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Tissue architecture and breast cancer: the role of extracellular matrix and steroid hormones

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Abstract

The changes in tissue architecture that accompany the development of breast cancer have been the focus of investigations aimed at developing new cancer therapeutics. As we learn more about the normal mammary gland, we have begun to understand the complex signaling pathways underlying the dramatic shifts in the structure and function of breast tissue. Integrin-, growth factor-, and steroid hormone-signaling pathways all play an important part in maintaining tissue architecture; disruption of the delicate balance of signaling results in dramatic changes in the way cells interact with each other and with the extracellular matrix, leading to breast cancer. The extracellular matrix itself plays a central role in coordinating these signaling processes. In this review, we consider the interrelationships between the extracellular matrix, integrins, growth factors, and steroid hormones in mammary gland development and function.

Introduction

Tissue architecture depends on proper cell-cell and cell-extracellular matrix interactions involving a reciprocal exchange of mechanical and biochemical information to maintain homeostasis (Grobstein 1967, Bissell *et al.* 1982, Sakakura 1983). Disruption of these delicately balanced interactions can result in loss of tissue structure, with severe consequences for the tissue as well as the organism. The mammary gland is a versatile model to investigate how functional and structural homeostasis is achieved and maintained because it undergoes cycles of development, differentiation, and apoptosis during the adult life of the organism. These cyclic changes involve dramatic shifts in the structure and function of the tissue, and are regulated through complex multi-hormonal and growth factor signaling pathways. Furthermore, the tumors of the breast provide examples of changes in signaling pathways and gene expression, and the corresponding loss in tissue structure and function.

In both the normal mammary gland and breast cancer, the steroid hormones, estrogen and progesterone, have important roles in tissue function (reviewed in Lapidus *et al.* 1998, Russo & Russo 1998). Estrogen and progesterone regulate mammary gland development at puberty and pregnancy, and are associated with the initiation, development, and progression of breast cancer. Numerous studies have established that the reproductive history of a woman is associated with the risk of developing breast cancer, and factors that inhibit or decrease exposure to steroid hormones reduce this risk. Early pregnancy, late menarche, and early menopause all provide a protective effect against breast cancer (Staszewski 1971, Trichopoulos *et al.* 1972, Lambe *et al.* 1996). In spite of this evidence and decades of examining the mechanisms of steroid hormone action, however, we have yet much to learn about how estrogen

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and progesterone integrate with other signals that contribute to homeostasis, and how they may be involved in processes that lead to breast cancer.

The identification of the extracellular and intracellular components that are responsible for the maintenance of mammary gland structure and function has been the focus of the work of many investigators, including our own. We now know that the tissue microenvironment plays a dominant role in this process, and that the extracellular matrix (ECM), an essential component of the microenvironment, is intimately involved in processes that determine tissue specificity (Fig. 1; reviewed in Bissell *et al.* 1999). We know that the tissue microenvironment has an impact also on the initiation and maintenance of estrogen and/or progesterone responsiveness (reviewed in Woodward *et al.* 1998). In this brief review, we discuss the importance of cell–cell and cell–ECM interactions in maintaining tissue structure and function, and hormone responsiveness.

The organization of the mammary gland

Normal mammary gland development

Mammary gland development occurs throughout the female life and can be divided into several stages that differ in morphology, function, and hormonal responsiveness (Fig. 2; reviewed in Ronnov-Jessen *et al.* 1996,Russo & Russo 1998). From birth to the onset of puberty, few changes occur in the mammary gland, and it is essentially the same in both males and females. During puberty, however, the female mammary gland responds to the production of the ovarian steroid hormone, estrogen, and the epithelium branches into numerous ducts with terminal endbuds or alveoli, collectively referred to as the terminal ductal lobular unit (TDLU). For humans, this fairly undifferentiated structure is composed of clusters of 6 to 11 ductules per lobule, and has been called lobule type 1 (or Lob 1) (Russo & Russo 1998). Lob 1 progresses to lobule type 2 (Lob 2) in the postpubertal virgin gland, with only modest alveolar proliferation producing a higher number of ductular structures per lobule during the menstrual cycle. Once pregnancy occurs, elevated levels of estrogen and progesterone, another ovarian steroid hormone, as well as pituitary hormones stimulate Lob 1 and Lob 2 to progress to lobule type 3 (Lob 3). Lob 3 is formed by epithelial expansion of existing pubertal alveoli to 80 small lobules per alveoli. These changes prime the mammary gland for milk secretion from the alveoli, now called secretory lobules type 4 (Lob 4). After parturition, the lactating mammary gland becomes insensitive to estrogen-dependent regulation of growth; during the post-weaning involution phase, responsiveness to estrogen is restored. Finally, with the cessation of lactation, the alveoli collapse and the mammary gland regresses apoptotically to its resting, pre-pregnancy state, reverting to Lob 3 and Lob 2, retaining a more extensive framework of branching than Lob 1. Thus the adult female mammary gland experiences recurrent cycles of regulated growth, differentiation, and apoptosis, and estrogen and progesterone play important roles in this process (Fig. 2; reviewed in Ronnov-Jessen *et al.* 1996,Russo & Russo 1998).

Mammary gland composition

Irrespective of the developmental status of the gland, the mammary epithelium is a bilayered structure consisting of an inner continuous layer of luminal epithelial cells and an outer layer of myoepithelial cells. The epithelial bilayer is polarized; the apical layer (luminal epithelial cells) faces the lumen of the ducts and the alveoli, and the basal layer (myoepithelial cells) is in close contact with a laminin-rich basement membrane (BM). The epithelium is embedded in the mammary stroma, which makes up >80% of the breast volume (Ronnov-Jessen *et al.* 1996). Types of breast stroma include fat tissue, interstitial/interlobular dense connective tissue, intralobular loose connective tissue, and blood vessels. In addition to contacting the BM, stromal cells are also surrounded by ECM that is sometimes referred to as `stromal ECM'.

For the purpose of this review, we will use the term ECM to describe all the acellular, insoluble proteinaceous components that exist in the mammary tissue, including BM and stromal ECM.

In the normal human breast, approximately 20% of the luminal epithelial cells are in direct contact with the BM; the remaining cells are adjacent to the myoepithelial cells (Gusterson *et al.* 1982, Petersen & van Deurs 1988). The precise relationship between the luminal epithelial cells and myoepithelial cells, and the origin of these cells is largely unknown, making this an important problem for developmental biology of the mammary gland. We have recently shown that a portion of luminal epithelial cells, cultivated in culture to maintain correct functional characteristics, give rise to myoepithelial cells in an appropriate medium, but myoepithelial cells do not produce luminal epithelial cells (Pechoux *et al.* 1999). This finding suggests a linear relationship between these two epithelial cell types, and may be important to tumor biology because most breast cancers are luminal rather than myoepithelial in origin (Wellings *et al.* 1975, Rudland 1993). Myoepithelial cells have been hypothesized to play a `tumor suppressive' role by maintaining the differentiated state of luminal epithelial cells (Bani *et al.* 1994, Liu *et al.* 1996). While we have also arrived at this hypothesis, we believe that luminal epithelial cell transformation may prevent conversion to myoepithelial cells. This may explain why in premalignant lesions there are fewer myoepithelial cells, and in invasive breast cancer, myoepithelial cells are either missing or less differentiated (Gusterson *et al.* 1982, Guelstein *et al.* 1988, Rudland *et al.* 1995). In >90% of cases, tumor cells are restricted to a luminal-like phenotype (Altmannsberger *et al.* 1986, Nagle *et al.* 1986, Dairkee *et al.* 1988, Guelstein *et al.* 1988, Bocker *et al.* 1992), and only a small proportion of these cells are in contact with myoepithelial cells (Gusterson *et al.* 1982, Petersen & van Deurs 1988).

