Published in final edited form as:
J Mammary Gland Biol Neoplasia. 1999 April ; 4(2): 193–201.

Functional Culture Models to Study Mechanisms Governing Apoptosis in Normal and Malignant Mammary Epithelial Cells

V. M. Weaver[1,2] and **M. J. Bissell**[1]

[1] Life Sciences Division, Lawrence Berkeley National Laboratory, University of California, Berkeley, California 94720

Abstract

Mammary tissue homeostasis depends upon dynamic interactions between the epithelial cells, their microenvironment (including the basement membrane and the stroma), and the tissue architecture, which influence each other reciprocally to regulate growth, death and differentiation in the gland. To study how apoptosis is regulated in normal mammary cells, and to understand its role in breast tumor pathogenesis, we need model systems that recapitulate breast tissue architecture and microenvironment in culture. We have established culture models of primary and established nonmalignant mammary cell lines from both rodent and human, and defined procedures to study how cell and tissue architecture affect signaling by the basement membrane. We show that both a basement membrane and an organized tissue structure are required to achieve sustained mammary cell survival. These models could now be used to investigate how the basement membrane represses apoptosis in normal cells, and how breast cancers become death-resistant.

Keywords

Cell death; tissue architecture; tumor; basement membrane; mammary epithelial cell

MODEL SYSTEMS TO STUDY APOPTOSIS

Apoptosis is a cellular process necessary for embryonic tissue development and for tissue maintenance in the adult organism. Perturbations in cell death underlie a number of tissue-specific pathologies including malignancy, and may explain the origins of at least some therapy-resistant tumors (1,2).

In reviewing the extensive literature on cell death, it is evident that the seminal observations were derived largely from the use of appropriate model systems to answer specific physiological questions. For example, genetically defined lower eukaryotic systems were used to identify the basic cell death components and delineate the stages of apoptosis (3,4). Likewise isolated nuclei and cell-free systems, reconstituted with cell extracts or genetically engineered proteins, were used to clarify how the cell death components function to regulate and execute apoptosis (5,6). Model systems such as transgenic mice or cultures of differentiated human neurons have helped elucidate how apoptosis can be regulated in higher organisms (2,4,7). What is becoming apparent from such studies is that cell death decisions are linked to those regulating tissue-specificity. Yet little is known about how cell and tissue-specific factors influence and integrate with the cell death pathways. It is thus important to study apoptosis regulation systematically using tractable, tissue-specific model systems.

[2]To whom correspondence should be addressed at vmweaver@lbl.gov.

THE MAMMARY GLAND AS A MODEL SYSTEM TO STUDY TISSUE-SPECIFIC APOPTOSIS

The mammary gland has been used extensively to study how apoptosis is regulated *in vivo*, and to examine the role of cell death in tumor progression (8). Studies in animal models have emphasized the complexity of systemic and breast-specific factors influencing death decisions in the mammary gland (9). The enormity of physiological, biomechanical and endocrine modifiers regulating mammary cell function *in vivo* makes these experiments challenging, the data analysis complicated, and requires a substantial commitment of time and expense. There are also certain types of experiments and manipulations that cannot be conducted easily *in vivo*. Thus model systems and 'designer microenvironments' that recapitulate aspects of the functional mammary gland in culture have been developed to study the underlying mechanisms that govern regulation of cell death in normal and malignant mammary cells (10–12). In addition a number of physical and biochemical manipulations that alter cell shape, tissue organization and gene expression have also been devised (12–14). The assays are highly reproducible, are fast and easy to perform, and yield clear, interpretable experimental results that correlate well with events *in vivo*.

In this brief review we describe some of these assays, and show how they have been adapted to study apoptosis in nonmalignant and malignant mammary cells. We argue that the mammary phenotype is dictated by the complex physical and biochemical interactions between the cellular and extracellular constituents of the tissue microenvironment, and is influenced by the cell and tissue architecture. We use our own work to illustrate that the choice of cell model, the manner in which the cells are spatially manipulated, and the type of extracellular matrix material and components used can and will influence the results obtained.

Reconstructing the Differentiated Mammary Cell in Culture: The Importance of the Cellular Microenvironment

The mammary gland is a secretory tissue with an extensively branched system of epithelial ducts terminating in multiple alveoli or acinar structures. Myoepithelial cells encase the luminal epithelial cells in the ducts, and are themselves in contact with a laminin and collagen IV-rich basement membrane. In the alveoli, both epithelial and myoepithelial cells contact the basement membrane. Surrounding the ductal network, and accounting for greater than 80% of the breast volume, is a highly compartmentalized stroma. The stromal tissue is composed of a mesenchymally-derived cellular complement of adipocytes and fibroblasts, and a proteinaceous network of stromal matrix proteins, consisting of glycosaminoglycans, collagens (mainly type I and III) and noncollagenous glycoproteins (15).

In this review, we use the term extracellular matrix to connote all the acellular, insoluble proteinaceous components that exist in the tissue, including the basement membrane. The term stromal extracellular matrix is used to describe the material surrounding the stromal cells. Basement membrane is used to denote the organized material between the stroma and the epithelial components of the gland. The term reconstituted basement membrane is used to describe the reconstituted gels prepared from Englelbreth-Holm-Swarm tumors (16; Matrigel™). When we refer to basement membrane in culture, it is the endogenous material secreted in response to reconstituted basement membrane, since we have shown that both human and mouse mammary cells synthesize their own basement membrane in response to appropriate extracellular matrix cues (17,18).

