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TITLE: Mammary Stromal Effects on Epithelial Differentiation and Expression of ESX and ErbB2 (HER2/neu)

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13. ABSTRACT *(Maximum 200 Words)*

A novel system for studying growth of normal human mammary epithelium in vivo as grafts in athymic nude mice has been developed. The key feature of this model is the reconstitution of the epithelial-stromal interactions required for normal growth and differentiation of the human mammary epithelium, which produces ducts that are comparable to the normal human mammary gland. Human breast epithelial organoids were combined with mammary fibroblasts from mouse or human origin in collagen gels, which were subsequently transplanted under the renal capsule of female nude mice hosts. The resulting grafts showed an increase in the ductal density than observed previously. These ducts expressed appropriate markers for luminal and myoepithelial cells and steroid receptors. This model allows for a variety of epithelial and stromal cells to be used in combination, which would aid in understanding key factors that regulate normal human mammary gland development. The manuscript detailing this data has been accepted for publication in the Decemeber 2002 issue of Endocrinology. The experiments currently in progress utilize both normal and tumor epithelium and carcinoma associated fibroblasts (CAF) in this model to assess the effect of CAF on the development of normal human mammary epithelium. CAF from a variety of tumors including ErbB2 positive tumors will be used.

14. SUBJECT TERMS
breast cancer, mammary, epithelial-stromal interactions, renal grafting

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INTRODUCTION:

The main focus of this research is studying the epithelial-stromal interactions that take place in the normal and the tumor mammary gland. To aid in this study we have developed a new model to study human mammary gland development in vivo using organoids from reduction mammoplasty specimens combined with fibroblasts from either the mouse or the human mammary gland. The recombinations are placed in a collagen gel, which is then grafted under the renal capsule of female nude mice. Using this strategy has resulted in ductal development resembling the normal human mammary gland. This model is now being used to assess the effect of using tumor fibroblasts in combination with the organoids to test whether they cause abnormal ductal development. Preliminary data suggests that the CAF does have an effect on normal development. This model provides a very powerful method in which to study normal mammary gland development and to study the changes that initiate tumor formation.

BODY:

Technical objective 1: Profile ESX expression in normal developmentally staged human breast epithelium.

Using both in situ hybridization and immunohistochemistry, ESX expression was determined in the sections of human breast epithelium. The expression was found in the epithelial cells of the ducts but work is underway to produce an antibody that can show more specific staining with less background. I have collaborated on a project studying the promoter region of the ESX transcription factor to define which regions are involved in the initiation of cancer using breast cancer cell lines. Clones were made in which regions of the ESX promoter were mutated and transfected into breast cancer cell lines. A manuscript is in preparation with this data (Neve et al., 2002 manuscript in preparation).

Technical objective 2.1: confirm that embryonic mammary mesenchyme is capable of inducing a mammary specific pattern of ESX expression.

Embryonic mammary mesenchyme and mammary epithelium was recombined and placed under the renal capsule of female nude mice (Cunha et al., 1995) where it developed into phenotypically normal mouse mammary gland. The gland was removed at various stages throughout the reproductive cycle; virgin, mid pregnant, late pregnant, lactation and involution. Tissues have been fixed and embedded in paraffin for sectioning and analyzing once the ESX antibody staining has been perfected.

Technical objective 2.2: testing the correlation between stromal age and tumor induction on epithelial ESX and ErbB2 expression.

Work has been initiated for age related mammary development. I have obtained organoids from reduction mammoplasty specimens (from J. Emerman) from women whose ages range from 20-50. These samples will be combined with mammary fibroblasts of a known and consistent passage number. To test the effects of the aged stroma, mammary fibroblasts have been cultured in vitro for up to 20+ generations (towards Hayflick limit and senescence). These have already been recombined with organoids and initial results show abnormal development.

Technical objective 2.3: Employ ESX-null epithelium to demonstrate its function in mesenchyme-induced mammary gland development.

