Precocious Mammary Gland Development in P-Cadherin–deficient Mice

Glenn L. Radice,*‡§ M. Celeste Ferreira-Cornwell,† Stephen D. Robinson,* Helen Rayburn,*
Lewis A. Chodosh,‡ Masatoshi Takeichi,§ and Richard O. Hynes*

*Howard Hughes Medical Institute, Center for Cancer Research, Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139; †Department of Biophysics, Faculty of Science, Kyoto University, Kitashirakawa, Sakyō-ku, Kyoto, 606-001, Japan; ‡Departments of Obstetrics and Gynecology; and §Molecular and Cellular Engineering, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania 19104

Abstract. To investigate the functions of P-cadherin in vivo, we have mutated the gene encoding this cell adhesion receptor in mice. In contrast to E- and N-cadherin–deficient mice, mice homozygous for the P-cadherin mutation are viable. Although P-cadherin is expressed at high levels in the placenta, P-cadherin–null females are fertile. P-cadherin expression is localized to the myoepithelial cells surrounding the luminal epithelial cells of the mammary gland. The role of the myoepithelium as a contractile tissue necessary for milk secretion is clear, but its function in the nonpregnant animal is unknown. The ability of the P-cadherin mutant female to nurse and maintain her litter indicates that the contractile function of the myoepithelium is not dependent on the cell adhesion molecule P-cadherin. The virgin P-cadherin–null females display precocious differentiation of the mammary gland. The alveolar-like buds in virgins resemble the glands of an early pregnant animal morphologically and biochemically (i.e., milk protein synthesis). The P-cadherin mutant mice develop hyperplasia and dysplasia of the mammary epithelium with age. In addition, abnormal lymphocyte infiltration was observed in the mammary glands of the mutant animals. These results indicate that P-cadherin–mediated adhesion and/or signals derived from cell–cell interactions are important determinants in negative growth control in the mammary gland. Furthermore, the loss of P-cadherin from the myoepithelium has uncovered a novel function for this tissue in maintaining the undifferentiated state of the underlying secretory epithelium.

Classical cadherins, such as E-, N-, and P-cadherin, play critical roles in tissue morphogenesis as evidenced by studies in Xenopus and mice (Kintner, 1992; Hermiston and Gordon, 1995). Cadherins are a family of glycoproteins involved in Ca++-dependent, homotypic cell–cell adhesion (Takeichi, 1995; Gumbiner, 1996). Classical cadherins have five extracellular domains, one transmembrane domain, and a highly conserved cytoplasmic domain. Two subclasses of cadherins, E- and P-cadherin, are detected in various epithelial tissues of mouse embryos (Nose and Takeichi, 1986). Antibody perturbation experiments have shown that E- and P-cadherin function cooperatively in the histogenesis of embryonic lung and lip skin in organ explant cultures (Hirai et al., 1989a, b). In the case of the lung primordia, addition of anti-E-cadherin antibodies resulted in collapsed lobules with little luminal space, while anti–P-cadherin antibodies had a less dramatic effect. However, a mixture of both antibodies had a synergistic effect resulting in a severely distorted epithelium that could not undergo the normal branching process. In similar experiments performed on embryonic skin, P-cadherin appears to be more important compared with E-cadherin, but again disruption of both E- and P-cadherin produced the more dramatic effect on skin morphogenesis. Taken together, these studies suggest that both E- and P-cadherin play important roles in maintaining the structural integrity of epithelial tissues. The cadherin cytoplasmic domain interacts with a group of proteins termed catenins, which link the cadherin to the actin cytoskeleton. Interaction with both the catenins and the actin cytoskeleton is necessary for full cadherin adhesive activity (Kemler, 1993). Either β-catenin or plakoglobin, which are members of the Armadillo family of proteins (Peifer, 1995), binds directly to the cadherin. In addition to playing structural roles in cell–cell adhesion, these two catenins, along with Armadillo, appear to have signaling roles, although the exact mechanism is not fully understood. Armadillo is the product of a Drosophila segment polarity gene and is part of the wingless signaling...
transcription factor, LEF-1, providing evidence that 
Rimm et al., 1995). Thus, 
formation of mesoderm and the anterior-posterior axis in the 
Xenopus embryo (Heasman et al., 1994; Funayama et al., 1995). Recently, β-catenin was shown to interact with the transcription factor, LEF-1, providing evidence that β-catenin can regulate gene expression (Behrens et al., 1996). α-catenin, which shares homology with the cytoskeleton-associated protein vinculin, binds the cadherin indirectly through β-catenin or plakoglobin. Like vinculin, α-catenin binds to both α-actinin and actin (Knudsen et al., 1995; Rimm et al., 1995). Thus, α-catenin serves to link the cadherin/catenin complex to the actin cytoskeleton.

