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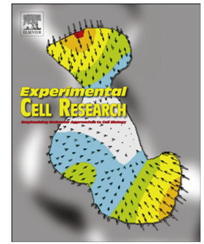

Research Article

Malignancy of bladder cancer cells is enhanced by tumor-associated fibroblasts through a multifaceted cytokine-chemokine loop

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ABSTRACT

The microenvironment of tumor cells is critically involved in tumor development and progression. Tumor-associated fibroblasts (TAFs) represent a major constituent of the tumor stroma. Tumor cells are operative in the activation of TAFs, whereas TAFs in turn contribute to tumor cell malignancy. This report describes mechanisms of communication between fibroblasts and urinary bladder cancer (UBC) cells.

Migration of bladder cancer cell lines RT112 and Cal-29, representing two different grades of dedifferentiation, was enhanced by cocultivation with TAFs. Conditioned medium from tumor cells induced the release of interleukin (IL)-8, hepatocyte growth factor (HGF), matrix metalloproteinase-2, granulocyte macrophage colony-stimulating factor, and monocyte chemotactic protein (MCP)-1 by TAFs. Tumor cell-derived IL-1α was identified as a major mediator of these stimulatory effects. Fibroblasts, on the other hand, exerted a migration and invasion stimulating influence on UBC cells. MCP-1 and HGF were shown to promote cell migration of both bladder cancer cell lines.

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Introduction

Normal stroma corresponds to a connective tissue arrangement that beholds an array of tissues and organs. Stromal fibroblasts deposit extracellular matrix (ECM) and form the basement membrane [1]. Under certain circumstances, they remold ECM by secreting proteases, for example, matrix metalloproteinase (MMPs), and participate in wound healing by transforming to myofibroblasts [2]. In recent years, it became evident that stromal fibroblasts are crucially involved in cancer progression. Through normal to preinvasive to invasive ductal carcinoma, tumor cells modify the characteristics of adjacent stroma for a supportive microenvironment. Of major importance in this transformation are tumor-associated fibroblasts (TAFs), which play a vital role in tumorigenesis and metastasis [3,4]. TAFs represent an inhomogeneous population and arise from normal fibroblasts, endothelial cells, or mesenchymal cells under the influence of diverse types of microenvironment. Irrespective of their heterologous origin, TAFs can be clearly distinguished from normal fibroblasts by specific

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features. Using immunohistochemistry, typical TAF proteins such as alpha smooth muscle actin, fibroblast activation protein, Thy-1, desmin, and S100A4 were identified, none of which, however, is a generally occurring surface marker [5].

Several functional studies in prostate, lung, breast, and colon cancer have confirmed that fibroblasts can determine the fate of epithelial cells because they are able to endorse malignant conversion as well as to revert cancer cells to regular phenotype [6].

Growth factors, cytokines, and chemokines are the mediators of the functional cross talk between tumor cells and fibroblasts. Interleukin (IL)-1β, for instance, has recently been shown to stimulate an inflammatory response in stromal fibroblast and concomitant ovarian tumorigenesis [7]. Granulocyte macrophage colony-stimulating factor (GM-CSF) was identified as a tumor-promoting cytokine in different cancers [8,9]. TAFs, in turn, secrete various growth factors, cytokines and chemokines [10], fibroblast specific proteins [11], and hormones inducing epithelial proliferation [12] and other parameters of malignancy. Colorectal liver metastasis, for example, appears to be supported by TAFs, which generate a prometastatic microenvironment through inflammatory activation of IL-6 and monocyte chemoattractant protein (MCP)-1, also termed chemokine (C–C motif) ligand 2 (CCL-2) [13]. Lastly, TAFs also have been described as sources of MMPs which can favor cancer cell dissemination by degradation of the ECM and whose expression is stimulated in the presence of tumor cells [14,15].

