

## Original Article

# Expression of TDP43 in cervical cancer

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**Abstract:** TDP43 is ubiquitously expressed in human tissues and plays a crucial role in a wide range of neurodegenerative diseases. However, the study in cancer especially in cervical cancer is less known. The aim of this study is to identify the expression and function of TDP43 in cervical cancer. Here we reported that protein and mRNA level of TDP43 expression was up-regulated in cervical cancer tissues. The status of TDP43 was correlated with T stage in cervical cancer. Moreover, we knocked down TDP43 by RNA interference and found that the inhibition of TDP43 suppressed the cervical cancer cell growth and induced cell cycle arrest. Over-expression of TDP43 promoted cell growth and drove cell cycle. Finally, silencing TDP43 reduced CCNA1 and CDK2 activity, and TDP43 overexpression enhanced CCNA1 and CDK2 activity. These results might provide a potential target to cancer treatment.

**Keywords:** TDP43, cervical cancer, cell growth, cell cycle

### Introduction

Cervical cancer as the third most common gynecological cancer has the fourth highest cancer-related death in women [1, 2]. In spite of the development of modern radiotherapy, chemotherapy, and surgery, the 5-year survival rate for advanced patients is still very low [3]. A better understanding the mechanisms of cervical cancer development is becoming essential.

TDP43 is a nuclear protein containing 414 amino acids with two RNA recognitions and a carboxy-terminal glycine-rich domain [4]. TDP43 belongs to a RNA/DNA binding protein initially as a regulator of HIV-1 gene expression [5]. TDP43 is highly conserved and exists in vertebrates, *Drosophila* and *C. elegans* [6].

TDP43, which is ubiquitously expressed in human tissues, plays an important role in gene transcription and RNA splicing [7-9]. Studies on TDP43 pathology were reported in a wide range of neurodegenerative diseases [10-12]. However, the study in cancer is still rare.

The aim of this study is to identify the expression and function of TDP43 in cervical cancer. Our findings might provide a potential target to cancer treatment.

