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Glycoprotein Contributions to Mammary Gland and Mammary Tumor Structure and Function: Roles of Adherens Junctions, ErbBs and Membrane MUCs

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Abstract Mammary function is dependent on its three-dimensional organization, which is established and maintained by cell adhesive junctions linked through the membrane to the cell cytoskeleton. These junctions serve not only as structural elements, but also function as initiators and integrators of cell signals. In this review we discuss three types of glycoproteins whose interactions impinge on the function of mammary cell–cell junctions, cadherins, ErbB receptor tyrosine kinases and membrane mucins, as a microcosm of events regulating mammary cell behaviors. Actions of these components are integrated by the critical signaling element β-catenin. When functioning properly, these glycoproteins, β-catenin and associated signaling pathways mesh into a highly structured program for development and function of the gland. However, disruption or dysfunction of these glycoproteins or the signaling elements can lead to disorganization of the epithelia and ultimately to neoplasia. J. Cell. Biochem. 96: 914–926, 2005.

Key words: mammary; adherens junctions; cadherin; β-catenin; ErbB; Muc4; MUC1

The mammary gland was considered so important by Linnaeus in his classification of species that it was chosen as the basis for defining mammals as a taxonomic group [Oftedal, 2002]. The primary function of the gland, of course, is to provide nutrition for the often fragile mammalian young. That function derives from a developmental patterning program, which is unusual in that most of it occurs in the adult animal [Medina, 1996], creating a branching system of ducts feeding product from alveoli at the branch ends to a nipple (Fig. 1A,B). This branching structure is surrounded by stroma consisting of fat cells and/or condensed mesenchymal elements, depending on the species. In the adult female the mammary gland, again depending on the species, may undergo multiple cycles of growth, lactation, and involution in response to pregnancy and weaning. In preparation for lactation, alveologenesis expands the number of alveoli to fill the stromal space (Fig. 1B). The signals driving these proliferative and regressive processes during the lactation cycle come from both the blood and the stroma. Thus, the functional cells of the mammary gland may be in a quiescent state through much of the animal's life, but must be organized in a way to receive, process, and integrate multiple signals in response to pregnancy. The thesis behind this review is that cell surface glycoproteins play critical roles in the cellular organization and responses of the gland that determine its functional states. This organization is necessary for integrating signals that control development and function of the gland and depends on the interactions between adhesion complexes regulating structure and signaling complexes regulating function. Given the extent and complexity of this subject, this review will seek to "teach by example," focusing particularly on three interacting classes of these glycoproteins, the adherens junction cadherins and their

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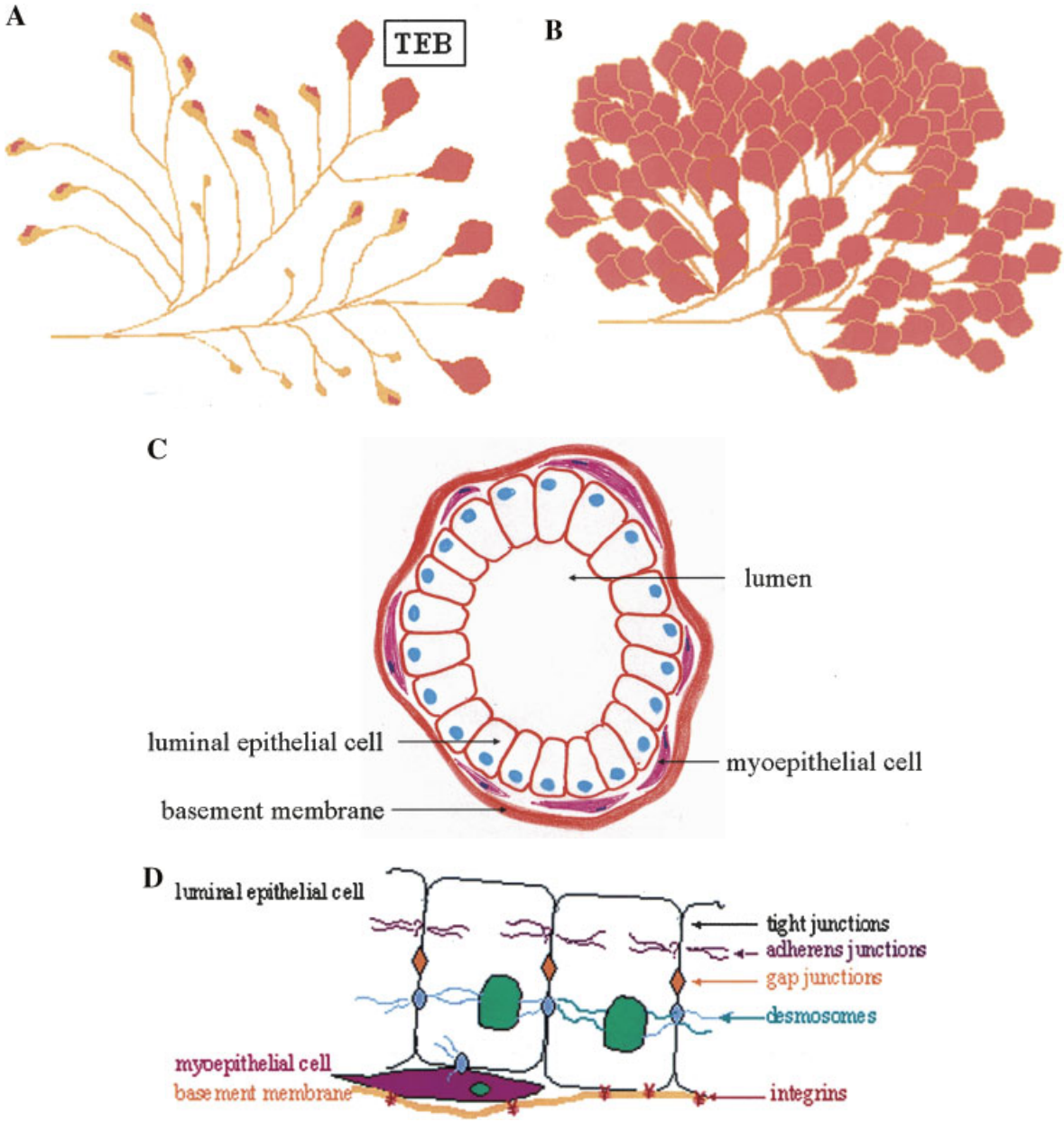

