rely on transgene promoters expressed in basal (myoepithelial) cells. This requires use of e.g., K5, K14 promoters (which often result in a skin phenotype), rather than mammary promoters (MMTV, WAP), which are expressed in luminal cells that do not contact the BM (this situation is different in alveolargenesis, see later discussion). Genetic studies on stromal-expressed matrix proteins and remodeling enzymes have yet to be executed, though the promoters are now available (Trimboli et al. 2009).

Proliferation and Migration

The main driver of ductal morphogenesis is epithelial cell proliferation and migration, and both are dependent on cell–matrix interactions.

These processes occur within the endbud, which is the engine of duct development. The endbud is surrounded by a thin BM, which is remodeled as the cells collectively invade the adjacent stroma. Although estrogen is a dominant regulator of MEC proliferation, local growth factor interactions between the epithelial and stromal cells are also crucial; e.g., stromal FGF and epithelial FGFR2 are required for MEC proliferation (Lu et al. 2008). Similarly the HGF-met axis has a key role.

β 1-integrins are required genetically for mammary ductal cells to proliferate in 2-dimensional or 3-dimensional (2D or 3D) culture (Jeanes et al., unpubl.), and function-perturbing anti- β 1-integrin antibodies largely eliminate endbuds (Klinowska et al. 1999). One possible mechanism to explain this requirement is that integrin activation may influence the expression of FGF receptors. LM-511 is a β 1-integrin ligand, which is required for FGFR expression in SMG, and similarly FGFR is needed for LM- α 5 expression, indicating a positive feedback loop for maintaining FGFR and competence to proliferate (Rebustini et al. 2007). Alternatively, integrins may cooperate directly with FGFR to activate signaling and cause cell cycles at the advancing endbud, though neither of these mechanisms have been tested in mammary ducts. The BM also regulates the delivery of growth factors such as FGF to the growing epithelium. In SMG, stromal FGF10 is captured by the heparan sulphate chains of BM perlecan, but only delivered to the epithelium after heparanase releases it (Patel et al. 2007). Similarly, in mammary gland, over-expressed heparanase leads to an excessively branched ductal network (Zcharia et al. 2004). Integrins may also regulate c-met signaling, by sensing laminin molecules within the BM to allow met signaling to take place and control morphogenesis (Liu et al. 2009). Laminin-related proteins such as netrin-1 also have a role in mammary morphogenesis, though the mechanisms for this are not known though they control cell survival and migration in other systems (Castets et al. 2009; Hagedorn et al. 2009; Strizzi et al. 2008).

How mammary ducts invade stroma is not clear. Most knowledge of cell movement comes from 2D cultures, where cells extend lamellipodia to provide traction, and at the same time release cell-matrix contacts at the rear (Ridley et al. 2003). Recent approaches to study epithelial migration in 3D are largely centered around the use of cancer cells, which can similarly extend lamellipodia, or alternatively migrate in an amoeboid fashion (Sahai 2005). Cancer cells also migrate collectively (Friedl and Gilmour 2009). One recent study has risen to the challenge of determining how normal mammary epithelium advances, and finds the process is different than in cancer (Ewald et al. 2008). Ducts form branching structures with primitive endbuds when cultured in a 3-dimensional BM matrix, and video imaging affords new insights into these mechanisms of morphogenesis. Lamellipodia do not form, rather the cells slowly advance at ductal tips via a rearranging, multilayered cell population. New ducts forming behind the endbud are ensheathed by myoepithelial cells, which appear to restrain outward movement, to form a tube. It is not clear whether the whole process is simply driven by cell proliferation, which pushes the cells into the neighboring matrix, or if there is active pulling involving integrin contacts and cytoskeletal rearrangement (Andrew and Ewald 2009).

