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Regulation between two alternative splicing isoforms ZNF148^{FL} and ZNF148$^{\Delta N}$, and their roles in the apoptosis and invasion of colorectal cancer

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ABSTRACT

Objective: To investigate the effect of two alternative splicing isoforms of zinc finger protein (*ZNF*) 148 gene on the invasion and metastasis of human colorectal cancer (CRC) cells and their related mechanisms.
Methods: Quantitative RT-PCR assays were used to detect the expression of two*ZNF148* alternative splicing isoforms in SW480 cells. *ZNF148*^{FL}-siRNA, *ZNF148*^{FL}-over express vector, *ZNF148*$^{\Delta N}$-siRNA, and *ZNF148*$^{\Delta N}$-over express vector were introduced into SW480 cells. The transfection efficiency was confirmed by RT-PCR. The proliferation, invasion, and migration in vitro as well as the apoptosis of SW480 cells were detected by MTT, transwell, scratch assay and flow cytometry, respectively.
Results: Both *ZNF148*^{FL} and *ZNF148*$^{\Delta N}$ were expressed in SW480 cells, and the level of ZNF148^{FL} protein was higher than ZNF148$^{\Delta N}$. After *ZNF148*^{FL}-siRNA and *ZNF148*$^{\Delta N}$-over express transfection, the expression level of *ZNF148*^{FL} and *ZNF148*Δ^{N} were significantly decreased and increased, respectively. In contrast, the expression of *ZNF148*^{FL} and *ZNF148*$^{\Delta N}$ were significantly increased and decreased, respectively, after *ZNF148*^{FL}-over express and *ZNF148*$^{\Delta N}$-siRNA transfection (all $P < 0.05$). The proliferation of SW480 cells was increased in *ZNF148*^{FL}-over express group and the *ZNF148*$^{\Delta N}$-siRNA group, while decreased in *ZNF148*^{FL}-siRNA group and ZNF148$^{\Delta N}$-over express group. The invaded cell number and migrated distance in *ZNF148*^{FL}-siRNA group and *ZNF148*$^{\Delta N}$-over express group were significantly decreased, but the apoptotic rate was significantly increased. In contrast, *ZNF148*^{FL}-over express and *ZNF148*$^{\Delta N}$-siRNA group showed the significantly increased ability of invasion and migration but decreased apoptosis rate (all $P < 0.05$).
Conclusion: *ZNF148*^{FL} could increase proliferation, invasion, and migration of CRC cells, while *ZNF148*$^{\Delta N}$ showed opposite effect; the two splicing isoforms of *ZNF148* may exert a mutual antagonistic effect to each other on the malignant biological activities.

1. Introduction

Colorectal cancer (CRC) is a common malignant tumor of the gastrointestinal tract [1,2]. The incidence of CRC has been increasing in recent years, was second only to that of gastric cancer, esophageal cancer and primary liver cancer in the digestive system [3]. Aberrant activation of the Wnt signaling pathway is a major molecular and genetic change of early malignant lesions in the small intestine [4], which has been reported to promote metastasis of CRC [5].

Zinc fingers proteins (ZNF) are the largest family of DNA binding proteins and can act as transcriptional factors in eukaryotes, and selectively binds to specific DNA sequences in the promoter of target genes via characteristic zinc finger domain [6]. ZNF interacts directly with the p53 through its zinc-finger domain, and indirectly with the histone acetyltransferase and transcriptional co-activator p300 through its amino terminus [7]. ZNF can promote binding of transcription factors to homologous DNA recognition sites or chromatin modification [8], and specific target genes of most ZNF genes have been identified. ZNF plays an important role in cell growth, proliferation, differentiation, apoptosis and other biological activities [9,10]. *ZNF148* participates in the regulation of cell proliferation and death, and is expressed at lower levels in mammalian somatic cells, and highly expressed in tumor cells [11]. Previous studies show that there was a significant correlation between ZNF148 expression and the invasion and metastasis of colorectal tumors [12,13]. *ZNF148* has two alternative splicing isoforms, which are *ZNF148*^{FL} and *ZNF148*$^{\Delta N}$. *ZNF148*^{FL} contains a complete 794 amino acids, *ZNF148*$^{\Delta N}$ lacks the amino-terminal 129 amino acids, and the mechanisms involved in the generation of these

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two splicing isoforms are not yet clear [14,15]. This study aimed to investigate the effects of two alternative splicing variants of *ZNF148* on proliferation, apoptosis, invasion, and metastasis of CRC cells and their corresponding mechanism.