Fig. 1. Models for mammary gland and mammary epithelial cell structure. **A**: Ductal structure of developing gland with branching structure and terminal end buds (TEB) surrounded by stroma. **B**: Mammary gland during lactation, exhibiting alveoli filling the stromal space. **C**: Cross section of alveolus with surrounding luminal epithelial cells encompassed by loose myoepithelial layer on basement membrane. **D**: Model showing adhesion junctions of alveolar cells. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

complexes, the ErbB family of receptor tyrosine kinases, and the membrane mucins.

ADHESION COMPLEXES AND MAMMARY EPITHELIAL CELL POLARITY

The secretory apparatus of the mammary gland is the alveolus, which consists of luminal epithelial cells around a central lumen at the ends of the ducts (Fig. 1B,C). Ductal luminal cells are surrounded by a thick insulating layer of myoepithelial cells whereas the alveolar lumenal cells are encircled by in a loose basket-weave structure of myoepithelial cell processes. These myoepithelial cells are situated on a basal lamina surrounding the acini and the ducts. The luminal acinar cells are in most respects typical polarized epithelial cells; however, because of the spaces between the myoepithelial cells, their basal surfaces may contact either the surrounding

TABLE I. Transmembrane Glycoproteins of Mammary Epithelial Cell Adhesion Complexes

Junction type	Principal glycoprotein(s)	Cellular location	Associated cytoskeleton
Adherens junctions	Cadherin	Lateral	MF
Desmosomes	Desmocollin, desmoglein	Basal, lateral	IF
Tight junctions	Claudin, occludin, JAM, CAR	Apico-lateral	MF
Gap junctions	Connexin	Lateral	
Hemidesmosomes	α6β4-integrin	Basal	IF
Focal adhesions	Integrin	Basal	MF
Proteoglycan adhesions	Dystroglycan	Basal	MF

MF, microfilaments; IF, intermediate filaments; JAM, junctional adhesion molecule; CAR, coxsackie virus and adenovirus receptor.

myoepithelial cells or the basal lamina (Fig. 1C). As with any polarized epithelium, the maintenance of the polarized structure of the mammary epithelial cells is critical to function. Key elements in maintaining that structure are the transmembrane glycoproteins of adhesion complexes that establish cell contacts, either with extracellular matrix (ECM) or with other cells. Table I lists the major adhesion complexes associated with the secretory mammary epithelial cell.

Traditionally, these epithelial adhesion complexes have been considered to be structural elements involved in stabilizing the cell layer and providing the permeability barrier between the epithelial lumen and the underlying stroma. More recently, it has become obvious that these complexes play dynamic roles in regulating cellular signaling and, particularly, in integrating signals that impinge on epithelial cell surfaces from exterior sources. Both cell–matrix and cell–cell junctions are essential to proper epithelial cell function. As indicated in Table I, cell–matrix adhesions are present at the basal surfaces of the epithelial cells and organized around integrins as transmembrane components. These integrin complexes are linked to the cytoskeleton at the cytoplasmic face of the membrane. Since cell–cell interaction complexes are also similarly linked, the cytoskeleton provides one means for integrating signals that impinge on the cell from different locations with cell structure. Such integration is particularly important in polarized cells, such as those of the mammary epithelium, in which extracellular inputs can modulate cell behaviors from independent compartments. For example, the luminal and stromal compartment of alveoli are not only separate, but also independent, and capable of delivering independent signals. Thus, cell polarity is a critical feature of mammary epithelial cells. Studies on the development of polarity of epithelial cells indicate that integrin-mediated cell–matrix interactions are sufficient to establish a polarized phenotype, and that determination of the apico-basal orientation occurs with development of cadherin junctions (see Table I) [Yeaman et al., 1999]. However, in the mammary gland, as in most simple epithelia, the polarized epithelial cell derives during development from a more complex multi-layered epithelium (terminal end bud in the case of the mammary gland, Fig. 1A) through a differentiation process. Recent studies on cell culture models suggest that myoepithelial cells, desmosomes and laminin 1 have important roles in this process [Bissell et al., 2002]. Regardless of the mechanism for establishing polarity, both the secretory and ductal epithelial cells of the mammary gland have multiple adhesion complexes centered around transmembrane glycoproteins important to maintaining the structure and functions of these cells. In this review, we will focus on the cell–cell interactions based on cadherins.

CELL–CELL INTERACTIONS, CADHERIN, AND β-CATENIN

Table I lists the four primary types of cell–cell junctions of mammary epithelial cells, three of which form links between the cell junction and the cellular cytoskeletons. These are illustrated in Figure 1D. Tight junctions in mammalian cells act as a seal between cells to form a barrier between the external (lumenal) and internal (stromal) space in the organism, thus helping to maintain homeostasis. They also form the membrane barriers which maintain the apical and basolateral domains as separate membrane compartments [D'atri and Citi, 2002]. In contrast, gap junctions provide channels for communication between cells in an epithelium. Desmosomes form extensive links connecting the surfaces of adjacent cells to stabilize their

interactions. This review will focus on the adherens junctions because of their essential contributions both to tissue structure and cell signaling.

