Report

Differential regulation of normal and tumoral breast epithelial cell growth by fibroblasts and 1,25-dihydroxyvitamin D$_3$

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Summary

Mesenchymal-epithelial interactions are of paramount importance during normal and tumoral breast developments. We have investigated the paracrine growth regulation of normal and tumoral breast epithelial cells by fibroblasts derived from normal or pathological breast tissues. In some cases, breast cancer MCF-7 cells or normal epithelial cells in primary culture were cocultured with fibroblasts in a Transwell system allowing diffusible factor exchanges. Alternatively, conditioned medium produced by fibroblast cultures was added to epithelial cell cultures. Fibroblasts were shown to stimulate the proliferation of normal and carcinoma cells through paracrine mechanisms. However, the paracrine exchanges appeared to be different in normal versus tumoral breast epithelial cell growth regulation. Moreover, vitamin D-related compounds that have been proposed as anti-tumoral drugs were studied for their ability to affect normal and tumoral mammary epithelial cell proliferation and to interfere with the growth-regulatory activity of fibroblasts. Whereas vitamin D compounds inhibited MCF-7 cell growth, they led to a marked stimulation of the proliferation of normal mammary epithelial cells. Moreover, it was shown that the vitamin D analog EB 1089 can block the mitogenic effect of fibroblast-conditioned medium on tumoral but not normal breast epithelial cells. The differential effects of vitamin D compounds on cell proliferation provide further data in favor of the different behaviours of normal and tumoral mammary epithelial cells. The potential therapeutic use of vitamin D derivatives in the treatment of breast cancer is supported by these results but their growth-stimulatory properties on normal epithelial cells cannot be overlooked.

Introduction

Mesenchymal-epithelial interactions play an essential role in epithelial cell proliferation and differentiation during normal breast development. Previous studies have suggested that stromal cells are important for the regulation of growth, differentiation, invasion, and metastatic processes during carcinogenesis [1, 2].

A number of in vivo and in vitro studies have shown that the stroma is able to produce many of the mediators involved in the paracrine regulation of normal and tumoral mammary cell growth, but few data exist on the potential interactions between fibroblasts and normal mammary epithelial cells. Conditioned medium (CM) from cultured mouse mammary fibroblasts has previously been reported to stimulate normal mouse mammary epithelial cell proliferation in vitro [3]. It has been shown that mammary fibroblasts are involved in the estrogen mitogenic response of normal mouse mammary epithelial cells [4, 5]. Also, we have recently demonstrated that human mammary fibroblasts are able to induce a very significant stimulation of tumoral as well as normal breast epithelial cell proliferation in a coculture system [6].

Many studies have focused on the regulation of breast adenocarcinoma cell proliferation by fibro-
blasts. Most of these studies demonstrate a paracrine growth stimulation of human breast cancer cells when they are cocultured with human fibroblasts [7–13]. Studies of the influence of breast fibroblasts from a variety of sources on mammary adenocarcinoma cell growth in vitro have provided conflicting data. Previously, Adams et al. [14] reported that CM produced by fibroblasts derived from both benign and malignant breast tumors had a growth-stimulatory effect on the breast cancer cell line MCF-7, whereas CM produced by fibroblasts derived from normal mammary tissue displayed a growth-inhibitory activity. Conversely, several authors reported that conditioned serum-free media from primary cultures of breast fibroblasts were able to stimulate the proliferation of a variety of breast carcinoma cell lines, whatever the origin of the fibroblasts, from normal or pathological breast tissue [15, 16]. Nonetheless, van Roozendaal et al. [16] suggested that the extent of the proliferative response depended on the source of the fibroblasts, tumor fibroblasts inducing a greater mitogenic response than those derived from normal breast tissue.

In spite of these controversies regarding the effects of stroma from a variety of sources on mammary epithelial cell growth under particular experimental conditions, it is well established that the stromal cells such as fibroblasts are required for growth and hormonal response of both the normal mammary gland and breast tumors [17]. These epithelial-mesenchymal interactions can be mediated by signals such as extracellular matrix components, cell membrane-associated molecules, and soluble factors, that is cytokines and growth factors [18]. Nonetheless, the precise basis of these interactions is far from being elucidated. Whereas there is evidence that fibroblasts derived from tumors, although not transformed themselves in breast tumors, are phenotypically different from normal fibroblasts in several ways [19], it is presently unknown whether normal and tumoral breast epithelial cell growth is differently affected by fibroblasts.

We were thus interested in comparatively testing the activity of fibroblasts from normal and tumor breast tissues on the proliferation of adenocarcinoma cell lines and normal mammary glandular cells in primary culture, in order to provide information about the normal and pathological regulation of epithelial cell growth by the stroma. Our main concern was to investigate the potential involvement of reciprocal paracrine exchanges between fibroblasts and epithelial cells. For this purpose, two in vitro models were employed. First, CM produced by fibroblasts cultured alone was used to evaluate the intrinsic ability of normal and tumoral fibroblasts to produce growth factors able to regulate breast epithelial cell growth. Alternatively, a coculture system was used, in which a permeable membrane prevents direct cell contacts between the two cell populations while permitting paracrine exchanges of diffusible factors. So, the comparison between these two models allowed us to develop the hypothesis that, whereas both normal and tumoral breast epithelial cell proliferation may be stimulated by fibroblasts, the mechanisms involved in these interactions differ in relation to the normal or the cancerous origin of the epithelial cells.

Since epithelial-mesenchymal interactions may be important in promoting tumor development, investigation on drugs able to antagonize the growth-stimulatory activity of fibroblasts on breast epithelial cancer cells may be of great interest. 1,25-dihydroxyvitamin D$_3$ (1, 25(OH)$_2$D$_3$), the biologically active form of vitamin D$_3$ (cholecalciferol), has been shown to counter the growth stimulatory effect of steroids [20–22] and growth factors like epidermal growth factor and insulin-like growth factor [23–26]. Moreover, it has been previously demonstrated that 1,25(OH)$_2$D$_3$ and its synthetic analogues were potent negative growth regulators of breast cancer cells, both in vivo and in vitro [27–30]. Furthermore, Lefebvre et al. [12] reported that 1, 25(OH)$_2$D$_3$ was able to reverse the stimulation of breast cancer epithelial cells related to the presence of fibroblasts, in a direct coculture system. In the present study, experiments were also designed to examine and compare the potential ability of 1, 25(OH)$_2$D$_3$ and its analogue EB 1089 to affect normal and tumoral cell growth stimulation induced by mammary fibroblasts.

Materials and methods

Cell cultures

Breast adenocarcinoma MCF-7 cell line

The MCF-7 cell line, derived from human breast adenocarcinoma, was obtained from Dr. M. Lippman (NIH, Bethesda). Cells were grown in 50% (vol/vol) Dulbecco’s modified Eagle medium (DMEM) and 50% (vol/vol) Ham’s F12 medium (F12), supplemented with 10% (vol/vol) heat-inactivated fetal bovine serum (FBS), 2 mM L-glutamine (Gibco, Cergy Pontoise, France), and 6 ng/ml human insulin. MCF-7 cells were maintained at 37°C in a humid atmosphere of 5% CO$_2$ in air.