Actin and tubulin networks have a central role in cell movement, so cytoskeletal polymerization within the cells at the end bud periphery might contribute to duct advancement by generating protrusive forces (Hall 2009; Pollard and Cooper 2009). Similarly, polarity needs to be established to orientate cell movement, and might provide guidance for a persistent direction for migration (Petrie et al. 2009). For example, the polarity protein Par3 is required for the normal formation of endbuds and proper ducts (McCaffrey and Macara 2009). Finally, the trafficking of integrins through early endosomes contributes to the formation of new matrix adhesions and controls cell migration in 3D matrices (Caswell et al. 2009). However, the role of the cytoskeleton, polarity proteins, and trafficking in mammary

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ductal morphogenesis has not yet been examined.

ECM Density and Remodeling

In addition to cell-autonomous controls on ductal morphogenesis, the composition and mechanical properties of the stromal ECM has a profound effect on mammary cell behavior (Butcher et al. 2009). For example, ECM density regulates cell fate decisions, which might direct ductal morphogenesis and the lineage of stem cell progenitors (Guilak et al. 2009). Integrin-associated scaffold proteins such as filamin constitute a mechanism for mammary cells to detect mechanical cues within the ECM, and thereby control morphogenesis (Gehler et al. 2009).

In addition, matrix remodeling is required for cells to sprout from the main ducts to form branches, and is important for endbud progression (Page-McCaw et al. 2007). The matrix metalloproteinase MMP-3 induces secondary and tertiary branching of mammary ducts, while MMP-2 promotes endbud invasion into the stroma by suppressing epithelial apoptosis (Wiseman et al. 2003). Membrane-bound MMPs are also expressed in mammary ducts. Interestingly, MT2-MMP is in the epithelia and MT3-MMP is in the adjacent stromal cells, while MT1-MMP appears to be present in both (Szabova et al. 2005). In SMG, MT2-MMP cleaves the BM collagen-IV to release its NC1 domain, which in turn promotes proliferation possibly by activating integrin signaling directly (Rebustini et al. 2009). MT-MMPs are likely to be required for mammary morphogenesis, and it will be important to elucidate how the epithelial and stromal proteases each contribute.

HOW CELL–MATRIX INTERACTIONS CONTROL THE FORMATION OF POLARIZED MAMMARY DUCTS

The polarized epithelial bilayer is established early in mammary development, as the bud emerges from the ectoderm (Jolicœur 2005). There are two aspects to the polarity of

mammary epithelium. The first is polarity of the bilayered structure consisting of the luminal and myoepithelial (basal) layers: i.e., the mechanisms establishing the orientation of two different cell types. The second is cell polarity: i.e., the mechanisms controlling how MECs establish an apical surface and thereby form lumens.

Establishing a Bilayer

Studies in adult tissues reveal that the spatial orientation of luminal and myoepithelial cells is largely controlled by differential adhesivity between the cell types. Both express the desmosomal cadherins Dsg2 and Dsc2, while the myoepithelial cells additionally contain Dsg3/Dsc3. The luminal cells are intrinsically more adhesive to one another, and thereby restrict the myoepithelial cells to a more external, basal location, an organization that is prevented in the absence of Dsg3/Dsc3 function (Runswick et al. 2001). Conventional cadherins also contribute, as function-blocking anti E-cadherin antibodies selectively perturb luminal cells and do not affect the myoepithelium, whereas P-cadherin antibodies only disrupt the basal cell layer (Daniel et al. 1995). The BM matrix contributes to the bilayered organization as well, because cocultures of luminal and myoepithelial cells in collagen gels form bilayers, but require laminin-111 production (by the latter cell type) to do so (Gudjonsson et al. 2002). Interestingly, although the myoepithelial cells contain hemidesmosomes, which might rivet the cells to BM, genetic deletion of $\alpha 6$ -integrin, which is required to assemble this type of adhesion complex, does not alter the relative positioning of basal and luminal cells, neither does the deletion of $\beta 1$ -integrin (Klinowska et al. 1999; Naylor et al. 2005). Further studies on other matrix receptors or BP180, might reveal the extent to which ECM plays a role here.