Although breast cancer cells originate mainly in the epithelium, evidence suggests that the stroma is an active participant in cancer progression (and possibly even induction), and constitutes the majority of the tumor mass (Dvorak 1986, Thomasset *et al.* 1998). Compared with normal mammary gland stroma, which mainly consists of fibroblasts and adipocytes, tumor stroma contains changes in the cellular composition and in the amounts of certain protein constituents, often referred to as `reactive stroma' or `desmoplasia'. For example, the most prominent cellular change in tumor stroma is the appearance of myofibroblasts, which are found in close proximity to tumor cell nests (reviewed in Ronnov-Jessen *et al.* 1996). Myofibroblasts produce proteases such as urokinase plasminogen activator and stromelysin-3, which degrade ECM and contribute to tumor cell invasion (Wolf *et al.* 1993, Unden *et al.* 1996).

The ECM of the normal mammary gland is a dense, sheetlike meshwork of collagens, fibrillar glycoproteins, and proteoglycans that are secreted by the cells, the composition of which changes during preadult development, estrous cycles, and pregnancy, lactation, and involution (reviewed in Lochter & Bissell 1995). In addition, there are both quantitative and qualitative modifications in ECM components in breast cancer (reviewed in Kosmehl *et al.* 1996). Since myofibroblasts produce proteases that degrade ECM, tumor stroma may alter the ECM surrounding a tumor and thus enhance the malignant potential of epithelial cells (Ronnov-Jessen *et al.* 1996, Thomasset *et al.* 1998). There are several lines of evidence that these changes in the epithelial cell microenvironment can lead to changes in cell behavior. First, cells grown in two-dimensional (2D) monolayers exhibit morphological differences compared with cells *in vivo* and fail appropriately to express tissue-specific genes (Emerman *et al.* 1977, reviewed in Stoker *et al.* 1990, Roskelley *et al.* 1995). Secondly, coculture of MCF-7 breast cancer cells with various types of fibroblasts (normal or tumor) alters the expression of genes such as estrogen receptor (ER) and progesterone receptor (PR) (Adam *et al.* 1994). And thirdly, three-dimensional (3D) cultured cells grown in reconstituted basement membrane (rBM) recapture some of the normal features lost in 2D cultures, such as expression of milk proteins, and the correct alveolar structure of the human breast (Barcellos-Hoff *et al.* 1989, Petersen *et al.*

1992). Thus cell–cell and cell–ECM interactions within the mammary gland are important to maintain normal epithelial structure and function.

Mammary gland morphology and breast cancer origin

The development of breast cancer is characterized by the acquisition or loss of discrete cellular functions, resulting in altered tissue organization, which has long been recognized by pathologists and used to classify breast tumors as specific morphological types (reviewed in Beckmann *et al.* 1997). Russo and Russo (1998) have observed that specific morphological types of breast cancer are associated with specific breast structures or developmental stages of the mammary gland. For example, the common breast malignancy, ductal carcinoma, which is thought to originate within the fairly undifferentiated epithelial cells of the TDLU, corresponds to Lob 1 (described in the first section; Fig. 2). Similarly, lobular carcinomas *in situ* are found in Lob 2, benign breast lesions originate in Lob 3, and lactating adenomas arise in Lob 4. Russo and Russo (1998) concluded from these observations that less functionally differentiated breast cells (Lob 1) are more susceptible to giving rise to the most undifferentiated, aggressive neoplasms. Thus the developmental stage of the breast appears to affect neoplastic transformation. Supporting this hypothesis are studies demonstrating the higher risk of malignancy in nulliparous and late parous women (Lambe *et al.* 1996). In spite of this evidence, we do not yet understand how the morphological and developmental stages of the mammary gland are associated with breast cancer.

It is generally accepted that development of invasive breast cancer occurs through the multistep transformation of epithelial cells via steps of hyperplasia, premalignant change, *in situ* carcinoma, and invasive carcinoma (reviewed in Wellings *et al.* 1975, Gould 1993, Beckmann *et al.* 1997). However, there is no evidence that each step is a necessary precursor of the next stage because it has been difficult to develop model systems with cells representing various types of breast lesions, from benign tumors to invasive carcinoma. Markers of malignant cells have been partially defined, but the characteristics of the precursor cells are less well-known, making identification difficult. Evidence does suggest, however, that certain regions of the mammary gland may be predisposed to tumor formation. Studies by Tsai *et al.* (1996) indicate that whole regions of the breast may originate from the same cells, i.e. that they are clonal. If these cells are `primed' for tumor formation by harboring genetic mutations, one might expect to find normal-appearing cells with genetic abnormalities in the region surrounding tumors. We now know that morphologically normal epithelia do contain many genetic mutations and that these mutations may give rise to cancer (Deng *et al.* 1996).

The role of steroid hormones

Mechanisms of steroid hormone action

Estrogen and progesterone promote proliferation and differentiation in the normal breast epithelium. They function via binding to their corresponding intracellular receptors, ER and PR, which are members of the nuclear hormone receptor superfamily (reviewed in Evans 1988). The process by which estrogen and progesterone interact with their receptors is similar for all members of the nuclear hormone receptor family (reviewed in White & Parker 1998). In the absence of hormones, the receptors are in an inactive complex with a number of other proteins, including heat shock proteins. When hormones pass through the cell membrane and bind the receptors, the inactive oligomeric complex dissociates and the receptors are transformed into an active state that regulates gene expression either directly as a transcription factor by binding DNA at a specific response-element (Beato & Sanchez-Pacheco 1996, Glass *et al.* 1996, Horwitz *et al.* 1996), or indirectly by cooperative interactions with other transcription factors (e.g. AP-1) (Gaub *et al.* 1990, Philips *et al.* 1993, Umayahara *et al.*

1994). As DNA-binding transcription factors, steroid hormone receptors do not function alone, but interact with general transcription factors and receptor interacting proteins.

In addition to this complexity, members of the nuclear hormone receptor superfamily are expressed in multiple forms. Two forms of ER are known to exist, ERα and ERβ (Green *et al.* 1986, Greene *et al.* 1986, Kuiper *et al.* 1996), and although most of the work described in this review concerns ERα, it is important to mention ERβ (reviewed in Enmark & Gustafsson 1998, Hansen & Fuqua 1999). ERβ is expressed in the mammary gland and in some breast cancers (Dotzlaw *et al.* 1997, Saji *et al.* 2000), but since it was very recently identified, its role in mammary gland function is largely unknown. In the rat mammary gland, the presence and cellular distribution of ERα and ERβ differs depending on the developmental stage, but during most stages there are more cells expressing ERβ than ERα (Saji *et al.* 2000). ERβ has a similar affinity for estradiol, and binds DNA in a manner similar to ERα, as a homodimer interacting with estrogen response elements (EREs) (Cowley *et al.* 1997, Kuiper *et al.* 1997, Pace *et al.* 1997, Pettersson *et al.* 1997). In addition, ERβ and ERα form functional heterodimers on EREs, suggesting the existence of a previously unrecognized pathway of estrogen signaling (Cowley *et al.* 1997, Pettersson *et al.* 1997). Estrogen signaling may occur through ERβ in cells expressing this subtype, through ERα in cells expressing this subtype, and through ERβ/ERα in cells expressing both subtypes. Furthermore, varying ratios of ERβ and ERα in cells expressing both subtypes may be important for estrogen responsiveness (Enmark & Gustafsson 1998).