2. Materials and methods

2.1. Reagent

Trizol and Lipofectamine™ 2000 were purchased from Invitrogen (Carlsbad, CA, USA). Dulbecco's Modified Eagle medium (DMEM) was purchased from Sigma (St. Louis, MO, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was bought from Promega (Madison, WI, USA). SYBR Premix Ex Taq and pMD18-T Vector were purchased from Takara (Dalian, China). Fetal bovine serum (FBS) was purchased from Sangon Biotech (Shanghai) Co., Ltd. Gel Extraction Kit, and Plasmid Isolation Kit were purchased from Promega (Madison, WI, USA). Cell lysate, Reverse Transcription Kit, and *Escherichia coli* DH5α were purchased from Dingguo Biological Co., Ltd. (Beijing, China). Mouse Anti-Human ZNF148^{FL}, ZNF148$^{\Delta N}$ antibody, and Goat Anti-Mouse HRP antibody were purchased from Abcam (Cambridge, MA, USA). The primers and recombinant plasmids carriers were purchased from Shanghai GenePharma Co., Ltd. Annexin V-FITC Apoptosis Detection Kit was bought from MultiSciences Biotech Co., Ltd.

2.2. Cell culture

Human CRC (SW480) cells were purchased from Shanghai Novobio Co., Ltd. Cells were resuscitated and cultured in DMEM medium supplemented with 10% fetal bovine serum at 37 °C under 5% CO_2, then passaged to the next generation every two to three days and digested with 0.25% trypsin. Logarithmic growing cells were prepared.

2.3. RT-PCR

The total cellular RNA was isolated using Trizol, and cDNA was synthesized and amplified using the Transcript First-strand cDNA Synthesis SuperMix in accordance with the manufacturer's protocol. The PCR primers were designed from the cDNA sequence of *ZNF148* gene (NM_001348424.1), and synthesized by the Shanghai Sangon Biological Engineering Technology Services (Shanghai, China). The primer sequences were as follows: *ZNF148*^{FL} forward, 5′-ATGAACATT GACGACAAACTGGA-3′; *ZNF148*$^{\Delta N}$ forward, 5′-ATGAGAGACAAAAAA CAAATCAGAGA-3′; *ZNF148*^{FL} and *ZNF148*$^{\Delta N}$ reverse, 5′-TTAGCCAAA AGTCTGGCCAGTTGT-3′. RT-PCR was performed using a Super Real PreMixPlus (SYBR Green) kit (Tiangen, Beijing, China) on ABI 7500 instrument (Applied Biosystems, Carlsbad, CA, USA). All data from each sample were assessed by $2^{-\Delta\Delta Ct}$ analysis.

2.4. Western blot

Cells were harvested and homogenized on ice in RIPA lysis buffer. Lysates were centrifuged and the protein concentrations were measured using a BCA kit. Equivalent amounts of protein extracts were separated by 10% SDS-poly-acrylamide gels electrophoresis and transferred onto polyvinylidene fluoride (PVDF) membranes. The membranes were blocked for 1 h with 5% non-fat dry milk in Tris-buffered saline/Tween-20 (TBST) buffer and incubated with primary antibodies against ZNF148^{FL} and ZNF148$^{\Delta N}$ at 4 °C overnight. Blots were washed three times and incubated with secondary antibodies (HRP-conjugated anti-mouse IgG) for 1 h at room temperature. The membranes were then visualized by the ECL-Plus Western detection system, the intensity of the respective signals in these blots was determined using Image J software.

2.5. Construction of ZNF148 interference and overexpression vector

According to the sequence of *ZNF148* gene the specific interference plasmids (*ZNF148*^{FL}-siRNA and *ZNF148*$^{\Delta N}$-siRNA) and the negative control (Control-siRNA) were synthesized by Shanghai GenePharma Co., Ltd (Shanghai, China). *ZNF148*^{FL} and *ZNF148*$^{\Delta N}$ gene were amplified by PCR, and then agarose gel electrophoresis was performed. The DNA fragment of *ZNF148*^{FL} and *ZNF148*$^{\Delta N}$ were excised from the agarose gel under the UV imager, purified by the gel extraction kit, and then cloned into a pMD18-T vector. The positive clones were obtained by screening following PCR, digestion, and sequencing. The recombinant vector digested with restriction enzymes BamHI and EcoRI were ligated with pLXSN at 16 °C overnight and transformed to DH5. Finally, the positive clones were identified by PCR and enzyme digestion. The recombinant *ZNF148*^{FL} and *ZNF148*$^{\Delta N}$-overexpress plasmid were obtained.

