Biochemical and Biophysical Research Communications xxx (2018) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

STAT3 activation by IL-6 from adipose-derived stem cells promotes endometrial carcinoma proliferation and metastasis

Yijing Chu [a, 1], Yan Wang [b, 1], Wei Peng [a], Lin Xu [a], Meixin Liu [a], Jing Li [a], Xiaoyu Hu [a], Yan Li [a], Jianxin Zuo [a, **], Yuanhua Ye [a, *]

[a] Department of Obstetrics and Gynecology, The Affiliated Hospital of Qingdao University, Qingdao, China
[b] Department of Orthopaedic Surgery, The Affiliated Hospital of Qingdao University, Qingdao, China

ARTICLE INFO

Article history:
Received 7 April 2018
Accepted 14 April 2018
Available online xxx

Keywords:
Endometrial carcinoma
Adipose-derived mesenchymal stem cells
Proliferation
Metastasis
STAT3 pathway

ABSTRACT

Endometrial cancer is the most common gynaecological cancer, and its incidence is increasing. Obesity is a well-recognized risk factor for endometrial cancer, and the mechanisms by which adipose tissue influences tumour development remain controversial. In this study, we examined the high IL-6 level in the ADSCs supernatant following treatment of endometrial cancer cell CM. Then, the activation of STAT3, a major tumourigenic IL-6 effector, was examined in ADSCs CM treated endometrial cancer cells. Conditioned ADSC medium was used to stimulate endometrial cancer cell growth in vitro. Similar to IL-6, ADSC-conditioned medium significantly promoted endometrial cancer growth and invasion. Furthermore, siRNA-mediated STAT3 inhibition in endometrial cancer cells decreased the ADSC-mediated promotion of cell proliferation and invasion. In addition, a subcutaneous nude mouse model of endometrial cancer was established to monitor the tumour-promoting effect of ADSCs. ADSC-conditioned medium promoted tumour growth, and STAT3 inhibition attenuated this effect. Based on these data, ADSCs promote endometrial cancer progression by the STAT3 signalling pathway.

1. Introduction

Adipose tissue is a complex organ with endocrine, metabolic and immune regulatory roles, and obesity-associated disease is a rapidly growing national and international public health concern [1]. In particular, obesity is highly associated with an increased risk for several chronic illnesses, including cancer development and reoccurrence [2,3]. The enhanced secretion of adipose-derived hormones, growth factors and pro-inflammatory cytokines is a major factor in the pathogenesis of tumour growth, increased cell migration and subsequently cancer metastasis [4].

Endometrial cancer is one of the most common gynaecological tumours in the world and causes about 92% of the hysterectomy. Although obesity has been identified as an associated factor, the precise cause of endometrial cancer is unknown [5,6]. As a type of mesenchymal stem cell, adipose-derived stem cells (ADSCs) exist widely in adipose tissue and have been shown to secrete various growth factors, tremendously affecting vascular growth, cell migration and differentiation [7]. Recent studies have shown that ADSCs may provide a suitable microenvironment for increasing the recurrence and metastasis of cancer and play a vital role in cancer progression [8–10]. Little information exists about the role of ADSCs in the process of endometrial cancer progression.

As one of the most important inflammatory factors, interleukin-6 (IL-6) activates signal transducer and activator of transcription 3 (STAT3), which is aberrantly activated in the majority of cancers [11]. Phosphorylated STAT3 mediates the up-regulation of key genes associated with tumourigenesis and tumour immune evasion [12–14]. In addition, we found that IL-6 secretion was significantly higher in the endometrial cancer cells CM treated ADSCs medium supernatant than in the medium supernatant of ADSCs cultured alone. In this study, we examined the effect of ADSC on endometrial cancer cell proliferation and invasion and the role of the STAT3 signalling pathway in ADSC-driven endometrial cancer cell growth and metastasis both in vitro and in vivo.

* Corresponding author. Department of Obstetrics and Gynecology, the Affiliated Hospital of Qingdao University, 16 Jiangsu Road, Qingdao 266061, China.
** Corresponding author. Department of Obstetrics and Gynecology, the Affiliated Hospital of Qingdao University, 16 Jiangsu Road, Qingdao 266061, China.
E-mail addresses: zuojianxin3012@126.com (J. Zuo), yeyuanhua8067@126.com (Y. Ye).
[1] These authors contributed equally to this work.

https://doi.org/10.1016/j.bbrc.2018.04.121
0006-291X/

2. Materials and methods

2.1. Cell culture and conditioned medium preparation

Human ADSCs from omentum tissues were obtained from healthy adult female donors who underwent abdominal surgery because of benign gynaecologic disease as previously described [10] according to the ethical guidelines of the Qingdao University Affiliated Hospital, Qingdao, China. The Ishikawa and KLE human endometrial cancer cell lines from the China Center for Type Culture Collection were used in this study. Ishikawa and KLE were cultured in medium (DMEM/F12, 10% FBS). All cell lines were grown in a humidified 5% CO_2 chamber at a temperature of 37 °C.

To prepare conditioned medium (CM), ADSCs were grown to 80% confluence in 10-cm dishes in DMEM/F12 with 10% FBS. The medium was discarded, and the cells were further cultured in serum-free DMEM/F12 for 24 h. The medium was then collected, centrifuged at 1000 × g for 10 min, and filtered through 0.22-μm filters (Millipore, Billerica, MA).

2.2. siRNA transfection

Ishikawa and KLE cells (5×10^5) were seeded in 10-cm plates for 24 h to reach a sub-confluent status and then were transfected with STAT3-specific siRNA or control scrambled siRNA (Santa Cruz Biotechnology) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. STAT3 siRNA is a mix of 3 target-specific 20- to 25-nucleotide-long siRNAs designed to knockdown gene expression.