Forming a Lumen

Mammary ducts contain a lumen, which is continuous from the nipple to the extremities of its branches, as shown by duct injection with a marker (Russell et al. 2003). The lumen forms

behind the endbud as the duct develops. Endbuds show signs of apoptosis at their distal edges, just where the lumen becomes visible (Humphreys et al. 1996). Apoptosis via the proapoptotic protein, Bim, causes cavitation to occur, thereby creating the lumen (Mailleux et al. 2007). Whether or not cell–matrix interactions are involved in this process is unclear (Green and Streuli 2004). It has been suggested that the cells inside the ducts might undergo apoptosis because they are not in contact with the ECM. However, they retain cell–cell interactions, which also protect MECs from apoptosis (Boussadia et al. 2002). Moreover, Bim detects loss of growth factor signals rather than altered integrin signaling (Wang et al. 2004). Thus, although ductal lumen formation requires apoptosis of endbud cells, the ECM may not be involved directly, although it may regulate apoptosis at this region by altering GF delivery to the endbud.

Similarly, the role of cell–matrix interactions in forming alveolar lumens in lactation is not established. Culture models of acinus formation in the breast cell line, MCF10A, suggest that apoptosis-driven cavitation might create the lumen, and that apoptosis results because the cells are spatially separated from the ECM (Debnath et al. 2002). However, lumens form in the absence of luminal cell apoptosis in primary MEC 3D cultures or in alveoli in vivo (Akhtar and Streuli, unpubl.). Moreover, lumens form by fluid movement rather than apoptosis in other epithelial cells (Pearson et al. 2009).

Establishing apical–basal polarity may provide the key mechanism for lumen formation. The apical surface of luminal epithelia is decorated with transmembrane mucins, e.g., Muc-1, which are heavily glycosylated and prevent cell adhesion at those surfaces. LM-111, dystroglycan, and β 1-integrins all have central roles in establishing polarity, as gleaned from 3D cultures of mammary and MDCK cells (O'Brien et al. 2001; Weir et al. 2006; Yu et al. 2005). Thus the matrix provides a guiding principle within the alveolar epithelium, to orient luminal cells, and create luminal surfaces and thus fluid-filled cavities (O'Brien et al. 2002).

The intracellular mechanisms for how integrins control the formation of the apical surface remains unknown.

HOW CELL–MATRIX INTERACTIONS CONTROL DUCTAL PATTERNING

An important aspect of ductal morphogenesis is patterning, which involves at least four distinct mechanisms: (1) a bifurcator, which controls endbud splitting; (2) a periodic device, which determines how far apart the branches occur; (3) a restriction collar, which causes the growing epithelium to form a tube rather than a ball (like a toothpaste tube); and (4) negative feedback to prevent ducts from colliding (Fig. 4). These mechanisms have not been studied extensively in the mammary gland. However, they are different from other branching networks, such as lung, which forms as the tissue grows, through an iterative set of rules involving the localized expression of FGF10, FGFR2, and Sprouty-1 (Metzger et al. 2008).

Bifurcation

ECM proteins accumulate at the cleft where endbuds form two branches, supplying a wedge to split growth into new directions. Highly localized expression of TGF β 1 in the endbud cells may control this by increasing ECM deposition within the cleft (Robinson et al. 1991; Silberstein et al. 1990). The mechanism of bifurcation at mammary endbuds is not known, but may be related to that in SMG, lung, and kidney. There, fibronectin fibrils accumulate at the clefts between new buds, and are required for branching to occur (Sakai et al. 2003). In addition, blocking of the α 5 β 1 integrin, a fibronectin receptor, disrupts branching of ex vivo mammary explants (Fata et al. 2007). Interestingly, the process of clefting does not depend on proliferation, but on cell movement (Larsen et al. 2006). E-cadherin expression is suppressed at these sites which, together with the wedge provided by fibronectin, enable cells to part and form the beginnings of new branches.

