

Research article

Open Access

Breast fibroblasts modulate epithelial cell proliferation in three-dimensional *in vitro* co-culture

Andrea Sadlonova¹, Zdenek Novak², Martin R Johnson³, Damon B Bowe³, Sandra R Gault¹, Grier P Page⁴, Jaideep V Thottassery⁵, Danny R Welch¹ and Andra R Frost¹

¹Department of Pathology, The University of Alabama at Birmingham, Alabama, USA

²Department of Pediatrics, The University of Alabama at Birmingham, Alabama, USA

³Department of Pharmacology and Toxicology, The University of Alabama at Birmingham, Alabama, USA

⁴Department of Biostatistics, The University of Alabama at Birmingham, Alabama, USA

⁵Drug Discovery Division, Southern Research Institute, Birmingham, Alabama, USA

Corresponding author: Andra R Frost, afrost@path.uab.edu

Received: 15 Dec 2003 Revisions requested: 26 Jan 2004 Revisions received: 2 Sep 2004 Accepted: 24 Sep 2004 Published: 8 Nov 2004

Breast Cancer Res 2005, **7**:R46-R59 (DOI 10.1186/bcr949)

© 2004 Sadlonova *et al.*, licensee BioMed Central Ltd.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is cited.

Abstract

Background Stromal fibroblasts associated with *in situ* and invasive breast carcinoma differ phenotypically from fibroblasts associated with normal breast epithelium, and these alterations in carcinoma-associated fibroblasts (CAF) may promote breast carcinogenesis and cancer progression. A better understanding of the changes that occur in fibroblasts during carcinogenesis and their influence on epithelial cell growth and behavior could lead to novel strategies for the prevention and treatment of breast cancer. To this end, the effect of CAF and normal breast-associated fibroblasts (NAF) on the growth of epithelial cells representative of pre-neoplastic breast disease was assessed.

Methods NAF and CAF were grown with the nontumorigenic MCF10A epithelial cells and their more transformed, tumorigenic derivative, MCF10AT cells, in direct three-dimensional co-cultures on basement membrane material. The proliferation and apoptosis of MCF10A cells and MCF10AT cells were assessed by 5-bromo-2'-deoxyuridine labeling and TUNEL assay, respectively. Additionally, NAF and CAF were compared for expression of insulin-like growth factor II as a potential mediator of their effects on epithelial cell growth, by ELISA and by quantitative, real-time PCR.

Results In relatively low numbers, both NAF and CAF suppressed proliferation of MCF10A cells. However, only NAF and not CAF significantly inhibited proliferation of the more transformed MCF10AT cells. The degree of growth inhibition varied among NAF or CAF from different individuals. In greater numbers, NAF and CAF have less inhibitory effect on epithelial cell growth. The rate of epithelial cell apoptosis was not affected by NAF or CAF. Mean insulin-like growth factor II levels were not significantly different in NAF versus CAF and did not correlate with the fibroblast effect on epithelial cell proliferation.

Conclusion Both NAF and CAF have the ability to inhibit the growth of pre-cancerous breast epithelial cells. NAF have greater inhibitory capacity than CAF, suggesting that the ability of fibroblasts to inhibit epithelial cell proliferation is lost during breast carcinogenesis. Furthermore, as the degree of transformation of the epithelial cells increased they became resistant to the growth-inhibitory effects of CAF. Insulin-like growth factor II could not be implicated as a contributor to this differential effect of NAF and CAF on epithelial cell growth.

Keywords: breast cancer, breast epithelium, fibroblast, insulin-like growth factor, stroma

Introduction

The structure and homeostasis of normal breast parenchyma is maintained by dynamic interactions between breast epithelial cells and their associated stroma. These

stromal elements include the vasculature, adipocytes, resident immune cells, and fibroblasts with their numerous cellular products, including various growth factors and extracellular matrix (ECM) components. In breast cancers,

3D = three-dimensional; BrdU = 5-bromo-2'-deoxyuridine; CAF = carcinoma-associated fibroblasts; CK = cytokeratin; DCIS = ductal carcinoma *in situ*; DMEM = Dulbecco's modified Eagle's medium; ECM = extracellular matrix; E:F = ratio of epithelial cells to fibroblasts; ELISA = enzyme-linked immunosorbent assay; FITC = fluorescein isothiocyanate; H&E = hematoxylin and eosin; HPLC = high-performance liquid chromatography; IGF = insulin-like growth factor; NAF = normal breast-associated fibroblasts; PBS = phosphate-buffered saline; PCR = polymerase chain reaction.