In addition to serving as a structural support system and a reservoir of soluble factors, culture assays have shown that the basement membrane, stromal extracellular matrix, and cells of the mammary stroma, modulate mammary cell growth, differentiation and death (10,14,15 and

refs. therein). Although not as well established *in vivo*, mammary differentiation correlates with the presence of an organized tissue structure and a polarized basement membrane, and can be modified by manipulating the ratio of matrix degrading enzymes to their inhibitors (for review see 9). Moreover, stromal-epithelial interactions have been implicated in the regulation of processes such as branching morphogenesis, involution and tumorigenesis (9,10,11,14 and refs. therein). Culture studies have shown this regulatory relay probably depends upon both matrix-directed biochemical and biomechanical signaling (13). Indeed, alterations in tissue organization and matrix composition accompany breast cancer (11,15,19). Conversely, if tumor-basement membrane interactions could be modified in such a way as to restore the organization of the breast tissue unit of structure, then the tumor phenotype might be reverted both in culture and *in vivo* (20,21). Thus the cytostructure and tissue architecture, the nature of the matrix, and the ability of the target cell to respond appropriately to the matrix stimuli, are all important experimental considerations when designing assays to examine mammary tissue-specific behavior in culture.

Modeling Mammary Phenotype in Culture

A caveat of traditional monolayer cultures of mammary cells is the aberrant cell shape and organization, inappropriate growth-rates that are similar to malignant cells, and the failure of the cells to exhibit tissue-specific gene expression (12,14). Biomatrices used to overcome these drawbacks include: collagen I matrices from rat tail collagen (22), reconstituted basement membrane from the mouse Englebreth-Holm-Swarm tumor (16), or from tissues such as the rat liver (12 and refs. therein), those deposited by confluent cell monolayers such as: endodermal PFHR9 cells (23), keratinocytes (24), or natural matrices such as intact amnion basement membranes (25), or sea urchin basement membranes (26). The biomatrices can be further processed to yield growth factor-depleted preparations (27); in addition, purified matrix proteins, or proteolytically-derivitized, bioactive fragments and functional competitors can be used (17). Finally, peptides specifying active domains of matrix proteins can be synthesized, and function-altering antibodies can be utilized for specific competition assays (17,20, for review see 12).

Using these cell adhesion tools, investigators have been able to recapitulate a number of mammary functions in culture which otherwise would be lost. For example, when mouse mammary cells are plated on plastic or onto attached type I collagen gels, they acquire a flat cuboidal morphology and are unable to synthesize a functional basement membrane, and thus fail to differentiate and express milk proteins, even in the presence of lactogenic hormones (13,22). By allowing the collagen gels to float, or by plating mammary cells on pliable tissue culture membranes, (which allows the cells to organize an endogenous basement membrane; 22), or by providing the cells with an exogenous reconstituted basement membrane (13), they are able to express β-casein, an important milk protein (for brief review see 10).

Altering Integrin Signaling to Modify Mammary Cell Differentiation

Extracellular matrix-directed effects are transmitted to cells predominantly by a family of integral transmembrane receptors called integrins (28). Integrins transduce matrix-derived signals through ligation-associated events and via clustering-mediated recruitment of cytosolic and cytoskeletal proteins (29). Integrin signaling activity can be manipulated by regulating the molecular activation state(s), by changing the extracellular cation concentration (30), or free energy availability (24), or by manipulating integrin conformation using integrin-specific function-altering antibodies or substrate competitor(s) (31). Alternately matrix-induced function can be mimicked by artificially clustering or activating integrins using polyclonal antibodies, bead cross-linked substrates or integrin-specific monoclonal antibodies, or by altering integrin function using dominant-negative integrin expression constructs or genetically modified downstream signaling molecules (32). By applying such methods, β1 and β4 integrin

heterdimers, and a third nonintegrin receptor have been implicated as the mediators of basement membrane-driven murine mammary epithelial cell β-casein expression (Muschler *et al.*, submitted).

Inducing a Tissue-Like Architecture in Culture

In addition to eliciting biochemical-signaling in mammary cells, a malleable reconstituted basement membrane induces cell rounding, drives cell-cell interactions and facilitates the assembly of a polarized tissue-like acinus structure, reminiscent of the alveolar sacs from which the cells are derived from *in vivo* (18). Following recapitulation of this acinus-like structure, the cells reconstitute their tissue-specific behavior, repress the expression of growth-associated genes, for example the cytokines TGFα and TGF-β, as well as transcription factors such as Id-1, and synthesize and vectorially-secrete most classes of milk proteins, including whey acidic protein (10,33). These findings imply that features of the organized mammary epithelial alveolar structure, such as cell rounding, cell-cell communication and tissue polarity, and the cell adhesion molecules and pathways which direct and maintain the acini (including integrins, adherens junction proteins and associated signaling molecules), modulate mammary gene expression. Recent evidence supporting this hypothesis has been obtained in human mammary cells, where adhesion and growth factor receptor pathways were shown to modulate the mammary phenotype through reciprocal connections (20,21).

