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TGF-β1 induced fascin1 expression facilitates the migration and invasion of kidney carcinoma cells through ERK and JNK signaling pathways

Jianyu Yang [a, b], Naiwen Zhang [a, b], Ruxu Gao [a, b], Yuyan Zhu [a, b], Zhe Zhang [a, b], Xiaolong Xu [a, b], Jianfeng Wang [a, b], Zeliang Li [a, b], Xiankui Liu [a, b], Zhenhua Li [a, b], Jun Li [a], Jianbin Bi [a, b, *], Chuize Kong [a, b, **]

[a] Department of Urology, The First Hospital of China Medical University, Shenyang, 110001, China
[b] Institude of Urology, The First Hospital of China Medical University, Shenyang, 110001, China

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ABSTRACT

Transforming growth factor-β1 (TGF-β1) plays a crucial role in the signaling network that controls cellular invasion and motility capability during tumor development. To investigate whether fascin1 plays a crucial role in TGF-β1-facilitated invasion and migration of kidney cancer cells (KCC), real-time PCR and western blotting were used to test the fascin1 expression after TGF-β1 treatment (10 ng/ml) in 769-P and OSRC cells. Fascin1 was silenced using the small interfering RNA (siRNA) technique. Cytoskeleton staining was used to test the change of Cytoskeleton. Cell migration and invasion changes were measured by wound-healing and Transwell assay. The results indicate that mRNA and protein levels of fascin1 were dramatically increased after treatment with 10 ng/ml TGF-β1 in 769-P and OSRC cells. TGF-β1 promoted the occurrence of EMT (Epithelial-Mesenchymal Transition) and the invasive and migratory capabilities of the two cell lines after treatment with 10 ng/ml TGF-β1. In addition, fascin1 siRNA dramatically attenuated the invasiveness and migration induced by TGF-β1. Furthermore, we identified that specific inhibitors of ERK and JNK signaling pathways, FR180204 and SP600125, can suppress TGF-β1-induced fascin1 expression. In conclusion, these results reveal that fascin1 is an important mediator of TGF-β1-induced invasion and migration of KCC through ERK and JNK signal pathways.

1. Introduction

Kidney cancer is the sixteenth most common urological malignancy and cause of cancer-related death worldwide [1]. In the past few years, the incidence and mortality rates have increased gradually. Recently, in China, there has been an annual increase of approximately 2% in the incidence [2]. The distant metastases and poor diagnosis of kidney cancer are the cause of treatment failure in patients with kidney cancer [1,3]. Therefore, studying the underlying molecular mechanisms of the initiation and development of kidney cancer is of great importance and could be used to help improve therapeutic strategies in KCC.

TGF-β1 is a multifunctional cytokine that plays a pivotal role in regulating a wide variety of cellular processes, including cell growth, differentiation, migration, motility and invasive capability [4]. Recent studies have shown that TGF-β1 can promote carcinogenesis and the invasion and migration of tumor cells [5,6]. Moreover, abrogation of the TGF-β1 signaling axis contributes to suppress tumor progression [7]. However, TGF-β1-mediated tumor cells metastasis and invasion in kidney cancer are not fully understood [1,7].

Fascin1 is a globular protein of approximately 55 kDa that belongs to a unique family of actin-bundling proteins [8]. Fascin1 is widely expressed by mesenchymal tissues and in the nervous system, which play a critical role in cancer cell migration and invasion [9,10]. Recent publications have shown that the up-

* Corresponding author. Department of Urology, Institude of Urology, The First Hospital of China Medical University, 155 North Nanjing Street, Heping, Shenyang, Liaoning, 110001, China.
** Corresponding author. Department of Urology, Institude of Urology, The First Hospital of China Medical University, 155 North Nanjing Street, Heping, Shenyang, Liaoning, 110001, China.
E-mail addresses: chaoyucmu@163.com (J. Yang), bijianbin@yahoo.com (J. Bi), kongchuize_cmu@sina.com (C. Kong).

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regulation of fascin1 can promote cancer cell invasion and tumor metastasis. On the other hand, through the inhibition of fascin1 expression, cancer cell invasion and cancer progression can be suppressed [11,12]. Previously, we found that fascin1 can facilitate the invasion and migration of urothelial cancer of the bladder. Meanwhile, we also confirmed that TGF-β1 can promote the invasion and migration of bladder cancer cells by increasing fascin1 expression [13–15].

2. Materials and methods

2.1. Cell culture

The human KCC 769-P and OSRC were obtained from the China Type Culture Center (Shanghai, China) and cultured following the manufacturer's protocol. The cells were cultured at 37 °C with an atmospheric composition of 5% CO_2 and 95% air using RPMI-1640 medium (Lonza, Verviers, Belgium) supplemented with 10% fetal bovine serum (FBS) (Euro Clone, West York, UK). When cells grew to 70% confluence, the culture medium was removed and replaced with 1% FBS RPMI-1640 medium with or without 10 ng/ml TGF-β1 (R&D Systems, USA) for 24 h.