At least two forms of PR are also known to exist, PR-A and PR-B, but unlike the ER subtypes, which are separate genes, these isoforms are transcribed from two distinct transcription start sites within the same gene (Rousseau-Merck *et al.* 1987, reviewed in Giangrande & McDonnell 1999). PR-A and PR-B have different transcriptional activation properties; their activity varies depending on the cell type (Tora *et al.* 1988, Tung *et al.* 1993, Vegeto *et al.* 1993). PR-A can either inhibit or enhance PR-B activity, depending on the cell type and promoter context (Vegeto *et al.* 1993). The ratio between PR forms also varies depending on cell type, and is believed to be an important factor for appropriate progesterone responsiveness (Tsai & O'Malley 1994, McDonnell 1995). For example, an imbalance in the expression and/or activities of PR-A and PR-B can result in inappropriate progesterone signaling and an aberration in mammary development (Shyamala *et al.* 1998, reviewed in Shyamala *et al.* 1999). Progesterone signaling may also be affected by the presence of a third PR, PR-C (Wei *et al.* 1990, 1996). PR-C is missing the first 594 amino acids at the N-terminus common to PR-A and PR-B, but still binds hormone and is capable of forming heterodimers with PR-B. Both PR-A and PR-B also modulate ER and estrogen-dependent gene expression (Chalbos & Galtier 1994, McDonnell & Goldman 1994, Kraus *et al.* 1995).

Finally, there are variant forms of ER and PR, which may have unique properties that influence hormone responsiveness (Fuqua *et al.* 1991, Leygue *et al.* 1999, reviewed in Lapidus *et al.* 1998). For ERα, variants with point mutations at a single amino acid have been identified, as well as numerous messenger RNA splice variants. ERα splice variants are usually coexpressed along with the wild-type ER message (Zhang *et al.* 1996), and may affect the activity of the wild-type receptor. For example, an ERα splice variant lacking exon 5 has constitutive and hormone-independent transcriptional activity (Fuqua *et al.* 1991). Other variants have also been identified with altered functional activities (reviewed in Hopp & Fuqua 1998).

Expression of steroid hormone receptors

The cellular distribution of ER and PR in the mammary gland is important in the complex relationships between the epithelial cells and stroma. Normal human mammary epithelial cells express very low levels of ER and PR, with only approximately 7–30% of these cells staining positive by immunohistochemistry, depending on the status of the mammary gland (Petersen

et al. 1987, Ricketts *et al.* 1991, reviewed in Ronnov-Jessen *et al.* 1996). These levels fluctuate in conjunction with the cyclic changes in estrogen and progesterone during the menstrual cycle (Markopoulos *et al.* 1988). ER has not been detected in human mammary stroma by immunohistochemistry, although primary cultures of human breast fibroblasts have been reported to express this receptor (McCarty *et al.* 1986, Malet *et al.* 1991). Similar to ER, PR has not been detected in human mammary stroma (Press & Greene 1988). In the mouse mammary gland, ER has been detected in both epithelial and stromal cells, with only approximately 4–20% of these cells staining positive by immunohistochemistry, depending on the status of the mammary gland (Haslam & Nummy 1992). Initial PR localization studies suggested that this receptor was also expressed by both epithelial and stromal cells (Haslam & Shyamala 1981). Subsequent studies, however, revealed that PR is expressed only by epithelial cells, as is the case in human tissues (Silberstein *et al.* 1996, Shyamala *et al.* 1997). The distribution of ER and PR within the mammary gland, and the function of the receptors expressed by epithelial versus stromal cells are critical for determining how cells in the mammary gland communicate.

Steroid hormones have long been implicated in the transformation of the normal mammary gland to invasive carcinoma. Although ER and PR are expressed by a small number of cells in the normal human mammary gland, approximately two-thirds of breast cancers express ER, and half of these express both ER and PR; only one-third of breast cancers lack both ER and PR (McGuire 1978). ER- and PR-positive tumors tend to be more differentiated and are often responsive to hormonal therapies, and ER- and PR-negative tumors are generally associated with poor differentiation, and rarely respond to hormonal therapies. Even though the majority of steroid receptor-positive tumors initially respond to hormonal therapies, invariably they progress from hormone dependence to independence. The presence and concentration of these receptors, therefore, are useful markers of prognosis and survival, and can be used as clinical indices of the effectiveness of potential therapeutic intervention (McGuire & Clark 1992, Mansour *et al.* 1994).

In addition to being useful markers for the treatment and outcome of breast cancer, steroid hormone receptors also appear to play a role in tumor initiation and/or progression to malignancy. The role of ER in breast cancer development is based on the ability of estrogens to stimulate proliferation of mammary epithelial cells (Osborne *et al.* 1985, Katzenellenbogen *et al.* 1987). Since premalignant breast lesions are often characterized by intense epithelial cell proliferation, the presence of ER (and estrogen) could confer a selective growth advantage to these cells. The role of PR in breast cancer has not been as clear because it is an estrogen-induced product, and because progesterone modulates ER and estrogen-dependent gene expression (Chalbos & Galtier 1994, McDonnell & Goldman 1994, Kraus *et al.* 1995). Evidence now suggests, however, that PR may also play an important role in the development of breast cancer (Groshong *et al.* 1997). The proliferative index for mammary epithelial cells parallels that of progesterone levels, and progesterone also stimulates epithelial cell proliferation (Masters *et al.* 1977, Meyer 1977, Hissom & Moore 1987, Robinson & Jordan 1987).

Although estrogen and progesterone are key hormones in the regulation of normal and malignant mammary cell growth, there are many additional contributing factors that also regulate mammary cell behavior. For example, the peptide hormone, prolactin, is intimately involved in mammary gland development and differentiation (reviewed in Vonderhaar 1999); we will not discuss prolactin further in this review. Growth factors and their receptors also regulate mammary cell behavior. The expression of epidermal growth factor receptor (EGFR) varies throughout the menstrual cycle, and is inversely related to ER expression (van Agthoven *et al.* 1994, Gompel *et al.* 1996). From these and many other studies, it has become clear that steroid hormones and their receptors do not act independently, but function with multiple other

regulatory factors through a maze of interrelated intracellular signaling pathways. The combined effect of these events may have an impact on the tissue architecture, as will be discussed in the following sections.

Models to study the mammary gland

Mammary cells require normal cell–cell and cell–ECM interactions for the maintenance of structure and function, and these interactions change during the conversion to malignancy (Lochter *et al.* 1997, Sternlicht *et al.* 1999). Steroid hormone receptor expression levels also change during the conversion to malignancy. Therefore, studies of the effect of these changes on the transformation process should accommodate the need for cells to interact with other cells and with the ECM. The ability to study how normal and malignant cells interact with each other and with the ECM, and the effect of steroid hormones on these interactions have been facilitated by the availability of ECM-like biomatrices, human breast cell lines, and animal models.