The central element in the adherens junction is cadherin, a type 1 transmembrane glycoprotein containing five extracellular repeats [Gooding et al., 2004]. Epithelial cadherin (E-cadherin) forms two types of homophilic interactions, a *cis* interaction between molecules in the same membrane and a *trans* interaction that forms the bridges between cells. As noted in Table I, one function of these junctional complexes is to link cell surface interactions to the cytoplasmic cytoskeleton, microfilaments in the case of the cadherin junctions. This linkage involves a chain of catenins. β-catenin is associated with the cytoplasmic domain of cadherin, and α-catenin bridges the β-catenin and microfilaments. This catenin chain and microfilament linkage contribute to the stability of the cadherin junction [Beavon, 2000]. Interestingly, assembly of the cadherin–catenin complex occurs in a stepwise fashion at two different locations. Cadherin and β-catenin associate on the endoplasmic reticulum, soon after the two components are synthesized [Ozawa and Kemler, 1992]. α-Catenin is added to the complex later, probably only after the complex has reached the plasma membrane. Two additional catenins can also participate in these complexes [Beavon, 2000]. γ-Catenin (plakoglobin) is an analog of β-catenin, which can bind to cadherin at its β-catenin-binding site and replace the β-catenin in some contexts. In contrast, p120-catenin binds separately to the juxtamembrane region of cadherins and has been implicated in signaling from the complex and recently in cadherin turnover [Reynolds and Carnahan, 2004].

Stability of the cadherin–catenin complex is regulated by tyrosine phosphorylation [Gumbiner, 2000]. Immunolocalization studies have shown that phosphotyrosine is concentrated at adherens junctions in polarized epithelial cells, as are some receptor tyrosine kinases and members of the Src family of tyrosine kinases. Moreover, introduction of active Src into such cells leads to breakdown of the cell–cell interactions. Both β-catenin and p120-catenin have been shown to be phosphorylated; phosphorylation of β-catenin Tyr-654 and Tyr-142 have been implicated in regulating its association with cadherin [Roura et al., 1999] and α-catenin [Piedra et al., 2003], respectively. Thus, there appear to be multiple mechanisms by which phosphorylation can lead to junction breakdown, which may include effects on cadherin turnover as well as effects on cadherin–catenin complex stability.

Regardless of the mechanisms by which cadherin–catenin complexes are regulated, it is clear that they have important roles in epithelial homeostasis, such as contact inhibition of cell proliferation. Contact inhibition, sometimes called density dependent inhibition, represses cell proliferation when cadherin junctions are formed. Several mechanisms have been suggested to explain contact inhibition, including sequestration of the EGF receptor to cadherin junctions [Takahashi and Suzuki, 1996], recruitment and activation of phosphoinositol-3-kinase [Pang et al., 2005] and inhibition of Rac signaling through the membrane-cytoskeleton linker Merlin [Jaffer and Chernoff, 2004]. These are not necessarily mutually exclusive. Undoubtedly, cell context plays a role in determining which of these are operative.

In addition to its role in cell–cell adhesion complexes with cadherin, β-catenin acts as a transcription factor, in association with the TCF/Lef DNA binding protein. Distinct molecular forms of β-catenin, regulated by Wnt signaling, appear to be associated with cadherin and TCF binding [Gottardi and Gumbiner, 2004]. As a transcription factor, β-catenin participates in the activation of cell proliferation events required for developmental processes or in neoplasia. Thus, the levels of β-catenin must be tightly regulated to prevent excessive cell proliferation and hyperplasia. This regulation is accomplished in the cytoplasm by the formation of a complex with axin, APC, and the protein kinases CK1α and GSK3β [Hatsell et al., 2003]. Phosphorylation of the β-catenin by these kinases targets it for ubiquitinylation and proteosomal degradation. The degradation process is regulated by signaling pathways that inhibit β-catenin phosphorylation, including those involving the FGF2, PKB/Akt, and Wnt pathways that are important in mammary development and implicated in breast cancer. Thus, β-catenin has the potential to integrate information from multiple signaling pathways that contribute to mammary biology.

β-Catenin is involved in multiple stages of mammary development [Hatsell et al., 2003],

many of which are responsive to control of β-catenin signaling by the Wnt pathway. Most of the evidence for the roles of β-catenin comes from studies on transgenic mice. For example, animals lacking the β-catenin transcription partner Lef-1 arrest in mammary development at the early end bud stage at E13 and fail to form mammary glands. Similarly, mice expressing dickkopf, an inhibitor of the Wnt signaling pathway that regulates β-catenin expression, also repress mammary gland formation at several stages. Most importantly, animals expressing the activated form of β-catenin display evidence of early alveologenesis and develop mammary adenocarcinomas with multiple pregnancies. These phenotypes are associated with upregulation of the β-catenin target genes cyclin D1 and c-myc. A role for endogenous beta-catenin in alveologenesis has been corroborated by findings that expression of the beta-catenin inhibitor axin or its transcriptional suppressor, beta-engrailed, result in impaired alveologenesis and alveolar apoptosis, respectively. These studies all provide a case for β-catenin as an integrative factor in mammary development, which functions both as a transcription factor in driving mammary cell proliferation and as an essential component of the cell surface adhesive complex, stabilizing the epithelium and repressing proliferation through contact inhibition. The balance of these activities is regulated by complex mechanisms for regulating the level of cellular β-catenin, including its association with adherens junction cadherin and its degradation by a proteosomal mechanism responsive to Wnt signaling [Hatsell et al., 2003].