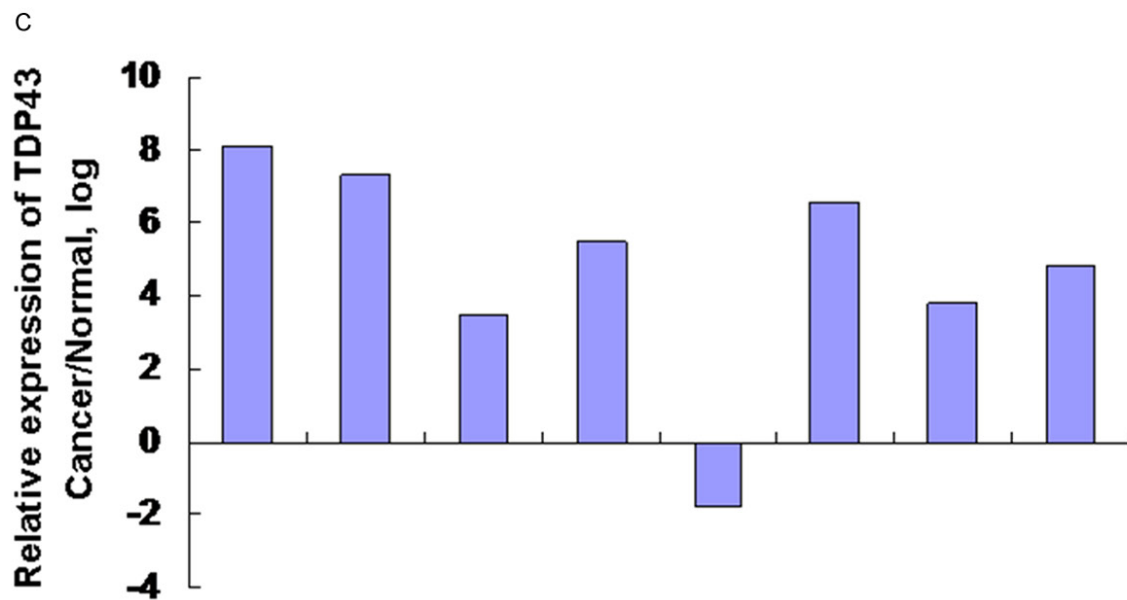
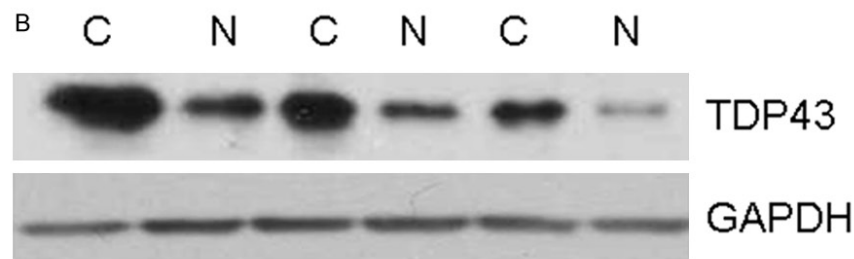
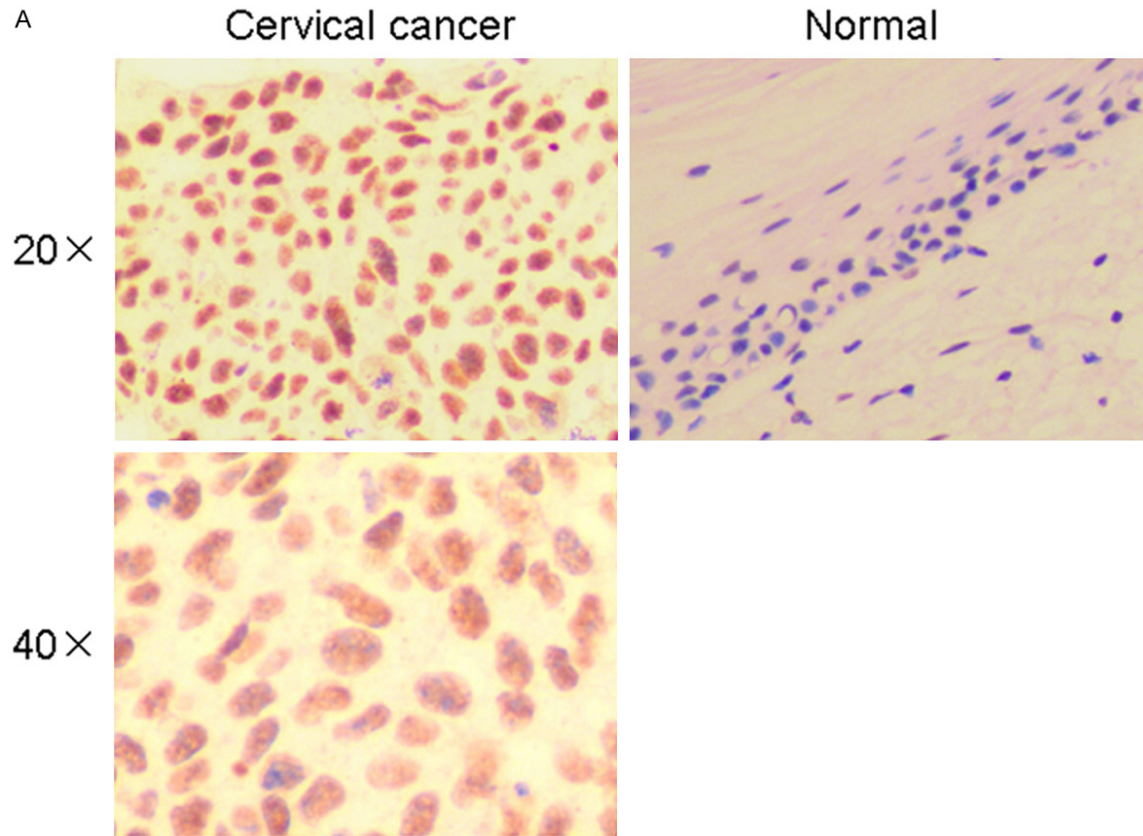
### Materials and methods