Cell adhesion molecules, including the cadherins, are known to play important roles in mammary gland morphogenesis. The mammary gland develops postnatally under the proper hormonal stimuli during puberty and adolescence. The morphogenesis of the mammary ductal tree occurs when the end buds invade the surrounding fatty stroma until they reach the edge of the fat pad. The end buds of the mammary ducts represent the growth points for ductal morphogenesis. The end buds consist of basally located cap cells and luminal epithelial cells (Williams and Daniel, 1983). The cap cells are loosely adhering epithelial cells that lack cytoplasmic polarity and a well organized cytoskeleton. During early pregnancy lateral buds differentiate from the ducts and during the second half of pregnancy these alveoli develop into fully differentiated secretory lobules. These morphogenetic events are accompanied by cellular differentiation leading to development of secretory epithelial cells which are capable of synthesizing and secreting milk proteins.

The mammary duct consists of two main cell types, the luminal epithelial cells and a surrounding monolayer of myoepithelial cells with a closely apposed basement membrane. The myoepithelial cells are thought to differentiate from the cap cells extending their cell processes laterally along the duct. In the pregnant animal, the myoepithelium is present all along the duct and in the alveoli, where myoepithelial cells are basket shaped resulting in space between the cells allowing direct contact between the alveolar epithelial cells and the basal lamina. In contrast with other tissues, the expression pattern of E- and P-cadherin in the mammary gland is very distinct. In the mouse, cap cells and myoepithelial cells express P-cadherin while the luminal epithelial cells express E-cadherin (Daniel et al., 1995). Function-blocking antibodies were used in situ to examine the role of E- and P-cadherin in maintaining the tissue integrity of the end bud (Daniel et al., 1995). Antibody to E-cadherin induced disruption of the epithelium resulting in freely floating epithelial cells in the lumen. In contrast, antibody to P-cadherin had no effect on the luminal layer but partially disrupted the basally located cap cell layer. These data show that E- and P-cadherin are important for maintaining the integrity of the different cell layers of the mammary duct.

The cell–cell and cell–matrix interactions of myoepithelial cells may play an important role in maintaining the structural integrity of the mammary duct. Myoepithelial cells are specialized contractile cells, whose ultrastructure is reminiscent of smooth muscle cells (Deugnier et al., 1995). They express smooth muscle contractile and cytoskeletal proteins such as α-smooth muscle actin (Radnor, 1972). However, they are true epithelial cells since cytokeratin is the major component of the intermediate filament system, they form desmosomes, hemidesmosomes, and adherens junctions, and are permanently separated from the connective tissue by the underlying basement membrane (Franke et al., 1980; Sonnenberg et al., 1986; Rasbridge et al., 1993).

Recent experiments suggest that myoepithelial cells may have an important role in branching morphogenesis in the mammary gland (Niranjan et al., 1995). Hepatocyte growth factor/scatter factor (HGF/SF) is produced by the fibroblasts in the breast. The luminal epithelium and myoepithelium respond differently to HGF/SF; which acts as a mitogen for the luminal cells while it behaves like a morphogenic factor for the myoepithelial cells. Human myoepithelial cells exposed to HGF/SF form extended branching tubules while luminal epithelial cells did not show any morphological changes (Niranjan et al., 1995). Furthermore, overexpression of HGF/SF in the mammary gland of transgenic mice leads to precocious alveolar differentiation (Takayama et al., 1997). These data suggest that myoepithelial cells may play an important role in branching morphogenesis in the mammary gland.