To study the contribution of cell shape, cell rounding can be induced by plating cells on a malleable matrix, or on a pliable porous filter (27), by de-polymerizing filamentous actin using cytochalasin D (34), or by preparing cell suspensions using polyHEMA-coated dishes (an inert nonadhesive compound which inhibits cell attachment) to prevent cell adhesion and spreading (35, for review see also 13). Using such strategies, it was shown that cell rounding was sufficient to induce growth-arrest and the expression of lactoferrin (34, for review see also 13).

To study biochemically-generated basement membrane signals, while minimizing effects on cell shape, investigators have cross-linked the reconstituted basement membrane with gluteraldehyde and air dried gels made of basement membrane materials (36). Alternately purified basement membrane components, such as laminin-1 or fibronectin have been dripped onto pre-clustered and pre-rounded cells, or cells and tissue structures have been ligated with basement membrane components or integrin-specific antibodies cross-linked to inert microbeads (13, Muschler *et al.*, submitted, see also 10 for review). Using these approaches, it was shown that laminin-directed signaling is not sufficient for β-casein induction, unless the cells possess the correct cell shape (34; for review see also 13).

Cell-cell adhesion in the mammary gland is mediated in part, although not exclusively, by adherens and tight junction proteins such as E cadherin, β-catenin, occludin and ZO-1, while basement membrane cues are transduced predominantly by the integrins α2/β1, α3/β1 and α6/β4, and their associated plaque proteins and cytoskeletal elements (37). The contribution of intercellular interactions to mammary cell phenotype can be studied by compromising E-cadherin function using either blocking antibodies (38) or dominant-negative E-cadherin expression constructs (39), incubating cells in a low calcium/EGTA containing medium (40), or employing single cell assays (41). Cells can also be grown in artificial microcarriers such as cytodex 3 or cultisphers, to enhance cell-cell interactions and maintain cell attachment, while reducing oxygen and nutrient gradients (42).

Mammary-specific tissue properties and cell phenotype can be studied further by manipulating the quality of cell-cell interactions and the nature of the tissue organization. For example, mechanical agitation and soft agar have been used to cluster mammary cells into disorganized aggregates to study branching morphogenesis (43), overexpression of the nonclassical homophillic adhesion molecule epCAM has helped clarify the role of cell-cell interactions in

mammary tumor invasion and growth (44), and released collagen I gels have shown integrin-specific effects of basement membrane on mammary morphogenesis and survival (45). Such manipulations have also been applied to demonstrate the formation of an organized, 3-D[3] acinus is necessary to repress TGFα expression and permit whey acidic milk protein expression in murine mammary cells (33 and refs. therein).

Adapting Biomatrix Assays to Study Mammary Cell Death in Culture

Experiments *in vivo* have that implied a basement membrane represses cell death in mammary cell (9). Yet it was results obtained using the 'differentiation' based mammary culture assays that provided the direct relationship between basement membrane-directed signaling and mammary survival (27,46–48). For example, in the absence of growth factors, primary and immortalized mammary cells remain viable when in contact with a laminin-rich basement membrane, yet undergo apoptosis on other matrices such as fibronectin or collagen I (27,47). The proof for involvement of endogenous basement membrane was provided by studies using murine mammary cells ectopically expressing the metalloproteinase stromeylsin-1, under an inducible promoter. When the activated proteinase was induced in growth-arrested and differentiated mammary cells, endogenous basement membrane was degraded leading to cell death (27).

Studies using biomatrix assays have suggested that adhesion-directed survival mechanisms that are linked to growth control may be distinct from those facilitating long-term survival in growth-arrested, differentiated mammary cells. For example, experiments in nondifferentiated kidney and endothelial cells showed that appropriate integrin ligation supports short-term survival, provided the cells exhibit a critical degree of spreading which is associated with actin stress fibers and focal adhesion-like structures (49, and refs. therein). In fact, cell spreading and integrin signaling appear necessary to support a growth response and maintain long-term viability in cultured nonmalignant cells (50, and refs. therein). Similar observations have also been made using cultured primary and immortalized human and murine mammary cells (40, 46,47,51). More recently cell rounding, which is associated with growth-arrest and cortical actin, was shown to lead directly to endothelial cell death, despite integrin ligation and cytokine stimulation (52). These results suggest adhesion-dependent growth and survival in nonmalignant cells probably requires integrin-directed actin stress fibers and focal adhesion-directed signaling (52, for review see 49). This argument is supported by studies that have shown cell-cell interactions and basement membrane ligation each enhance cell survival in growth-arrested cells, but only for short periods of time, unless focal adhesions, a degree of cell stretching and integrin signaling are present (40,50).

This conclusion however, does not explain cellular survival *in vivo*. For example, the majority of the mammary epithelium *in vivo* is growth-arrested and exhibits cortically organized actin, yet remains viable for extended periods of time. Moreover, focal adhesions are well-characterized structures found in fibroblasts and growing epithelial cells that are spread on rigid substrata in culture, and are essentially absent in mammary epithelium *in vivo* (unpublished observations). As such, we were interested in determining how cells maintain long-term survival in the mammary gland, in the absence of adhesion-directed stress fibers and detectable focal adhesions. (see Fig. 1) Since mammary cells *in vivo* are incorporated into complex organized tissue structures, we predict that their viability depends upon tissue-specific features peculiar to the organized mammary acini. Consistent with this argument, when murine mammary cells were cultured on a reconstituted basement membrane and allowed to form differentiated structures, they survived for extended periods of time, despite growth-arrest,

[3]Abbreviations: two-dimensiona l, (2-D); three-dimensional, (3-D); epidermal growth factor, (EGF); epidermal growth factor receptor, (EGFR); mitogen activated kinase, (MAPK).

cortically organized actin and the absence of cytokine stimulation, as well as discernible focal adhesions (51). We are currently investigating how this organized mammary tissue-like structure represses cell death.