ERBB RECEPTORS AND LIGANDS

The discovery of the overexpression of ErbB2/HER2/Neu in about 25% of breast cancers has focused attention on the ErbB family of receptor tyrosine kinases as a significant contributor to tumor progression [Yarden and Sliwkowski, 2001]. However, the implication of the ErbBs and their ligands in epithelial proliferation and differentiation, including in the mammary gland and its tumors, has an even longer history. The prototypic ErbB, the epidermal growth factor receptor, EGFR or ErbB1, was discovered in a search for a receptor for the growth factor EGF [Carpenter, 1983]. Analyses of its mechanism of action led to the discovery that the ligand EGF induced phosphorylation of specific tyrosines in the cytoplasmic tail of the receptor via a homodimerization (and/or homomultimerization) of the receptor [Ullrich and Schlessinger, 1990]. The phosphorylated tyrosines then recruited cytoplasmic factors which initiated cellular signaling pathways. The discovery of three additional ErbB family members (ErbB2, ErbB3, ErbB4) created a conundrum. In spite of extensive searches, no soluble high affinity ligand has been found for ErbB2, and ErbB3 has amino acid changes (compared to ErbB1) in its active site region which render it catalytically silent [Carraway and Cantley, 1994]. Thus, only ErbB1 and ErbB4 can be activated by homodimerization. The resolution to the conundrum was provided by the discovery that all of the ErbB receptors can be activated by a heterodimerization mechanism, pairing different receptors. In fact, ErbB2 is the preferred partner for the other receptors in many physiological contexts; the ErbB2/ErbB3 heterodimer is the most active of all in promoting cell growth [Stern, 2003].

Approximately a dozen ligands have been identified for the ErbBs, about half of which bind ErbB1 and one-third bind both ErbB1 and ErbB4 [Yarden and Sliwkowski, 2001). The neuregulins comprise a large family of ErbB3 and ErbB4 ligands [Carraway and Cantley, 1994]. In tissues such as the mammary gland, the key to the actions of these ligands and receptors is their locations. Obviously, the ligand must be able to associate with the receptor for the activation process. Thus, the sites of production of the ligand and receptor are critical. Moreover, specific sequestration and release of ligand provides a powerful mechanism for initiating cellular responses. Importantly, the barriers created by polarized epithelia can separate ligands and receptors in normal tissues, but not in damaged or neoplastic tissues [Ramsauer et al., 2003]. Thus, understanding the roles of ErbBs and their ligands in the mammary gland requires knowledge of the biosynthesis and locations of these molecules. Unfortunately, research in this area has been confounded with technical problems associated with protein localization and development of appropriate models [Stern, 2003]. Recent improvements in localization methods, better antibodies and the extensive use of transgenic animals promise more reliable results in the future.

As the founding member of the EGF family, a role for EGF and its receptor in mitogenesis of the mammary gland was appreciated early [Stern, 2003]. Estrogen, a prime mediator of mammary development, induces production of EGFR agonists, which stimulate tyrosine phosphorylation of ErbB1 and ErbB2. Thus, ErbB1 and ErbB2 have been implicated in ductal proliferation in the mammary gland, a critical aspect of mammary development during puberty and pregnancy (Fig. 1A). The specific roles of these ErbBs in mammary development and function still need to be defined. Analyses of the expression and phosphorylation of the ErbBs, though complicated by some inconsistencies, suggest that ErbB1 and ErbB2 act at puberty, late pregnancy and lactation, while ErbB3 and ErbB4 act in pregnancy and lactation.

Most of our understanding of the roles of ErbBs has come from studies of transgenic animals in which the genes for the ErbBs in the mammary gland have been inactivated or the receptor functions repressed by dominant negative analogs. Such studies have indicated that neither ErbB1 nor ErbB2 is required for embryonic mammary development [Stern, 2003]. ErbB1 is involved in pubertal ductal morphogenesis; tissue transplantation/recombinants indicate that the active ErbB1 is present in stromal cells rather than the epithelium. ErbB2 has also been implicated in ductal morphogenesis, possibly in combination with ErbB3 or ErbB4, since their ligand neuregulin appears to act at this stage. The EGFR ligand amphiregulin is a potent inducer of ductal growth [Kenney et al., 1996]. One possible scenario is that amphiregulin activates EGFR in stromal cells to produce factors that then stimulate growth from the terminal end bud cap cells responsible for duct elongation.

Pregnancy leads to ductal side-branching and ultimately alveologenesis of the mammary gland (Fig. 1B), with increased expression of ErbB3 and ErbB4 and tyrosine phosphorylation of all of the receptors. ErbB4 appears to be particularly important in pregnancy and lactation, based on results from knockout and dominant negative transgenic animals [Stern, 2003]. This conclusion is supported by studies on the ErbB ligands. Neuregulin-α, an ErbB4 ligand, has been implicated in alveologenesis and the early phase of lactation. Heparin-binding EGF, a ligand for both ErbB1 and ErbB4, may be important for late phase lactation. Interestingly, HB-EGF is produced "on demand" by cleavage from a cell surface precursor by a matrix metalloprotease matrilysin [Yu et al., 2002]. It can also be sequestered by binding to the ECM, thus providing a potential mechanism for "activation by release." Other ErbB ligands, including EGF, are produced during pregnancy and lactation. EGF is abundant in milk, raising the question of whether it is active in the mammary gland of the mother or whether it functions in the gut of the neonate. Clearly, we have much to learn about the roles of the ErbBs and their ligands in mammary development and function and, particularly, about their mechanisms of action.

