Biomedicine & Pharmacotherapy 104 (2018) 576–581

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

Knockdown of TMEM45B inhibits cell proliferation and invasion in gastric cancer

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ARTICLE INFO

Keywords:
TMEM45B
Gastric cancer
Proliferation
Invasion

ABSTRACT

Transmembrane protein 45B (TMEM45B), belonging to the TMEM family, has been found abnormally expressed in several types of tumors and can play an important role in tumorigenesis. However, the role of TMEM45B in gastric cancer remains unclear. Therefore, the current study was designed to examine the effects of TMEM45B on gastric cancer cell proliferation, migration and invasion *in vitro*, and to explore the potential molecular mechanisms. The presented study is the first demonstration that TMEM45B was highly expressed in human gastric cancer tissues and cell lines. In addition, knockdown of TMEM45B significantly inhibited cell proliferation, migration/invasion and EMT phenotype in gastric cancer cells. Furthermore, knockdown of TMEM45B efficiently inhibited the expression of p-JAK2 and p-STAT3 in gastric cancer cells. Taken together, our findings indicate that knockdown of TMEM45B suppresses the proliferation, migration and invasion of gastric cancer cells, at least partly, *via* the inhibition of JAK2/STAT3 signaling pathway. Therefore, TMEM45B may be a new potent therapeutic molecule for the treatment of gastric cancer.

1. Introduction

Gastric cancer is the most prevalent cancer type and the second largest contributor to cancer-related deaths in the world [1]. Despite significant improvements in the surgery and radiotherapy/chemotherapeutic treatment, the 5 year survival rate of patients with gastric cancer is poor because most gastric carcinoma cases are diagnosed in advanced stages [2,3,4]. Therefore, exploring the molecular mechanisms underlying gastric cancer progression is of significance to the development of therapeutic strategies for gastric cancer patients.

The transmembrane (TMEM) family of proteins plays critical roles in regulating fibrogenesis, transepithelial ion transport, neuronal excitability, smooth muscle contraction and nociception [5,6,7]. Transmembrane protein 45B (TMEM45B), belonging to the TMEM family, has been found abnormally expressed in several types of tumors and can play an important role in tumorigenesis [8,9,10]. For example, a study by Li et al. reported that TMEM45B was highly expressed in human osteosarcoma cell lines, and down-regulation of TMEM45B significantly suppressed the proliferation, migration, and invasion of osteosarcoma cells [11]. However, the role of TMEM45B in gastric cancer remains unclear. Therefore, the current study was designed to examine the effects of TMEM45B on gastric cancer cell proliferation, migration and invasion *in vitro*, and to explore the potential molecular mechanisms. Our findings provide evidence that knockdown of TMEM45B inhibited the proliferation, migration and invasion of gastric cancer cells through modulating the JAK2/STAT3 signaling pathway.

2. Materials and methods

2.1. Human gastric cancer samples and cell culture

Frozen normal gastric tissues (10 cases) and gastric cancer tissue samples (10 cases) were obtained from China-Japan Union Hospital of Jilin University (China). The protocol was approved by the Ethics Committee of China-Japan Union Hospital of Jilin University, and informed consent was obtained from all patients.

Human gastric adenocarcinoma cell lines (BGC-823, MGC-803, SGC-7901 and HGC-27) and a normal gastric epithelial cell line (RGM-1) were purchased from Shanghai Institutes for Biological Sciences (Shanghai, China). The cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (FBS), 100 mg/mL streptomycin, and

Abbreviations: TMEM45B, transmembrane protein 45B; FBS, fetal bovine serum; qRT-PCR, quantitative real-time polymerase chain reaction; ECL, enhanced chemiluminescence; DMSO, dimethyl sulfoxide; PVDF, polyvinylidene difluoride

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https://doi.org/10.1016/j.biopha.2018.05.016
Received 8 February 2018; Received in revised form 4 May 2018; Accepted 7 May 2018

