Biochemical and Biophysical Research Communications xxx (2018) 1–7

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

SDF-1/CXCR4 axis promotes the growth and sphere formation of hypoxic breast cancer SP cells by c-Jun/ABCG2 pathway

Chenyang He [a, b], Huimin Zhang [a], Bin Wang [a], Jianjun He [a, **], Guanqun Ge [a, *]

[a] Department of Breast Surgery, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, 710061, China
[b] Department of Vascular and Endocrine Surgery, Xijing Hospital, The Air Force Military Medical University, Xi'an, 710032, China

ARTICLE INFO

Article history:
Received 18 September 2018
Accepted 20 September 2018
Available online xxx

Keywords:
CXCR4
ABCG2
SP cells
JNK/c-Jun
Hypoxia
Breast cancer

ABSTRACT

ATP-binding cassette sub-family G member 2 (ABCG2) confers to the major phenotypes of side population (SP) cells, the cancer stem-like cells. In this study, the SP cells displayed a distinctly higher ABCG2 expression level, sphere formation efficiency (SFE) and growth rate even under hypoxia condition. CXCR4 overexpression by pcDNA-CXCR4 transfection robustly increased ABCG2 expression, and promoted SFE and growth of hypoxic SP cells, while CXCR4 inhibitor AMD3100 could suppress the promotion. Additionally, we found that CXCR4 promoted the expression of c-Jun, a major gene in the oncogenic JNK/c-Jun pathway. Our data on electrophoretic mobility shift assay (EMSA) and chromatin immunoprecipitation (ChIP) assays both showed that c-Jun directly bound with the ABCG2 promoter sequence. Moreover, overexpression of JNK/c-Jun promoted ABCG2 expression, SFE, and growth of hypoxic SP cells and the promotion could be rescued by c-Jun inhibitor SP600125. In conclusion, CXCR4 increases the growth and SFE of breast cancer SP cells under hypoxia through c-Jun-mediated transcriptional activation of ABCG2.

1. Introduction

Currently, breast cancer has become one of the most common cancers and leading death cases among female cancers [1–3]. Tumor side population (SP) cells are regarded as the "seed" of tumor growth, invasion, metastasis, and recurrence and have become a hotspot in tumor growth and metastasis research [4,5]. The hypoxic environment is one of the basic characteristics of the solid tumor microenvironment, which is closely related to the survival of tumor cells, invasion and metastasis [6–8]. However, it remains largely unclear that hypoxia affects the biological behaviors of breast cancer SP or stem cells from what mechanisms.

ATP-binding cassette subfamily G member 2 (ABCG2) is a eukaryotic member of the high-capacity ABC transporter family, which confers to the major phenotype of SP cells. It plays an important role in the regulation of stem-like behaviors, such as cell promotion, cell metastasis and sphere formation [9–11]. ABCG2 could enhance cell survival and drug resistance by pumping the exogenous substances such as drugs, poisons, toxins, and others outside the cells [12–14]. It was also found that SP cell ratio would be reduced when the half-life of ABCG2 protein was shorter, and suppression of ABCG2 would reduce cell growth capacity [15]. JNK/c-Jun is an important cell survival signaling pathway that regulates cell cycle, cell proliferation and transforming growth. Activated JNK induces the phosphorylation of serine residues in the amino-terminal of nuclear transcription factor c-Jun and promotes transcription of downstream genes and secretion of multiple cytokines [16,17]. It is reported that the activation of JNK pathway could induce the expression of ABCG2 in acute lymphoblastic leukemia [18]. Other studies also demonstrated that increased activation of the JNK pathway enhanced the multi-drug resistant in colon tumor companied with upregulation of ABCG2 [19,20]. Additionally, some drugs could inhibit the proliferation of cardiac SP cells through the JNK pathway, and that may be related to the inhibited expression of ABCG2 [21]. These clues suggest that ABCG2 is likely downstream the JNK/c-Jun pathway. However, the exact way that JNK/c-Jun regulates ABCG2 expression is still unknown.