Much of the attention on ErbBs has been focused on breast cancer. Though such studies have contributed enormously to our understanding of ErbB functions and mechanisms of action, they can also be confusing because of the complexities introduced by the great heterogeneity of tumors. Whereas normal cells have very strictly regulated and defined signaling pathways that act in a paracrine fashion to convey and confer morphogenic information, tumor cells, because of their genetic plasticity, seem to have an almost infinite variability. Studies on tumor cell lines suggest that all combinations of ErbB receptors are possible. Moreover, many tumor cells also produce ErbB ligands that act in an autocrine fashion. Given this complexity, the question of importance is how the ErbBs actually contribute to human breast cancer etiology and progression in vivo, as opposed to how they affect isolated aberrant cell lines. The best answers to this question have come from two types of models: transgenic animals [Hutchinson and Muller, 2000) and 3-dimensional cultures of mammary epithelial cells [Bissell et al., 2002; Shaw et al., 2004].

Both ErbB1 and ErbB2 have been implicated in breast cancer through transgenic animals. Mammary expression of the ErbB1 ligand TGFα induces mammary adenocarcinomas [Muller, 2003]. Mammary expression of the ErbB3 ligand neuregulin or of ErbB2 does likewise. When an activated form of ErbB2 with a mutation in its transmembrane domain is expressed, the latency period is short compared to that observed with unactivated c-ErbB2. Interestingly, most of these latter tumors have acquired mutations in their juxtamembrane domains which lead to covalent, disulfide-linked ErbB2 dimers, suggesting that expression of wild type-nonmutated

ErbB2 is insufficient to drive tumor progression. Bitransgenic animals expressing both TGFα and ErbB2 in mammary glands developed tumors faster than single transgenic animals of either, suggesting a direct or indirect synergistic contribution from ErbB1–ErbB2 interactions. ErbB2 transgenic strains also exhibited increased ErbB3 expression. Since the ErbB2–ErbB3 heterodimer is the most potent activator of cell growth, these results suggest that it may be a robust contributor to tumor progression.

The role of ErbB2 in tumor formation has also been investigated in 3-dimensional cultures of MCF-10A cells [Muthuswamy et al., 2001]. These cells form acinar-like structures when cultured in Matrigel, a laminin-rich ECM material similar to the basement membrane. Chimeric derivatives of ErbB1 and ErbB2 that could be activated by specific crosslinking agents were expressed in the epithelial cells. Activation of ErbB2, but not ErbB1, induced disruption of cell–cell junctions, cell proliferation and cell accumulation in the acinar lumens [Muthuswamy et al., 2001]. Thus, the cell culture models mimic carcinoma in situ of breast cancer. These studies suggest that cell–cell junction disruption is one of the early events in cancer development. Why ErbB2, and not ErbB1, activation promotes such transformations is unclear. Both are primarily located in the lateral surfaces of these epithelial cells and both have been proposed to directly associate with β-catenin [Hoschuetzky et al., 1994; Kanai et al., 1995], which plays a critical role in integrating cell signaling in mammary cells. Thus, it is important to understand how the interactions and activities of these receptors are regulated in polarized epithelial cells.

MUC4 AS A MODULATOR OF ERBB2

ErbB2 is recognized as an important contributor to some types of breast cancer. Thus, there is interest in understanding how it is regulated in normal mammary gland and how that regulation is disrupted in oncogenesis. As mentioned above, no soluble, high affinity ligand has been described for ErbB2. However, the membrane mucin Muc4 has been shown to act as an intramembrane ligand for ErbB2, which can regulate both its localization and signaling [Carraway et al., 2002]. Thus, Muc4 acts as an unorthodox ligand and chaperone for ErbB2.

Muc4 was originally discovered as a cell surface, heterodimeric glycoprotein in highly metastatic rat mammary adenocarcinoma cells [Sherblom and Carraway, 1980]. Subsequently, the human analogue was cloned from a human tracheal cDNA library [Porchet et al., 1991]. Most functional and mechanistic studies have been done on rat Muc4. Human MUC4 contains two types of mucin repeat domains; the rat contains only one. Thus, the human mucin is 2–3 times larger than the rat. The salient features of the Muc4 are its mucin subunit, called ASGP-1 in the rat and MUC4α in the human, its transmembrane domain and two EGF-like domains (Fig. 2A) [Sheng et al., 1992]. One of those EGF-like domains, EGF1, the most N-terminal (Fig. 2A), is involved in specific complex formation between Muc4 and ErbB2.

Two important functions have been attributed to Muc4, anti-adhesion and modulation of ErbB2 signaling. The former is clearly resident in the mucin subunit, whose large size (potentially >1 μm for the human MUC4) and rigid structure provide an imposing steric barrier as part of the glycocalyx at the apical surfaces of epithelial cells [McNeer et al., 1998]. A soluble form of Muc4 is produced by intracellular proteolysis [Komatsu et al., 2002] and may also contribute to the glycocalyx and surface barrier as a loosely bound component [McNeer et al., 1998]. Interestingly, soluble Muc4 is copiously secreted into milk [Rossi et al., 1996], where it may function in the protection of the intestine of the neonate, though this role has not been investigated. The dark side of anti-adhesive Muc4 is that the barrier function can also protect tumor cells from killing by immune cells when Muc4 is overexpressed by the tumor [Komatsu et al., 1999]. Anti-adhesive release of epithelial or epithelial-like carcinoma cells from their attachments probably also contributes to tumor progression. This release should induce anoikis (apoptosis). However, Muc4 can also act as an anti-apoptotic, allowing tumor cells to escape their adhesive interactions without incurring cell death [Komatsu et al., 2001], further contributing to tumor progression.