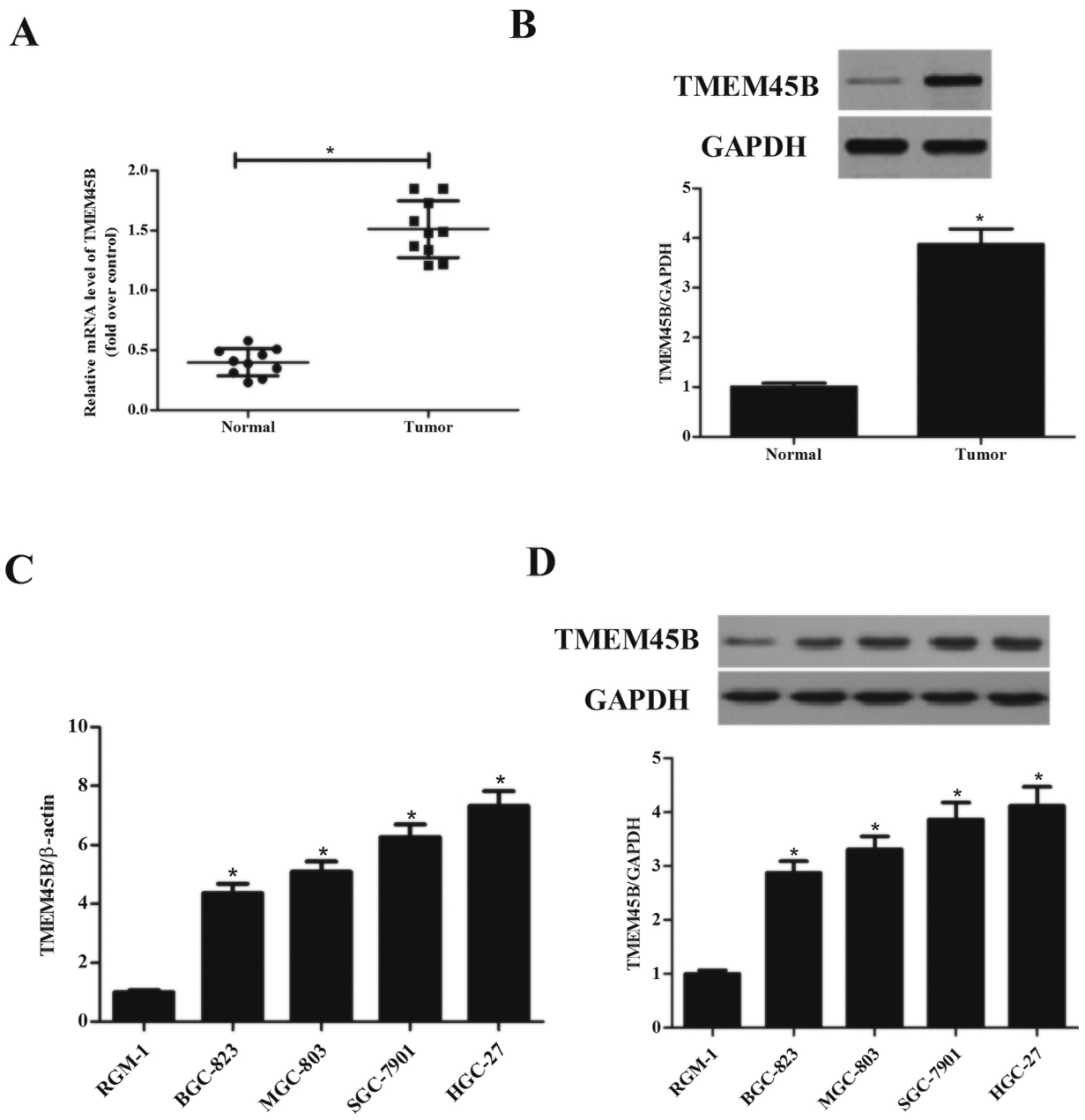

Fig. 1. TMEM45B is overexpressed in human gastric cancer tissue and cell lines. A, The mRNA expression profile of TMEM45B in gastric cancer tissues were detected using qRT-PCR analysis. B, The protein expression profile of TMEM45B in gastric cancer tissues were detected using western blot analysis. $^{*}P < 0.05$, *vs*. Normal group. C, The mRNA expression profile of TMEM45B in human gastric cancer cell lines was evaluated by qRT-PCR. D, The protein expression profile of TMEM45B in human gastric cancer cell lines was evaluated by western blot. The results were reproduced in three independent experiments. $^{*}P < 0.05$, *vs*. RGM-1 group.

100 U/mL penicillin in a humidified incubator with an atmosphere of 95% air-5% CO_2 at 37 °C.

2.2. RNA interference and transfection

The specific siRNA against human TMEM45B and its negative control were synthesized by Invitrogen (Carlsbad, CA, USA). For *in vitro* transfection, HGC-27 cells (5×10^4 per well) were transfected with siRNA-TMEM45B or scramble using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA), according to the manufacturer's instructions.

2.3. Quantitative real-time polymerase chain reaction (qRT-PCR) analysis

Total RNA was isolated from gastric cancer tissues and cells using the RNA plus kit (Invitrogen, Carlsbad, CA, USA). Five microgram of total RNA from each sample was reverse transcribed into first-strand cDNA for RT-PCR analysis using a High-Capacity RNA-to-cDNA kit (Applied Biosystems, Foster City, CA, USA). All qRT-PCR tests were performed by the ABI Step One Plus real-time PCR-System (Applied Biosystems) according to the manufacturer's instructions. The sequences of primers were as follows: TMEM45B, forward 5′-TCCTTCA CCGCGCCTATAATC-3′ and reverse 5′-TACCGGGTTCATGCCATT CTC-3′; β-actin forward 5′-GATCATTGCTCCTCCTGAGC-3′ and reverse 5′-ACTCCTGCTTGCTGATCCAC-3′. β-actin served as internal control, and the differential expression of these genes was analyzed by the ΔCt method [12].

2.4. Western blot

Total protein was extracted from gastric cancer tissues and cells