The mechanism(s) whereby the basement membrane regulates mammary cell death either *in vivo* or in culture still remains poorly defined. Nevertheless some key pathways have been identified and characterized. For example, it was shown that mammary cell-matrix interactions repress mammary apoptosis in culture through β1-integrin signaling, and that α3-integrins mediate survival in basement membranes (20,27,46,47) whereas α2-integrins direct survival on collagen I (46,53). Analogous to cell death *in vivo* (involution), mammary apoptosis in culture is associated with bax expression (40,54), and can be prevented by bcl-2, both *in vivo* (55) and in culture (unpublished observations, N. Boudreau personal communication). Furthermore, a failure to repress ice caspase protein and gene expression was associated with a lack of sustained mammary survival in culture (51), as has been documented for the early stages of breast involution *in vivo* (9). However, a more detailed understanding of the underlying mechanisms for these observations awaits further study.

Tumor Progression and Cell Death

Altered adhesion-dependent growth and survival constitute a necessary step for malignant progression in the breast. Moreover, malignant mammary cells consistently exhibit alterations in the expression and organization of their basement membrane integrin receptors *in vivo* and in culture (11,56), and show perturbations in their growth, apoptosis and differentiation behavior in a reconstituted basement membrane culture assay (18). Whereas nonmalignant mammary cells die following loss of adhesion or treatment with a β1-integrin function-blocking antibody, many malignant mammary cells appear resistant to this treatment (20,46). Thus understanding the mechanism used by mammary tumor cells to bypass their basement membrane-adhesion dependence for survival constitutes an important question in tumor biology. The challenge is how to overcome the inherent difficulties involved in deriving definitive experimental conclusions when comparing normal and tumor cells from different origins.

We have been studying adhesion-linked growth and survival regulation in selected passages of the HMT-3522 tumor progression series (11,56, and refs. therein). In this model, loss of growth regulation, changes in morphogenesis and basement membrane-independent survival precede malignant transformation. For example, the nonmalignant cells which are epidermal growth factor (EGF)-dependent (lines S-1 50, 110, 175) from this series, all form organized, 3-D acini and arrest their growth in response to cues from a reconstituted basement membrane, yet the pre-malignant (S-2) and tumorigenic (T4-2) cells form continuously growing, disorganized colonies under the same circumstances (11,56). Concomitantly, these cells acquire the ability to survive blockage of β1-integrin-basement membrane interactions as they progress towards malignancy, such that the tumor progeny from this series do not die when their β1-integrin-basement membrane interactions are inhibited (20; Fig. 2, Weaver *et al.*, unpublished observations).

We asked whether the substantially increased expression and activity of β1-integrins in these tumor cells could account for their death resistance and if this was associated with their malignant behavior. We found that treatment of HMT-3522 tumor cells with β1-integrin function-blocking antibody, in the presence of a reconstituted basement membrane, led to profound morphological and behavioral changes in the cells including: re-assembly of a polarized basement membrane, reestablishment of E-cadherin-catenin complexes, reorganization of the actin and cytokeratin cytoskeletons, relocalization of α6/β4-integrins and growth-arrest (20). These are all features associated with a nonmalignant phenotype. Moreover these ‘reverted’ structures exhibited decreased tumorigenicity *in vivo*, which was reversible

upon removal of antibodies. Since the genotype of the tumor cells was constant these results show that tissue organization, directed by cell-matrix signaling, can act as a dominant regulator of tissue-specific gene expression, even in cells which are already tumorigenic. Furthermore, since these tumor cells do not die, but instead revert towards a nonmalignant phenotype, these results suggest a link between integrin signaling, death resistance and tissue structure.

The Link Between Growth and Adhesion in Mammary Cells

In exploring how inhibition with β1-integrin function-blocking antibodies led to growth-arrest and morphological reversion, we have recently demonstrated a reciprocal crosstalk between EGFR and β1-integrin cell-adhesion signaling in mammary cells via the mitogen-activated kinase (MAPK) pathway (21). Antibody-mediated inhibition of either the β1-integrin or EGFR receptor in the HMT-3522 tumor cells, or inhibition of MAPK kinase activation, induced a concomitant decrease in the expression of both receptors and a reduction in their downstream signaling. Proliferation was arrested and tissue morphology was restored with each treatment by itself, when the cells were polarized in the presence of a reconstituted basement membrane. In contrast, the effects were not observed when tumor cells were kept flat on tissue culture plastic, even in the presence of a reconstituted basement membrane, or when they were rounded, in the absence of a reconstituted basement membrane. Conversely, overexpression of EGFR in the nonmalignant cell line led to disruption of basement membrane-directed acinus formation. EGFR overexpression in turn produced a compensatory up-regulation of β1-integrin expression in the nonmalignant cells, but only when the cells were polarized and in contact with a reconstituted basement membrane and not on tissue culture plastic. Our results indicate that cell shape and the nature of the extracellular matrix can, and do, affect the cell's response to external stimuli. They further imply that both basement membrane signaling and changes in tissue architecture integrate to coordinate growth, adhesion and morphogenesis in mammary cells. These findings further suggest that valid culture models must recapitulate the normal *in vivo* cell shape, cell-cell interactions and polarity. (See Fig. 3.)