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Figure 4. Mammary gland patterning. (A) Bifurcation and restriction collar. This whole-mount image of a gland from a 6-wk virgin mouse shows one advancing endbud, and another in the process of splitting to form two new ducts (arrow). Notice how the new duct is restricted just behind the endbud, forming a narrow tube (blue arrows). (B) Periodicity and open architecture. Branching in a 6-wk gland occurs at discrete intervals (arrows). The new ducts do not bump into one another, so they retain an even network throughout the tissue. Photos courtesy Julia Cheung.

Periodicity

Presumably, distinct mechanisms control the timing of endbud branching during the growth of major ducts, and the periodicity of side branch eruption from pre-existing main ducts. Currently neither is understood, and it is possible they occur stochastically. Side branches appear intermittently along ducts, particularly during estrus cycles and in pregnancy, and are the precursors of alveoli (Metcalf et al. 1999). Branch spacing is likely to depend on the periodic location of progenitor cells, which may be controlled by lateral inhibition signals such as Notch (Bouras et al. 2008). Progenitors are maintained within a stem cell niche, which depends on integrin–matrix interactions. $\beta 1$ -integrins are required to maintain mammary stem cells, and deleting them from basal cells causes morphogenetic disorders and perturbed branching (Taddei et al. 2008). The emergence of cells at side branches is controlled by morphogenetic signals such as progesterone, RankL and Wnts, but also requires coordinated events of ECM turnover (Fernandez-Valdivia et al. 2009; Mulac-Jericevic et al. 2003; Roarty and Serra 2007). The latter is accompanied by diminished TGF β in the vicinity of the newly forming branch, which leads to reduced ECM expression, and increased MMPs. Notably, overexpressed TGF $\beta 1$ and deleted MMP-3 abrogate side branches (Pierce

et al. 1993; Wiseman et al. 2003). It will be interesting to learn whether ECM changes are deterministic and occur before branches emerging, or if they are consequent on other events that specify evagination.

Patterning

The patterning of mammary gland is characterized by long, thin ducts (Lu and Werb 2008). A dominant mechanism to restrict the epithelium laterally might involve two possible mechanisms, but these remain speculations at this time. One results from planar cell polarity, where intracellular forces provided by localized cadherin expression and cytoskeleton contraction, impede cell expansion perpendicular to the long axis of the duct, but permit it longitudinally (Nishimura and Takeichi 2009). Wnts are classic regulators of planar cell polarity, and are central in mammary morphogenesis (Brennan and Brown 2004). The other mechanism is physical restriction by an ECM sheath, which is initially deposited just behind the emerging endbud and retained for the length of the mature duct, and is under the control of TGF β (Silberstein and Daniel 1982). The topography of the collagen fibrils around ducts is not known (e.g., a mesh or parallel fibrils), but they are presumably synthesized by stromal fibroblasts: Here the use of electron microscope

tomography will be useful in unraveling the details of the matrix sheath (Starborg et al. 2008).

Open Architecture

Finally, the open architecture of the network depends on ducts maintaining their distance from each other, and on epithelial cells remaining within the ducts themselves. TGF β is sequestered within the duct-ensheathing ECM, and prevents new ducts colliding into them (Silberstein 2001). Although epithelia are strongly cohesive via cadherin contacts, individual cells that migrate into the stroma are deleted by apoptosis because their matrix interactions change from BM to collagen (Pullan et al. 1996).

Ductal architecture is visually elegant, involving a limited number of cell types and simple patterns. However, the formation of this structure is molecularly complex, involving stromal–epithelial conversation and an ultimate control by cell–matrix interactions. A detailed understanding of this highly dynamic process is currently limited, and will require considerable emphasis on 4D imaging combined with genetic analysis to fully dissect.

HOW CELL–MATRIX INTERACTIONS INFLUENCE MAMMARY DIFFERENTIATION

The eventual function of the mammary gland is enabled by a dramatic and rapid outgrowth of the gland during pregnancy that includes increased tertiary branching, invasion of the mammary fat pad, and the formation of milk-secreting acinar units, termed alveologenesis (Fig. 1). The major cues for these events are hormones produced outside the gland, including estrogen and progesterone from the ovaries, prolactin from the pituitary gland, lactogens from the placenta, and growth hormone from multiple sources including the liver (Oakes et al. 2006). All of these signals must not only cross the BM, but they are regulated by it. Thus, the ECM modulates estrogen, progesterone, and prolactin signaling in MECs, though whether the matrix also influences steroid responsiveness in stromal cells is not yet known

(Haslam and Woodward 2003; Streuli and Akhtar 2009).