#### *Human tissue specimens and cell culture*

Cervical cancer and adjacent normal tissues were collected from the First Affiliated Hospital, Xi'an Medical University. Informed consent was obtained from all patients and the study was approved by the local Ethics Committee. The cervical cancer Caski cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco) supplemented with 10% bovine calf serum (BCS) (Gibco) at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>.

#### *Western blot analysis*

The tissues or cells were lysed in Laemmli buffer. The lysates were analyzed by SDS/PAGE and transferred electrophoretically to polyvinylidene difluoride membrane. And then the blots were tested with specific antibodies. The immunoreactive proteins were revealed using an ECL kit. The following antibodies were used: Rabbit anti-TDP43 antibody (Abcam), Rabbit anti-CCNA1 antibody (Santa Cruz Biotechnology), Mouse anti-CDK2 antibody (Santa Cruz Biotechnology), Rabbit anti-GAPDH antibody (Santa Cruz Biotechnology).



## TDP43 and cervical cancer

**Figure 1.** TDP43 expression was up-regulated in cervical cancer tissues. A. The expression of TDP43 in cervical cancer tissues was tested by immunohistochemistry (IHC). B. TDP43 protein expression level was detected by western blot analysis. C. TDP43 mRNA level was analyzed by real time PCR. Data are expressed as mean  $\pm$  standard deviation.

**Table 1.** Relationship between expression of TDP43 and T stage in cervical cancer

| T stage | Cases | IHC score       | P values |
|---------|-------|-----------------|----------|
| T1/T2   | 12    | 1.55 $\pm$ 1.18 | 0.015    |
| T3/T4   | 18    | 2.50 $\pm$ 1.01 |          |

### Real-time PCR

The procedures were previously described in detail [13].

### Cell transfection

For transfection, tumor cells were seeded in 6-well plates and transfected with 10  $\mu$ l (20  $\mu$ M) siRNA or 2  $\mu$ g plasmid using Lipofectamine™ 2000 reagent (Invitrogen). Each experiment was done in triplicate and at least three times independently.

Small interfering RNAs (siRNAs) targeting TDP43 or their corresponding negative controls were designed and synthesized by Guangzhou RiboBio (Guangzhou, China). TDP43 expression plasmid was generated and stored in our laboratory [13].

### MTT and colony formation assay

Cell growth was assessed by the 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) reduction assay as described previously [14]. Briefly, cells were seeded in 96-well plates. After transfection, the quantity of viable cells was estimated using MTT (Sigma, St. Louis, MO). The absorbance was read at 490 nm. Cell viability was defined as the relative absorbance of transfected cells versus the control cells. For colony formation assay, we performed as reported previously [14].

### Flow cytometry analysis of cell cycle

Cell cycle was analyzed as described previously [15]. Briefly, cells were harvested at 70-80% confluence and resuspended in fixation fluid at a density of  $10^6$ /ml. Propidium iodide (PI) solution was then added, and the cell cycle was detected by FACS Caliber (Becton-Dickinson).

### Statistical analysis

Data are expressed as mean  $\pm$  standard deviation and analyzed with ANOVA using SPSS software. A value of  $P < 0.05$  was considered as statistical significance.

