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Research Article

Mortalin antibody-conjugated quantum dot transfer from human mesenchymal stromal cells to breast cancer cells requires cell–cell interaction

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ABSTRACT

The role of tumor stroma in regulation of breast cancer growth has been widely studied. However, the details on the type of heterocellular cross-talk between stromal and breast cancer cells (BCCs) are still poorly known. In the present study, in order to investigate the intercellular communication between human mesenchymal stromal cells (hMSCs) and breast cancer cells (BCCs, MDA-MB-231), we recruited cell-internalizing quantum dots (i-QD) generated by conjugation of cell-internalizing anti-mortalin antibody and quantum dots (QD). Co-culture of illuminated and color-coded hMSCs (QD655) and BCCs (QD585) revealed the intercellular transfer of QD655 signal from hMSCs to BCCs. The amount of QD double positive BCCs increased gradually within 48 h of co-culture. We found prominent intercellular transfer of QD655 in hanging drop co-culture system and it was non-existent when hMSCs and BBCs cells were co-cultured in trans-well system lacking imminent cell–cell contact. Fluorescent and electron microscope analyses also supported that the direct cell-to-cell interactions may be required for the intercellular transfer of QD655 from hMSCs to BCCs. To the best of our knowledge, the study provides a first demonstration of transcellular crosstalk between stromal cells and BCCs that involve direct contact and may also include a transfer of mortalin, an anti-apoptotic and growth-promoting factor enriched in cancer cells.

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Introduction

Breast cancer is one of the most frequent malignancies among females accounting for 23% of the total diagnosed cancer cases and 14% of the total cancer deaths in 2008 [1]. The role of complex microenvironment in normal mammary development and function is critical and several differences have been shown between malignant and normal mammary stroma [2–3]. Tumor microenvironment consists of extra cellular matrix (ECM) and cellular components such as immune cells, stromal fibroblasts, myofibroblasts, endothelial cells, adipocytes and also bone marrow derived cell types [2–3]. The cancer microenvironment has been widely studied and the supporting stromal cells have been shown to induce epithelial-to-mesenchymal transition (EMT), supporting the survival of cancer cells by providing nutrients, inducing invasiveness and increasing radiation and drug resistance of cancer cells [4–7].

Heterogenic crosstalk between stromal cells and breast cancer cells (BCCs) has been shown to occur by several different mainly soluble mechanisms. Cancer associated fibroblasts and human mesenchymal stem/stromal cells (hMSCs) secrete growth factors and cytokines such as transforming growth factor-beta (TGF-β), interleukin-6 (IL-6), vascular endothelial growth factor A (VEGF), platelet-derived growth factor-D (PDGF-D) and prostaglandin E2 which have been shown to induce migration, growth and stem cell properties of BCCs [8–12]. However, the role of direct cell-to-cell interactions is yet poorly known, even though several studies have revealed the role of direct cell contacts between stromal cells and BCCs in the regulation of function of BCCs [13–17]. Most recently, it has been shown that spontaneous fusions are occurring between different subpopulations of BCCs but also between BCCs and hMSCs [14,15]. Moreover, the fusions between BCCs and tumor associated macrophages have shown to induce metastasis and cancer stem cell properties of BCCs [16].

Bone marrow-derived hMSCs have been shown to home to the site of damaged tissues, site of inflammation but also to the site of tumors [18–21]. Several studies have been conducted to elucidate the role of hMSCs in the breast cancer growth. However, the results show some inconsistency and therefore the exact role of hMSCs in controlling tumor growth is still unclear. The following *in vivo* and *in vitro* studies have shown that hMSC promote breast cancer tumor growth [19,22], whereas others have shown opposite results [23–24]. Most recently, the role of metabolic coupling between stromal cells, including hMSCs, and breast cancer cells has been demonstrated. It has been postulated that the BCCs secrete hydrogen peroxide to trigger autophagy and oxidative stress in neighboring stromal cells which lead to secretion of ketones, lactate and other highly energetic molecules that are utilized by BCCs [6,25–26]. In addition, starvation deprived hMSCs have also been shown to secrete tumor promoting factors and induce breast cancer growth more actively than normal hMCSs [17,22]. These studies could partly explain how cancer cells survive in a hostile tumor environment where the lack of vascularization causes hypoxia and lack of nutrients.

Mortalin, also known as heat shock protein A 9 (HSPA9), glucose regulated protein 75 (GRP75) or mitochondrial heat shock protein 70 (mtHSP70), is the member of the heat shock protein (HSP) 70 superfamily and is highly expressed in cancers [27–29]. Mortalin is present in variety of different cellular compartments, such as mitochondria, cytosol, endosomes and endoplasmic reticulum [30]. It has been shown to interact with p53 and restrict its nuclear localization leading to the inhibition of apoptosis and thus supports tumor growth [31]. Overexpression of mortalin has also been linked to the increased malignancy of cancer cells and hence its use as a biomarker of metastatic cancer has been suggested [32–33]. The detailed role of mortalin in a regulation of metastasis or malignancy is mainly unknown, but its well-known anti-apoptotic function may offer an advantage towards cellular stress encountered by metastatic cells.

Cell internalizing anti-mortalin antibodies have been reported previously [34–35]. It was shown that these antibodies have unique feature of cell internalization and hence were used as nano-carriers for delivery of genes and quantum dots into the cells [34–35]. The latter, due to their inert nature and illumination, could be used for multicolor long-term real time imaging. In the present study, we aimed to investigate whether the intercellular transfer of cytosolic proteins occur between mesenchymal stromal cells and BCC, and whether the transfer requires direct cell-to-cell interactions by using this well-established internalizing quantum dots (QD) cell labeling system [34].

Materials and methods

hMSCs culture

hMSC line from Lonza (PT-2501; LOT# 6F3974) was cultured following the manufacturer's instruction (Lonza, Walkersville, MD). Briefly, hMSCs were thawn and suspended to the proliferation medium consisting of Mesenchymal Stem Cell Basal Medium, Mesenchymal Cell Growth Supplement and L-glutamine and GA-1000 (Lonza, Walkersville, MD). Cells were plated on culture flasks and cultured at 37 °C under 5% CO_2 until reaching 70–80% confluency after which the cells were either used in experiments or passaged further. The proliferation medium was changed twice a week and Lonza hMSCs from passages 2–5 were used in experiments.

Patient derived hMSC lines (hMSC 1, hMSC 2, hMSC 3 and hMSC 4) were derived and cultured as described earlier [36]. Briefly, hMSCs were isolated from unaffected bone site of patients who were operated for osteoarthritis at Oulu University Hospital, Finland. These cell lines have been approved for research use by permission of the Northern Ostrobothnia Hospital District Ethical committee. Samples from bone marrow were suspended in a proliferation medium containing alpha minimum essential medium (αMEM, Sigma Aldrich) and containing 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin, 0.1 mg/ml streptomycin and 2 mM L-glutamine. The suspension was transferred to cell culture flasks and the BMMSCs were allowed to attach for 48 h at 37 °C under 5% CO_2. Nonattached cells were removed by changing fresh medium and attached cells were cultured at the bottom of the flask until they reached a 70–80% confluence.

Transfection of MDA-MB-231 cells with GFP tagged γ-actin

Recombinant Semliki Forest Virus (SFV) encoding GFP-γ-actin [37] was used to infect MDA-MB-231 cells. After few hours of adsorption the virus media was removed and infection was allowed to proceed. On the next day of infection the cell cultures were