Culture models to study normal breast and malignant tumors

As discussed in the previous section, certain features of normal mammary cell cytoarchitecture are lost in 2D cultures. To simulate the 3D condition of the normal mammary gland for culture experiments, several ECM-like biomatrices have been used, including collagen I matrices (rat tail collagen) (Emerman & Pitelka 1977, Hallowes *et al.* 1980, Yang *et al.* 1980), and reconstituted basement membrane (rBM; Englebreth-Holm-Swarm tumor or EHS; Matrigel) (Barcellos-Hoff *et al.* 1989, Petersen *et al.* 1992). Using these biomatrices, we asked whether normal human breast epithelial cells could respond and recapitulate certain aspects of their normal growth and differentiation program. Normal breast epithelial cells proliferate inside collagen matrices attached to a rigid surface, and form duct-like structures but do not differentiate (Hallowes *et al.* 1980, Taylor-Papadimitriou *et al.* 1980, Foster *et al.* 1983). In contrast, if grown in rBM (EHS; Matrigel), these cells form polarized ducts surrounded by an endogenous BM, with the size of these structures reaching the physiological size of TDLUs, thus simulating the structure of the normal mammary gland (Petersen *et al.* 1992). Armed with this culture system, we tested breast carcinoma cells to determine whether they react differently from normal cells to the 3D rBM microenvironment (Petersen *et al.* 1992). We found that these cells did not form duct-like structures and did not growth arrest, but instead formed large clusters of cells that were not polarized or surrounded by an organized BM. Since the phenotypes of normal and tumorigenic breast cells are apparent only in 3D cultures, we hypothesized that cell–ECM interactions are important for growth and differentiation, and that changes in these interactions might influence breast cancer initiation and/or progression. Therefore, we have recently combined the 3D rBM culture system with a breast cancer progression series (reviewed in Weaver *et al.* 1996) to create tools to study cell–ECM interactions during breast cancer progression.

When rodent or human breast epithelial cells are cultured, they rapidly lose their functional and morphological characteristics including all steroid hormone receptor expression, in spite of the development of advanced strategies for epithelial cell isolation and culture (Emerman & Pitelka 1977, reviewed in Ronnov-Jessen *et al.* 1996). Most culture studies of mammary function have depended on the availability of breast cancer cell lines and immortalized nonmalignant mammary epithelial cells.

There are at least 90 malignant human breast cancer cell lines originating from primary, recurrent, or metastatic carcinomas (e.g. MCF-7, MDA-231, MDA-435, T47D, BT-20), and at least 20 nonmalignant `normal' breast epithelial cell lines immortalized spontaneously, chemically, or virally which are available for study (reviewed in Ronnov-Jessen *et al.* 1996). Some of these cell lines have been extensively characterized, and have been valuable tools for

breast cancer studies. However, there are some limitations to the information obtained from cell lines. First, the nonmalignant cells are not exactly normal because they are immortal, and often have a number of genetic lesions. Secondly, functional comparisons between these unpaired nonmalignant and malignant cell types are limited by the possibility that the observed differences may be due to variability in cell type and not restricted to the conversion process from a normal to malignant phenotype. Thirdly, there is an enormous gap in our knowledge of the steps between normal breast epithelial cells and breast carcinoma, as discussed in the previous section. To address the second and third of these problems, several breast cancer progression models derived from single cell lineages have been developed (Stampfer & Bartley 1985, Clark *et al.* 1988, Band *et al.* 1990, Bartek *et al.* 1991, Shearer *et al.* 1992, Miller *et al.* 1993, Stampfer & Yaswen 1993, Wazer *et al.* 1994). We have chosen to use the human mammary epithelial cell (MEC) model, HMT-3522, for the reasons discussed below.

The HMT-3522 series was derived from a reduction mammoplasty of a woman with benign fibrocystic disease (Briand *et al.* 1987, 1996). This cell line (known as HMT-3522 S1) was established from a purified luminal epithelial cell population, and has been in culture for over 10 years (i.e. 500+ passages) without becoming tumorigenic. HMT-3522 S1 cell culture medium is completely defined and contains insulin, transferrin, epidermal growth factor (EGF), hydrocortisone, estradiol, prolactin, and sodium selenite (Briand *et al.* 1987). At passage 118, EGF was removed from HMT-3522 medium to test whether S1 cells could become EGF-independent and progress to malignancy. After 120 passages in the absence of EGF, the cells became tumorigenic; the cells explanted from one of these tumors were named HMT-3522 T4-2 (Briand *et al.* 1996). Nontumorigenic EGF-independent cells from an intermediate stage between HMT-3522 S1 and T4-2 were called S2.

During the past five years, we have been characterizing the cells of the HMT-3522 series in 2D and 3D cultures. In 2D monolayers on plastic, S1 and T4-2 cells display similar morphology, but in 3D rBM, they look very different, as predicted from our initial studies in 3D (Fig. 3; Petersen *et al.* 1992,Weaver *et al.* 1997). S1 cells form phenotypically normal structures reminiscent of TDLUs, with regular boundaries. In contrast, T4-2 cells form disorganized clusters, with irregular boundaries and dissemination into the EHS. The first objection raised above, i.e. that established cell lines are not genetically normal, applies also to HMT-3522. However, since the HMT-3522 series appears to be a useful model system, and early passages are phenotypically normal in 3D, we have used these cells to address the role of cell adhesion, growth factor signaling, and tissue architecture in normal and malignant breast (discussed in the following sections) (Weaver *et al.* 1996,Wang *et al.* 1998, reviewed in Bissell *et al.* 1999).

Animal and culture models to study steroid hormone receptors

Animal models are yet another powerful tool for studying the mammary gland. Although the system of implanting intact human breast tissue into athymic nude mice has been used to investigate the effect of steroid hormones on tumor growth for many years, the recent development of transgenic and knockout mouse models has allowed us more specifically to address molecular mechanisms of steroid hormone action (Fig. 3). For example, ERα (ERKO) and PR (PRKO) knockout mice provide definitive evidence that these hormones play an important role in mammary gland development (Lydon *et al.* 1995,Bocchinfuso & Korach 1997). ERKO mice develop mammary glands with only vestigial ducts present at the nipples, resembling the mammary glands found in wild-type (WT) mice before puberty (Bocchinfuso & Korach 1997). Unlike WT mice, however, there is no further development of the ERKO gland during adulthood, suggesting that estrogen signaling through ERα is critical for ductal morphogenesis. ERα is apparently not required for lobuloalveolar development, since it can be initiated in mammary glands of ERKO mice. These results contrast with those from the

recently generated ERβ (BERKO) knockout mice, in which mammary development was unaffected (Krege *et al.* 1998).

ERα is present in both the epithelial and stromal cell components of normal mice. Therefore, it was not clear whether the lack of mammary development in the ERKO mice was due to a lack of epithelial or stromal ER. This question was addressed by performing surgical recombinations of WT ER-positive epithelium and ERKO-negative stroma or ERKO-negative epithelium and WT ER-positive stroma (Cunha *et al.* 1997). Ductal morphogenesis was only observed when ER-positive stroma was combined with either ER-positive or ER-negative epithelium, supporting yet another role for stroma in mammary ductal proliferation. Stromal ER is necessary for estrogen-induced epithelial proliferation, but epithelial ER is neither necessary nor sufficient for this process. Thus paracrine mechanisms play a role in ductal morphogenesis in the mammary gland.

To exclude the possibility that the lack of mammary development in ERKO mice was due to downregulation of estrogen-inducible PR, ovariectomized WT and ERKO females were treated with estradiol and the mammary glands were analyzed for PR mRNA (Bocchinfuso & Korach 1997). PR mRNA was induced 3-fold by estradiol treatment in WT mice, but was not induced in ERKO mice, suggesting that loss of ER may result in loss of PR in the mammary gland. Further insights into the role of PR in the mammary gland have been gained from analysis of PRKO mice.

