Commentary

New highlights on stroma–epithelial interactions in breast cancer

Mary Helen Barcellos-Hoff[1] and Daniel Medina[2]

[1]Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, California, USA
[2]Department of Molecular and Cellular Biology, Baylor School of Medicine, Houston, Texas, USA

Corresponding author: Mary Helen Barcellos-Hoff, mhbarcellos-hoff@lbl.gov

Published: 25 November 2004

Breast Cancer Res 2005, **7**:33-36 (DOI 10.1186/bcr972)
© 2004 BioMed Central Ltd

Abstract

Although the stroma in which carcinomas arise has been previously regarded as a bystander to the clonal expansion and acquisition of malignant characteristics of tumor cells, it is now generally acknowledged that stromal changes are required for the establishment of cancer. In the present article, we discuss three recent publications that highlight the complex role the stroma has during the development of cancer and the potential for targeting the stroma by therapeutic approaches.

Keywords: breast, carcinogenesis, chemical carcinogen, radiation, stroma, transforming growth factor beta 1

Introduction

Epithelial–stromal interactions, mediated by the extracellular matrix, play a pivotal role in normal mammary gland function [1]. These interactions, along with epithelial–epithelial interactions, actively suppress the expression of the preneoplastic phenotype in epithelial cells [2–7]. It is now recognized that a specific environment is necessary for tumorigenesis; indeed, it has been postulated that cancer can be a physiological response to an abnormal stromal environment (reviewed in [8–11]).

An abnormal stroma can be regarded as a classical promoter in the terminology of carcinogenesis, in that the dysfunction of normal epithelial–mesenchymal interactions increases the probability that the preneoplastic lesion will progress to malignancy. On the other hand, in the terminology of development, the environment provided by the abnormal stroma may be considered 'permissive' for tumorigenesis by leading to the selection of cells with altered survival characteristics.

Both these views signify that an aberrant stroma predisposes tissue to cancer by increasing the frequency with which an initiated cell proceeds to neoplasia, rather than by increasing the frequency of initiation. Although few studies have attempted to define the particular point at which the stroma actively participates in multistep carcinogenesis, several important concepts have been put forward [12–14]. Importantly, normal stroma can efficiently inhibit the expression of neoplastic characteristics of tumor cells. Conversely, perturbations in the epithelial–stromal interaction may accelerate the process of carcinogenesis, especially since carcinogen exposure elicits persistent phenotypic changes in stromal cells. These non-neoplastic stromal effects induced by carcinogens can be conducive to the expression of or to the progression of preneoplastic phenotypes, just as carcinoma-associated fibroblasts support malignant behaviors. Together stromal alterations are likely to be essential to the development of frank, neoplastic disease. Understanding them requires additional experimental models.

Among the many mediators of stromal–epithelial interactions, the extracellular cytokine transforming growth factor beta 1 (TGF-β) is perhaps one of the best studied, and most perplexing. TGF-β has been widely implicated in mammary epithelial growth (reviewed in [15]), in cancer (reviewed in [16]), and in response to estrogen and progesterone in breast cancer cells and the mammary gland (reviewed respectively in [17,18]). As such, TGF-β is a focus of research highlighted in these recent reports.

HGF = hepatocyte growth factor; TGF-β = transforming growth factor beta 1.

Recent studies in stromal–epithelial interactions

Several noteworthy studies of stromal effects on carcinogenesis have recently appeared in the literature. First, Moses and colleagues have shown that TGF-β signaling in fibroblasts modulates the growth and oncogenesis of adjacent epithelia [19]. These studies used conditional inactivation of the TGF-β type II receptor gene in fibroblasts (Tgfβr2fspKO mice), which rapidly led to development of intraepithelial neoplasia in prostate and invasive squamous cell carcinoma of the forestomach, both accompanied by increased abundance of stromal cells. The authors suggest that this is due in part to the dysregulation of hepatocyte growth factor (HGF). The TGF-β signaling pathway, whether activated in epithelial cells or in adjacent stromal fibroblasts, therefore does have an inhibitory effect on epithelial proliferation. Loss of this inhibitory pathway may facilitate progression to invasive carcinoma in some tissues. The question of the initiating events in the neoplasms and the growth autonomy of the neoplasms were not addressed in these studies.