Muc4 plays a dual role in ErbB2 signaling, as an unorthodox chaperone and ligand, regulating both localization and phosphorylation, respectively. The Muc4–ErbB2 complex is formed very early after synthesis of the two proteins, well before either transits to the cell surface. In polarized epithelial cells, this complex localizes to the apical surface, in contrast to

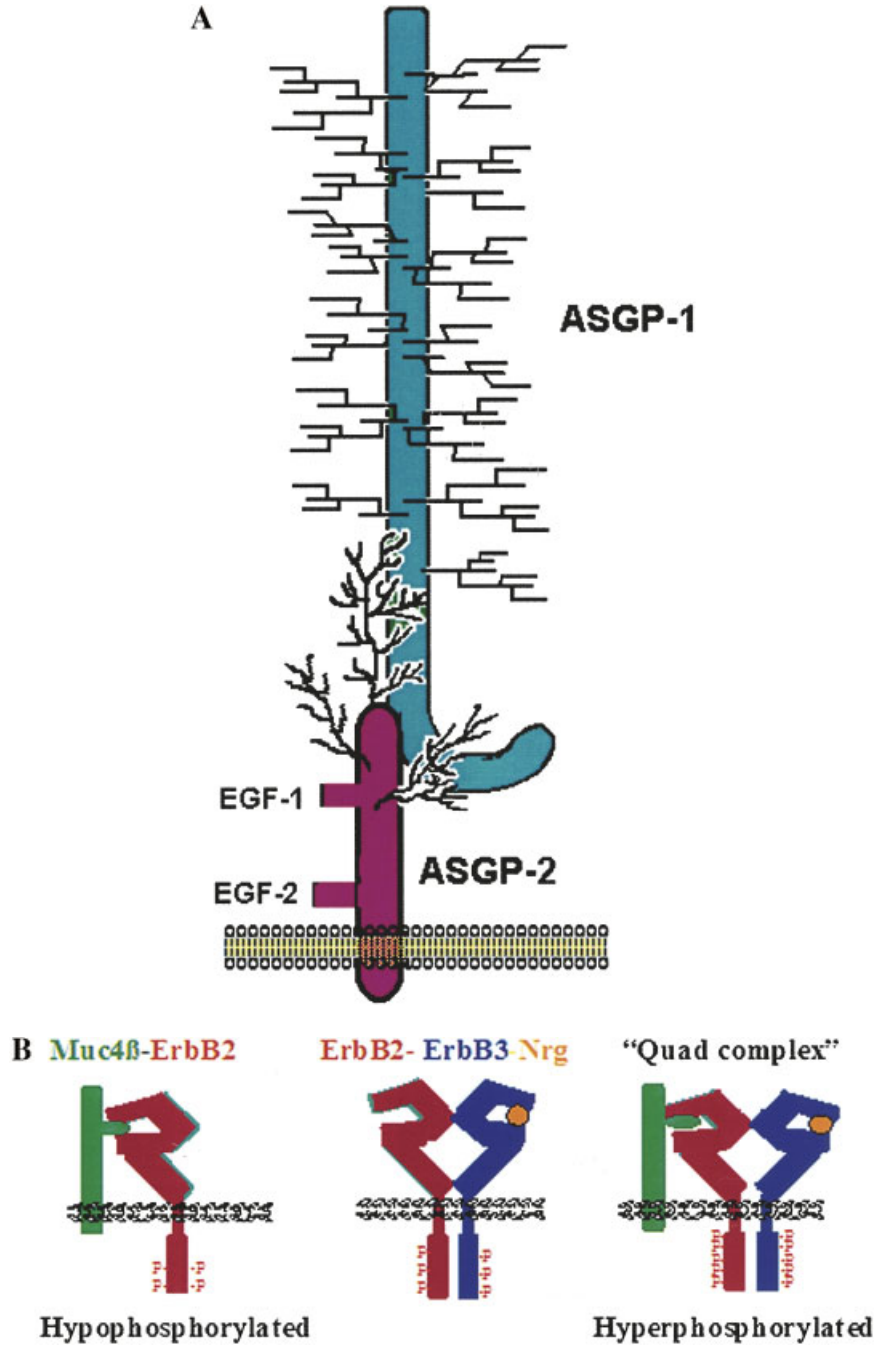

Fig. 2. Models for Muc4 and its complexes with ErbB2. **A**: Model for heterodimeric structure of Muc4, with mucin subunit ASGP-1 (MUC4α) and transmembrane subunit ASGP-2 (MUC4β). Note two EGF-like domains in transmembrane subunit. **B**: Phosphorylation states of Muc4 complexes with ErbB2 and ErbB3 and neuregulin. The Muc4 is shown as a simplified structure without the mucin subunit (ASGP-1, Muc4α) or EGF2 domain. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

localization of ErbB2 at the lateral surface of polarized cells without Muc4 [Ramsauer et al., 2003]. Since ErbB2 has been shown to bind β-catenin and cadherin forms a complex with β-catenin soon after their synthesis, ErbB2 localization may be determined by a partition between Muc4 and β-catenin in the endoplasmic reticulum. According to this scenario, in cells without Muc4, ErbB2 would accompany the cadherin-catenin complex to the lateral junctions. In cells expressing abundant Muc4, the ErbB2 would be directed by the Muc4 to the

apical surface (Fig. 3A), with Muc4 acting as a type of chaperone or chauffeur. This apical localization of ErbB2 can have profound effects on cell signaling, because it effectively segregates ErbB2 from ErbB3, its most productive heterodimeric ErbB partner for activation of proliferation, which remains at the lateral surfaces of the polarized cells expressing Muc4 (Fig. 3A). This segregation prevents ErbB2–ErbB3 heterodimer signaling, which has been implicated strongly in developmental processes and breast cancer. Effectively, this segregation also represses activation of the phosphoinositol-3-kinase-Akt/PKB pathway, which is most frequently induced through ErbB3 and which can contribute to tumor progression by promoting proliferation and repressing apoptosis. Loss of polarization, as occurs in neoplastic transformation and damage to the epithelium, breaks the segregation of ErbB2 and ErbB3 (Fig. 3A) and permits a panoply of signaling events, including activation of the Ras-Erk canonical mitogenesis pathway and the PI3K-Akt anti-apoptotic pathway.

