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Expression profile of IL-8 and growth factors in breast cancer cells and adipose-derived stem cells (ASCs) isolated from breast carcinoma

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ABSTRACT

Adipose-derived stem cells (ASCs) are regarded as a major player of breast cancer microenvironment. By production of various growth factors and expression of regulatory molecules, it is postulated that ASCs protect breast cancer cells from the host immune responses. In this study, the expressions of insulin-like growth factor-1 (IGF-1), hepatocyte growth factor (HGF), vascular endothelial growth factor (VEGF), CXCL8 (IL-8) in breast cancer cells and adipose-derived stem cells isolated from breast tissue of women with breast cancer were investigated. The results were analyzed comparatively in normal ASCs isolated from healthy normal women. In case of breast cancer tissues, results were analyzed between high stage and low stage patients. The expressions of extracted mRNAs were determined using real-time quantitative RT-PCR. As a result, in breast cancer tissues, IGF-1 and IL-8 mRNAs had 28.6 and 56-fold more expressions in high stage compared to low stage patients. In ASCs, relative quantifications (RQ) of VEGF, IL-8, HGF and IGF-1 was about 2-fold higher in patients than controls. Data of this study conclude that presence of resident ASCs within the scaffold of breast tissue may support breast tumor growth and progression through the expressions of tumor promoting factors.

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1. Introduction

There are a plenty of studies investigated the important factors in breast tumor progression and metastasis [1]. Recently, it has been demonstrated that the production of growth and angiogenic factors in tumor microenvironment is one of the most important predictors of breast cancer progression [2–4]. For instance, the productions of matrix metalloproteinases (MMPs) such as MMP2 and MMP9 [4,5] and vascular endothelial growth factor (VEGF) [6] have been reported to be involved in the growth and metastasis of human malignant tumors. In addition, Linderholm BK et al. have implicated that the higher levels of VEGF in triple-negative breast tumors is significantly correlated with shorter survival times [6]. Interestingly, it has been shown that the released VEGF induces resident endothelial cells to secrete CXCL8 (IL-8), which further promotes endothelial cell branching [7]. Several investigators have recently demonstrated the expression and role of IL-8 (CXCL8) in breast tumor invasion and progression [1]. Moreover, production of hepatocyte growth factor (HGF) can cause the upregulation of adhesion molecules on breast cancer cells and lead to their adhesion to endothelial cells and consequently to their invasion and metastasis [8]. Moreover, IGF-1 is reported to be associated with the malignant properties of tumor cells such as proliferation and differentiation [9]. Besides tumor cells, mesenchymal stem cells (MSCs) have been introduced as one of the potential cell sources of angiogenic and growth factors in tumor sites [10,11]. It has been shown that MSCs have the ability to recruit to the tumor microenvironment and injury sites after intravenous (i.v.) injection [12] which then can lead to the development of cancer such as epithelial cancers [13]. Also, Rubio D and collogues have reported that adipose-derived human MSCs can be immortalized and transformed spontaneously after long-term in vitro expansion [14]. Thus, "mesenchymal stem cells may seed cancer" [15]. The migration of MSCs to the tumor site is strongly depends on the expression of several chemokine receptors, especially CXCR4 (SDF-1 receptor) on these cells [16]. The expression of CXCR4 is regulated in many tumors by hypoxia mediators such as VEGF [17]. Interestingly, MSCs are reported to produce tumor promoting factors such as VEGF, IL-6 [10], HGF [18] and also matrix-degrading enzymes such as MMPs. Thus, MSCs might be predicted to promote tumor formation and growth [19,20]. Moreover, it has currently been

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determined that adipose-derived stem cells (ASCs) may also enter the tumor vessels and differentiate into endothelial cells [21]. Based on these reports, we aimed to determine the expressions of IGF-1, HGF, VEGF and IL-8 in breast cancer tissues and together with MMP2 in ASCs isolated from breast adipose tissue of patients with breast cancer.

2. Materials and methods

2.1. Subjects

To assessment the mRNA expressions of IGF-1, HGF, VEGF, IL-8 and MMP2, ASCs and breast cancer tissues were isolated from 21 Iranian breast cancer patients from the southern part of Iran. Data of mRNA expression levels in ASCs were compared to 15 sex matched healthy female from the same region that did not have any evidence of malignancy or autoimmune disease. Data of breast cancer tissues were compared between high stage and low stage patients. All patients and normal samples were obtained and used after giving consent. The mean ages of patients and controls were 49 and 33 years, respectively. Clinicopathological characteristics of breast cancer patients were presented in Table 1.