Unlike ERKO mice, PRKO mice did not initially exhibit changes in the mammary gland; PRKO mice have normal ductal morphogenesis (Lydon *et al.* 1995). However, when ovariectomized WT and PRKO mice were treated with exogenous estrogen and progesterone, a dramatic difference was observed. WT mice developed a fully differentiated mammary gland, the typical pregnancy-associated phenotype. PRKO mice, however, exhibited limited ductal branching and lobuloalveolar maturation, suggesting that PR has a role in both ductal branching and lobuloalveolar development. As mentioned above, PR is now known to be in the epithelial and not stromal mammary components of mice. Surgical recombination of WT PR-positive epithelium and PRKO-negative stroma indicated that lobuloalveolar morphogenesis was normal in these mice, confirming that epithelial PR is required for lobuloalveolar maturation (Humphreys *et al.* 1997).

In addition to PRKO mice, PR-A transgenic mice have been made to simulate an imbalanced ratio of PR-A to PR-B (Shyamala *et al.* 1998, reviewed in Shyamala *et al.* 1999). These mice exhibit aberrant mammary development, as characterized by extensive lateral branching and very thick ducts. The lateral branches resembled those found in an early pregnant female except that they terminated in bulbous structures, and the ducts were composed of multilayered luminal epithelial cells, in contrast to the single layer found in normal ducts. The morphology of these mice resembles that of mice transgenic for stromelysin-1, a matrix metalloproteinase (MMP) that destroys basement membrane and is usually expressed in the glands of involuting animals (Sympson *et al.* 1994, Witty *et al.* 1995, Thomasset *et al.* 1998, Sternlicht *et al.* 1999). In PR-A transgenic mice, disorganized masses of epithelial cells accompanied by an indistinct epithelial–stromal boundary were also seen at the tip of some ducts, suggesting there was a disruption of the basement membrane, and an alteration of MMP expression and/or activity. This observation is especially relevant to this review because it suggests that steroid hormone signaling also plays a role in regulating cell–cell and cell–ECM interactions (Simian *et al.* 2000).

In addition to the animal models discussed above, culture systems have also been utilized in studies of steroid hormone receptors, although these studies have been challenging because receptor expression is rapidly lost in normal mammary cells that are placed in culture. There

are only a few reports of the isolation of steroid hormone receptor expressing nonmalignant mammary epithelial cells. One group reported the isolation of epithelial cells expressing both ER and PR that were stimulated to proliferate by estradiol, although there was some question as to whether the cells were derived from normal epithelium or a fibrocystic lesion (Malet *et al.* 1991). Recently, another group has reported the successful culture of ER- and PR-positive human luminal epithelial cells (Yang *et al.* 2000). Cells obtained from reduction mammoplasty specimens were cultured inside 3D collagen matrices and immunostained for ER and PR, or cultured inside matrices then implanted in nude mice before immunostaining; both methods produced ER- and PR-positive cells.

Other attempts to produce receptor-positive cells have focused on transfection of immortalized human breast epithelial cells (Zajchowski *et al.* 1993, Lundholt *et al.* 1996, Pilat *et al.* 1996). For example, HMT-3522 S1 cells, which are ER-negative, were stably transfected with ER (ERα) to study the effect of estrogen on nonmalignant human breast epithelial cells (Lundholt *et al.* 1996). These ER-transfected cells were growth inhibited by both estrogen and antiestrogens, in agreement with results from another group (Zajchowski *et al.* 1993), suggesting that the introduction of ER into cells is not sufficient for restoring estrogen responsiveness. ER activity may depend on the availability of receptor interacting proteins and other regulatory factors, and on cellular context (reviewed in White & Parker 1998). For example, isolated mammary epithelial cells in culture do not grow in response to estrogen, but when epithelial and stromal cells are combined in co-cultures, they do respond (McGrath 1983, Haslam 1986). Our preliminary data indicate that, in addition to these cell–cell interactions, cell–ECM interactions may also be important for ER activity; we have found that the ERE is activated by ECM in a mouse epithelial cell line (C D Roskelley, V Novaro & M J Bissell, unpublished observations).

Integrating mammary gland communication

Connections between ECM and steroid hormones

Collectively, the studies utilizing ECM-like biomatrices, mammary cell lines, co-cultures, and animal models indicate that the relationships between epithelial cells, stroma, and ECM are important for tissue structure and steroid hormone responsiveness. We have demonstrated that the ECM surrounding epithelial and stromal cells is more than an acellular, insoluble proteinaceous mix of components; it coordinates diverse cellular behaviors via a combination of cell adhesion and growth factor connections between the different cells that make up the mammary gland (reviewed in Howlett & Bissell 1993). Therefore, the hormone responsiveness of mammary epithelial cells may be influenced by stromal cell production of soluble factors and/or specific ECM molecules.

Cell adhesion connections

Cell–cell and cell–ECM connections involve adhesion molecules, which when engaged, initiate cell adhesion-mediated signals that are transduced from the cell membrane to the nucleus. The bulk of studies of cell adhesion proteins have concentrated on integrins (reviewed in Hynes 1992) and cadherins (reviewed in Knudsen *et al.* 1998), although other molecules such as dystroglycan (reviewed in Durbeej *et al.* 1998, Henry & Campbell 1999) and CD44 (reviewed in Herrera-Gayol & Jothy 1999) are also important. Here we will briefly summarize integrin-related studies in the mammary gland.

The integrins are a major family of transmembrane receptors that connect the cell to the ECM on the outside, and anchor the cytoskeleton to the plasma membrane on the inside of cells (reviewed in Clark & Brugge 1995, Giancotti & Ruoslahti 1999). There are more than 20 integrin receptors, formed by heterodimerization between different α and β subunits. Normal

human breast epithelial cells express at least two β-integrins (β1 and β4) and four α-integrins (α1, α2, α3, α6), which dimerize to form α1β1, α2β1, α3β1, and α6β4 receptors (Glukhova *et al.* 1995, Alford & Taylor-Papadimitriou 1996). Mouse epithelial cells also express at least two β-integrins (β1 and β4), and possibly as many as 7 α-integrins (α1, α2, α3, α5, α6, α7, αv) (Delcommenne & Streuli 1995). These transmembrane receptors bind the ECM glycoproteins laminin and/or collagens, and associate with components of the cytoskeleton, forming an `extracellular–intracellular bridge' involved in the transmission of signals (Clark & Brugge 1995). The integrin bridge is a bi-directional conduit for the transfer of information, as signals can be transmitted from the outside to the inside of the cell and *vice versa* (O'Toole *et al.* 1991, Du *et al.* 1993, Dedhar & Hannigan 1996). Much of what we know about integrin-mediated signaling has been determined through analysis of biochemical events triggered by integrin engagement. These investigations indicate that the signaling pathways stimulated by integrins overlap with those identified for growth factors, suggesting a role for integrins in tumor progression.

Both quantitative and qualitative changes in integrin expression have been associated with breast cancer, and changes in β1-, β4-, α2-, α3-, and α6-integrins have all been reported in breast cancer cell lines and mammary tumor tissue sections (Natali *et al.* 1992, Berdichevsky *et al.* 1994, Gui *et al.* 1995, Zutter *et al.* 1995). These changes in expression may result in altered cell surface ratios of individual integrins, which could affect tissue organization and tumor progression via altered intracellular signaling (Schoenenberger *et al.* 1994, Sastry *et al.* 1996). For example, in normal breast epithelial cells, signaling via α6β4 plays a role in cell morphology and growth arrest, but in malignant cells, signaling through α6β4 is impaired (Spinardi *et al.* 1995). Instead, tumor cells express high levels of β1 integrin, which promotes growth and may contribute to the tumorigenic phenotype of these cells (Howlett *et al.* 1995, Weaver *et al.* 1997).

