IDENTIFICATION, PARACRINE GENERATION, AND POSSIBLE FUNCTION OF HUMAN BREAST CARCINOMA MYOFIBROBLASTS IN CULTURE

LONE RØNNOV-JESSEN, BO VAN DEURS, MAJA NIELSEN, AND OLE W. PETERSEN

Structural Cell Biology Unit, Department of Anatomy, The Panum Institute, University of Copenhagen, DK-2200 Copenhagen N (L. R.-J., B. v. D., O. W. P); Laboratory of Tumor Endocrinology, The Fibiger Institute of The Danish Cancer Society, DK-2100 Copenhagen Ø (L. R.-J., O. W. P); Department of Pathology, Bispebjerg Hospital, DK-2400 Copenhagen NV (M. N.), Denmark.

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SUMMARY

Myofibroblasts from human breast carcinomas were identified and experimentally generated in culture, and a possible function was examined. The frequency of α-smooth muscle actin immunoreactive cells was evaluated as a measure of myofibroblast differentiation in primary culture. Few or no α-smooth muscle actin-positive stromal cells (6.1 ± 8.4%) were identified in primary cultures from normal breast tissue (n = 9). In contrast, high frequencies (68.8 ± 15.1%) were observed in primary cultures from carcinomas (n = 19). The frequencies of myofibroblasts in primary cultures were almost identical to those obtained in the corresponding cryostat sections (69.1 vs. 68.8%). A possible precursor cell to the myofibroblast was looked for among typical fibroblasts and vascular smooth muscle cells. Purified blood vessels containing both fibroblasts and vascular smooth muscle cells were embedded in collagen gel and incubated with medium conditioned by breast epithelial cells. Fibroblasts rather than smooth muscle cells were recruited from the blood vessels. In medium conditioned by carcinoma cell lines or in co-cultures of carcinoma cell lines and purified fibroblasts, α-smooth muscle actin and the typical myofibroblast phenotype were induced in otherwise α-smooth muscle actin-negative fibroblasts. The effect of myofibroblasts on cellular movement—essential to neoplastic cells—was analyzed. Spontaneous motility of tumor cells (MCF-7) was entirely suppressed in a collagen gel assay. Under these conditions tumor cell motility was selectively mediated by direct cell-to-cell interaction between tumor cells and myofibroblasts. Under chemically defined conditions, interaction was dependent on the presence of plasminogen. Anti-plasminogen, soybean trypsin inhibitor, and anti-fibronectin partly neutralized the effect of plasminogen. It is concluded that elements of myofibroblast differentiation and function may be studied in culture.

Key words: myofibroblasts; paracrine attraction; tumor cell motility.

INTRODUCTION

In human breast carcinomas, epithelial neoplasia is associated with the appearance of so-called myofibroblasts believed to be responsible for the frequent, excessive collagen deposition and tissue contraction referred to as desmoplasia (38,39,45). Recently, more than 80% of the stromal cells in breast carcinomas have been identified as myofibroblasts based on immunoreactivity to α-smooth muscle (α-sm) actin (36). No such stromal cells were found in normal tissue (36). However, apart from the cytoskeleton (44), little is known about the myofibroblast phenotype, about the origin of myofibroblasts, and about their biological relevance to epithelial neoplasia in breast carcinomas (35).

The aim of the present study has therefore been: a) to identify the myofibroblast in culture, b) to experimentally generate myofibroblasts, and c) to elucidate elements of their possible function.

1 To whom correspondence should be addressed at Laboratory of Tumor Endocrinology, The Fibiger Institute of the Danish Cancer Society, Ndr. Frihavnsgade 70, Copenhagen Ø, Dk-2100, Denmark.
plated on either T-25 flasks (Nunc, Roskilde, Denmark) coated with 8 µg/cm² of Vitrogen 100 (Collagen Corporation, Palo Alto, CA) in DME-F12 supplemented with 20% fetal bovine serum (FBS) or in DME-F12 on T-25 Primaria flasks (Falcon no. 3013, Becton Dickerson, Albertslund, Denmark). The cells were used for co-cultivation with either MCF-7 (5% FBS) (Human Cell Culture Bank, Mason Research Institute, Rockville, MD, and cultured at the Fibiger Institute since 1977) or MCF-7 subline 9 in serum-free medium (4). In addition, fibroblasts were co-cultured with another breast carcinoma cell line, HMT-3909 S13, which is a tumorigenic subline of HMT-3909 S8 (32).

Epithelial cells. Some of the epithelial organoids from nine biopsies served as controls for the biopsies from carcinomas. These were seeded on T-25 flasks (Nunc), and cultured in DME-F12 supplemented with 20% FBS. Others were washed free of collagenase for another 24 h on the rotary shaker (60 rpm) at 37 °C. The following day, some of the organoids were trypsinized in 2 ml of 0.25% trypsin + 1 mM EDTA for 10 rain and seeded in T-25 Primaria flasks (Falcon) after addition of 80 µl of the protease inhibitor, soybean trypsin inhibitor (SBTI) (0.1%), while others were embedded in collagen gels (see below). The medium from these cultures was used for conditioning the blood vessels in collagen gels.

Blood vessels. After separation from the epithelial organoids, some of the blood vessels were plated in T-25 flasks (Nunc) and analyzed by immunofluorescence (see below). Most of the blood vessels from 14 of the biopsies were, however, embedded in collagen gels. Vitrogen 100 (Collagen Corporation) was diluted in sterile water to make a concentration of 2.5 mg/ml. On ice, a volume of 10 X phosphate buffered saline (PBS) corresponding to 1/10 of the collagen volume was added. The PBS contained 0.5 ml phenol red/100 ml. The pH of the gel was adjusted with 0.1 N NaOH, until the gel was slightly red. The purified blood vessel fraction was pelleted and resuspended in medium corresponding to 0.1 ml suspension per well. The blood vessels were added to the gel at room temperature and seeded in six wells (Nunc) with 2 ml of gel in each well. After 45 min of gelification 5 ml of DME-F12 was added.

Media from the following cell lines were used for studying the effect on blood vessels: the nontumorigenic cell line HMT-3522 (5), and the tumorigenic cell lines MCF-7 S9 (4) and HMT-3909 S9, which is a subline of HMT-3909 S8 (32). The cultures of HMT-3522 were changed twice with