Second, an orthotopic xenograft breast model that uses human fibroblasts to condition the mouse mammary gland so that it supports growth of primary human breast organoids was introduced by the Weinberg laboratory [20]. Similar to the experiments of Parmar and colleagues that demonstrated a novel method using reconstituted mammary epithelium and mouse-derived or human-derived fibroblasts in collagen gels as the transplanted material for examining the growth of human mammary epithelium in nude mice [21], these models provide another approach to understanding important factors that play a regulatory role in both normal and neoplastic development. The 'humanized' fat pads provided the necessary microenvironment to promote outgrowths of remarkably differentiated epithelial structures capable of at least rudimentary secretory differentiation. The specific cellular components of human stroma, in contrast to what is contained in mouse stroma, therefore provided an essential signal for growth and differentiation of normal epithelial behavior. This model is an important addition to the arsenal of the experimentalist since it provides a means of recreating the critical physiological environment, albeit in a mouse host, for human breast epithelium in the context of at least a partial human stroma. Future models in which the humanized fat pad is generated in the context of a humanized bone marrow could further species-specific interactions.

Equally notable in this study was that perturbation of stromal signaling by human fibroblasts engineered to overexpress active TGF-β or HGF promoted the development of histologically malignant lesions from ostensibly 'normal' human mammary epithelial cells. The behavior of this one specimen is consistent with the interpretation that this specimen contained occult initiated cells. The different effect of TGF-β signaling on truly normal cells versus initiated cells observed in this study speaks to the stage-specific effects reported in both human and mouse mammary models of progression [22,23].

Where and when TGF-β is active in the mammary gland is still fairly undefined due to multiple controls on its bioavailability. In classic experiments by Silberstein and Daniel to define the effects of TGF-β on the mammary gland, pellets containing TGF-β were implanted in the highly proliferative pubertal mouse mammary gland. This implantation resulted in regression of the terminal endbuds [24]. Interestingly, similar experiments during early pregnancy showed little effect on the proliferating alveolar epithelium. Two possibilities may explain these differential effects. First, the epithelium is refractory to TGF-β during pregnancy. However, recent studies indicate that the epithelium is responsive to TGF-β during this period since its depletion results in significantly increased proliferation in the *Tgfβ1* heterozygote mammary gland [25]. In this case, there might exist a maximum threshold for TGF-β function – thus, added TGF-β (supplied by the implant) would have no discernible effect. Alternatively, it may be that the effect of exogenous TGF-β during puberty is mediated via the stroma. A recent study using the same implant experimental design in prepubertal heifers provides support for this possibility. Short-term TGF-β exposure (<24 hours) resulted in increased stromal proliferation and fibronectin mRNA abundance, without affecting epithelial proliferation [26].

The experiments in humanized mammary gland, in which fibroblasts producing TGF-β or HGF promoted malignant progression in susceptible epithelium [20], also indicate that stromal TGF-β acting on the epithelium in a paracrine fashion may have very different effects on the epithelium than autocrine sources (i.e. TGF-β-producing fibroblasts stimulated proliferation rather than inhibited proliferation). However, this differential effect may also be a function of the developmental or premalignant state of the epithelial cells. Consistent with this, loss of mammary stroma responsiveness to TGF-β via expression of a dominant-negative receptor resulted in increased mammary epithelial branching and expression of HGF [27]. These studies indicate that the tissue compartment source of TGF-β is a significant factor in assessing how TGF-β may contribute to breast composition and breast cancer risk.