## Results

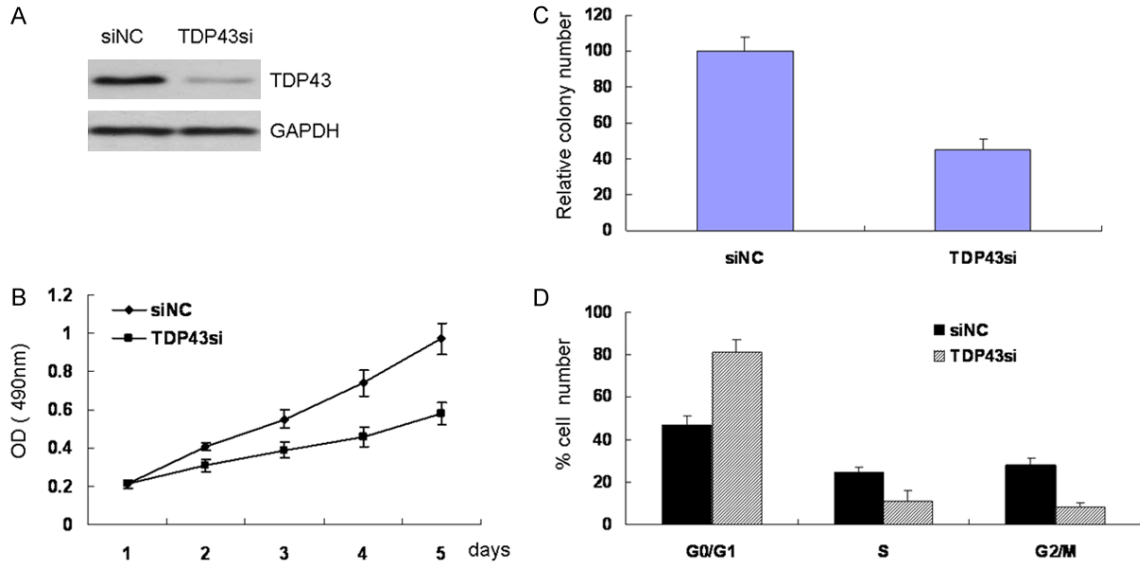
### TDP43 expression was up-regulated in cervical cancer tissues

To investigate the role of TDP43 in cervical cancer, we firstly examined the expression of TDP43 in cervical cancer tissues by immunohistochemistry (IHC). We found that TDP43 mainly located in the nucleus of cervical cells. IHC showed that the expression of TDP43 was increased in cervical cancer tissues compared with normal tissues (**Figure 1A**). The analysis of clinicopathological factors showed that the status of TDP43 was correlated with T stage ( $P < 0.05$ ) (**Table 1**). In addition, TDP43 protein expression level was significantly higher than normal tissues by Western blot analysis (**Figure 1B**). We also found that mRNA level of TDP43 in cervical cancer tissues was upregulated by real time PCR (**Figure 1C**). The above results indicated that TDP43 expression was up-regulated in cervical cancer tissues and might play an important role in cervical cancer progression.

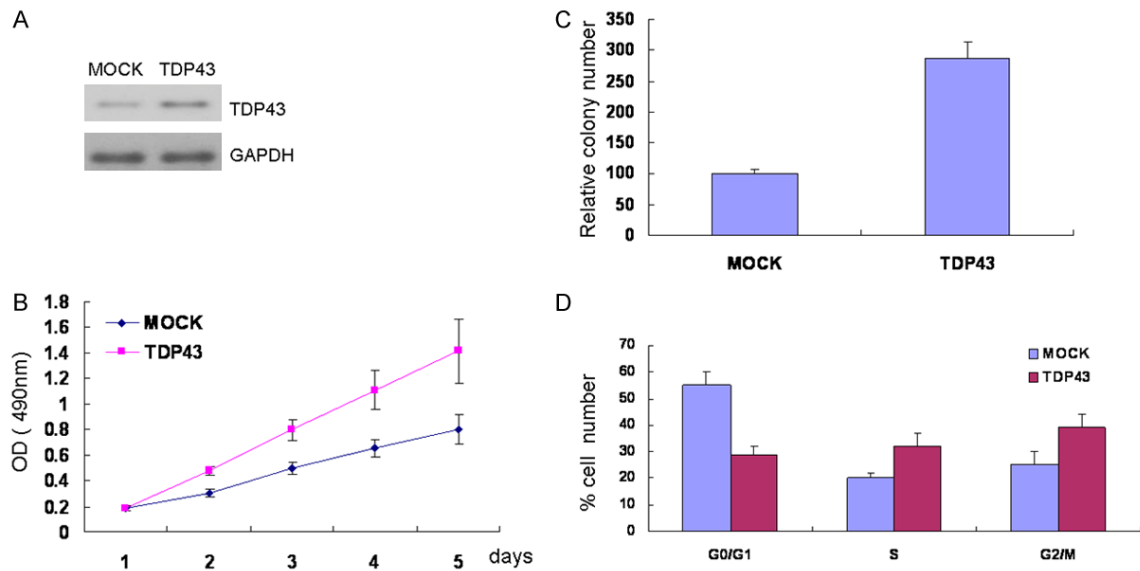
### Knockdown of TDP43 inhibited cell growth and induced cell cycle arrest