In conclusion, both the literature and our studies show that the use of appropriate model systems can reveal new principles of tissue biology. To understand signal transduction in epithelial tissue, we need to understand signal integration. Our results suggest that the tissue structure is perhaps the integrator of dynamic interactions that must continually exist between the microenvironment and the nucleus (57). An understanding of the molecular mechanisms mediating basement membrane-directed tissue organization should help elucidate how apoptosis is controlled in the mammary gland.

Acknowledgments

This work was supported by the U.S. Department of Energy, Office of Biological and Environmental Research (contract DE-AC03-76SF00098), by the National Institutes of Health (grants CA64786 and CA57621), and a University of California Breast Cancer Research Program (IFB-0400) to VMW.

References

1. Evan G, Littlewood T. A matter of life and cell death. Science 1998;281:1317–1322. [PubMed: 9721090]
2. Adams JM, Cory S. The Bcl-2 protein family: Arbiters of cell survival. Science 1998;281:1322–1326. [PubMed: 9735050]
3. Steller H. Mechanisms and genes of cellular suicide. Science 1995;267:1445–1449. [PubMed: 7878463]
4. Jacobson MD, Weil M, Raff MC. Programmed cell death in animal development. Cell 1997;88:347–354. [PubMed: 9039261]
5. Green DR, Reed JC. Mitochondria and apoptosis. Science 1998;281:1309–1312. [PubMed: 9721092]

6. Thornberry NA, Lazebnik Y. Caspases: Enemies within. Science 1998;281:1312–1316. [PubMed: 9721091]
7. Ashkenazi A, Dixit VM. Death receptors: Signaling and modulation. Science 1998;281:1305–1308. [PubMed: 9721089]
8. Schedin PJ, Thackray LB, Malone P, Fontaine SC, Friis RR, Strange R. Programmed cell death and mammary neoplasia. Cancer Treat Res 1996;83:3–22. [PubMed: 8826639]
9. Werb Z, Sympson CJ, Alexander CM, Thomasset N, Lund LR, MacAuley A, Ashkenas J, Bissell MJ. Extracellular matrix remodeling and the regulation of epithelial-stromal interactions during differentiation and involution. Kidney Int Suppl 1996;54:S68–74. [PubMed: 8731199]
10. Lelievre S, Weaver VM, Bissell MJ. Extracellular matrix signaling from the cellular membrane skeleton to the nuclear skeleton: A model of gene regulation. Recent Prog Horm Res 1996;51:417–432. [PubMed: 8701089]
11. Weaver VM, Fischer AH, Peterson OW, Bissell MJ. The importance of the microenvironment in breast cancer progression: Recapitulation of mammary tumorigenesis using a unique human mammary epithelial cell model and a three-dimensional culture assay. Biochem Cell Biol 1996;74:833–851. [PubMed: 9164652]
12. Stoker AW, Streuli CH, Martins-Green M, Bissell MJ. Designer microenvironments for the analysis of cell and tissue function. Curr Opin Cell Biol 1990;2:864–874. [PubMed: 2083086]
13. Roskelley CD, Bissell MJ. Dynamic reciprocity revisited: A continuous, bidirectional flow of information between cells and the extracellular matrix regulates mammary epithelial cell function. Biochem Cell Biol 1995;73:391–397. [PubMed: 8703411]
14. Bissell, MJ. The central role of basement membrane in functional differentiation, apoptosis, and cancer. In: Tilly, JL.; Strauss, JF., III; Tenniswood, M., editors. Cell Death in Reproductive Physiology. Springer-Verlag; New York: 1997. p. 125-140.
15. Ronnov-Jessen L, Petersen OW, Bissell MJ. Cellular changes involved in conversion of normal to malignant breast: Importance of the stromal reaction. Physiol Rev 1996;76:69–125. [PubMed: 8592733]
16. Kleinman HK, McGarvey ML, Hassell JR, Star VL, Cannon FB, Laurie GW, Martin GR. Basement membrane complexes with biological activity. Biochemistry 1986;25:312–318. [PubMed: 2937447]
17. Streuli CH, Schmidhauser C, Bailey N, Yurchenco P, Skubitz AP, Roskelley C, Bissell MJ. Laminin mediates tissue-specific gene expression in mammary epithelia. J Cell Biol 1995;129:591–603. [PubMed: 7730398]
18. Petersen OW, Ronnov-Jessen L, Howlett AR, Bissell MJ. Interaction with basement membrane serves to rapidly distinguish growth and differentiation pattern of normal and malignant human breast epithelial cells. Proc Natl Acad Sci USA 1992;89:9064–9068. [PubMed: 1384042]
19. Lochter A, Bissell MJ. Involvement of extracellular matrix constituents in breast cancer. Semin Cancer Biol 1995;6:165–173. [PubMed: 7495985]
20. Weaver VM, Petersen OW, Wang F, Larabell CA, Briand P, Damsky C, Bissell MJ. Reversion of the malignant phenotype of human breast cells in three-dimensional culture and *in vivo* by integrin blocking antibodies. J Cell Biol 1997;137:231–245. [PubMed: 9105051]
21. Wang F, Weaver VM, Petersen OW, Larabell CA, Dedhar S, Briand P, Lupu R, Bissell MJ. Reciprocal interactions between β1-integrin and EGF-R in three-dimensional basement membrane cultures: A new perspective in normal and malignant breast epithelial biology. Proc Natl Acad Sci USA 1998;95:14821–14826. [PubMed: 9843973]
22. Emerman JT, Pitelka DR. Maintenance and induction of morphological differentiation in dissociated mammary epithelium on floating collagen membranes. In Vitro 1977;13:316–328. [PubMed: 559643]
23. Gospodarowicz D, Lepine J, Massoglia S, Wood I. Comparison of the ability of basement membranes produced by corneal endothelial and mouse-derived Endodermal PF-HR-9 cells to support the proliferation and differentiation of bovine kidney tubule epithelial cells *in vitro*. J Cell Biol 1984;99:947–961. [PubMed: 6470046]
24. Xia Y, Gil SG, Carter WG. Anchorage mediated by integrin α6β4 to laminin 5 (epiligrin) regulates tyrosine phosphorylation of a membrane-associated 80-kD protein. J Cell Biol 1996;132:727–740. [PubMed: 8647901]

