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Tissue resident stem cells produce CCL5 under the influence of cancer cells and thereby promote breast cancer cell invasion

Severin Pinilla [1], Eckhard Alt [1], F.J. Abdul Khalek, Constantin Jotzu, Fabian Muehlberg, Christoph Beckmann, Yao-Hua Song *

Department of Molecular Pathology, University of Texas, M.D. Anderson Cancer Center, Houston, TX 77030, United States

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ABSTRACT

In the present study, we investigated whether human adipose tissue derived stem cells (hASCs) could enhance tumor invasion and whether these hASCs could be a potential source of CCL5. We observed a significant increase in the number of breast cancer cells that invaded the matrigel when Co-cultured with hASCs. We found that hASCs produce CCL5 in the Co-culture and cancer cell invasion was diminished by an antibody against CCL5. Furthermore, cancer cell invasion in the Co-culture was associated with an elevated level of MMP-9 activity. We conclude that CCL5 plays a crucial role for tumor invasion in the interplay of tissue resident stem cells from the fat tissue and breast cancer cells.

1. Introduction

Stromal cells contribute to the development of a wide variety of tumors. There is a higher incidence of tumor formation in tissues exhibiting a chronically inflamed stroma as well as those undergoing various types of wound healing, in which the stroma plays a central role [1,2]. Stromal cell compartments contain a variety of cell types such as fibroblasts, myofibroblasts, endothelial cells, pericytes as well as inflammatory cells. Tumor stromal cells produce CCL5 – a chemokine which is involved in tumor progression [3–6]. Recent studies demonstrated that bone marrow derived stem cells (BMSCs) are involved in tumor stroma formation [7,8]. Furthermore BMSCs produce CCL5 when in direct Co-culture with breast cancer cells. However, the involvement of tissue resident stem cells in tumor stroma formation has not been investigated so far. We and others have shown that adipose tissues contain multipotent stem cells which secrete various paracrine factors. Since breast tissue contains large amounts of adipose tissue, we sought to investigate the potential contribution of hASCs to breast cancer invasion.

2. Materials and methods

2.1. Isolation and culture of cells

Human adipose tissue was obtained from elective body contouring procedures in compliance with the guidelines of the M.D. Anderson Cancer Center Institutional Review Board. Tissue was minced by sharp dissection. Minced specimens were added to a solution of 0.07% blendzyme 3 (F. Hoffman-La Roche Ltd., Basel, Switzerland), digested with mild agitation at 37 °C for 60 min, passed through a 40 μm filter and finally selected based on adherence to T75 tissue culture flasks at 24 h. Cells were grown in alpha MEM medium supplemented with 20% fetal bovine serum, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml

* Corresponding author. Address: University of Texas, M.D. Anderson Cancer Center, 2SCR3.3024, Box 0951, 7435 Fannin Street, Houston, TX 77054, United States. Tel.: +1 713 834 6117; fax: +1 713 834 6105.
E-mail address: ysong@mdanderson.org (Y.-H. Song).
[1] These authors contributed equally to this work.

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streptomycin. Cells were incubated in a 5% CO_2-containing chamber at 37 °C with medium changed every 3 days. hASCs between Passages 1 and 8 were used for all experiments. hASCs used in these experiments have previously been characterized by Bay et al. group [9].

MDA MB 231 (American Type Culture Collection) were grown in MEM (Life Technologies) supplemented with 10% FBS and penicillin–streptomycin at 37 °C in 5% CO_2.

2.2. Lentiviral GFP transfection

Stable GFP labeling was performed with a third-generation lentivirus system as described previously by our group [10,11].

2.3. Cell staining

Cells that were 80% confluent in a T25 flask were incubated at 37 °C in 5% CO_2 with 3 ml of their regular culture-medium, containing 15 μl of DiI (or 21 μl of DiO, respectively) for 1 h. In the next step cells were washed twice with PBS, before regular medium was added for a 5 min incubation time. This step was repeated and cells were then harvested and seeded for the following experiment.

2.4. Proliferation assay

MDA MB 231 $\times$ 10^4 cells were seeded in 6-well plates in 2 ml of MEM (10% FBS). After 24 h medium was exchanged to MEM (5% FBS) in the control group and to stem cell conditioned medium (conditioning time 48 h) in the treatment group. Cell number was counted using a hemocytometer and viability assessed with Trypan Blue exclusion method.