Because TDP43 expression was increased in cervical cancer, we wonder the possible function of the increased TDP43 in tumorigenesis and development. Firstly we knocked down TDP43 in Caski cells by RNA interference to evaluate the biological behaviors. **Figure 2A** showed the knockdown efficiency. MTT assay revealed the cell proliferation ability of TDP43 knockdown group was decreased compared with control group (**Figure 2B**). Subsequently, consistent with cell proliferation assay, colony formation also showed that the colony number after knockdown of TDP43 was attenuated relative to control group (**Figure 2C**). These sug-

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**Figure 2.** Knockdown of TDP43 inhibited the cell growth and induced cell cycle arrest. A. Western blot was used to analyze the knockdown efficiency. B. The cell proliferation ability was performed with the MTT assay. C. Colony formation was conducted. D. Cell cycle was analyzed. Data are expressed as mean  $\pm$  standard deviation and analyzed with ANOVA using SPSS software. A value of  $P < 0.05$  was considered as statistical significance.



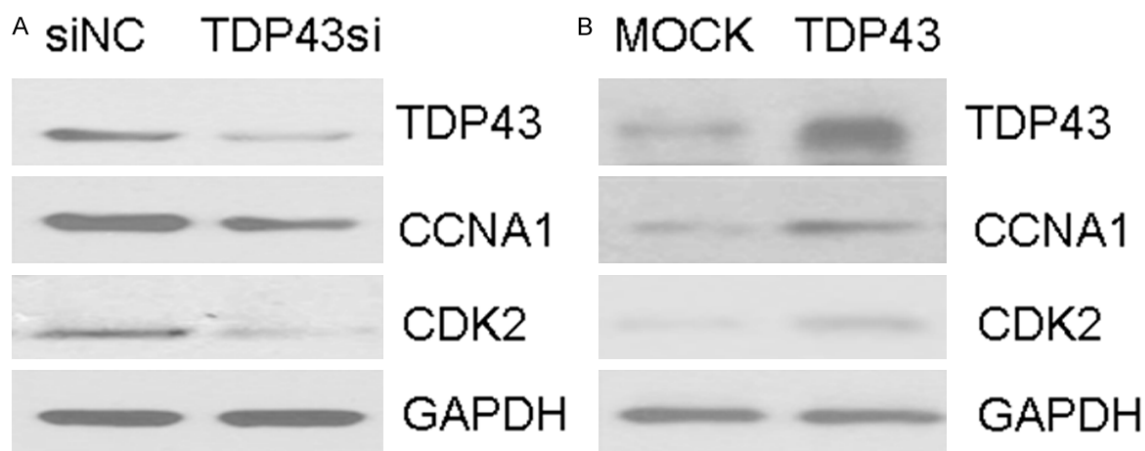
**Figure 3.** Overexpression of TDP43 promoted the cell growth and drove cell cycle. A. Western blot was used to analyze the protein level. B. The cell proliferation ability was performed with the MTT assay. C. Colony formation was performed. D. Cell cycle was analyzed. Data are expressed as mean  $\pm$  standard deviation and analyzed with ANOVA using SPSS software. A value of  $P < 0.05$  was considered as statistical significance.

gested that TDP43 gene silencing significantly inhibited the growth of cervical cancer cell.