25. Ingber DE, Madri JA, Jamieson JD. Basement membrane as a spatial organizer of polarized epithelia. Exogenous basement membrane reorients pancreatic epithelial tumor cells *in vitro*. Am J Pathol 1986;122:129–139. [PubMed: 3942197]
26. Livant DL, Linn S, Markwart S, Shuster J. Invasion of selectively permeable sea urchin embryo basement membranes by metastatic tumor cells, but not by their normal counterparts. Cancer Res 1995;55:5085–5093. [PubMed: 7585556]
27. Boudreau N, Sympson CJ, Werb Z, Bissell MJ. Suppression of ICE and apoptosis in mammary epithelial cells by extracellular matrix. Science 1995;267:891–893. [PubMed: 7531366]
28. Clarke EA, Brugge JS. Integrins and signal transduction pathways: The road taken. Science 1995;268:233–239. [PubMed: 7716514]
29. Shattil SJ, Ginsberg MH, Brugge JS. Adhesive signaling in platelets. Curr Opin Cell Biol 1994;6:695–704. [PubMed: 7530462]
30. Haas TA, Plow EF. Integrin-ligand interactions: A year in review. Curr Opin Cell Biol 1994;6:656–662. [PubMed: 7833046]
31. Takada Y, Puzon W. Identification of a regulatory region of integrin β1 subunit using activating and inhibiting antibodies. J Biol Chem 1993;268:17597–17601. [PubMed: 7688727]
32. Yamada KM, Miyamoto S. Integrin transmembrane signaling and cytoskeletal control. Curr Opin Cell Biol 1995;7:681–689. [PubMed: 8573343]
33. Lin CQ, Bissell MJ. Multi-faceted regulation of cell differentiation by extracellular matrix. FASEB J 1993;7:737–743. [PubMed: 8330681]
34. Close MJ, Howlett AR, Roskelley CD, Desprez PY, Bailey N, Rowning B, Teng CT, Stampfer MR, Yaswen P. Lactoferrin expression in mammary epithelial cells is mediated by changes in cell shape and actin cytoskeleton. J Cell Sci 1997;110:2861–2871. [PubMed: 9427294]
35. Roskelley CD, Desprez PY, Bissell MJ. Extra-cellular matrix-dependent tissue-specific gene expression in mammary epithelial cells requires both physical and biochemical signal transduction. Proc Natl Acad Sci USA 1994;91:12378–12382. [PubMed: 7528920]
36. Chen LH, Bissell MJ. A novel regulatory mechanism for whey acidic protein gene expression. Cell Regul 1989;1:45–54. [PubMed: 2519617]
37. Alford D, Taylor-Papadimitriou P. Adhesion molecules in normal and cancerous mammary gland. J Mamm Gland Biol Neoplasia 1996;1:207–218.
38. Daniel CW, Strickland P, Friedmann Y. Expression and functional role of E- and P-cadherins in mouse mammary ductal morphogenesis and growth. Dev Biol 1995;69:511–519. [PubMed: 7781895]
39. Zhu AJ, Watt FM. Expression of a dominant negative cadherin mutant inhibits proliferation and stimulates terminal differentiation of human epidermal keratinocytes. J Cell Sci 1996;109:3013–3023. [PubMed: 9004036]
40. Merlo GR, Cella N, Hynes NE. Apoptosis is accompanied by changes in Bcl-2 and Bax expression, induced by loss of attachment, and inhibited by specific extracellular matrix proteins in mammary epithelial cells. Cell Growth Differ 1997;8:251–260. [PubMed: 9040947]
41. Streuli CH, Bailey N, Bissell MJ. Control of mammary epithelial differentiation: Basement membrane induces tissue-specific gene expression in the absence of cell-cell interaction and morphological polarity. J Cell Biol 1991;115:1383–1395. [PubMed: 1955479]
42. Kmicikiewicz I, Koziel E, Pelczar U, Pierscinski A, Bilinska B. Mouse Leydig cell culture on microcarriers. Acta Histochem 1998;100:297–307. [PubMed: 9717567]
43. Hirai Y, Lochter A, Galosy S, Koshida S, Niwa S, Bissell MJ. Epimorphin functions as a key morphoregulator for mammary epithelial cells. J Cell Biol 1998;140:159–169. [PubMed: 9425164]
44. Litvinov SV, Velders MP, Bakker HA, Fleuren GJ, Warnaar SO. Ep-CAM: A human epithelial antigen is a homophilic cell-cell adhesion molecule. J Cell Biol 1994;125:437–446. [PubMed: 8163559]
45. Berdichevsky F, Alford D, D'Souza B, Taylor-Papadimitriou J. Branching morphogenesis of human mammary epithelial cells in collagen gels. J Cell Sci 1994;107:3557–3568. [PubMed: 7535787]
46. Howlett AR, Bailey N, Damsky C, Petersen OW, Bissell MJ. Cellular growth and survival are mediated by β1 integrins in normal human breast epithelium but not in breast carcinoma. J Cell Sci 1995;108:1945–1957. [PubMed: 7544798]