We have focussed on urinary bladder cancer (UBC) to study the communication between tumor cells and TAFs. UBC is the prevalent malignant epithelial tumor of the human bladder and, according to the current diagnostic scheme, can be subdivided into low-grade and high-grade carcinomas [16]. Bladder cancer is notable for profound influence of the microenvironment, underscored by the fact that, in the vast majority of UBC biopsies, myofibroblasts are present [17]. Some cytokines and chemokines have been functionally associated with UBC and with tumor–stroma interactions in bladder tumors. For instance, we were able to show that transforming growth factor (TGF)-β promotes transdifferentiation of fibroblasts into a phenotype capable of supporting invasiveness of UBC cells [18]. Elevated IL-8 expression has been linked to bladder cancer and is discussed as a biomarker [19,20]. It has also been shown that hepatocyte growth factor (HGF) is able to increase tumor cell invasion in the context of tumor–stroma interaction in bladder cancer [21].

Although TAFs represent the predominant cell type of the neoplastic stroma of solid tumors in general and in UBC in particular, the molecular nature of their malignancy promoting communication with tumor cells is only partly understood. The purpose of this study is therefore to analyze the reciprocal communication between UBC cells and fibroblasts. In this context, we also explicitly addressed the question of how far the quality of cross talk between UBC cells and fibroblasts is determined by the dedifferentiation (grading) status of the cancer cells involved.

Materials and methods

Cells and cell culture

Human foreskin fibroblasts (HFFs) and TAFs were isolated as described [22]. Human carcinoma cell lines RT112 (G2, DSMZ ACC 418) and Cal-29 (G4, DSMZ ACC 515) were purchased from the German Collection of Microorganisms and Cell Lines (DSMZ) (Braunschweig, Germany) and routinely cultivated in Dulbecco's minimum essential medium (DMEM) with 10% fetal calf serum (FCS) and 1% penicillin/streptomycin. Media were obtained from GIBCO Invitrogen Corp. (Carlsbad, CA).

Immunocytochemistry

Tumor cells were seeded onto cell culture slides (BD Falcon; BD Biosciences, Franklin Lakes, NJ) and cultured until subconfluence, washed in PBS, fixed in methanol:acetone (1:2) for 2 min, and subjected to immunocytochemistry.

Immunocytochemistry was performed as recently described [18]. After blocking of endogenous biotin by applying biotin blocking, cell phenotype was assessed using the following antibodies: anti-E-cadherin (clone NCH-38; dilution 1:1000), anti-N-cadherin (clone 6G11; dilution 1:200), and antivimentin (clone V9; dilution 1:1500). Bound antibodies were visualized using the DAKO REAL detection system, alkaline phosphatase/RED, rabbit/mouse. All reagents and antibodies were from DAKO Deutschland GmbH (Hamburg, Germany).

Cytokines and chemokines

GM-CSF, IL-1α, and IL-β were obtained from INVIGATE GmbH (Jena, Germany); MCP-1 was purchased from ImmunoTools GmbH (Friesoythe, Germany); and TGF-β was from PeproTech GmbH (Hamburg, Germany).

Cell coculture, cell migration, and invasion assays

Coculture experiments and cell migration assays were performed by Boyden chamber assays using 12-well culture chambers with 8-μm pore inserts from BD Biosciences. Fibroblasts were seeded into the lower compartments and grown to confluence in DMEM/10% FCS, then washed with PBS and incubated in DMEM without FCS. A total of 250,000 tumor cells in DMEM without FCS were seeded into the upper chambers. Tumor cells were allowed to migrate towards fibroblasts (or medium control). After an incubation time of 24 h for RT112 cells or 8 h for Cal-29 cells, respectively, medium was collected and tumor cells on the membranes separating the two compartments were fixed in 4% paraformaldehyde. Membranes were washed with distilled water and stained with haematoxylin as described [23]. Membranes were inspected by phase contrast microscopy. Cells in three arbitrarily chosen view fields were counted, and determined cell numbers were used as a measure for migratory activity.

Alternatively, cell migration was quantified by a scratch wound healing assay: Cells were grown in 24-well dishes in DMEM containing 10% FCS. After reaching confluence, two scratches were made crosswise into the cell layers using a pipette tip. Fresh medium without FCS, optionally supplemented with cytokine/growth factor at varied concentrations, was added to the cells. After incubation for 24 h (RT112) or 8 h (Cal-29), dishes were photographed at ×10 magnification using an inverted microscope The wound area was measured, and the percentage of closed wound area was determined by using the TScratch software package (developed by Tobias Gebäck and Martin Schulz and provided as freeware by the Computational Science & Engineering Laboratory of the ETH Zurich, Switzerland). All assays were performed in triplicate.

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