To determine whether cell cycle contribute to cell growth, we analyzed the cell cycle after knockdown of TDP43. The results showed the inhibition of TDP43 expression caused the G2/M arrest (Figure 2D).

*Overexpression of TDP43 promoted cell growth and drove cell cycle*

Next, we further explored the effect of TDP43 on cell biology by overexpression. TDP43 expression vector (TDP43-pcDNA3.1, TDP43) was generated and transfected into Caski cells. The levels of TDP43 protein was determined by



**Figure 4.** Cell cycle protein was detected by western blot. A. The activity of CCNA1 and CDK2 decreased after knockdown of TDP43 in cervical cancer cells. B. The activity of CCNA1 and CDK2 increased after over-expression of TDP43 in cervical cancer cells.

western blot (**Figure 3A**). Following a 5-day period, MTT assay showed that the growth of TDP43 overexpressed cells was faster than control (**Figure 3B**). A similar pattern of promote effect of increased TDP43 expression on Caski cells was got by colony formation assay (**Figure 3C**). Frequency of Caski-TDP43 cells at G<sub>0</sub>/G<sub>1</sub> phase decrease and G<sub>2</sub>/S phase increased as compared with that in control cells (**Figure 3D**). Together with these data, TDP43 regulated cervical cancer cell progression.

#### *Effect of TDP43 on cell cycle protein activity*

Furthermore, we investigated cell cycle protein and found that the activity of CCNA1 and CDK2 decreased after knockdown of TDP43 expression in cervical cancer Caski cells (**Figure 4A**). Overexpression of TDP43 enhanced CCNA1 and CDK2 activity (**Figure 4B**).

Collectively, these data evidenced that TDP43 expression regulated the tumorigenicity of cervical cancer cells in vitro.

#### **Discussion**

TDP-43 plays a role in neurodegeneration and is also crucial in mammals' development [16, 17]. The loss of TDP-43 results in dysmorphic nuclear shape, misregulation of the cell cycle, and apoptosis [18]. In the tested acute myeloid leukemia cell lines strong nucleolar expression of TDP-43 was showed [19]. Our results showed that protein and mRNA of TDP43 expression was up-regulated in cervical cancer tissues. The immunoreactivity of pTDP43 was found to

be significantly higher in gangliogliomas than in dysembryoplastic neuroepithelial tumours [20]. The variants in the TARDBP regions are strong Ewing sarcoma susceptibility loci [21]. It is reported that TDP-43 is a novel regulator of glycolysis in HCC cells and silencing of TDP-43 expression in multiple HCC cell lines leads to impaired glucose metabolism and growth inhibition of HCC cells [22]. We also reported that TDP-43 promoted the migration and invasion of non-small cell lung cancer [13]. In this study, the knockdown of TDP43 inhibited the cell growth and induced cell cycle arrest. Overexpression of TDP43 promoted the cell growth and drove cell cycle. TDP-43 positively contributes to the anticancer activity of curcumin in MCF-7 cells [23]. TDP-43 in glioma cells may be protective for cancer cells under apoptotic insult [24]. Our results were consistent with these reports. CCNA1 and CDK2 are important cell cycle proteins in cancer progression [25-27]. We also found that the activity of CCNA1 and CDK2 also changed after modulation of TDP43 expression.

Although the knowledge of TDP43 is currently still less in cancer development, TDP43 will become interesting with the increasing investigating in TDP43. TDP43 might be a potential target to cancer treatment.

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#### Disclosure of conflict of interest

None.

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