47. Pullan S, Wilson J, Metcalfe A, Edwards GM, Goberdhan N, Tilly J, Hickman JA, Dive C, Streuli CH. Requirement of basement membrane for the suppression of programmed cell death in mammary epithelium. J Cell Sci 1996;109:631–642. [PubMed: 8907708]

48. Streuli CH, Gilmore AP. Adhesion-medal regarding in the regulation of mammary epithelial cell-survival. J Mam Gland Biol Neoplasia 1999;4:183–191.

49. Frisch SM, Ruoslahti E. Integrins and anoikis. Curr Opin Cell Biol 1997;9:701–706. [PubMed: 9330874]

50. Schwartz MA. Integrins, oncogenes, and anchorage independence. J Cell Biol 1997;139:575–578. [PubMed: 9348275]

51. Boudreau N, Werb Z, Bissell MJ. Suppression of apoptosis by basement membrane requires three-dimensional tissue organization and withdrawal from the cell cycle. Proc Natl Acad Sci USA 1996;93:3509–3513. [PubMed: 8622967]

52. Chen CS, Mrksich M, Huang S, Whitesides GM, Ingber DE. Geometric control of cell life and death. Science 1997;276:1425–1428. [PubMed: 9162012]

53. Alford D, Baeckstrom D, Geyp M, Pitha P, Taylor-Papadimitriou P. Integrin-matrix interactions affect the form of the structures developing from human mammary epithelial cells in collagen or fibrin gels. J Cell Sci 1998;111:521–532. [PubMed: 9443900]

54. Schorr K, Li M, Krajewski S, Reed JC, Furth PA. *Bcl-2* gene family and related proteins in mammary gland involution and breast cancer. J Mam Gland Biol Neoplasia 1999;4:153–164.

55. Jager R, Herzer U, Schenkel J, Weiher H. Overexpression of Bcl-2 inhibits alveolar cell apoptosis during involution and accelerates c-*myc*-induced tumorigenesis of the mammary gland in transgenic mice. Oncogene 1997;15:1787–1795. [PubMed: 9362445]

56. Petersen OW, Ronnov-Jessen L, Weaver VM, Bissell MJ. Differentiation and cancer in the mammary gland: Shedding light on an old dichotomy. Adv Cancer Res 1998;75:135–161. [PubMed: 9709809]

57. Lelièvre SA, Weaver VM, Nickerson JA, Larabell CA, Bhaumik A, Petersen OW, Bissell MJ. Tissue phenotype depends on reciprocal interactions between the extracellular matrix and the structural organization of the nucleus. Proc Natl Acad Sci USA 1998;95:14711–14716. [PubMed: 9843954]

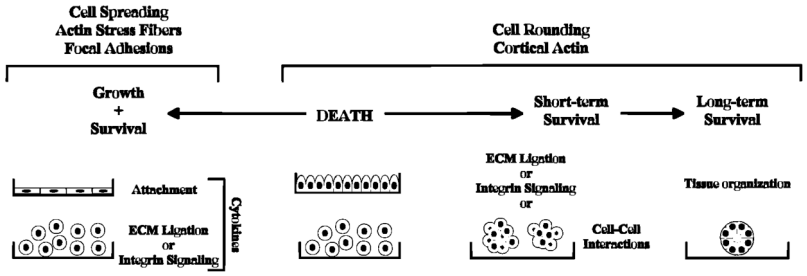

Fig. 1.

An organized tissue structure is required to mediate long-term mammary cell survival. Matrix attachment and integrin signaling are critical for mammary cell growth, differentiation and survival. Cells that are attached and ligated to a rigid matrix, or cells which maintain activated integrin signaling remain viable and could grow for extended periods of time in the presence of cytokines. Alternatively, cell-cell interactions, integrin ligation or matrix attachment each will support short-term survival in nongrowing mammary cells. However, long-term survival in growth-arrested mammary cells is critically dependent upon the formation of a basement membrane-directed, acinar structure.