2.2. Cytoskeleton staining

According to the instructions of Rhodamine Phalloidin (YEASEN, Shanghai, China), 769-P and OSRC cells was seeded on 24 well plates, and when the cell density reached 50%, the old culture medium was removed, and cells were washed twice with 37 °C phosphate-buffered saline (PBS). Afterwards, 4% formaldehyde was used to fix the cells for 10 min at room temperature. Next, cells were washed three times with PBS for 10 min each time. After treatment with 0.5% Triton X-100 for 5 min at room temperature, cells were washed three times with PBS and incubated with 200 μl Rhodamine Phalloidin per well in the dark conditions for 30 min at room temperature. After incubation, cells were washed three times with PBS for 5 min each time. Finally, approximately 200 μl of DAPI (4′, 6-diamidino-2-phenylindole) (Beyotime, Shanghai, China) were used to counterstain the cells for 30 s, and imaging was performed using a fluorescence microscope.

2.3. Cell transfection

The 769-P and OSRC cells were cultured to 80% confluence in RPMI-1640 medium supplemented with 10% FBS. The fascin1 siRNA (GenePharma, Shanghai, China) was diluted according to the manufacturer's instructions. Cell transfection was performed using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) according to the product's protocol. After 6 h, the medium was replaced with RPMI-1640 containing 10% FBS and then cultured for another 24 or 48 h with or without 10 ng/ml TGF-β1 for further assays.

2.4. Real-time RT-PCR

Total RNA was extracted by using ice-cold Trizol reagent (Invitrogen), following the manufacturer's protocol. The concentration of total RNA was tested using a Thermo Scientific NanoDrop ND-100 (Wilmington, DE, USA). Total RNA was subjected to reverse transcription using the SYBR PrimeScript RT- PCR kit (Perfect Real-Time) (Takara, Kyoto, Japan). Real-time PCR was carried out using a Thermal Cycler Dice™ Real-Time system TP800 (Takara). The sequence of primers designed for fascin1 and GAPDH were listed as follows: fascin1, 5′-CTGCTACTTTGACATCGAGTGG-3′ and 5′-GGGCGGTTGATGAGCTTCA-3'; GAPDH, 5′- ACAACTTTGGTATCGTGGAAGG-3′ and 5′- GCCATCACGCCACAGTTTC-3'. The mRNA expression of the target gene was analyzed using the $2^{-\Delta\Delta Ct}$ method.

2.5. Western blotting analysis

Monolayer cell cultures at 90% confluence were harvested in RIPA lysis buffer (Beyotime, Shanghai, China) with a cocktail of protease inhibitors (Sigma, St, Louis, MO, USA). Protein concentrations were measured using the BCA assay kit (Sigma) and boiled for 15 min at 100 °C. A sample consisting of 15 μg of protein was separated by 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and electrotransferred onto polyvinylidene difluoride (PVDF) membranes (Millipore, Billerica, MA, USA). The membranes were subsequently blocked with 5% nonfat milk in Tris-buffered saline and Tween-20 (TBS-T) for 90 min at 37 °C and incubated with specific primary antibodies overnight at 4 °C. Next, the membranes were washed and incubated at 37 °C for 90 min with horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse secondary antibodies. The bands were then visualized by ECL detection reagents (Transgen Biotechnology, Beijing, China) on a MiroChemi™ Chemiluminescent Imaging System (DNR Bio-Imaging Systems, Mahale HaHamisha Jerusalem, Israel). An antibody against fascin1 was obtained from Abcam (1:5000; Hong Kong, China). Antibodies against E-cadherin, N-cadherin and Vimentin were purchased from Santa Cruz Biotechnology (1:2000). Antibodies against *p*-JNK and *p*-ERK were supplied by EnoGene (Nan Jing, China). The housekeeping protein GAPDH (1:2000; Sigma-Aldrich, USA) was used as an internal control.

2.6. Cell migration assay

The migration capacities of 769-P and OSRC were investigated by wound-healing assay. The 769-P and OSRC cells were seeded in 6-well plates and incubated in RPMI-1640 medium supplemented with 10% FBS. When cells grew to 90% confluence, the medium was replaced with 1% FBS RPMI-1640 medium without or with 10 ng/ml TGF-β1 for 24 h. Subsequently, a wound was performed by scraping using a 10-μl pipette tip, and then, the wells were washed one time with PBS. The cells were left to grow for 24 or 48 h in serum-free medium. The scratch spaces were measured using photomicrographs.

2.7. Cell invasion assay

Cell invasion was carried out using Transwell methods. Cells (1×10^4) in serum-free medium were applied onto the upper chamber of a 24-well Matrigel-coated micropore membrane filter with 8-μm pores (Corning, NY, USA) and the bottom chambers were loaded with RPMI-1640 supplemented with 10% FBS 600 μl. After 24 h incubation, the cells on the upper surface were removed with a cotton swab, and those that invaded the under surface were fixed with 4% paraformaldehyde and stained with crystal violet. Cells were observed under a light microscope and five randomly selected fields were selected at × 200 magnification. The mean value was calculated from data obtained from three independent experiments.

2.8. Inhibitor treatment

The specific protein kinase inhibitors FR180204 and SP600125 can inhibit ERK and JNK, respectively, and then block the downstream signaling pathway. FR180204 and SP600125 (Selleck, USA) were dissolved in DMSO. The 769-P and OSRC cell lines were pretreated with 20 μM SP600125, 10 μM FR180204 for 1 h and then treated with FBS-free RPMI-1640 medium with TGF-β1 (10 ng/ml)

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