The significance of β1 integrin overexpression in the phenotype of breast cancer cells was examined in our laboratory using the HMT-3522 breast cancer progression model described in the previous section (Weaver *et al.* 1997). β1 integrin expression was higher in tumorigenic T4-2 cells than in nontumorigenic S1 cells, and its cell surface distribution was altered. In S1 cells, integrin distribution was restricted to the basal or basolateral surface, but in T4-2 cells, it was random and disorganized. To address the significance of increased β1 integrin signaling in the phenotype of these cells, inhibitory antibodies were used to attenuate β1 integrin function in T4-2 cells. When β1 signaling was reduced, the tumorigenic T4-2 cells reverted to morphologically normal structures (resembling S1 cells), stopped growing, produced a basement membrane, and reorganized their cytoskeleton, supporting the concept that the relative distribution and proportion of integrins helps maintain normal breast structure and function. We have also shown that the integration of downstream signaling events is important in this process (Weaver *et al.* 1997, Wang *et al.* 1998). These results have led us to hypothesize that not all tumor cells necessarily lose their ability to respond to ECM-generated signals, but instead may have alterations in the level of signaling that, if attenuated, could lead to normal behavior.

Although integrins themselves lack intrinsic tyrosine kinase activity, integrin–ECM interactions recruit a number of proteins involved in signal transduction, including focal adhesion kinase, mitogen-activated protein kinase, and Rho GTPases (Miyamoto *et al.* 1995, reviewed in Giancotti & Ruoslahti 1999). In addition, integrins appear to be linked to activation of growth factor receptors, such as EGFR, as discussed below.

Growth factor connections

Stromal cells secrete a variety of growth factors, many of which have been shown to interact with ECM in at least three ways (reviewed in Adams & Watt 1993, Streuli 1999). First, growth

factors bind the glycosaminoglycan side chains or the protein cores of specific ECM molecules. For example, platelet-derived growth factor-B binds heparin/heparan sulfate chains (LaRochelle *et al.* 1991), and transforming growth factor-β (TGFβ) binds the proteoglycan core proteins betaglycan and decorin (Andres *et al.* 1989, Yamaguchi *et al.* 1990). These binding interactions result in a local source of growth factors within the ECM that persists after growth factor production has ceased. Secondly, growth factors can induce production of ECM proteins, and ECM in return can affect growth factor production. TGFβ upregulates the expression of a variety of ECM components (Ignotz & Massague 1986), and ECM downregulates TGFβ expression (Streuli *et al.* 1993). Thirdly, cell–ECM and growth factor signaling pathways can converge or overlap. One growth factor family that is believed to play an important role in mammary gland proliferation and differentiation as well as breast cancer pathogenesis, and shares signaling components with integrins is EGF.

The EGF family consists of a subfamily of at least three neuregulins and a subfamily of at least six different growth factors, including EGF (reviewed in Normanno & Ciardiello 1997, Pinkas-Kramarski *et al.* 1997, Rosfjord & Dickson 1999). EGF family expression changes throughout mammary gland development, pregnancy, and lactation (Schroeder & Lee 1998, Sebastian *et al.* 1998). For example, expression of EGF is low in the virgin and pregnant mammary gland, but increases dramatically towards the end of pregnancy, peaking during lactation with high levels of EGF in milk, and decreases in the involuting mammary gland (Carpenter 1980, Beardmore & Richards 1983, Kenney & Dickson 1996). EGF mRNA has been detected in 83% of human breast cancer biopsy samples (Dotzlaw *et al.* 1990), and EGF protein has been detected by immunohistochemistry in 15–30% of human primary breast carcinomas (Mizukami *et al.* 1991, Umekita *et al.* 1992). EGF, as well as a number of other ligands, binds the EGFR, and EGFR forms homodimers or heterodimers with the ErbB2, ErbB3, and ErbB4 receptors (reviewed in Earp *et al.* 1995, Pinkas-Kramarski *et al.* 1997). Studies using EGFR knockout mice have shown that EGFR is essential for ductal growth and branching morphogenesis, but not lobuloalveolar development, in the mammary gland (Wiesen *et al.* 1999). The EGFR is overexpressed in approximately 14–93% of breast carcinomas (Koenders *et al.* 1992), and is associated with poor patient prognosis (reviewed in Fox & Harris 1997). Since the majority of breast cancers that overexpress growth factors also overexpress EGFR, signaling through this receptor may be important in breast tumor development and acquisition of hormone independence (Lundy *et al.* 1991, Umekita *et al.* 1992, reviewed in Normanno & Ciardiello 1997, Fox & Harris 1997). For example, overexpression of EGFR and the ErbB2 receptor are associated with progression to hormone independence in human breast cancer (Fitzpatrick *et al.* 1984, Sainsbury *et al.* 1985, Klijn *et al.* 1992), and both receptors are being explored as potential targets for cancer therapy.

Crosstalk between integrins and growth factor receptors

Integrins and growth factor receptors (i.e. EGFR) are interconnected in at least two ways (reviewed in Giancotti & Ruoslahti 1999). First, integrins are necessary for optimal activation of growth factor receptors. Although a systematic analysis has not been performed, certain integrins appear preferentially to associate with specific growth factor receptors. For example, β1 integrin appears to associate with EGFR in fibroblasts and they form a complex on the cell membrane (Miyamoto *et al.* 1996, Moro *et al.* 1998). The formation of integrin–growth factor receptor complexes enhances growth factor-dependent autophosphorylation and activation of downstream substrates. Secondly, there are reciprocal interactions between integrins and EGFR, and coupling of their signal transduction pathways. Using the HMT-3522 breast cancer progression model we examined crosstalk between β1 integrin and EGFR (Wang *et al.* 1998). Similar to β1 integrin, EGFR is expressed at a higher level in T4-2 cells. When either β1 or EGFR inhibitory antibodies were used to treat the cells, both β1 integrin and EGFR protein expression decreased, and the cells formed morphologically normal structures.

Additional experiments determined that these changes were mediated by bidirectional integrin–growth factor receptor crosstalk via the mitogen-activated protein kinase pathway.

Crosstalk between growth factors, integrins, steroid hormones, and ECM

In addition to crosstalk between growth factor receptors and integrins, there is evidence for crosstalk between growth factors and steroid hormones (reviewed in Nicholson *et al.* 1999). Estrogen and progesterone can regulate the synthesis of EGFR (Berthois *et al.* 1989, Haslam *et al.* 1992, Chrysogelos *et al.* 1994). Numerous studies have also shown that steroid hormone receptors can be activated by growth factors. For example, ER is phosphorylated and activated by EGF, and is a target for several growth factor-induced kinases, including mitogen-activated protein kinase, casein kinase II, and p60c-src (Ignar-Trowbridge *et al.* 1992, Ali *et al.* 1993, Arnold *et al.* 1994, Le Goff *et al.* 1994, Bunone *et al.* 1996, Arnold *et al.* 1997). Growth factors appear to function cooperatively with steroid hormones to regulate mammary gland structure and function. For example, EGF, estrogen, and progesterone regulate ductal sidebranching in the mature mammary gland (Haslam *et al.* 1993). Mammary epithelial cells may require cooperative interactions between growth factors and estrogen for estrogen-induced responsiveness, but may not require these interactions for progesterone-induced responsiveness (Imagawa *et al.* 1990, Xie & Haslam 1997). Thus steroid hormone receptors may function not only as direct transducers of hormonal effects, but also as key points for the convergence of multiple signal transduction pathways (Nicholson *et al.* 1999). Since integrins share some of the same signal transduction pathways with growth factors, we can hypothesize that there may also be crosstalk between integrins and steroid hormones.