A third study from the laboratories of Soto and Sonnenschein suggest that the stroma is one of the targets of the chemical carcinogen *N*-methylynitrosurea in the rat mammary gland [28]. As shown with ionizing radiation [29], exposing the rat mammary stroma to *N*-methylynitrosurea promoted tumorigenesis of mammary

epithelial cells that were not treated with the chemical carcinogen. Interestingly, a similar but not identical experiment using 7,12-dimethylbenzanthracene-treated mouse mammary stroma did not promote tumorigenesis of untreated TM10 preneoplastic mouse mammary outgrowths [30]. In the case of ionizing radiation the cell line COMMA-1D was used because it could either generate a normal epithelial outgrowth or produce small tumors in a normal stroma [31]. In the irradiated stroma, the cells produced predominantly tumors that were quite large and fast growing, indicating that radiation alters the stroma in a manner that promoted the neoplastic potential. Notably, TGF-β activation is induced by ionizing radiation [32]. Differences between carcinogen action and the effect on stroma and the host, in addition to differences in the 'initiated' populations in each of these models, may thus account for different outcomes, and remain to be resolved. However, these studies emphasize that the interactions between the epithelium and the stroma evolve dynamically, and thus will require considerable study if they are to be manipulated therapeutically.

Indeed, the mouse mammary gland is well suited for such studies because of the ease of recombining the epithelium and the stroma, and because of the availability of a wide variety of engineered mouse models. For example, Jessani and colleagues have recently used functional proteomic techniques to show that the *in vivo* environment of the mouse mammary fat pad cultivates the growth of human breast cancer cells with distinct molecular and cellular properties [33]. Man and colleagues examined the role of a specific transcription factor, ets2, in the stroma using transplantation of initiated cells into mammary fat pads of mice that had altered ets2 activity [34]. Tumor growth was decreased relative to the stroma of wild-type mice, which was associated with increased p21Cip1 expression and with decreased MMP-3 and MMP-9 mRNA expression.

Future research targeting stroma therapeutically

Together, these recent studies underscore that the stromal–epithelial interface is a critical mediator of oncogenic potential. The heightened awareness of the stroma as an active participant in carcinogenesis has led to ideas for intervening in breast cancer progression by manipulating the stroma [35–38]. Compared with the multiple routes taken by cells to become cancers, the response of tissues to cancer is relatively predictable. Controlling the early stages of invasive cancer growth may therefore be more readily achieved indirectly via the stroma.

As cancer progresses, it is clear that normal cells are recruited by the tumor and are subverted in a manner that warps the phenotype, sometimes resulting in persistent phenotypic change (e.g. myofibroblasts, tumor endothelium). In this scenario, the therapeutic potential lies in the juxtaposition of novel events that may lead to novel targets, such as the revelation of cryptic epitopes, fetal protein forms, or matrikines [39]. Some stromal targets (e.g. the induction of proteases, growth factors and extracellular matrix proteins) are a reaction to the disruption of tissue architecture by malignant cells, in a manner akin to 'the wound that does not heal' [40]. Since normal cells have a restricted repertoire of possible responses, it is likely that there will be common events that underpin the production of tumors that can be widely targeted. Angiogenesis inhibitors are an excellent example of the type of target that may be most suitable for manipulation early in carcinogenesis, or as a chemopreventative strategy [41]. Viewing the opportunities for inhibiting cancer progression as a dynamic process, in which tumor control may be mediated by more than cell kill as a consequence of single and multiple anticancer agent exposures, may uncover additional tissue processes that are susceptible to intervention.

To exploit these possibilities, we need to better understand the dynamic interactions between the epithelium and the stroma, and between cancer and the stroma, which in turn will help define the windows of opportunity in the stroma for cancer suppression and repression. The recent development of stroma-specific promoters for use in genetically engineered mouse mammary models and the humanized mouse stroma model provide additional experimental manipulations to define these interactions. The recent publications in this area are provocative evidence that manipulating stroma may be of benefit in cancer treatment.

Competing interests

The author(s) declare that they have no competing interests.

Acknowledgments

This publication was made possible by grant number U01 ES012801 from the National Institute of Environmental Health Sciences, NIH and the National Cancer Institute, NIH (MHBH).

