Biomedicine & Pharmacotherapy 104 (2018) 119–126

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

Down-regulation of ETS2 inhibits the invasion and metastasis of renal cell carcinoma cells by inducing EMT *via* the PI3K/Akt signaling pathway

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ARTICLE INFO

Keywords:
ETS2
Renal cell carcinoma (RCC)
Invasion
Metastasis
Epithelial-Mesenchymal transition (EMT)

ABSTRACT

V-ets erythroblastosis virus E26 oncogene homolog 2 (ETS2), belonging to the ETS family of transcription factors, is implicated in a broad range of cellular functions. Recently, ETS2 has been found playing an important role in the progression of some types of cancers. However, it remains unclear whether ETS2 has any effects on renal cell carcinoma (RCC). In this study, we investigated the biological functions of ETS2 in RCC. The results showed that ETS2 was highly expressed in RCC tissues and cell lines and its expression had an association with clinicopathological characteristics of RCC patients. In addition, down-regulation of ETS2 significantly inhibited RCC cell invasion *in vitro* and metastasis *in vivo* as well as suppressed the epithelial-mesenchymal transition (EMT) process. We also found that ETS2 down-regulation significantly reduced the levels of PI3K and Akt phosphorylation in RCC cells. Taken together, we suggest that ETS2 is of potential value as a molecular target for RCC treatment.

1. Introduction

Renal cell carcinoma (RCC), a common subtype of kidney cancer, accounts for almost 95% of all kidney neoplasms and primarily occurs in the renal parenchyma [1,2]. According to statistics, more than 200,000 people are annually diagnosed with RCC and this incidence is increasing year by year [3,4]. RCC may be caused by multiple risk factors related to genes or environments [5]. So far, a large number of RCC patients with localized tumors can be cured after complete surgical resection [6]. But about 25% of the patients may develop distant metastasis and their five-year survival rate is even less than 10% [7,8]. The high mortality is mainly due to the typical resistance of metastatic RCC to conventional radiotherapy, chemotherapy and hormonal therapy [9,10]. Therefore, it is urgently needed to explore novel therapeutic markers for the purpose of facilitating our understanding of the progression of RCC.

The E26 transformation-specific (ETS) family contains 28 members [11]. These members are highly conserved in the aspect of function and can be further divided, according to the structure, into 12 subgroups (ETS, PEA3, ESE, TCF, ELF, TEL, ERG, ERF, SPI, ELG, PDEF and ER71) [12–14]. Most of the ETS members are transcription factors, but some of them act as repressors and some of them both repressors and activators [15–18]. Based on investigations, the ETS members are expressed in a wide range of tissues and are implicated in diverse pathological and physiological processes including embryonic development, hematopoietic differentiation and angiogenesis [19,20]. Recent studies have shown that some of the ETS members play an important role in tumorigenesis. For example, ETS2 has been found overexpressed in breast and prostate cancer cells and its deletion inhibits the growth and survival of these cells [21–23]. However, it remains unclear whether ETS2 has any effects on RCC cells.

In this study, we showed that ETS2 was up-regulated in RCC tissues and cell lines. Down-regulation of ETS2 inhibited RCC cell invasion *in vitro* and metastasis *in vivo*. In addition, the decrease in ETS2 expression suppressed the epithelial-mesenchymal transition (EMT) process in RCC. We also found that the PI3K/Akt signaling pathway played a key role in si-ETS2-inhibited RCC cell invasion and EMT.

2. Materials and methods

2.1. Patients and tissue samples

A total of 112 patients participated in the study with informed consent. These patients provided RCC tissues and matched adjacent tumor-free tissues when they received radical or partial nephrectomy at Huaihe Hospital of Henan University (Kaifeng, China). None of the patients underwent chemotherapy, radiotherapy or other tumor-related interventions. The pathologic grading of tumors was based on the WHO

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https://doi.org/10.1016/j.biopha.2018.05.029
Received 22 January 2018; Received in revised form 2 May 2018; Accepted 8 May 2018