To understand the role of the P-cadherin adhesion receptor in mouse development, we mutated the P-cadherin gene in embryonic stem (ES) cells and introduced the mutation into the mouse germ line (Capellachi, 1989). In contrast to E- and N-cadherin knockout mice, the P-cadherin-deficient mice are viable and fertile. However, the P-cadherin-null females exhibit precocious differentiation of the mammary gland and display mammary hyperplasia later in life. The P-cadherin-deficient mice provide us with a valuable model to determine the role of P-cadherin in cell proliferation and differentiation in the mammary gland.

Materials and Methods

Derivation of Mutant Mice

The genomic structure of the mouse P-cadherin gene was reported previously (Hatta et al., 1991). An EcoRI–EcoRI DNA fragment (8.5 kb) was isolated from lambda clone, PG21, and subcloned into Bluescript KS (Stratagene, La Jolla, CA). The neo expression cassette, pMC1neo (Thomas and Capecchi, 1987), was digested with XhoI-Sall, and subcloned into a unique XhoI site in exon 11. The BamHI–BamHI DNA fragment was subcloned into a plasmid containing the pMC1–HSVTK cassette (Mansour et al., 1988). The construct was linearized with NotI and electroporated into ES cells (Doetschman et al., 1985), and colonies were selected with G418 and gancyclovir (a gift from Syntex Corp.) as described (George et al., 1993). Homologous recombination events were screened by Southern blot analysis (Sambrook et al., 1989). An EcoRI–BamHI genomic DNA fragment (0.7 kb) located immediately 5′ to the short arm of 11q was used in the PCR reactions (Hanley and Merlie, 1991). Temperature cycling conditions for the Perkin Elmer 480 PCR machine were 94°C for 1 min; 65°C for 2 min; and 72°C for 3 min for 40 cycles. PCR products were resolved on a 1.6% agarose gel.

1. Abbreviations used in this paper: APC, adenomatous polyposis coli; ECM, extracellular matrix; ES, embryonic stem; HGF/SF, hepatocyte growth factor/scatter factor; HSV, Herpes simplex virus; PCD-1, P-cadherin antibody.
Western Immunoblotting
Decidual tissue from wild-type, heterozygous, and homozygous conceptuses was isolated on day 8 of gestation. The protein lysates were subjected to SDS-PAGE (Laemmli, 1970), and the resolved proteins were transferred electrophoretically to nitrocellulose. The blot was reacted with antibodies to P-cadherin (PCD-1; Nose and Takeichi, 1986) and protein bands were visualized using the ECL (Amersham Corp., Arlington Heights, IL) detection system.

Morphological and Histological Analysis
The thoracic no. 3 and/or inguinal no. 4 mammary glands were examined. Whole mount staining of the glands was performed as previously described (Williams and Daniel, 1983). The mammary glands were fixed overnight in Tellyesniczky's fixative (5% formalin, 5% acetic acid, 70% EtOH). The fixed glands were defatted in acetone, stained with: hematoxylin (0.65 g FeCl₃.67.5 ml H₂O, 8.7 ml stock hematoxylin [10% in 95% ethanol], and 1,000 ml 95% ethanol, adjust pH 1.25 with concentrated HCl), and then rinsed in tap water, dehydrated in increasing concentrations of ethanol, and then rinsed in 10% formalin, processed for paraffin sectioning, and stained with hematoxylin and eosin.

Immunostaining for P- and E-cadherin, a-smooth muscle actin, and caseins was performed on frozen sections of mammary tissue as described previously (Daniel et al., 1995). Tissue was embedded in Tissue-Tek OCT compound (Miles Diagnostic Division, Elkhart, IN) and frozen in an isopentane/dry ice bath. 8-μm sections were cut with a Zeiss cryostate, placed on Superfrost Plus slides (Fisher Scientific, Pittsburgh, PA), post-fixed in 1:1 acetone/methanol at −20°C for 10 min, air dried, and stored at −20°C. Antibodies were diluted in 5% skim milk/PBS except for casein antibody (1% goat serum/PBS) as follows: rat monoclonal PCD-1 (1:200), rat monoclonal ECD-2 (1:500; Shirayoshi et al., 1986), mouse monoclonal a-smooth muscle actin clone 1A4, 1:400; Sigma Chemical Co., St. Louis, MO), and rabbit polyclonal casein antibody (1:1,000; a gift from Charles Daniel, University of California, Santa Cruz). The samples were incubated overnight with primary antibodies except casein antibody (1 h), washed with PBS, incubated with species specific biotinylated secondary antibodies (Amersham), washed with PBS, and processed with Vectastain ABC reagents (Vector Labs, Burlingame, CA), regular or elite, and washed with PBS. The casein antibody washes contained 1% goat serum. The peroxidase substrate was applied, sections were lightly counterstained with the mAb PCD-1. No P-cadherin protein was detected in the homozygous mice. kb, kilobases; m, markers.