References

1. Bissell MJ, Barcellos-Hoff MH: **The influence of extracellular matrix on gene expression: is structure the message?** *J Cell Sci* 1987, **8:**327-343.
2. Decosse JJ, Gossens CL, Kuzma JF, Unsworth D: **Breast cancer: induction of differentiation by embryonic tissue**. *Science* 1973, **181:**1057-1058.
3. Cooper M, Pinkus H: **Intrauterine transplantation of rat basal cell carcinoma as a model for reconversion of malignant to benign growth.** *Cancer Res* 1977, **37:**2544-2552.
4. Fujii H, Cunha GR, Norman JT: **The induction of adenocarinomatous differentiation in neoplastic bladder epithelium by an embryonic prostatic inducer.** *J Urol* 1982, **128:**858-861.
5. Kamiya K, Yasukawa-Barnes J, Mitchen JM, Gould MN, Clifton KH: **Evidence that carcinogenesis involves an imbalance between epigenetic high-frequency initiation and suppression of promotion.** *Proc Natl Acad Sci USA* 1995, **92:**1332-1336.
6. Medina D, Shepherd F, Gropp T: **Enhancement of the tumorigenicity of preneoplastic mammary nodule lines by enzymatic dissociation.** *J Natl Cancer Inst* 1978, **60:**1121-1126.