2.5. Invasion assays

The chemoinvasion assay was performed using a Boyden chamber with filter inserts (pore size, 8 μm) coated with Matrigel in 24-well dishes (BD Biosciences Bedford, MA) as described previously [12]. Before performing the invasion assay, cells were mixed together in 5% FBS containing MEM to be seeded in direct Co-culture. 35×10^3 cells (MDA MB 231), 70×10^3 cells (hASCs/WI-38/HMECs) or in direct Co-culture (ratio of 1:2, MDA MB 231:hASCs), respectively were placed in 600 μl of 5% FBS containing MEM in the upper chamber, and 750 μl of the same medium containing 10% FBS was placed in the lower chamber. The plates were incubated for 48 h at 37 °C in 5% CO_2. Cells on the upper side of the filters were removed with cotton-tipped swabs, and the filters were washed with PBS. Cells on the underside of the filters were examined and counted under a microscope. Green fluorescent cell signal of GFP labeled MDA MB 231 cells was counted in five randomly chosen view fields at a 10× magnification of every insert. Each experiment was repeated at least three times.

2.6. ELISA

The amount of CCL5, secreted by MDA MB 231 and hASCs was measured using Quantakine ELISA kit (R&D Systems). Secreted cytokines were standardized to the amount of cells counted in each well and expressed as pg cytokine per ml.

2.7. Exposure of hASCs and MDA MB 231 to conditioned medium (CM)

MDA MB 231 alone and hASCs (Passages 2–4) alone were grown in MEM + 5% heat-inactivated FBS culture medium and conditioned medium from these both cell types was harvested after 48 h and centrifuged at 1500 rpm for 5 min and supernatant was passed through Millipore sterile 50 ml filtration system with 0.45-μm polyvinylidene difluoride membrane. CM was stored at −20 °C until hASCs or MDA MB 231 cells were exposed to CM.

2.8. Anti-CCL5 treatment

GFP labeled MDA MB 231 co-seeded with hASCs was incubated with neutralizing CCL5-antibody (3 μg/ml) in the upper chamber of the invasion assays. Anti-human RANTES Antibody (Cat. Number: AF-278-NA) and the control Normal Goat IgG (Cat. Number: Ab-108-C) was purchased from R&D Systems, Minneapolis.

2.9. Zymography

MMP-9 activity was determined using a 10% zymogram (gelatin) precats gel (Invitrogen Cat Number: EC6175). Preparation of cell lines was conducted by seeding the appropriate amount of cells (50,000) into each respective well of a 6-well plate. Cells were cultured at 37 °C (5% CO_2 atmosphere) until 80% confluency. Upon confluence media was changed to serum-free media and cells were cultured for an additional 48 h. After 48 h conditioned media was collected and mixed with equal volumes of 2× SDS (15 μl conditioned media: 15 μl 2× SDS). (Note: Preparation of conditioned media was not heated or reduced for detection of MMP-9 activity using zymography.) The electrical running apparatus was then prepared containing the 10% zymogram (gelatin) pre-cats gel and 1× running buffer (12 g Tris, 57.6 g Glycine, 10% SDS, 4 L distilled water). Samples were then loaded accordingly (15 μl Invitrogen pre-stained protein ladder-cat no. 10748010) into the 10% zymogram (gelatin) pre-cat gel and allowed to run at room temperature at 125 V for 1.5 h. After electrophoresis the zymogram gel was removed and incubated (30 min with gentle agitation) at room temperature in a zymogram renaturing buffer (1:9 with deionized water). Upon incubation, the renaturing buffer was decanted and further incubated at room temperature in 1× developing buffer (1:9 with deionized water) for 30 min. After 30 min the developing buffer was then decanted and replaced with fresh developing buffer. After equilibration with fresh developing buffer the zymogram gel was allowed to incubate overnight at 37 °C. After overnight incubation the developing buffer was decanted and replaced with Coomassie staining solution (25% MeOH, 10% acetic Acid, and 0.1% Coomassie brilliant blue-dissolved in water before adding) for 2 h at room temperature. Upon staining the zymogram gel was de-