2.3. RNA isolation and real-time PCR

Ishikawa and KLE cells were transfected with STAT3 siRNA for 48 h. Total RNA was extracted by using TRIzol reagent (Invitrogen), and complementary DNA was synthesized using a reverse transcription kit (Toyobo, Osaka, Japan). Subsequently, real-time PCR was performed using an ABI 7500 Sequencing Detection System and SYBR Premix Ex Taq (Takara, Japan). All the procedures were performed according to the manufacturer's protocols. The following primer sequences were used: GAPDH, 5′-ATGGGGAAGGTGAAGGTCG-3' (forward) and 5′-GGGGTCATTGATGGCAACAATA-3' (reverse); and STAT3, 5′-CACCTTGGATTGAGAGTCAAGAC-3' (forward) and 5′-AGGAATCGGCTATATTGCTGGT-3' (reverse). All the reactions were performed in triplicate.

2.4. Cancer cell invasion assay

CFSE-labelled Ishikawa and KLE cells (5×10^5) were cultured in 24-well Transwell plates (Corning, NY, USA) containing 8-μm pore membranes that were coated with 50 μL or BD Matrigel™ matrix (1:8 dilution). After a 6-h incubation, the lower chamber was filled with 600 μL of DMEM/F12 containing 10% FBS or CM from ADSCs. The cells on the lower surface of the membrane were then fixed in 4% paraformaldehyde. To avoid influences due to differences in cell proliferation, all cells were cultured in medium without FBS for one day prior to the experiments. The number of invading cells per high field was quantified to determine the invasive capability of Ishikawa and KLE cells. All assays were performed in triplicate.

2.5. Western blotting

The cells were lysed in NP40 buffer (Beyotime, Shanghai, China) for 10 min on ice and centrifuged at 10,000 g and 4 °C to remove cell debris. Equal amounts (30 μg) of cell extract were resolved by SDS-PAGE and transferred to polyvinylidene fluoride (PVDF) membranes (Bio-Rad, Hercules, CA), which were incubated with a primary rabbit monoclonal antibody against human STAT3 (1:1000 dilution; Cell Signaling Technology, MA, USA), p-STAT3 (1:500 dilution; Cell Signaling Technology, MA, USA) or GAPDH (1:1000 dilution; Proteintech, Chicago, IL). The membranes were then incubated in peroxidase-conjugated AffiniPure secondary IgG antibodies (H + L) (1:2000; Proteintech, Chicago, IL). The proteins were detected using a chemiluminescence detection system, and bands were quantitated using Image Lab™ version 5.1 software (both from Bio-Rad, Hercules, CA).

2.6. Cell proliferation analysis

We investigated the proliferative activity of Ishikawa and KLE cells treated with ADSC-CM for 24 h using a Cell-Light 5-Ethynyl-2′-deoxyuridine (EdU) cell proliferation kit according to the manufacturer's instructions (Guangzhou RiboBio, Guangzhou, China) [15]. Briefly, Ishikawa and KLE cells were seeded in a 96-well plate, and the medium in each well was replaced with 100 μL of 10 μM EdU medium. After a 2-h incubation, the cells were fixed with 4% paraformaldehyde and neutralized in formaldehyde containing 2 mg/mL glycine. The cells were then permeabilized with 0.5% Triton X-100 and incubated with Apollo® 567 for 30 min, followed by a second 0.5% Triton X-100 permeabilization step. The cells were then counterstained with Hoechst 33342 and imaged by fluorescence microscopy. EdU-labelled cells were manually counted in ten randomly selected fields of view from each well, and the percentage of EdU-positive cells was used to evaluate proliferative activity. All assays were performed in triplicate.

2.7. Multiplex analysis

Cell culture supernatants from the ADSCs treated with Ishikawa cells-CM for 2 d and from the ADSCs cultured alone were collected and assayed using a Luminex 200 system on a Bio-Plex (Bio-Rad) cytometer. In this study, we used a Bio-Plex Pro Human Cytokine 17-plex Assay, which contains beads for IL-1b, IL-4, IL-6, IL-10, IL-17A, IL-17F, IL-21, IL-22, IL-23, IL-25, IL-31, IL-33, IFN-g, sCD40L and TNF-a. Assays were performed following the manufacturer's instructions. Briefly, cell culture supernatants were incubated with the beads for 30 min, washed twice in washing buffer, exposed to the detection antibodies for 60 min, and washed thrice. Then, the supernatants were mixed with streptavidin-phycoerythrin (PE) for 10 min and washed thrice in assay buffer at room temperature. Finally, after using a Luminex 200 system to measure and record the fluorescence intensity, Bio-Plex Manager software was used to analyse the results.

2.8. Animals and tumour transplantation

We established a subcutaneous xenotransplanted tumour model of human endometrial cancer in nude mice to monitor the tumour cell response to ADSCs and the role of STAT3 in this response. Female 4-week-old BALB/c-nu mice (Beijing Vital River, China) were housed in a specific pathogen-free environment in the experimental animal centre of Qingdao University Affiliated Hospital. For in vivo tumour transplantation experiments, Ishikawa cells (5×10^5) were injected subcutaneously. One week after the Ishikawa cell injection, ADSC-CM (twice per week) or STAT3 siRNA (once per week) was injected into the tumour. Each of the three groups included six mice, which were injected with Ishikawa cells, Ishikawa cells + CM, or Ishikawa cells + siRNA + CM. All mice were sacrificed after 15 days, and then, the tumour volume and weight were measured (tumour volume (mm [3]) = length × width

Please cite this article in press as: Y. Chu, et al., STAT3 activation by IL-6 from adipose-derived stem cells promotes endometrial carcinoma proliferation and metastasis, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.04.121