As a ligand, Muc4 alone induces a limited phosphorylation of ErbB2 (Fig. 2B), particularly on tyrosines 1139 and 1248 if the ErbB2 is not otherwise activated [Jepson et al., 2002]. However, ligation of ErbB3 with neuregulin in the presence of the Muc4–ErbB2 complex leads to a hyperphosphorylation of both ErbB2 and ErbB3 (Fig. 2B) [Carraway et al., 1999], with pronounced activation of downstream pathways, including the Erk and PI3K pathways promoting oncogenesis. As noted above, this hyperactivation can only occur when ErbB2 and ErbB3 are not segregated, as in nonpolarized breast cancer cells. Thus, there can be a synergy between the effects of Muc4 on localization and phosphorylation of ErbB2 associated with either differentiation or oncogenesis. ErbB2 serves as part of a differentiation program in epithelia, and Muc4 enforces that program by its effects on ErbB2 localization and phosphorylation. A plausible scenario for ErbB2-driven breast cancer then is that amplification of the *ERBB2* gene with overexpression of ErbB2 initiates a breakdown of cell-cell junctions [Muthuswamy et al., 2001], which breaks the segregation barrier between the Muc4-ErbB2 complex and ErbB3 (Fig. 3A). Both the anti-adhesion effects of Muc4 and its ability to promote hyperphosphorylation may then promote malignant progression.

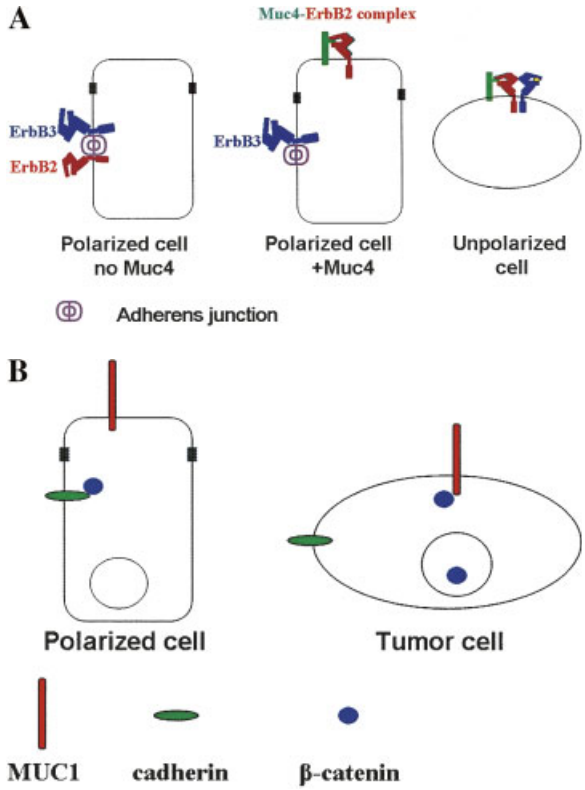

Fig. 3. Models for localization of MUC1, MUC4, ErbBs and β-catenin in mammary luminal epithelial cells and tumor cells. **A**: Effect of Muc4 on segregation and activation of ErbB2 and ErbB3. ErbB2 and ErbB3 are located on the lateral surfaces at the adherens junction (see Fig. 1D) of polarized cells not expressing Muc4. Expression of Muc4 localizes ErbB2 to the apical surface, effectively segregating ErbB2 and ErbB3. The ErbB3 localization does not change in cells expressing Muc4. Loss of polarization removes barriers segregating the receptors and permits ErbB2-ErbB3 heterodimerization, including formation of the hyperphosphorylated ''quad complex'' containing ErbB2, ErbB3, Muc4, and neuregulin (see Fig. 2B), which activates proliferation and represses apoptosis. The Muc4 is shown as a simplified structure without the mucin subunit (ASGP-1, Muc4α) or EGF2 domain. **B**: MUC1 association with β-catenin. MUC1 is apically located in polarized cells, segregated from β-catenin. Loss of polarity in tumor formation permits formation of MUC1–β-catenin complex and transfer of β-catenin to nucleus to act as a transcription factor. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Two further aspects of Muc4 are important to understanding its roles in the mammary gland, cellular consequences and regulation of expression. As noted above, the hyperphosphorylation of ErbB2 and ErbB3 promoted by Muc4 in tumor cells activates pathways which trigger cell proliferation and which also repress apoptosis. In contrast, the apical localization and limited phosphorylation of ErbB2 can potentially have other signaling consequences. In the polarized epithelial cell, Muc4 promotes activation of p38

MAPK phosphorylation, a signaling component of a pathway frequently associated with cell differentiation. Moreover, Muc4 expression can also repress apoptosis without activation of hyperphosphorylation [Komatsu et al., 2001]; though the mechanism is still uncertain, it is different from that induced by hyperphosphorylation. Regardless of the mechanisms involved, Muc4 appears to act as an intrinsic survival factor, whether for differentiated cells or tumor cells, once again emphasizing its dual capabilities. This dual nature is further emphasized by the fact that Muc4, as an anti-adhesive, should promote anoikis (apoptosis by release of adhesions), but in fact acts as a repressor of anoikis, as noted above.