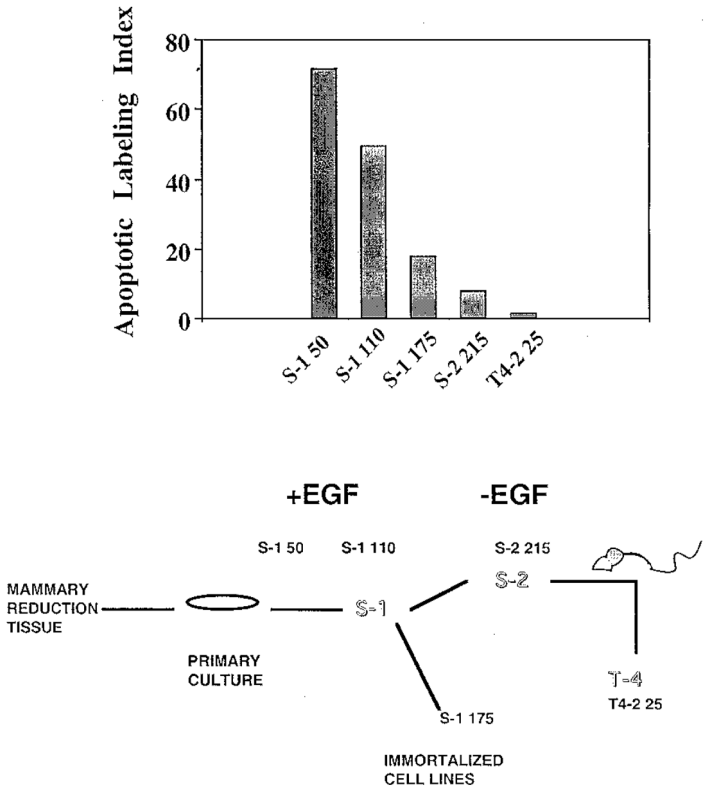

Fig. 2.

β_1-integrin-survival dependence in the HMT-3522 human mammary cell tumor progression series is lost upon malignant transformation. The HMT-3522 human mammary tumor cell series was established from a luminal epithelial cell population, isolated from mammary reduction mammoplasty. Following removal of epidermal growth factor, and prolonged culturing in defined media, these cells gave rise to tumors when injected into nude mice (for discussion of HMT-3522 tumor progression series see 11 and 54; see bottom for schemata). Alterations in cell-extracellular matrix responsiveness (11) and cell survival dependence (top) precede malignant transformation in this model. When these cells are grown within a reconstituted basement membrane in the presence of a β1-integrin function-blocking antibody (AIIB2; 20), greater than 75 percent of the epidermal growth factor-dependent passage S1-50 cells died within four days of culturing. The percent cell death induction by this treatment was 47 for the S-1 110, 17 for the S-1 175, 9 for the S-2 215 cells and less than 5 for the tumor cells (top, Weaver *et al.*, unpublished results). Thus as the cells advance towards malignancy, they become progressively independent of basement membrane-β1-integrin-interactions for growth and survival.

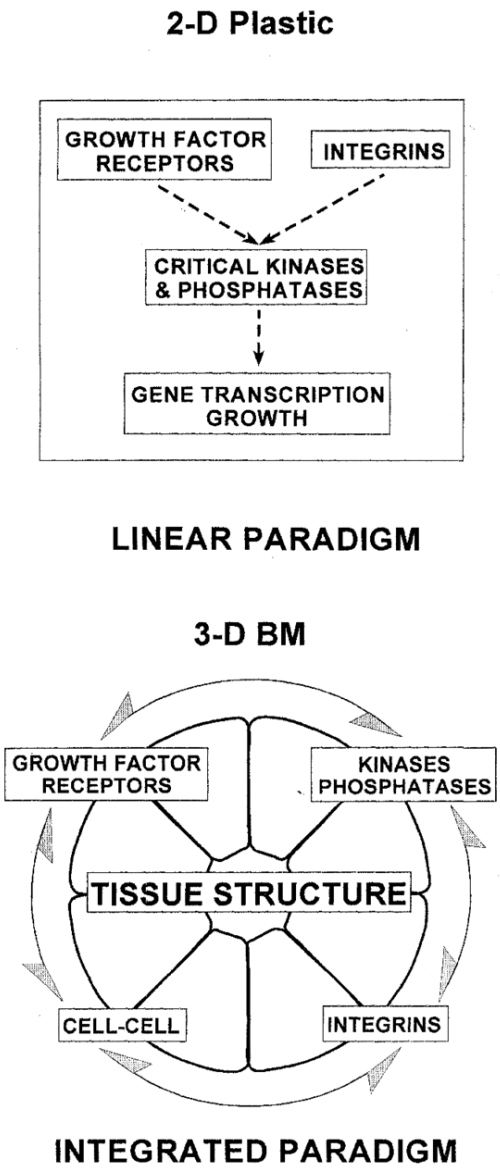

Fig. 3.

Basement membrane and three-dimensional spatial organization direct integration of growth and adhesion pathways in mammary epithelial cells. Cell shape, cell-cell interactions and the nature and context of the extracellular matrix, affect the manner in which mammary epithelial cells sense and respond to external stimuli. When cells are spread and grown as monolayers on various inert substrata, their adhesion and growth pathways are depicted as linear processes (for review see 49). Following the formation of basement membrane-directed acini however, mammary cells respond to either growth or adhesion signals as an integrated unit. This unified response is characterized by a significant degree of reciprocal cross-modulation between the various signaling pathways (21).