Integrin-Prolactin Cross Talk

By far the best elucidated of these matrix-dependent cues is the integrin requirement for prolactin receptor signaling in luminal cells from alveoli. Integrins act as microenvironmental checkpoints and only permit sustained prolactin signals when the epithelial cells are in the correct spatial location within the tissue (Katz and Streuli 2007). This is known experimentally from culture models, where cells placed in or on stromal matrix lose their responsiveness to lactogenic hormones, but regain this ability in the presence of BM proteins, with laminin-111 being critical (Barcellos-Hoff et al. 1989; Streuli and Bissell 1990; Streuli et al. 1995).

The pathway connecting cell–matrix interactions to prolactin receptor signaling has been the subject of many investigations and, with recent advances, a detailed picture is emerging. The critical convergence point of laminin and prolactin signaling resides in the sustained activation of the Jak2-to-STAT5 pathway, which mediates prolactin and growth hormone signaling. Laminin signaling is not required for the initial activation of STAT5, but maintains a sustained signal (Xu et al. 2009). Antibody and peptide blocking experiments implicate the β 1-integrins in this control, together with a nonintegrin receptor that binds the globular domains of the laminin α 1 subunit (Muschler et al. 1999; Streuli et al. 1991). Genetic dissections in cultured cells and in vivo point to cooperation among β 1-integrins and dystroglycan in prolactin signaling. Thus, conditional deletion of the β 1-integrin gene leads to reduced mammary outgrowth and a failure of lactation that coincides with a loss of STAT5 activity (Li et al. 2005a; Naylor et al. 2005). Similarly, deletion of dystroglycan in cultured mammary cells prevents sustained STAT5 activation and milk-protein expression, whereas dystroglycan knockout also results in defective gland outgrowth, lactation, and STAT5 activity (Weir et al. 2006; Leonoudakis and

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Muschler, unpubl.). The nature of the cooperation between two laminin receptors resides in the ability of dystroglycan to initiate anchoring and assembly of laminin-111 at the cell surface, thereby functioning as an integrin coreceptor.

Integrins are the key signaling receptors for the matrix regulation of STAT5 activation, and downstream they require the adaptor and signaling abilities of integrin-linked kinase and Rac, but not focal adhesion kinase (Akhtar et al. 2009; Akhtar and Streuli 2006). Although $\beta 1$ -, but not $\beta 4$ -, integrins specifically regulate prolactin signaling, which of the laminin-binding integrin heterodimers are involved is still an open question. Deletion of $\alpha 3$ - or $\alpha 6$ -integrins do not cause a lactation defect like that observed for loss of the entire $\beta 1$ -integrin family (Klinowska et al. 2001). Current challenges are to determine precisely how the integrin-assembled adhesion signaling complex controls the prolactin-STAT5 axis, and whether other adhesion-mediated signals directly affect the transcription machinery for differentiation.

Involution

Following the cessation of suckling, milk proteins accumulate in the mammary gland and, coupled with alveolar swelling, induce the process of involution. This is the dramatic deconstruction of the lactating mammary gland, restoring it to a state that resembles pre-pregnancy (Watson and Khaled 2008). The process includes dismantling the epithelial arbor via cell death, and remodeling the stroma. Cell–matrix interactions have an integral role in controlling involution and restoration of the ductal tree, as they modulate apoptosis and preserve the stem-cell niche. However, the precise role of the ECM in involution remains unclear. The induction or inhibition of ECM degradation affects the progression of involution, with a variety of MMPs, serine proteases, and TIMPs significantly affecting its kinetics (Green and Streuli 2004). But so far, it has proved difficult to distinguish between the impact of matrix degradation and the many other signaling events converging on the gland at this stage of development, including the release of soluble

factors from the degraded matrix. With the introduction of genetic tools to manipulate matrix protein expression in the mammary gland, these questions may soon be addressed.