Muc4 is present at the apical surface of the mammary ductal epithelium in virgin adult rats [Price-Schiavi et al., 2005], as is ErbB2. There is a pronounced increase in both during mid to late pregnancy, when Muc4 is present at the apical surface and secreted into the lumen, while ErbB2 is at both apical and lateral surfaces. Studies on isolated rat mammary epithelial cells indicate that they are regulated differently [Price-Schiavi et al., 2005], in particular differentially responsive to matrix adhesion effects. Interestingly, Muc4 is regulated post-transcriptionally. TGFβ appears to be particularly important in its regulation in alveolar cells, signaling through SMAD2 to modulate an essential post-translational cleavage of the Muc4 precursor to yield its two subunits [Soto et al., 2003]. This mechanism may play an important role in breast tumor progression, since most tumors lose their ability to respond to TGFβ. So, as discussed above, overexpression of ErbB2 in breast ductal epithelial cells may collaborate with MUC4 and ErbB3 to induce a neoplastic phenotype by disrupting cadherin–catenin junctions. TGFβ may also play a role in this early stage of neoplasia, since it promotes epithelial–mesenchymal transitions through Smad-interacting protein (SIP)-mediated downregulation of E-cadherin transcription. Since ErbB2 appears to induce further genetic or epigenetic changes leading to loss of TGFβ responsiveness of the neoplastic cells, ultimately ErbB2 overexpression will lead to an increase in MUC4 expression. The overexpressed MUC4 will then promote tumor progression through its anti-adhesive, anti-apoptotic and proliferative mechanisms. Consistent with the hypothesis that Muc4 promotes tumor progression without being oncogenic is the observation that an MMTV-Muc4 transgenic mouse inappropriately expressing mammary Muc4 exhibits hyperplasia and an unusual invasive phenotype, but no tumors [Price-Schiavi et al., 2005]. Finally, the progression from *ERBB2* amplification through MUC4 overexpression has serious consequences for breast cancer therapy, as Muc4 represses the apoptotic killing capacity of many chemotherapeutic drugs [Hu et al., 2003] and can also block the effects of the anti-ErbB2 monoclonal antibody Herceptin [Nagy et al., 2005].

MUC1, β-CATENIN, AND ERBBS

MUC1 is a heterodimeric membrane mucin present in simple epithelia, including the mammary gland, and in many carcinomas [Gendler, 2001]. It is present in about 90% of breast cancers. Transgenic mice overexpressing mammary Muc1 develop tumors, though with a long latency period [Schroeder et al., 2004]. MUC1 has anti-adhesive properties, though less potent than Muc4 because its mucin subunit is smaller. In spite of this effect on adhesion, which should induce anoikis, MUC1 can repress apoptosis [Raina et al., 2004]. Significantly, MUC1 may also participate in cellular signaling through the ability of its cytoplasmic tail to act as a docking site for components of cytoplasmic signaling pathways and through its interaction with β-catenin [Carraway et al., 2003]. MUC1 has six conserved tyrosines which are potential sites for phosphorylation and signaling. All four ErbBs have been reported to bind to the cytoplasmic tail of MUC1. A YEKV site is phosphorylated by either Src or by EGF-activated ErbB1. A second site (YTNP) binds the cytoplasmic Grb–Sos complex and can initiate mitogenic signaling through the canonical Ras–Erk pathway [Carraway et al., 2003].

MUC1 can repress cadherin-dependent cell–cell interactions by either a direct steric effect or by binding to β-catenin [Carraway et al., 2003]. The β-catenin binding site is an SAGNGGSSLS sequence in the cytoplasmic tail of MUC1. Binding is enhanced by Src or EGFR phosphorylation of the YEKV site adjacent to this sequence. Binding is also enhanced by protein kinase C phosphorylation of a threonine nine residues removed from the sequence. In contrast, glycogen synthase 3β, an important

element in Wnt signaling, inhibits the β-catenin–MUC1 interaction by phosphorylation of a serine six residues from the sequence. These results raise the question how MUC1 influences β-catenin functions. Since MUC1 is present at apical surfaces of polarized epithelial cells and β-catenin is at the lateral surface (Fig. 3B), no interaction and contribution to β-catenin function is expected in normal epithelia. However, loss of junctional barriers in cancer cells will permit this MUC1–β-catenin interaction (Fig. 3B). Moreover, the interaction appears to be favored under conditions, e.g. Src activation, which disrupt cadherin junctions. Finally, glycogen synthase 3β, which targets β-catenin for proteosomal degradation, is frequently repressed in cancer cells, thus unable to block formation of the β-catenin–MUC1 complex. These results suggest that conditions in cancer cells are appropriate for formation of the β-catenin–MUC1 complex, but what is its role in these cells? One possible answer is that MUC1 acts as a type of chaperone or chauffeur to escort β-catenin to the nucleus, where it acts as a transcription factor. Previous studies have shown a neuregulin-induced MUC1–ErbB2 association linked to nucleolar localization of plakoglobin, which in turn can promote or antagonize beta-catenin transcriptional activity. These studies also showed an EGF-induced nuclear association of MUC1 and β-catenin [Li et al., 2003].

PERSPECTIVE

Adherens junctions are critical to homeostasis of epithelia, involved in epithelial cell layer stabilization, contact inhibition and repression of apoptosis. A key component of the junction, the linking protein β-catenin, acts as an integrator of cell functions by serving both as an element of cell junctions and a transcription factor. We propose that regulation of its localization and function involves its association with three classes of membrane glycoproteins, cadherins, ErbBs, and membrane mucins. In particular, the membrane mucins MUC1 and MUC4 cooperate to regulate the localization and signaling of β-catenin and the ErbBs as critical functional elements of both mammary epithelial and mammary cancer cells. Though the specifics of these mechanisms are still being deciphered, the integration mechanism provides a powerful paradigm for understanding important aspects of mammary cell biology.

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