* Corresponding author. Department of Breast Surgery, the First Affiliated Hospital of Xi'an Jiaotong University, Yanta West Road 277, Xi'an, 710061, China.
** Corresponding author. Department of Breast Surgery, the First Affiliated Hospital of Xi'an Jiaotong University, Yanta West Road 277, Xi'an, 710061, China.
E-mail addresses: chinahjj@163.com (J. He), geguanqun@xjtu.edu.cn (G. Ge).

https://doi.org/10.1016/j.bbrc.2018.09.130
0006-291X/

Recently, SDF-1/CXCR4 axis was shown to have a regulatory effect on JNK/c-Jun pathway in a variety of cells [22]. Endothelial stromal cell-derived factor-1 (SDF-1) is robustly increased in local tumor under hypoxia condition [23]. CXCR4 is the natural receptor of SDF-1, which was involved in tumor growth and metastasis, apoptosis dysregulation and drug resistance [24]. Some researchers noticed that migration inhibitory factor (MIF) could activate JNK pathway in a CXCR4-dependent manner in fibroblasts and macrophages [22]. Other researchers found that blockade of CXCR4 inhibited the activation of JNK [25]. Therefore, we hypothesize that CXCR4 have a promotive effect on SP cell growth under hypoxic condition and its effect is associated with JNK/c-Jun-induced ABCG2 expression.

In this study, we found that CXCR4 expression was induced in hypoxic SP cells and had a promotive effect on the growth and sphere formation efficiency (SFE) of MCF-7 breast cancer SP cells. Moreover, our results revealed that the regulation effects of CXCR4 on SP cells were associated with JNK/c-Jun-induced ABCG2 expression in the transcriptional level.

2. Material and methods

2.1. Fluorescence-activated cell sorter (FACS)

Breast cancer cell line MCF-7 (ATCC, Manassas, VA, USA) was cultured in the DMEM-F12 medium (Invitrogen, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS, Invitrogen) in the humid atmosphere of 5% CO_2 at 37 °C. FACS was performed to sort the SP cells and was divided into two groups: the control group and verapamil group. The MCF-7 was resuspended (1×10^6/mL) followed by the treatment of 5 μg/mL Hoechst 33342 (Sigma, St. Louis, MO, USA) and 2 μg/mL propidium iodide (PI, Sigma) in two groups, while 5 mmol/L verapamil was added in the verapamil group. The cells were then incubated at 37 °C for 90 min and the staining was stopped by putting the tubes on the ice. The Hoechst dye is excited with the UV laser at 375 nm. The Hoechst dye is excited with the UV laser at 375 nm, and its fluorescence is measured with a 450/20 BP filter (Hoechst Blue) and a 675 EFLP optical filter (Hoechst Red).

2.2. Real-time quantitative polymerase chain reaction (qPCR) assay

Total RNA was extracted from the cells with the treatment of Trizol (Invitrogen, Carlsbad, CA, USA) and then be synthesized into cDNA with Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA). Subsequently, the qPCR assay was conducted in the 20-μL reaction system containing 10-μL SYBR Premix Ex Taq II (Takara Biotechnology, Dalian, China). The primers of genes are as follows: ABCG2, F 5′−CCA TAG CCA CAG GCC AAA GT-3′ and R 5′-GGG CCA CAT GAT TCT TCC AC-3′, β-actin, F 5′-TAC CCA GGC ATT GCT GAC AGG-3′ and R 5′-ACT TGC GGT GCA CGA TGG A-3’. The qPCR was performed according to the manufacturer's protocol.