2.2. ASCs isolation and culture

Fragments of adipose tissue isolated from breast tissue of breast cancer patients and normal individuals in mamoplasty surgery, were washed with phosphate buffered saline (PBS), minced in small pieces and digested with 0.2% collagenase type I (GIBCO, USA) at 37 °C on a shaker for 2 h. The resulted soup was centrifuged at 400*g* for 10 min. The pellet including the adherent stromal cells was carefully put on Ficoll solution (Biosera, UK) and centrifuged at 400*g* for 30 min. The second white layer, stromal vascular fraction (SVF), was transferred into a tube and washed with PBS. The SVF pellet was resuspended in DMEM culture medium (GIBCO, USA) containing 10% fetal bovine serum (GIBCO, USA) and 1% penicillin/streptomycin (Biosera, UK). Non-adherent cells were discarded after 24 h culturing. The adherent cells were cultured by changing medium every 4 days and harvested in passage 3 nearly after 30 days culture.

Table 1
Clinicopathological characteristics of breast cancer patients.

Characteristics	Number (%)
Tumor type	
Infiltrative ductal carcinoma	14 (66.7)
Non- infiltrative ductal carcinoma	7 (33.3)
Estrogen receptor	
Positive	16(76.2)
Negative	4 (19)
Missing	1 (4.8)
Progesterone receptor	
Positive	13 (61.9)
Negative	7 (33.3)
Missing	1 (4.8)
HER2[*]	
Positive	15 (71.4)
Negative	4 (19.1)
Missing	2 (9.5)
Lymph node involvement	
Positive	13 (61.9)
Negative	8 (38.1)
Tumor size [cm]	
T1 (⩽ 2)	8 (38.1)
T2 (2 – 5)	9 (42.9)
T3 (> 5)	0 (0)
Missing	4 (19)
Stage	
0	0 (0)
I	2 (9.5)
II	11 (52.4)
III	7 (33.3)
IV	0 (0)
Missing	1 (4.8)
Histological grade	
Low grade (I and II)	15 (71.4)
High grade (III)	2 (9.5)
Missing	4 (19.1)

[*] HER2 = human epidermal growth factor receptor 2.

2.3. ASCs characterization

2.3.1. Flow cytometry analysis

ASCs were harvested by treatment with 1% trypsin–EDTA (Biosera, UK). Trypsinized cells (5×10^6) were washed twice with PBS and stained with fluorescein isothiocyanate (FITC)-conjugated mouse anti-human CD14, CD34 and CD45 (BD Biosciences, USA) and phycoerythrin (PE)-conjugated mouse anti-human CD44, CD105 and CD166 (BD Biosciences, USA). Cells were stained with FITC- or PE-labeled mouse IgG as negative controls. After 30 min incubation at room temperature, cells were washed twice with PBS. Approximately 20,000 events were collected and further analyzed with the use of WinMDI 2.5 software.

2.3.2. ASCs differentiation to chondrocytes

To show the stem cell characteristics of ASCs, 1×10^5 passage 3 ASCs were cultured in 24-well tissue-culture plate and used for chondrogenic differentiation. When ASCs culture were confluent to 60–80%, they were used for chondrogenic differentiation using chondrogenesis differentiation kit (STEMPRO Chondrogenesis Differentiation Kit, GIBCO, USA) and stained with 0.2% Safranin O (Merck, Germany) in PBS after 2 weeks.

2.3.3. RNA extraction and cDNA synthesis

Breast cancer tissues isolated from breast cancer patients were cut into the small pieces and more minced on ice using a homogenizer device (Hettich, Germany). Total RNA was extracted from both breast cancer tissues and ASCs using TRizol Reagent (Invitrogen, Germany) and Chloroform (Merck, Germany). cDNA was generated from the extracted RNAs using the cDNA synthesis kit based on the manuscript (Fermentas, Canada).

2.3.4. Quantitative real time polymerase chain reaction (Q-PCR)

Q-PCR method was performed in triplicate for each sample using a Bio-Rad thermal cycler. Approximately 2 μl cDNA was amplified in each 25 μl PCR reaction mix containing 12.5 μl of 2X SYBR Green Master Mix (Fermentas, Canada), 0.2 μl of each 10 pmol forward and reverse primers (designed in primer 3 software, Table 2) and 10.1 μl DEPC treated water. PCR amplification was done in 40 cycles using the following program: 95 °C for 10 min, 95 °C for 15 s, 56 °C for 30 s and 60 °C for 34 s. All data were compared to those from beta actin housekeeping gene.

2.4. Statistical analysis

The expressions of mRNAs were determined using $2^{-\Delta CT}$ method. Analysis of gene expression between patients and controls and with the pathological information of the patients was considered significant if P value < 0.05 using the Mann–Whitney nonparametric and Kruskal–Wallis H tests, respectively. Graphs were presented using GraphPad Prism 5.

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