HOW THE BREAKDOWN OF NORMAL CELL–MATRIX INTERACTIONS INFLUENCES BREAST CANCER

In Situ Carcinoma

Changes in cell–matrix interactions are integral to the development and progression of cancers at every stage, from premalignancy to invasion, and the seeding, survival, and growth of metastases (Bissell and Radisky 2001). Early lesions of the breast epithelium develop in the context of an intact basement membrane, which normally exerts tumor suppressing functions by controlling tissue architecture. Signaling for epithelial polarity is one key to the BM's role in tumor suppression. A loss of polarity through altered cell–cell or cell–BM interactions can unleash the tumor phenotype, whereas restoration of polarity by manipulating BM signaling can suppress tumorigenicity (Bissell et al. 2005; Weaver et al. 1997; Zhan et al. 2008). The gatekeeper function of the BM is also involved in tumor suppression, by retaining nascent in situ carcinomas within its boundaries. Extensive genomic alterations and variations in histological grade can accumulate within in situ carcinomas, but the cells remain caged as a benign lesion by the presence of the BM (Allred et al. 2008; Chin et al. 2004; Hwang et al. 2004). Because the existence of a BM distinguishes pre-cancerous lesions from invasive cancers, its gatekeeper function is paramount as a determinant of cancer progression. Moreover, it might provide an opportunity for new therapies to block progression to invasive disease.

Crossing the BM

Despite the importance of the transition from in situ to malignant breast cancer, the mechanisms controlling invasion through the BM are uncertain. One central factor is the changing properties of the tumor cell itself, including

the increased synthesis of matrix degrading enzymes, and altered cell adhesion and ECM signaling mechanisms (Hood and Cheresch 2002; Mercurio et al. 2001). In addition, a collaboration between tumor cells and the tumor microenvironment is required to breach the BM. Microenvironmental factors recognized to drive cancer progression include changes to the myoepithelial cell population, alterations in the cellular components of the stroma, such as cancer associated fibroblasts, macrophages, and other infiltrating leukocytes, and changes to the stromal matrix itself (Butcher et al. 2009; Hu et al. 2008; Joyce and Pollard 2009; Orimo and Weinberg 2006).

The complexity of tumor–stromal interactions underlie the diverse manifestations of early breast cancer lesions, which are reflected by different mechanisms of BM invasion (Bergamaschi et al. 2008). Increased protease production and BM remodeling occurs at invasive sites, and there is little doubt that proteolysis of the BM plays an important role (Rowe and Weiss 2008). However, proteolysis alone is not sufficient to explain the loss of BMs at the invasive front, because matrix degradation is only one facet in a cycle of BM formation and remodeling, which is tightly coordinated with the synthesis of matrix components and their assembly, and turnover by endocytosis (Sottile and Chandler 2005). Receptor-facilitated laminin assembly and laminin endocytosis occur in MECs, but have not yet been carefully investigated in the context of breast cancer invasion (Coopman et al. 1991; Weir et al. 2006).

Myoepithelial cells are key producers of BM proteins, and changes in the synthesis of BM proteins, including a loss of laminin-111 production, are evident in cancer-associated myoepithelial cells (Allinen et al. 2004; Gudjonsson et al. 2002). The tumor suppressing activity of normal myoepithelial cells relies in part on their role in BM synthesis, but they also secrete inhibitors of ECM-degrading proteases such as maspin, which are reduced in breast cancer (Streuli 2002). Intriguingly, the myoepithelial population itself has a major role in the conversion of in situ carcinomas to invasive breast cancers (Clarke et al. 2005).