2.6. Cell transfection

ZNF148^{FL}-siRNA (Sequence: 5′-CTAGCCGGGACATTGATCAGGTTG ATAATCTCGGATTATCAACCTGATCAATGTCTTTTTTAAT-3′), *ZNF148*$^{\Delta N}$-siRNA (Sequence: 5′-AAAAAACCTGTGCATAGTAGTACTAA TCTCGAGATTAGTACTACTATGCACAGGCCGG-3′), *ZNF148*^{FL}-over express (F: 5′-TCAGGGATCCATGAACATTGACGACAACTGG-3′ R: 5′-TTT TCCCGGGGCCAAAAGTCTGGCCAGTTG-3′), *ZNF148*$^{\Delta N}$-over express (F: 5′-GGCAGTCCACAGCAAGAAC-3′ R: 5′-TTGGTATCCGAGAAAGTCAG AAG-3′) or corresponding negative control vectors were transiently transfected into SW480 cells using Lipofectamine 2000. After transfection, RT-PCR was performed to evaluate the transfection efficiency.

2.7. Cell proliferation

Cell proliferation was evaluated using MTT assays. 2×10^3 cells were seeded into each well of a 96-well plate, and incubated for 24, 48, and 72 h, respectively. Then, 20 μL MTT (0.5 mg/mL) was added to each well. After removal of the medium, absorbance was then measured spectrophotometrically at 492 nm.

2.8. Transwell assay

Cell invasion was assayed using a transwell chamber. The filters used were precoated with Matrigel in the upper compartment before cell seeding. 1×10^5 cells were seeded into the upper chamber, and the lower chamber was filled with 600 μL culture medium supplemented with 10% FBS. Cells were incubated at 37 °C under 5% CO_2 for 48 h, and the invaded cells were fixed with 4% paraformaldehyde, and stained with 0.1% crystal violet. Five random fields from each membrane were photographed and counted.

2.9. Scratch wound healing assay

Cells were seeded in fibronectin-coated 6-wells plates at a concentration of 1.0×10^5 per well. After an overnight incubation, the cell monolayer was scraped in a straight line to create a “scratch” with a p10 pipet tip. Remove the debris and smooth the edge of the scratch by washing the cells three times with PBS and then replace with DMEM medium supplemented with 5% FBS. Cells were incubated at 37 °C under 5% CO_2 for 12, 24, 48, and 72 h. The migrated cells were photographed using an inverted phase contrast microscope and the average distance between the upper and lower limits of the scratch was calculated.

2.10. Cell apoptosis

After digestion with trypsin, the cells were harvested, washed twice with ice-cold PBS, and then fixed in ice-cold ethanol. Cells were

resuspended in 100 μL Annexin binding buffer containing 5 μL of Alexa Fluor 488 Annexin V and 1 μL of PI (100 μg/mL) for 15 min at room temperature. Subsequently, 400 μL of Annexin-binding buffer was added in each reaction, mixed gently and kept the samples on ice until the cells were analyzed by flow cytometry (Becton Dickinson). Results were obtained by analyzing data with FlowJo Version 7.6.1 software (TreeStar). Cells that showed green fluorescence were early apoptotic (Annexin V-FITC + /PI-) while those that showed red and green fluorescence were classified as late apoptotic or necrotic (Annexin V-FITC + /PI +) cells.

2.11. Statistical analysis

Data were presented as the mean ± standard deviation (SD). Statistical differences were analyzed by using SPSS 19.0. Comparisons between two groups were made by Student's *t*-test. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Detection of two alternative splicing isoforms of ZNF148

In this study, two PCR primer sets were designed to obtain the full-length of *ZNF148*^{FL} (2385 bp) and *ZNF148*$^{\Delta N}$ (2004 bp) gene, respectively. The products of RT-PCR were detected by electrophoresis in agarose gel, and two fragments of different lengths could be clearly identified (Fig. 1A). The results of Western blot showed that the level of ZNF148^{FL} protein in human CRC SW480 cells was significantly higher than that of ZNF148$^{\Delta N}$ (Fig. 1B).

3.2. Inhibition and overexpression of *ZNF148*^{FL} and *ZNF148*$^{\Delta N}$

Compared with normal control group, the expression of *ZNF148*^{FL} was significantly decreased in *ZNF148*^{FL}-siRNA group and *ZNF148*$^{\Delta N}$-over express group, while remarkably elevated in *ZNF148*^{FL}-over express group and *ZNF148*$^{\Delta N}$-siRNA group ($P < 0.05$). However, the expression of *ZNF148*$^{\Delta N}$ showed an opposite tendency to *ZNF148*^{FL}. After transfection, the level of *ZNF148*$^{\Delta N}$ was significantly upregulated in *ZNF148*$^{\Delta N}$-over express group and *ZNF148*^{FL}-siRNA group, and downregulated in *ZNF148*$^{\Delta N}$-siRNA and *ZNF148*^{FL}-over express group ($P < 0.05$) (Fig. 2).