Results
Generation of P-Cadherin–deficient Mice
To construct a targeting vector for the P-cadherin gene (Fig. 1 A), a neomycin phosphotransferase (neo) expression cassette (pMC1neo; Thomas and Capecchi, 1987) was inserted into a unique XhoI site in exon 11 (Nose et al., 1987; Hatta et al., 1991). This disrupts the open reading frame of the P-cadherin gene in the extracellular domain of the protein. The neo cassette, containing a polyadenylation signal, was placed in the same transcriptional orientation as the P-cadherin gene thus serving to truncate any P-cadherin/neo fusion transcript. The vector includes 2.5 kb of genomic sequences 5′ of the neo gene, 5 kb on the 3′ side, and a flanking Herpes simplex virus (HSV) thymi- dine kinase expression cassette (pMC1-tk; Mansour et al., 1988). A similar construct was used successfully to inactivate the N-cadherin gene in mice (Radice et al., 1997).

The linearized targeting vector was electroporated into D3 ES cells (Doetschman et al., 1985), and the electroporated cells were subjected to positive/negative selection (Mansour et al., 1988) using G418 and gancyclovir. One of 86 double-resistant ES cell clones (P78) underwent homologous recombination at the P-cadherin locus as determined by Southern blot analysis (Fig. 1 B). Hybridization with a neo probe failed to detect any additional sites of integration (data not shown). The targeted clone was injected into host C57BL/6 blastocysts, and the blastocysts were transferred to the uteri of pseudopregnant females. Germine transmission of the mutant allele was achieved with the targeted ES clone. The heterozygous mice did not display any obvious abnormalities in comparison with their wild-type littermates.

P-Cadherin Mutant Mice Are Viable and Fertile
To examine whether animals homozygous for the P-cadherin mutation were viable, heterozygous animals were intercrossed and genotypes of the progeny were determined by Southern blot or by PCR analysis. Mice homozygous for the P-cadherin mutation were detected among the intercross progeny (Fig. 1 C). The genotypes of the progeny showed a good fit to Mendelian distribution (107 +/- : 211 +/- : 114 +/- : 107 +/- ). Homozygous P-cadherin–deficient mice did not show any overt developmental abnormalities and were indistinguishable from their heterozygous or wild-type littermates on the basis of size, activity, or fertility. To determine whether wild-type P-cadherin protein was present in homozygous mice, we examined the uterine de-
cidua of pregnant mice since P-cadherin protein is very abundant in this maternal tissue. The decidual tissue without the embryo and extraembryonic membranes was isolated from day 8 conceptuses of wild-type, heterozygous, and homozygous mice. Western blot analysis of decidual lysates was performed using monoclonal antibody, PCD-1, which recognizes the amino terminus of the protein (Nose et al., 1990). No full-length or truncated P-cadherin protein was detected in the mutant, while a reduced amount of P-cadherin protein was present in the heterozygote (Fig. 1D). In addition, a pan-cadherin polyclonal antibody (Takeichi et al., 1990) which recognizes the conserved cytoplasmic domain of classical cadherins did not detect P-cadherin in the mutant decidual tissue (data not shown).

To determine if loss of P-cadherin affected litter size, hybrid 129Sv/C57BL mutant males and females were mated. The P-cadherin–null females had litter sizes (average 8.8, 114 pups/13 litters) comparable to their wild-type littermates. The ability of the P-cadherin mutant females to nurse and maintain a normal size litter indicates that the contractile function of the myoepithelium is not dependent on the cell adhesion molecule P-cadherin.