7. DeOme KB, Miyamoto MJ, Osborn RC, Guzman RC, Lum K: **Detection of inapparent nodule transformed cells in the mammary gland tissues of virgin female BALB/cfC3H mice.** *Cancer Res* 1978, **38:**2103-2111.
8. Farber E: **Pre-cancerous steps in carcinogenesis. Their physiological adaptive nature.** *Biochem Biophys Acta* 1984, **738:** 171-180.
9. Barcellos-Hoff MH: **The potential influence of radiation-induced microenvironments in neoplastic progression**. *J Mammary Gland Biol Neoplasia* 1998, **3:**165-175.
10. Bissell MJ, Radisky D: **Putting tumours in context.** *Nat Rev Cancer* 2001, **1:**1-11.
11. Tlsty TD: **Cell-adhesion-dependent influences on genomic instability and carcinogenesis.** *Curr Opin Cell Biol* 1998, **10:** 647-653.
12. Farber E: **The multistep nature of cancer development.** *Cancer Res* 1984, **44:**4217-4223.
13. van den Hoof A: **Stromal involvement in malignant growth.** *Adv Cancer Res* 1988, **50:**159-196.
14. Barcellos-Hoff MH: **It takes a tissue to make a tumor: epigenetics, cancer and the microenvironment.** *J Mammary Gland Biol Neoplasia* 2001, **6:**213-221.
15. Daniel CW, Robinson SD: **Regulation of mammary growth and function by TGF-β.** *Mol Reprod Dev* 1992, **32:**145-151.
16. Derynck R, Ackhurst RJ, Balmain A: **TGF-β signaling in tumor suppression and cancer progression.** *Nat Genet* 2001, **29:**117-129.
17. Wakefield L, Colletta AA, McCune BK, Sporn MB: **Roles for transforming growth factors-β in the genesis, prevention and treatment of breast cancer.** In *Genes, Oncogenes, and Hormones: Advances in Cellular and Molecular Biology of Breast Cancer*. Edited by RB Dickson, ME Lippman. Boston: Kluwer Academic Publishers; 1991:97-136.
18. Barcellos-Hoff MH, Ewan KB: **TGF-β and mammary gland development.** *Breast Cancer Res* 2000, **2:**92-100.
19. Bhowmick NA, Chytil A, Plieth D, Gorska AE, Dumont N, Shappell S, Washington MK, Neilson EG, Moses HL: **TGF-β signaling in fibroblasts modulates the oncogenic potential of adjacent epithelia.** *Science* 2004, **303:**848-851.
20. Kuperwasser C, Chavarria T, Wu M, Magrane G, Gray JW, Carey L, Richardson A, Weinberg RA: **From the cover: reconstruction of functionally normal and malignant human breast tissues in mice.** *Proc Natl Acad Sci USA* 2004, **101:**4966-4971.
21. Parmar H, Young P, Emerman JT, Neve RM, Dairkee S, Cunha GR: **A novel method for growing human breast epithelium in vivo using mouse and human mammary fibroblasts.** *Endocrinology* 2002, **143:**4886-4896.
22. Roberts AB, Wakefield L: **TGF-beta signaling: positive and negative effects on tumorigenesis.** *Curr Opin Genet Dev* 2002, **12:** 22-29.
23. Dumont N, Arteaga CL: **Targeting the TGF beta signaling network in human neoplasia.** *Cancer Cell* 2003, **3:**531-536.
24. Silberstein GB, Daniel CW: **Reversible inhibition of mammary gland growth by transforming growth factor-β.** *Science* 1987, **237:**291-293.
25. Ewan KB, Shyamala G, Ravani SA, Tang Y, Akhurst RJ, Wakefield L, Barcellos-Hoff MH: **Latent TGF-β activation in mammary gland: regulation by ovarian hormones affects ductal and alveolar proliferation**. *Am J Pathol* 2002, **160:**2081-2093.
26. Musters S, Coughlan K, McFadden T, Maple R, Mulvey T, Plaut K: **Exogenous TGF-beta1 promotes stromal development in the heifer mammary gland.** *J Dairy Sci* 2004, **87:**896-904.
27. Joseph H, Gorska AE, Sohn P, Moses HL, Serra R: **Overexpression of a kinase-deficient transforming growth factor-β type II receptor in mouse mammary stroma results in increased epithelial branching.** *Mol Biol Cell* 1999, **10:**1221-1234.
28. Maffini MV, Soto AM, Calabro JM, Ucci AA, Sonnenschein C: **The stroma as a crucial target in rat mammary gland carcinogenesis.** *J Cell Sci* 2004, **117:**1495-1502.
29. Barcellos-Hoff MH, Ravani SA: **Irradiated mammary gland stroma promotes the expression of tumorigenic potential by unirradiated epithelial cells.** *Cancer Res* 2000, **60:**1254-1260.
30. Medina D, Kittrell FS: **Stroma is not a major target in 7,12-dimethlybenzanthracene mediated tumorigenesis of mouse mammary preneoplasia.** *J Cell Sci* in press.
31. Campbell SM, Taha MM, Medina D, Rosen JM: **A clonal derivative of mammary epithelial cell line COMMA-D retains stem cell characteristics of unique morphological and functional heterogeneity.** *Exp Cell Res* 1988, **177:**109-121.
32. Barcellos-Hoff MH, Derynck R, Tsang ML-S, Weatherbee JA: **Transforming growth factor-β activation in irradiated murine mammary gland.** *J Clin Invest* 1994, **93:**892-899.
33. Jessani N, Humphrey M, McDonald WH, Niessen S, Masuda K, Gangadharan B, Yates JR, III, Mueller BM, Cravatt BF: **Carcinoma and stromal enzyme activity profiles associated with breast tumor growth in vivo.** *Proc Natl Acad Sci USA* 2004, **101:** 13756-13761.
34. Man AK, Young LJ, Tynan JA, Lesperance J, Egeblad M, Werb Z, Hauser CA, Muller WJ, Cardiff RD, Oshima RG: **Ets2-dependent stromal regulation of mouse mammary tumors.** *Mol Cell Biol* 2003, **23:**8614-8625.
35. Folkman J, Hahnfeldt P, Hlatky L: **Cancer: looking outside the genome.** *Nat Rev Mol Cell Biol* 2000, **1:**76-79.
36. Wiseman BS, Werb Z: **Stromal effects on mammary gland development and breast cancer.** *Science* 2002, **296:**1046-1049.
37. Coussens LM, Werb Z: **Inflammatory cells and cancer: think different!** *J Exp Med* 2001, **193:**23F-26F.
38. Erickson AC, Barcellos-Hoff MH: **The not-so innocent bystander: microenvironment as a target of cancer therapy.** *Expert Opin Ther Targets* 2003, **7:**71-88.
39. Schor S, Schor A: **Phenotypic and genetic alterations in mammary stroma: implications for tumour progression.** *Breast Cancer Res* 2001, **3:**373-379.
40. Dvorak HF: **Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing.** *N Engl J Med* 1986, **315:**1650-1659.
41. Kerbel R, Folkman J: **Clinical translation of angiogenesis inhibitors.** *Nat Rev Cancer* 2002, **2:**727-739.