2.3. Western blotting

The proteins were extracted from cells with Lysis Buffer and Phenylmethanesulfonyl fluoride (Beyotime; 100:1). Subsequently, 25 μg of protein was separated in the 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis following the detection of protein concentration using a BCA kit (Beyotime). The separated proteins were then electrophoretic transferred to polyvinylidene fluoride (PVDF) membrane (Bio-Rad, Hercules, CA, USA) by semi-dry blotting apparatus (Bio-Rad). Next, the blots were blocked with 5% skim milk dissolved in Tris-buffered saline (TBS) for 2 h followed by the incubation with the primary antibodies (CST, Danvers, MA, USA) diluted in TBS (1:500) at 4 °C overnight. Afterward, the blots were incubated with horseradish peroxidase-conjugated secondary antibody (Abcam, Cambridge, UK) at room temperature for 1 h. Finally, the protein was detected using an enhanced chemiluminescence method.

2.4. Sphere formation assay

Single cells were cultured in DMEM-F12 supplemented with 10 ng/mL basic fibroblast growth factor (bFGF), 20 ng/mL epidermal growth factor (EGF), 2% of B27 supplement, 0.4% bovine serum albumin and 5 μg/mL insulin (all from Invitrogen). Cells were plated in 96-well Ultra-low attachment plates (Corning) as a density of 500 per well with 200 μL culture medium, and 20 μL medium per well was added every 2 days. Spheres in the plate were then counted under the microscope 8 days later. The passage culture was performed with cells in low attachment 10 cm plate. Spheres were enzymatically dissociated every 3 days by treated with a trypsin-EDTA solution (Cambrex) for 2 min and seeded in another 10 cm dish.

2.5. Cell proliferation

Cell proliferation was measured using the 3-(4,5-Dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) assay according to the manufacturer's instructions. The absorbance was detected by a microplate reader (Thermo Fisher Scientific) at 550 nm.

2.6. Cell transfection

The cells were cultured in the 6-well plate with DMEM-F12 supplemented with 10% FBS in a humidified atmosphere of 5% CO_2 at 37 °C until the cells reached 70% fusion. The ABCG2 siRNA (Ribobio, Guangzhou, China) and the plasmids pcDNA-JNK (Dharmacon, Lafayette, CO), pcDNA-ABCG2 (Invivogen, San Diego, CA), or pcDNA-CXCR4 (Invivogen) were transfected with Lipofectamine 3000 as per the standard instruction. The vectors were separately diluted in 200 μL FBS-free DMEM/F12 medium with 6 μL TurboFect (Thermo Fisher Scientific, Waltham, MA, USA) per well and incubated under hypoxic condition (2.5% O_2) at 37 °C for 24 h.

2.7. Chromatin immunoprecipitation assay

Chromatin Immunoprecipitation (ChIP) assay was conducted using a ChIP Kit (Upstate Biotechnology, Lake Placid, NY, USA). The cells were treated with 1% formaldehyde solution for 10 min at 37 °C followed by lysed in SDS buffer and sonicated. After centrifugation, the liquid supernatant was diluted 10-fold in ChIP buffer and then incubated with anti-JNK (CST) or anti-IgG (CST) overnight at 4 °C. Subsequently, Immune complexes were precipitated, washed, and eluted. DNA fragments were separated from the protein and dissolved in water. The amount of immunoprecipitated DNA was assessed by qPCR.

2.8. Electrophoretic mobility shift assay (EMSA)

Electrophoretic mobility shift assay (EMSA) was conducted on the basis of the instructions of the kit (Pierce, Rockford, IL, USA). Briefly, a probe containing a potential c-Jun-binding motif and a scrambled probe was synthesized and be labeled using biotin. Besides, an oligonucleotide identical to the sequence from (−1266) to (−973) in the ABCG2 promoter were synthesized, which contains the c-Jun responsive elements. The nuclear protein of SP cells was extracted using the nuclear extract kit (Pierce) followed by measurement of

Please cite this article in press as: C. He, et al., SDF-1/CXCR4 axis promotes the growth and sphere formation of hypoxic breast cancer SP cells by c-Jun/ABCG2 pathway, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.09.130