As discussed in the previous section, changes in integrin expression may result in altered cell surface ratios of individual integrins, which could affect tissue organization and tumor progression via altered intracellular signaling (Schoenenberger *et al.* 1994, Sastry *et al.* 1996). Since α6 integrin can dimerize with β1 and β4 integrins, and α2 and α3 can also dimerize with β1, there is a competition for β1 binding by the α integrins. Generally in this competition, α2 is the preferred β1 partner over α3, which is preferred over α6 (Schoenenberger *et al.* 1994); however alterations in α2, α3, or α6 expression can shift this balance. In this respect, it is very interesting that the α2 gene promoter contains ER binding sites (EREs) (Zutter *et al.* 1994), and the α6 gene promoter contains PR binding sites (progesterone response elements (PREs)) (Nishida *et al.* 1997), suggesting that ER and PR may play a role in regulation of integrin expression. ER has been correlated with α2β1 expression in breast carcinomas; in 82 ductal carcinomas of no special type, lack of ER corresponded to low or absent α2β1 expression (Lanzafame *et al.* 1996). α2β1 does not appear to be a major ECM receptor in ER-negative breast cancer cell lines, but it does function in ER-positive cell lines (Maemura *et al.* 1995).

Finally, there is a connection between growth factors, integrins, steroid hormones/steroid hormone receptors and ECM. ECM composition can be altered by a large number of proteolytic enzymes, including tissue serine proteases and the large family of matrix metalloproteinases (MMPs), in a process called ECM remodeling (reviewed in Schroen & Brinckerhoff 1996, Chambers & Matrisian 1997, Benaud *et al.* 1998, Lochter *et al.* 1998). In the normal mammary gland, MMPs cooperate with hormonal stimuli to induce some of the morphological and functional changes required at various stages, and in breast cancer, MMPs have been associated with tumor growth, invasion, metastasis, and angiogenesis (reviewed in Chambers & Matrisian 1997, Benaud *et al.* 1998, Lochter *et al.* 1998). In general, the number of different MMP family members expressed and the relative expression level of individual MMP family members tends to increase with tumor progression (Benaud *et al.* 1998). MMP regulation provides another link between growth factors, integrins, and steroid hormones/steroid hormone receptors. MMP expression can be regulated by growth factors, such as EGF. In response to EGF, the transcription factors Ets and AP-1 cooperate to stimulate transcription of matrilysin and

stromelysins (Wasylyk *et al.* 1991, Gaire *et al.* 1994). MMP expression can also be regulated by integrins. For example, α2β1 integrin has been implicated in MMP-1 production, and α3β1 integrin is associated with MMP-2 production (Langholz *et al.* 1995, Kubota *et al.* 1997, Sugiura & Berditchevski 1999). Finally, MMP expression may be regulated by steroid hormones. Progesterone inhibits MMP-1, -2, and -9 expression (Marbaix *et al.* 1992), although, in a similar manner to growth factors, the effect of steroid hormones on MMP expression may be indirect, through regulation of AP-1 binding (Schroen & Brinckerhoff 1996). Finally, as discussed above, PR overexpression affects mammary gland organization by disrupting basement membrane organization, possibly through altering MMP expression and/or activity (Shyamala *et al.* 1998, Simian *et al.* 2000).

Conclusions

Years of investigations have established that the normal structure and function of the mammary gland depends on the reciprocal exchange of information between the different types of mammary cells and between these cells and the ECM. The studies reviewed in this article suggest that integrin-, growth factor-, and steroid hormone-signaling pathways play an important part in maintaining the structure and function of the mammary gland (Fig. 4); disruption of the delicate balance of signaling results in dramatic changes in the way cells interact with each other and with the ECM. Therefore, the integrity of the mammary gland depends on the correct integration of signaling pathways. While we do not yet know how these signals are integrated, it is clear that the ECM, its receptors, and ECM-degrading enzymes play essential roles in this process.

We have previously hypothesized that the unit of function in higher organisms is not the individual cell, but the tissue itself (Bissell & Hall 1987). Tissue architecture appears to be intimately involved in controlling the signaling processes within cells, and although we are just beginning to investigate how tissue morphology and signaling are connected, our current information supports this hypothesis. Future studies will provide a better understanding of tissue architecture, and they may reveal new concepts of tissue function. Experimental approaches aimed at determining the mechanisms underlying structure and function in the normal mammary gland and the changes that occur in the malignant mammary gland may provide the basis for a revolutionary approach to the prevention and treatment of breast cancer.

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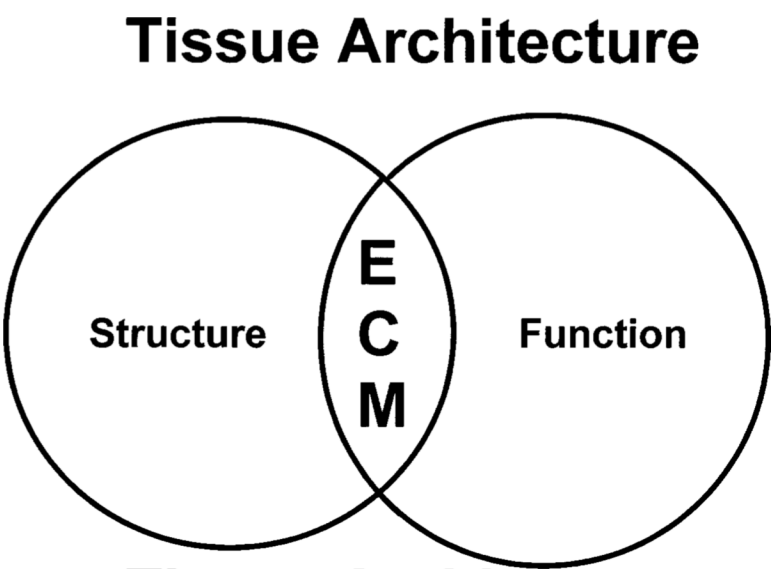

Figure 1.
Integration of structure and function by the ECM. The ECM, an essential component of the mammary gland, is intimately involved in processes that determine tissue architecture (reviewed in Bissell *et al.* 1999).