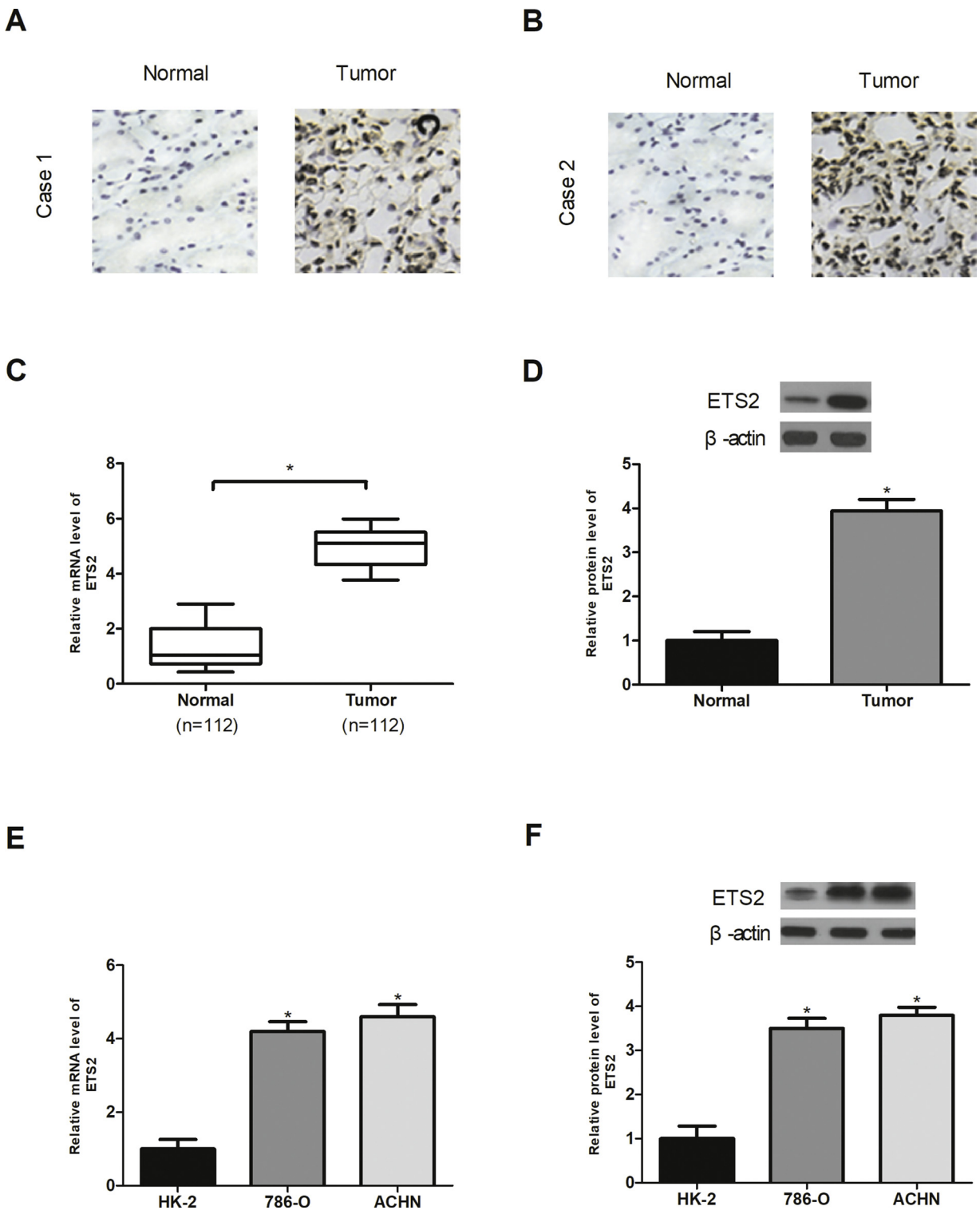

Fig. 1. Expression of ETS2 is up-regulated in RCC tissues and cell lines and correlates with clinicopathological characteristics of RCC patients. (A, B) Representative immunostaining of ETS2 expression in RCC tissues and adjacent normal tissues. Magnification: 400×. (C, D) Expression of ETS2 mRNA and protein in 112 pairs of RCC tissues and matched normal tissues was assessed by RT-PCR and western blot analysis. (E, F) Expression of ETS2 mRNA and protein in RCC cell lines (786-O and ACHN) and the normal renal tubular epithelial cell line HK-2 was assessed by RT-PCR and western blot analysis. (*$p < 0.05$).

criteria. All tissue samples were frozen in liquid nitrogen and stored at −80 °C immediately after collection. This study was performed with the approval of the Ethics Committee of Henan University.

2.2. Cell lines and cell culture

Human RCC cell lines (786-O and ACHN) and human renal tubular epithelial cell line HK-2 were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). All cell lines were cultured in Dulbecco's modified Eagle medium (DMEM; Sigma, St.