Experimental Cell Research xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Experimental Cell Research

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

Cancer-associated fibroblasts-stimulated interleukin-11 promotes metastasis of gastric cancer cells mediated by upregulation of MUC1

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ARTICLE INFO

Keywords:
IL-11
Invasion
Migration
MUC1
CAFs

ABSTRACT

Cancer-associated fibroblasts (CAFs) are major components of the tumor stroma and regulators of tumor progression. However, the molecular mechanism by which CAFs promote gastric cancer progression should be further explored. In our study, we found that interleukin-11 (IL-11) secretion was significantly increased when CAFs were co-cultured with gastric cancer cells. Co-culture system-derived IL-11 promoted the migration and invasion of gastric cancer cells, whereas the increase of migration and invasion was attenuated by a neutralizing antibody of IL-11 or inhibition of JAK/STAT3 and MAPK/ERK pathways with specific inhibitors. Taken together, these results revealed that CAFs play a significant role in the gastric cancer progression in the tumor microenvironment through IL-11-STAT3/ERK signaling by upregulating MUC1. Also, IL-11 targeted therapy can achieve an effective treatment against gastric cancer indirectly by exerting their action on stromal fibroblasts.

1. Introduction

Gastric cancer is one of the most widespread primary malignancies, and is the third-largest cause of cancer deaths worldwide [1]. Although early diagnosis and treatment have made some progress in gastric cancer, the overall survival remains disappointing. The 5-year overall survival has remained only 20–25% due to the postoperative recurrence and metastasis [2]. Therefore, a deeper understanding of the molecular mechanisms on gastric cancer progression would help to ameliorate the treatment and prognosis of gastric cancer.

Increased evidence suggested that a unique microenvironment created by the interaction between stromal and tumor cells is crucial for tumor progression [3,4]. Cancer-associated fibroblasts (CAFs), the major components of tumor stroma, play a key role in the tumor progression [3,4]. CAFs were known to promote tumor cell invasion and metastasis by overexpression of a variety of factors, such as chemokines, cytokines and growth factors (HGF, VEGF, TGF-β, IL-22, TGF-α, IL-6) that trigger the degradation of ECM, regulate metabolic reprogramming, enhance EMT and angiogenesis, and promote proliferation and chemotherapy resistance. However, current understanding about the mechanisms of CAFs on the promotion of tumor cell invasion and metastasis is still limited in gastric cancer. In order to investigate the mechanism of gastric cancer metastasis induced by CAFs, we co-cultured fibroblast TIG-3–20 with gastric cancer cells, and found that the ability of migration and invasion was enhanced by fibroblasts in gastric cancer cells. Further gene array analyses revealed that IL-11 was significantly increased in both CAFs and gastric cancer cells of the co-culture system. Therefore, we suspect that there is a positive feedback between CAFs and gastric cancer cells, and IL-11 promoted by the interaction of CAFs and gastric cancer cells in the microenvironment is important on the gastric cancer metastasis.

Interleukin-11 (IL-11) is initially regarded as a mediator of inflammatory and immune responses, and a multifunctional cytokine. Recently, IL-11 was reported to participate in multiple inflammation-associated cancers, including gastric cancer [5,6]. IL-11 could result in an aggressive phenotype by activation of JAK/STAT3, PI3K/AKT and RAS/ERK pathways to promote cancer motility and invasion [7–9]. Although it was reported that CAFs derived-IL-11 enhances metastasis initiation in colorectal cancer [10], the exact mechanism of IL-11 in the microenvironment on gastric cancer metastasis was not well addressed.

Abbreviations: IL-11, Interleukin 11; GC cells, gastric cancer cells; CAFs, cancer associated fibroblasts; STAT3, Signal Transducer and Activator of Transcription3; ERK, extracellular regulated protein kinases; KM-Plotter, Kaplan-Meier Plotter; EMT, epithelial to mesenchymal transition; MUC1, mucin 1 (also called CA 15-3, KL-6 and BM7); CD, cytoplasmic domain; FGF, Fibroblast growth factors; SDF-1, stromal-derived factor-1; VEGF, Vascular endothelial growth factor; IL-6, interleukin 6; IFNγ, interferon gamma

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https://doi.org/10.1016/j.yexcr.2018.04.028
Received 28 January 2018; Received in revised form 25 April 2018; Accepted 26 April 2018

In our study, we found that IL-11 derived from both CAFs and gastric cancer cells enhanced the ability of migration and invasion by activating JAK/STAT3 and MAPK/ERK pathways, and the following upregulation of MUC1 in gastric cancer cells. Our results revealed that inhibition of IL-11 or its downstream signals might provide a promising therapeutic target through exerting its effect on stromal fibroblasts.

2. Martials and methods

2.1. Cell culture and reagents

Gastric cancer cell lines MGC-803 and SGC-7901, obtained from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China), were cultured in RPMI-1640 with 10% fetal bovine serum and maintained at 37 ˚C containing 5% CO_2. Recombinant Human IL-11 Protein and human IL-11 Antibody were obtained from R & D (R&D Systems, MN, USA). The primary antibodies against AKT, phosphorylated AKT (Ser473), MUC1, phosphorylated ERK (Thr202/Tyr204), Stat3, Phospho-Stat3 (Tyr705) were bought from Cell Signaling Technology (Danvers, MA, United States); ERK, anti-β-actin, secondary goat anti-rabbit and goat anti-mouse antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). MEK inhibitor, PD 98059 was purchased from Promega (Wisconsin, USA, Cat. V119A), Stattic was obtained from Sigma (Sigma-Aldrich, USA).

2.2. Enzyme-linked immunosorbent assay (ELISA)

The protein level of IL-11 in Cell Culture Supernates was measured by IL-11 ELISA kit (R&D Systems, MN, USA) according to the manufacturer's instructions.