Loss of P-Cadherin from Myoepithelial Cells Leads to Alveolar Differentiation in Virgin Females

To examine cadherin expression in the mutant mammary duct, immunohistochemistry was performed on sections of mammary tissue. P-cadherin was localized to the myoepithelial cells surrounding the luminal epithelial cells in the wild-type duct (Fig. 2A). A diffuse nonspecific background staining was observed in the mutant (D), but the characteristic dark linear staining of the myoepithelial cells (A) was absent in the mutant. Myoepithelial cells are present in both the wild-type (B) and mutant (E) as shown by the smooth muscle actin staining. E-cadherin expression appears similar in wild-type (C) and mutant (F) ducts. The arrowheads indicate myoepithelial cells. Original magnification 125×.

During puberty the end buds of the mammary ductal tree actively penetrate the surrounding adipose stroma, P-cadherin has been shown to be expressed in the cap cells of the terminal end bud (Daniel et al., 1995). No difference in the extent of branching morphogenesis was observed in the mammary glands of mutant mice compared with their wild-type littermates at 5 wk of age, before puberty is complete (data not shown). However, we observed an unexpected phenotype when mammary glands from postpubescent virgin females (10 wk old) were examined by whole-mount staining. Wild-type animals had normal ductal morphogenesis, while the mutant animals displayed precocious alveolar differentiation resembling an early pregnant gland (Fig. 3A). No alveolar differentiation was ob-

Figure 2. Cadherin expression in wild-type and mutant mammary ducts. Mammary gland sections of wild-type (A–C) and mutant (D–F) animals were immunostained for P-cadherin (A and D), smooth muscle actin (B and E), and E-cadherin (C and F). P-cadherin is present in the myoepithelial cells surrounding the luminal epithelial cells in the wild-type duct (A) and absent in the mutant duct (D). A diffuse nonspecific background staining was observed in the mutant (D), but the characteristic dark linear staining of the myoepithelial cells (A) was absent in the mutant. Myoepithelial cells are present in both the wild-type (B) and mutant (E) as shown by the smooth muscle actin staining. E-cadherin expression appears similar in wild-type (C) and mutant (F) ducts. The arrowheads indicate myoepithelial cells. Original magnification 125×.

Figure 3. Structure of normal and mutant mammary glands in virgin female mice. Whole-gland stain of (A) wild-type and (B) mutant mammary gland from 10-wk-old virgin animals. P-cadherin–deficient virgin females display precocious alveolar differentiation. Original magnification 10×.
served (data not shown). To determine whether the mutant mammary glands exhibited biochemical changes associated with differentiation, milk protein production was examined using a polyclonal antibody against caseins. The wild-type virgin animal did not express casein(s) while the mutant expressed casein(s) at levels similar to a pregnant animal (Fig. 4, A–C). These data indicate that loss of P-cadherin leads to precocious alveolar differentiation which is morphologically and biochemically similar to an early pregnant wild-type gland.

A Mutation in the P-Cadherin Gene Predisposes Mice to Focal Mammary Hyperplasia and Dysplasia

As demonstrated in various animal models of mammary tumorigenesis, precocious alveolar differentiation is often associated with neoplastic lesions, an initial step in tumor development (Cardiff and Muller, 1993). Therefore, the P-cadherin mutant mice and their heterozygous and wild-type littermates were maintained until 2 yr of age. No palpable tumors were observed in the aged P-cadherin mutant animals. However, upon histological examination of the mammary glands, focal alveolar hyperplasia and ductal dysplasia were observed in the mutants. Comparison of a 20-mo-old wild-type and mutant virgin female gland reveals the extent of alveolar hyperplasia observed in the mutants (Fig. 5, A and B). The clusters of alveoli are shown at higher magnification (Fig. 5 C). A 24-mo-old virgin mutant animal displays ductal dysplasia and stromal/fibroblastic hyperplasia (Fig. 5 D). Secretory vacuoles were observed in the alveoli of a 20-mo-old virgin animal consistent with a differentiated hyperplastic phenotype (Fig. 5 E). In addition, extensive periductal lymphocyte infiltration was observed in the interstitial space of the mutant glands (Fig. 5 F). Furthermore, several multiparous mutant females (15 mo old) exhibited extensive mammary hyperplasia and dysplasia, however, palpable mammary tumors were not observed (data not shown). It should be noted that P-cadherin is not expressed in the epithelium which becomes hyperplastic, but confined to the surrounding myoepithei-