We have attempted to create ESX null mutants using pronuclear microinjection methodology. The results have not been successful so far but a new strategy is being utilized in collaboration with other groups.

Technical objective 3: Demonstrate that the abnormal stromal microenvironment provided by CAF perturbs ESX and/or ErbB2 induction in mammary epithelial cells differently from that of heterotypic stromas; in particular, determine if CAF from ErbB2 tumors can produce and exaggerated induction in non-malignant mammary epithelial cells.

To address this question, a novel model of growing human mammary epithelium in vivo was developed (Parmar et al., 2002. In press). This model allows the epithelial -stromal interactions to be studied which is advancement over previous models (Yang et al.,1994). Human organoids from reduction mammoplasty were combined with 250,000 mammary fibroblasts from either mouse or human origin. These were placed in a collagen gel and then grafted under the renal capsule of female nude mice for a month. The resulting grafts showed an increase in the ductal density than observed previously. These ducts expressed appropriate markers for luminal and myoepithelial cells and steroid receptors (Fig. 1 appendix). Treatment with estrogen and estrogen+progesterone resulted in an increase in ductal density and cell proliferation (Fig. 2 and 3 appendix). CAF has been combined with organoids and tested in this model. Preliminary work involved using CAF from mouse models of mammary cancer. The MMTV-Wnt-1 mouse (Tsukamoto et al., 1988) produces mammary gland tumors after 6 months. Fibroblasts from these tumors caused abnormal development of the organoids after recombination and development under the renal capsule. Also, fibroblasts from the MMTV c-neu (ErbB2) (Muller et al., 1989) mouse were utilized in this model. In combination with the mouse c-neu overexpressing tumors, I plan to use fibroblasts from human ErbB2 overexpressing tumors.

KEY RESEARCH ACCOMPLISHMENTS:

- Development of a novel method of growing human breast epithelium in vivo.
- Learnt renal grafting procedure
- Grown mouse embryonic mammary buds successfully under the renal capsule
- Successful tissue analysis using immunohistochemistry
- Publication of a manuscript based on work done developing in vivo model
- Manuscript in preparation on ESX data
- Invited to speak at prestigious research meetings

REPORTABLE OUTCOMES:

Invited speaker: Gordon research conference. Rhode Island 2002.

Poster and abstract. UCSF joint breast and prostate meeting. San Francisco 2002.

Poster: Gordon Research Conference. Il Ciocco. Italy 2002.

Invited speaker and poster: Era Of Hope Department of Defense breast cancer research program meeting. Orlando, Florida. 2002.

Papers in press and in preparation:

H. Parmar, P. Young, J. Emerman, R. M. Neve, S. Dairkee and G. R. Cunha. A novel method for growing human breast epithelium in vivo using mouse and human mammary fibroblasts. Accepted for publication in Endocrinology (Dec 2002 issue).

Neve R M, **Parmar H**, Mesa J and Benz C. Defining the regulatory factors controlling ESX epithelial-specific expression during differentiation and cancer. Manuscript in preparation for The Journal of Biological Chemistry.

CONCLUSIONS

The implications of the in vivo mammary gland model are vast. It is now possible to manipulate both the epithelium and fibroblast components of the mammary gland to better understand mammary development with an emphasis on the human mammary gland. It also serves as a model by which to study the initial changes that occur in normal mammary epithelium when in contact with fibroblast from the tumor. Are the tumor fibroblasts different from normal fibroblasts and do they contribute to abnormal epithelial development.? What happens if epithelium from a breast tumor is recombined with normal fibroblasts?

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H. Parmar, P. Young, J. Emerman, R. M. Neve, S. Dairkee and G. R. Cunha. A novel method for growing human breast epithelium in vivo using mouse and human mammary fibroblasts. Accepted for publication in Endocrinology (Dec 2002 issue).