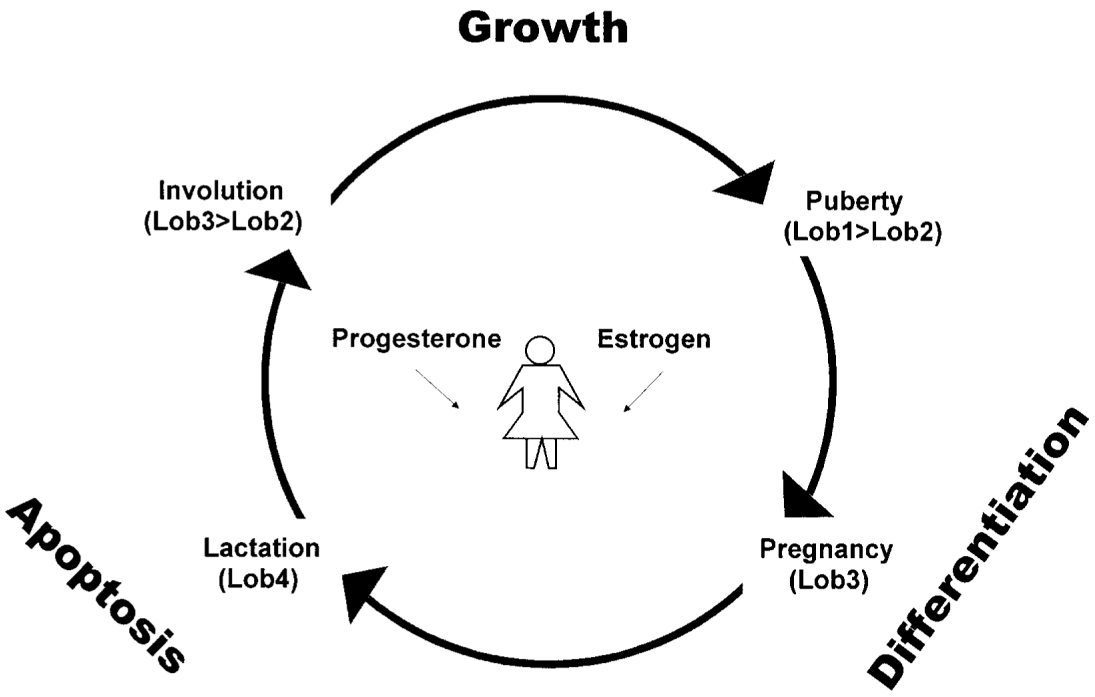

Figure 2.
The structure of the mammary gland is dependent on the developmental stage. The adult female mammary gland experiences recurrent cycles of regulated growth, differentiation, and apoptosis, and estrogen and progesterone play a central role in this process. The cycles that occur in the mammary gland can be divided into several stages: puberty, pregnancy, lactation, and involution. Each stage can be further described by the structure of the gland, called lobules or Lobs (reviewed in Russo & Russo 1998).

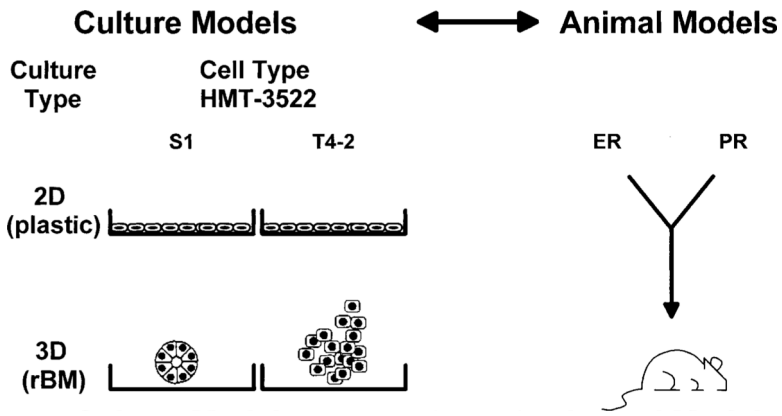

Figure 3.

Models to study the role of ECM and steroid hormones. Culture and animal models provide complementary systems for the study of breast structure and function. Cells from the HMT-3522 human breast cancer series exhibit similar phenotypes in 2D cultures on plastic, but exhibit very different phenotypes in 3D cultures in rBM. S1 cells form phenotypically normal structures reminiscent of terminal ductal lobular units, with regular boundaries, and T4-2 cells form disorganized clusters with irregular boundaries and dissemination into the EHS (reviewed in Bissell *et al.* 1999). Although mouse mammary tissue varies somewhat with respect to the overall mammary gland organization, it is now clear that there are many similarities. As such, the mouse has been a valuable model for studying the effects of ER and PR function.

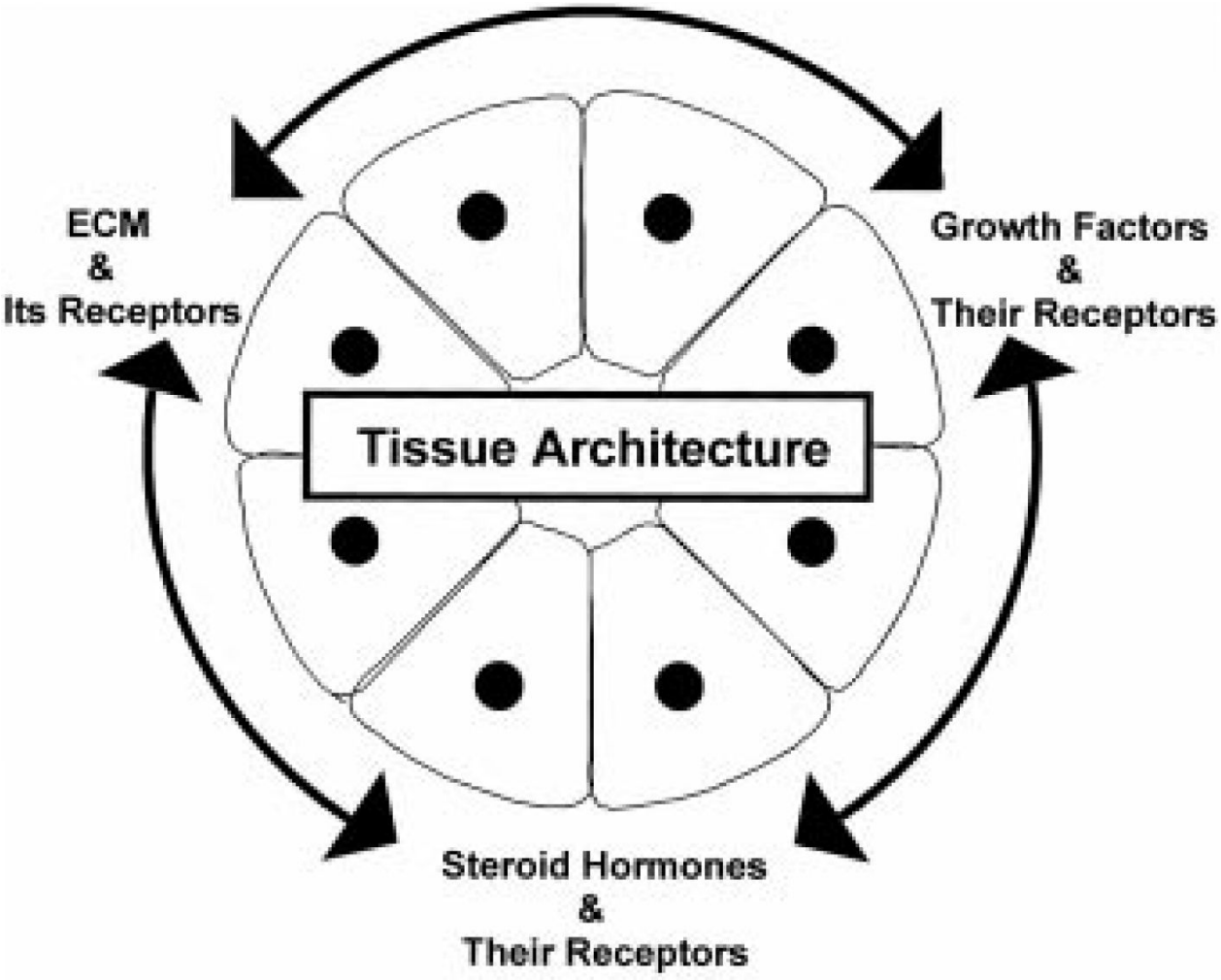

Figure 4.
Tissue architecture integrates, and in turn depends on, the integration of signals from ECM and its receptors, growth factors and their receptors, and steroid hormones and their receptors.