Figure 4. Expression of milk protein in P-cadherin-null virgin mice. Mammary gland sections of wild-type virgin (A), mutant virgin (B), and wild-type pregnant day 14 (C) animals were immunostained with a polyclonal antibody against caseins. The P-cadherin-deficient virgin mammary gland express casein(s) similarly to a pregnant animal. The wild-type virgin gland was counterstained to distinguish the ducts. Original magnification 62×.

Figure 5. P-cadherin mutant mice develop focal hyperplasia and dysplasia with age. Histology of virgin mammary tissue from wild-type (A) and mutant (B–F) animals, 19–24 mo of age. The mutant mice (B) exhibit extensive alveolar differentiation compared with their control littermates (A). Higher magnification of the alveoli (C). P-cadherin mutant animals develop fibroblastic (D) and secretory hyperplasia (E) and exhibit extensive lymphocyte infiltration (F). The lymphocytes are concentrated around the ducts (arrowhead). A portion of the lymph node is shown (arrow). Original magnifications: (A and B) 12.5×; (C–F) 31×.
Epithelial cells with a closely apposed basement membrane. The lumenal epithelial cells and an outer monolayer of myoepithelial cells. We observed an unexpected phenotype. As in vertebrates, it also interacts with cadherins and segment polarity gene acting in the wingless signaling pathway. The loss of P-cadherin may affect the function of other cell adhesion molecules, for example, integrin-mediated ECM interactions may be compromised leading to changes in cell morphology of the myoepithelial cells. In Drosophila, armadillo, the β-catenin homologue, is a segment polarity gene acting in the wingless signaling pathway. As in vertebrates, it also interacts with cadherins and is localized to adherens junctions. In Drosophila, the functions of armadillo in adherens junctions and wingless signaling are competitive. The segment polarity defect of a weak armadillo allele can be partially rescued by making the flies heterozygous for DE-cadherin (Cox et al., 1996).

Figure 6. Possible models for regulation of mammary gland differentiation by P-cadherin. (A) The indirect regulation model is based on a cell adhesion defect. For example, inductive signals from the surrounding ECM (basal lamina) and/or stroma may more easily permeate a less cohesive myoepithelium leading to differentiation of the epithelium. (B) The direct regulation model is based on a P-cadherin-mediated cell signaling defect. For example, loss of P-cadherin may cause the myoepithelium to relay a positive signal(s), or alternatively, it may relieve an inhibitory signal(s) resulting in differentiation of the luminal epithelium. The cell adhesion and cell signaling models are not mutually exclusive.
The suppression of the armadillo mutation is thought to occur because there is a reduction in the amount of DE-cadherin thus freeing up some of the wild-type maternal store of armadillo protein, allowing it to function in the wingless pathway. Similar competitive interactions have been seen in Xenopus, where increases in cadherin levels can block action of β-catenin in Wnt signaling as monitored by inhibition of axis duplication (Fagotto et al., 1996). In both these cases, there appears to be a competition between the β-catenin bound to the cytoplasmic tail of cadherin and the free cytoplasmic β-catenin involved in the wingless/Wnt signaling pathway.