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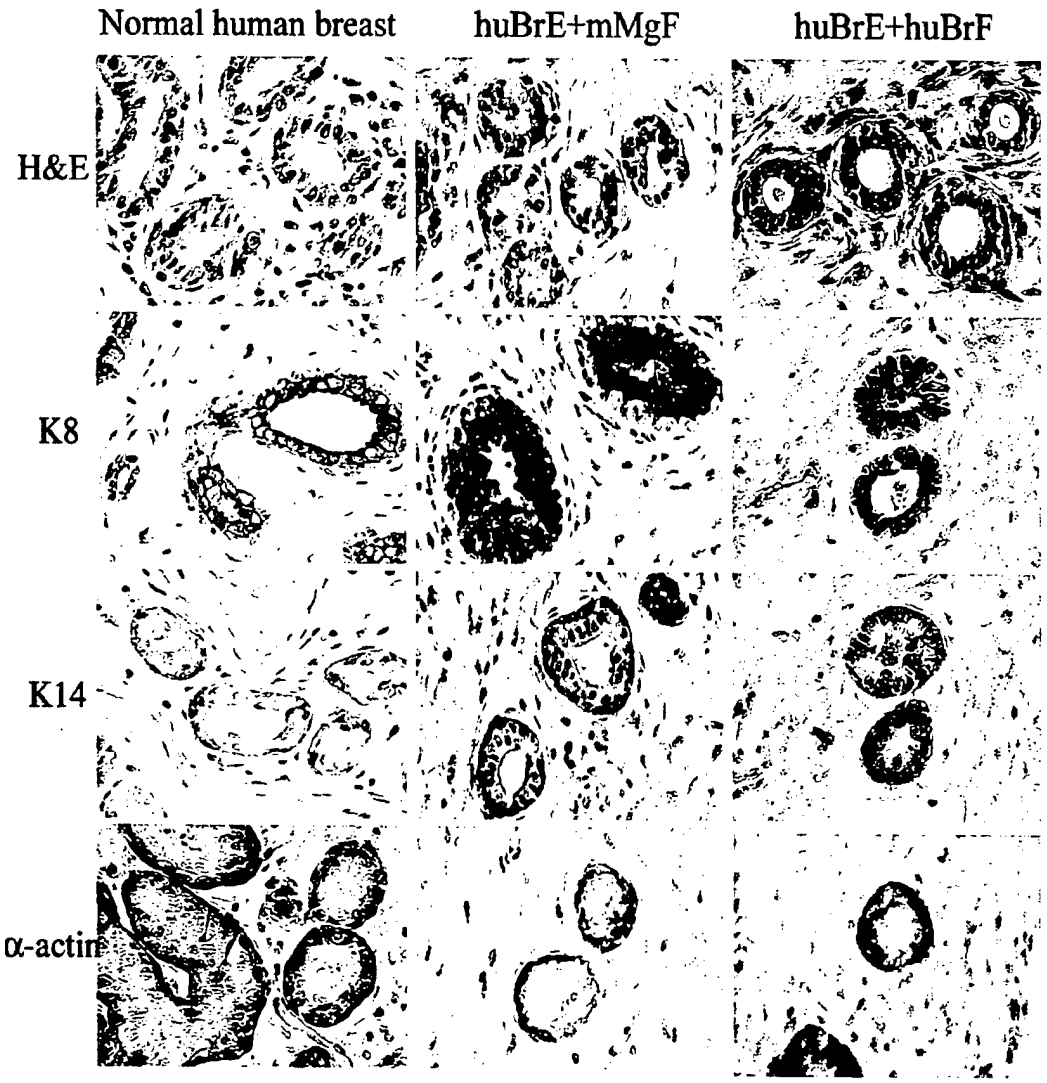

Fig.1 Histology of normal human breast with human breast epithelium (huBrE) + mouse mammary gland fibroblasts (mMgF) and human breast fibroblasts (huBrF)is the same.