Stromal Invasion

Once the regulatory influence of the BM has been evaded, the nascent cancer encounters a radically different environment of the stromal matrix. The invading tumor cell becomes exposed to a distinct array of matrix molecules and a milieu of proteases and cytokines that are no longer filtered by a protective BM. This conspicuous change is compounded by the increased cellularity of the tumor microenvironment and changes to the stromal matrix itself. Elevated production of matrix components such as collagen and hyaluronan are prominent in tumor stromal matrix, and they influence breast cancer cell invasion and metastasis (Itano and Kimata 2008; Provenzano et al. 2008). Lysyl oxidase activity, which mediates collagen cross-linking, also contributes to breast cancer progression (Levental et al. 2009). Importantly, increases in collagen synthesis and cross-linking produces a stiffer matrix that imparts distinct biochemical and mechanical influences, which can foster malignancy (Kumar and Weaver 2009). Breast density is a significant risk factor for cancer progression and may be linked with increased collagen deposition and stiffness of the stromal matrix, although this putative relationship requires further investigation (Li et al. 2005b).

Metastasis

The varied paths taken by malignant cells are paved with ECM molecules, which have a central role in tumor-cell dissemination and growth of metastases. Passage into and out of the vasculature requires crossing the endothelial BM, perhaps at regions where the integrity of the matrix has natural imperfections (Voisin et al. 2010). Indeed, changes in the peritumoral vasculature and endothelial BM facilitate increased cellular traffic into and out of the vasculature, and may be caused by similar factors that contribute to the invasion of the epithelial BM (McDonald and Baluk 2002). ECM molecules also participate in the preparation of the premetastatic niche, and in the survival and growth of metastases in assorted tissues

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(Kaplan et al. 2005; Psaila and Lyden 2009). One of the greatest challenges will be to understand precisely how the stromal microenvironment at metastatic sites provides a suitable home for tumor cells, in terms of both the address and the factors permitting survival of foreign cells. Although the role of chemokines is becoming clear, more needs to be learnt about the interactions of metastatic cells with the niche cells and its ECM, as well as ECM-dependent survival signaling pathways, such as those involving c-Src (Kim et al. 2009; Zhang et al. 2009).

HOW TARGETING CELL–MATRIX INTERACTIONS WILL IMPROVE CANCER THERAPY

The diverse and defining roles of ECMs in breast cancer progression present many opportunities for therapeutic intervention. Targets include: disrupted polarity; altered differentiation; defects in integrin–GFR crosstalk; invasion through the BM; survival and migration within the stromal matrix; and homing and survival in the metastatic niche. The manipulation of ECM receptors on tumor cells has proven to be a powerful means of controlling cancer-cell behavior in experimental models, and may turn out to be valuable in a clinical setting (Giancotti 2007; Pontier and Muller 2009). Matrix-degrading proteinase inhibitors have been devised to target ECM modifications, and are likely to be useful once the diverse activities of these proteases are better understood (Overall and Kleinfeld 2006). Moreover, ECM-modifying enzymes such as lysyl oxidase and hyaluronan synthase may become therapeutic targets aimed at reverting the ECM of the tumor stroma, or disrupting the metastatic bed (Erler et al. 2009).

Cell–ECM interactions have long been recognized as potent targets for the inhibition of angiogenesis in cancer therapy. Many matrix fragments possess antiangiogenic activities, for example endostatin, a fragment of collagen XVIII (Eble and Niland 2009). Targeting of the integrins has reached clinical trials for the inhibition of angiogenesis (Avraamides et al. 2008). Recent reports have also shown that

blocking integrin functions can enhance the responsiveness of breast tumor cells to therapies, including radiation and Her-2 targeting (Lesniak et al. 2009; Park et al. 2008).

Because of the many critical roles played by cell–ECM interactions in controlling normal tissue architecture, homeostasis and cancer progression, the targeting of cell–ECM interactions is destined to become a standard component of the oncologist's therapeutic arsenal. We assert that, in the mammary gland, these targets will prove particularly valuable in the three-pronged approach of attacking defective endocrine, growth factor signaling and cell–ECM signaling, and in combination with radiation and chemotherapies to overcome tumor cell resistance.

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