The cadherin/catenin adhesion complex and the Wnt signaling pathway share a common component, β-catenin. Data from transgenic and mutant mice suggest that the P-cadherin–null phenotype may be due to activation of the Wnt signaling pathway. Transgenic mice that overexpress Wnt-1 in the mammary gland develop extensive alveolar hyperplasia and adenocarcinomas (Tsukamoto et al., 1998). In addition, mutation of another member of the Wnt signaling pathway, Adenomatous polyposis coli (APC), predisposes mice to focal alveolar hyperplasia and carcinomas (Moser et al., 1993). APC is a negative regulator of the intracellular β-catenin pool, hence when APC is active the cytoplasmic pool of β-catenin is very low. When the Wnt pathway is activated, APC is turned off, and β-catenin accumulates in the cytoplasm. β-catenin can then interact with the DNA binding proteins of the T cell factor-lymphoid enhancer factor (Tcf-Lef) family to activate transcription of target genes (Behrens et al., 1996). The loss of P-cadherin may increase the available cytoplasmic β-catenin thus activating the Wnt signaling pathway resulting in transcription of growth factor genes whose gene products induce differentiation of the neighboring epithelial cells, the direct regulation model (Fig. 6B). This scenario assumes that APC cannot efficiently inactivate the excess β-catenin present in the P-cadherin–deficient mammary gland.

Ectopic or overexpression of several proteins including Wnt1 (Tsukamoto et al., 1998), growth hormone (Bchini et al., 1991), HGF/SF (Takayama et al., 1997), and stromelysin1 (Sympton et al., 1994) lead to extensive alveolar hyperplasia and mammary tumors in transgenic mice. The extent of precocious alveolar differentiation observed in the P-cadherin–deficient mice appears less dramatic in comparison to these other transgenic strains. No palpable tumors were observed in the mutant animals, although, focal hyperplastic and dysplastic lesions were observed in the mutant mammary glands upon necropsy. Hyperplasia was also observed in the salivary gland where P-cadherin is normally expressed in the myoepithelial cells (Ferreira-Cornwell, M.C. and G.L. Radice, unpublished data). The finding that disruption of P-cadherin function alone is not sufficient to induce mammary tumors, suggests that additional genetic lesion(s) are necessary to progress beyond the hyperplastic phenotype.

P-cadherin is also expressed in myoepithelial cells of the human mammary gland suggesting that loss of function of this cell adhesion molecule may promote cell growth and differentiation in the human breast. While most attention has focused on the role of E-cadherin in breast cancer, our data suggest that P-cadherin may also be involved. Loss of heterozygosity (LOH) of 16q22.1 has been implicated in many types of cancer including breast (Birchmeier and Behrens, 1994). E- and P-cadherin are tandemly arranged at this locus separated by only 32 kb of genomic DNA (Bussemakers et al., 1994). While cadherin expression has been examined extensively in the tumor tissue itself, perturbation of cadherin function in the surrounding myoepithelium has not been addressed. Germline mutations in the human P-cadherin gene may predispose women to breast cancer since hyperplastic growth is an early step in tumor development. Recently, a novel cadherin, H-cadherin, was found to be expressed in human mammary epithelial cells (Lee, 1996). H-cadherin lacks a cytoplasmic domain and is most similar to T-cadherin (Ranscht and Dours-Zimmermann, 1991). Its expression was found to be significantly reduced in human breast carcinoma cell lines and primary breast tumors (Lee, 1996). Furthermore, transfaction of H-cadherin into the breast cancer cell lines led to a decreased cell growth rate and loss of anchorage-independent growth in soft agar. These data suggest that two distinct cadherins, H- and P-cadherin, are involved in negative growth control in the mammary gland.

Several loss of function mutations in the mouse result in disruption of mammary gland development and function. Mutations in cyclin D1 (Sicinski et al., 1995), activin/inhibin βB (Vassalli et al., 1994), progestosterone (Lydon et al., 1995), and prolactin receptors (Ormandy et al., 1997), and Stat5a (Liu et al., 1997) all result in inhibition of lobuloalveolar outgrowth in the mammary gland. All these gene products are positive effectors in a signaling pathway(s) leading to alveolar cell proliferation and differentiation. In contrast, P-cadherin appears to act as a negative regulator of cell growth and differentiation in the mammary gland. Its absence leads to precocious alveolar differentiation in virgin animals.

In conclusion, gene targeting of the cell adhesion receptor, P-cadherin, has uncovered a novel function for this molecule in negative growth control of the mammary gland. The mechanism by which P-cadherin acts to control cell growth remains to be determined. No obvious cell adhesion defect has been observed in the myoepithelium of the mutant animals so far, suggesting the mammary gland phenotype may result from perturbation of cellular signals involved in negative growth control.

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