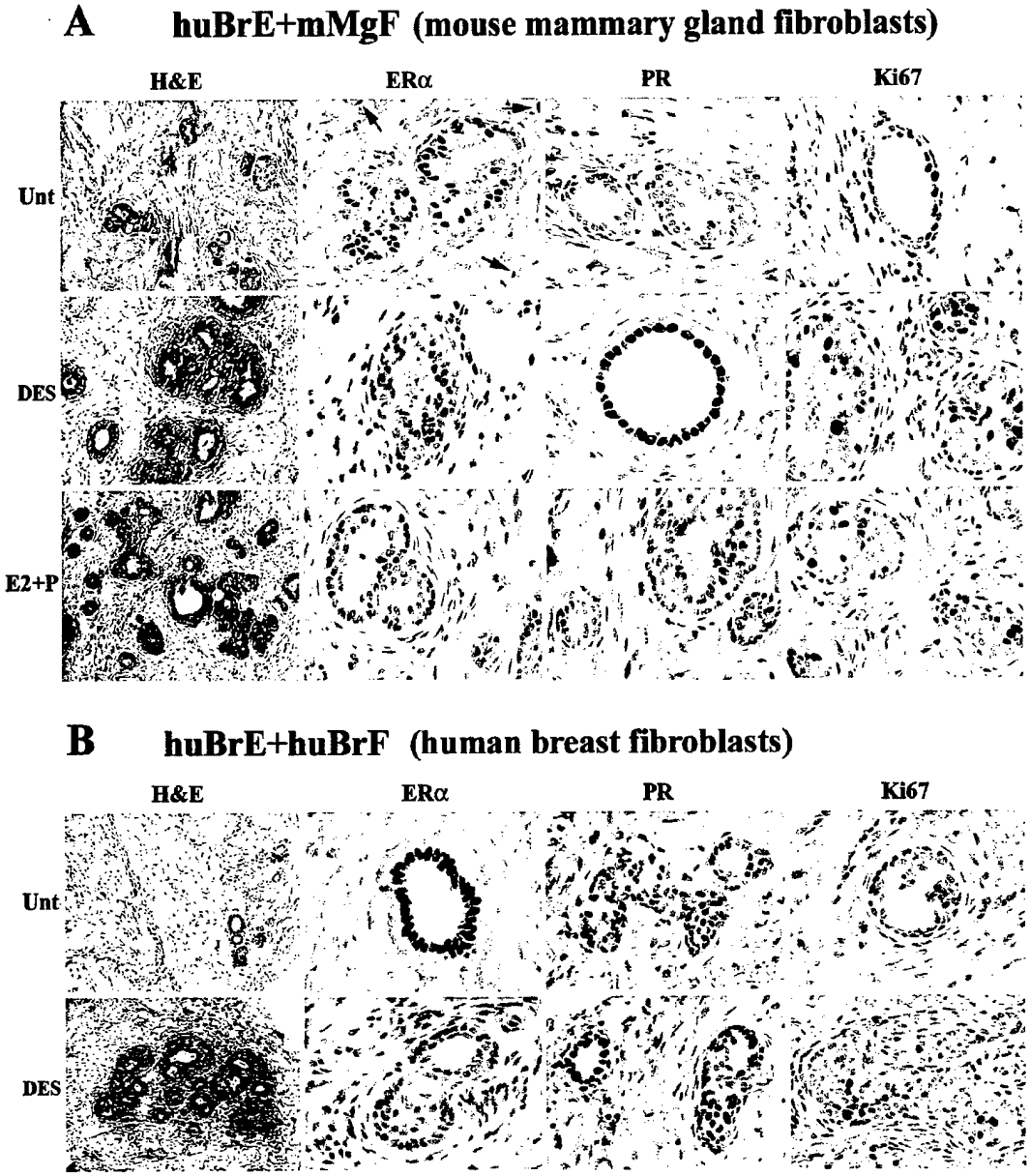

Fig. 2 Effect of hormones on estrogen receptor (ER) and progesterone receptors(PR) in human breast epithelium (huBrE)+ mammary fibroblast recombinations. Treatment with estrogen and estrogen+progesterone result in increased Ki67 labeling index and steroid receptor response. DES downregulates the ER and induces PR. Estrogen+progesteron downregulates both the ER and PR.