2.3. Western blot analysis

The concentrations of total protein were quantified using a BCA kit (ab102536; Abcam) after extracted in lysis buffer (1% Triton X-100, 50 mM Tris-HCl pH 7.4, 150 mM NaCl, 10 mM EDTA, 100 mM NaF, 1 mM Na3VO4, 1 mM PMSF, 2 μg/ml aprotinin). The samples (30 μg protein/lane) loading on SDS-PAGE (Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis) were separated and transferred to nitrocellulose membrane (Immoblin-P, Millipore; Merck KGaA). 5% skim milk dissolved in tris-buffered saline Tween-20 (TBST) buffer (10 mM Tris-HCl pH 7.4, 150 mM NaCl, 0.1% Tween 20) were used for blocking the blots at room temperature for 1 h. The membranes were incubated with the primary antibodies at 4 ˚C overnight. Three washes with TBST buffer was accomplished before the corresponding secondary antibodies incubation, which was performed at room temperature for 30 min. Following three washes with TBST, the protein bands were detected using enhanced chemiluminescence reagent (SuperSignal™ Western Pico Chemiluminescent Substrate; Pierce; Thermo Fisher Scientific, Inc.) and scanned using the Electrophoresis Gel Imaging Analysis System (DNR Bio-Imaging Systems, Neve Yamin, Israel).

2.4. Quantitative real-time PCR (QRT-PCR)

Total RNA was extracted with TRIZOL (Invitrogen, Carlsbad, CA) reagent. For mRNA detection, reverse transcription was performed using the PrimeScript® RT reagent Kit with gDNA Eraser (Takara, Japan). The cDNA generated from 800 ng total RNA using SYBR® Premix EX TaqTM II (Tli RNaseH Plus, Takara, Japan). RT-qPCR was run on Applied Biosystems® 7500 Real-Time PCR Systems (Thermo Fisher SCIENTIFIC, USA). The PCR conditions were 30 s at 95 ˚C, followed by 45 cycles at 95 ˚C for 5 s, and 58 ˚C for 34 s. Data were analyzed using the Applied Biosystems 7500 software program (version 2.3) with the automatic Ct setting for adapting baseline and threshold for Ct determination. The threshold cycle and $2^{-\Delta\Delta Ct}$ method were used for calculating the relative amount of the target RNA. Transcripts of 18 S in the same incubations were used as internal control. Primer sequences for IL11: Forward (5′-TCTCTCCTGGCGGACACG-3′), Reverse (5′-AATCCAGGTTGTGGTCCCC-3′). MUC1: Forward (5′-TGTCAGTGCC GCCGAAAGAA-3′), Reverse (5′-CTACAAGTTGGCAGAAGTGG-3′). 18S: Forward (5′-CCCGGGGAGGTAGTGACGAAAAAT-3′), Reverse (5′-CGCC CGCCCGCTCCCAAGAT-3′).

2.5. In vitro migration and invasion assays

The migration and invasion assays were performed using 8 μm-transwell chambers (Corning Life Science, MA, USA). Cells were added to the upper chamber, and 1.5×10^4 CAFs in 500 μl in FBM medium (Lonza, Walkersville, MD, USA) containing 2% FBS were added to the lower chamber. The cells could migrate through the membrane and a Matrigel-coated membrane (Corning Life Science, MA, USA) (for the migration assay and the invasion assay, respectively). Non-migrating cells from the interior of the inserts were removed with cotton-tipped swabs 24 h later, and cells that migrated to the bottom of the membranes were stained with Reiter dying method for 1 min, followed by mixing diluted Giemsa redyeing for 40 min. The stained cells were counted and photographed. At least four randomly selected fields were counted and the average number was presented.

2.6. RNA interference

Human MUC1 siRNA (VIEWSOLID BIOTECH, BeiJing, China) at the final concentration of 200 ng/ml were transfected into GC cells with Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA, USA). Cells were collected for further assay at 48 h after transfection.

2.7. Peritoneal metastasis in mouse models

Female BALB/c nude mice at the age of 4 weeks were purchased from Beijing Vital River Laboratory Animal Technology Co, Ltd. and housed at a specific pathogen-free environment in the Animal Laboratory Unit of the first hospital affiliated to China medical university. MGC-803 cells alone, TIG-3–20 alone or MGC-803 and TIG-3–20 cells mixed at the ratio of 1:1 in 300 μl PBS were inoculated peritoneally. All mice were sacrificed after 16 days and tumor peritoneal metastasis nodules were counted and analyzed. All experiments on animal were given permission by the Committee on Animal Care in China Medical University.

2.8. Statistical analysis

The experimental results were reported as mean ± standard deviation and analyzed by SPSS 16.0 software (IBM, USA). The graphics were performed with GraphPad 6.0 (GraphPad Software, USA). One-way ANOVA analysis of difference was used for comparisons among multiple groups. Student's *t*-tests were used for comparisons between two groups. p-values < 0.05 were considered that there existed statistically differences.

3. Results

3.1. CAFs enhance the migration and invasion of gastric cancer cells

To investigate the mechanism of CAF-induced metastasis on gastric cancer cells, the co-cultivation system consisting of gastric cancer cells (MGC-803 or SGC-7901), and fibroblast (TIG-3–20) cells were used in this study. Fig. 1**A** showed that the markers of cancer-associated fibroblasts (α-SMA, Fibronectin and Vimentin) were highly expressed in TIG-3–20 cells both alone and in co-culture with gastric cancer cells, indicating that TIG-3–20 cells have CAF characters. Therefore, TIG-3–20 cells were used as CAFs in the following study experiments. Then,