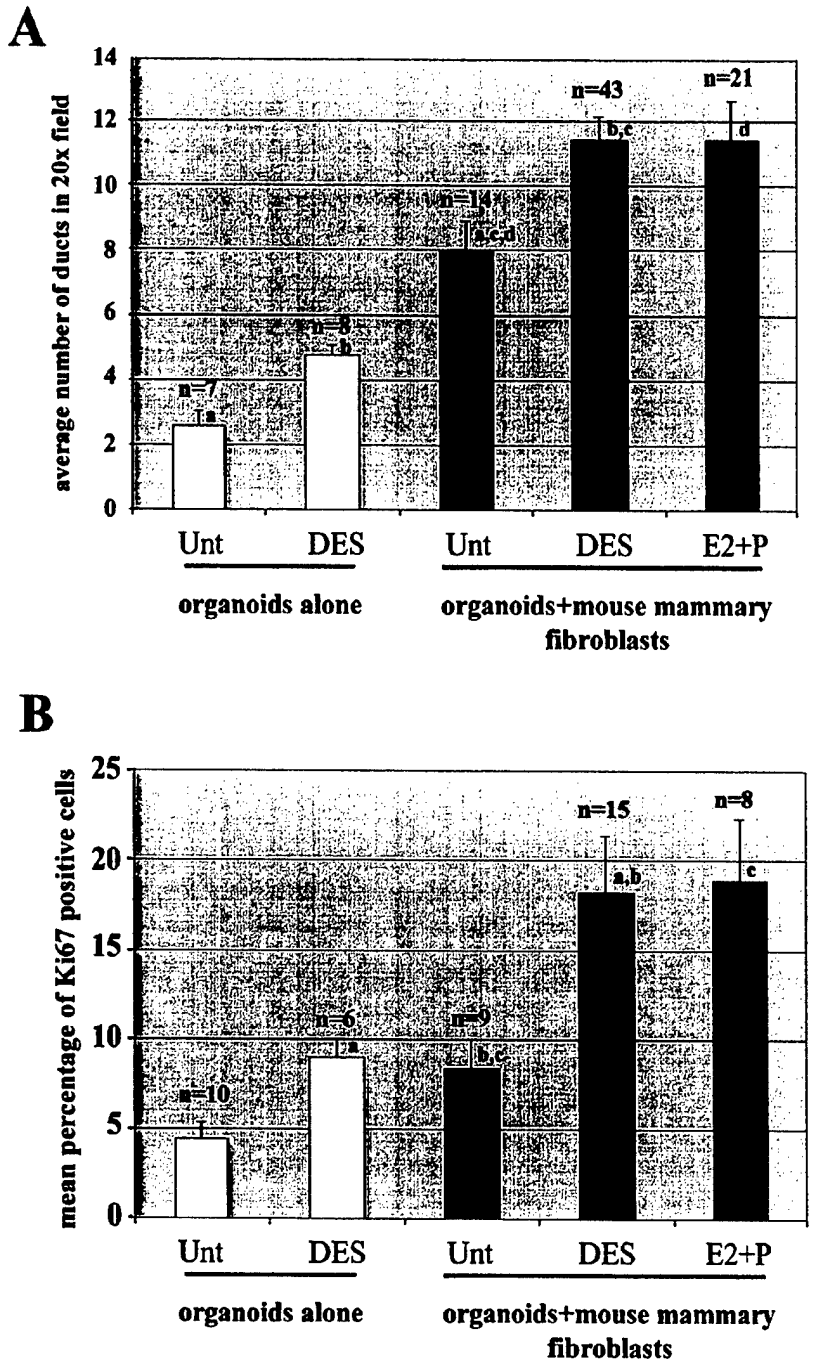

Fig. 3 Organoids + mammary fibroblasts have an increased Ki67 labeling index and increased ductal density after hormone treatment compared to organoids alone