3.3. Effect of *ZNF148*^{FL} and *ZNF148*$^{\Delta N}$ on cell proliferation

The cell proliferation was detected at 24, 48, and 72 h after transfection in each group. The proliferation of SW480 cells was significantly increased in *ZNF148*^{FL}-over express group and *ZNF148*$^{\Delta N}$-siRNA group, while decreased in *ZNF148*^{FL}-siRNA and *ZNF148*$^{\Delta N}$-over express group ($P < 0.05$). And the proliferation of SW480 cells in *ZNF148*^{FL}-over express group and the *ZNF148*$^{\Delta N}$-siRNA group was significantly different from that in *ZNF148*^{FL}-siRNA group and *ZNF148*$^{\Delta N}$-over express group ($P < 0.05$), indicating that *ZNF148*^{FL} promoted the proliferation of SW480 cells, whereas *ZNF148*$^{\Delta N}$ exerted an inhibitory effect on the proliferation of SW480 cells (Fig. 3).

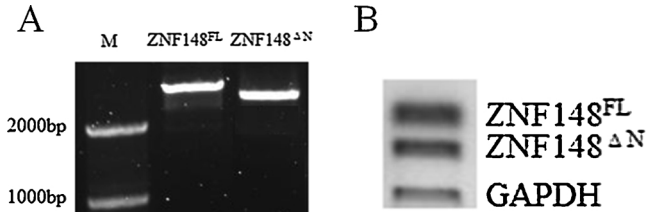

Fig. 1. The PCR amplification (A) and protein (B) expressions of two ZNF148 splicing isoforms in CRC SW480 cells. M, Marker.

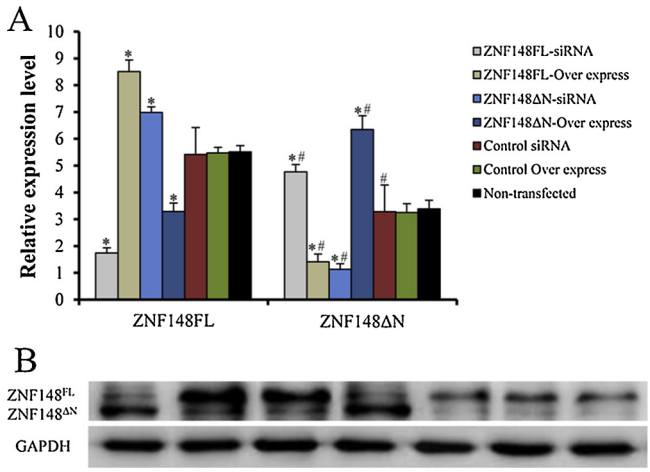

Fig. 2. Inhibition and over-expression of two ZNF148 splicing isoforms in CRC cells. (A) The transfect efficiency of the vectors targeting ZNF148^{FL} and ZNF148$^{\Delta N}$ was verified by RT-PCR. (B) Detection of the protein levels of ZNF148^{FL} and ZNF148$^{\Delta N}$ by western blotting. Data were obtained in three independent experiments. * $P < 0.05$ *vs* the non-transfected group; # $P < 0.05$ *vs* ZNF148^{FL} in the same group.

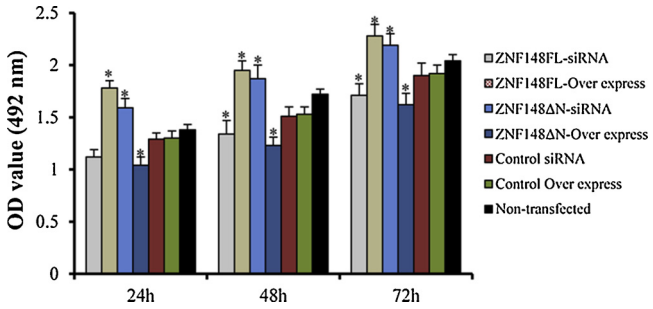

Fig. 3. Effect of two ZNF148 splicing isoforms on the proliferation of CRC cells. Data were obtained in three independent experiments. * $P < 0.05$ *vs* the normal control group; * $P < 0.05$ *vs* the non-transfected group.

3.4. Effect of *ZNF148*^{FL} and *ZNF148*$^{\Delta N}$ on cell invasion

As shown in Fig. 4, the average number of cells invaded through the membrane in *ZNF148*^{FL}-siRNA group and *ZNF148*$^{\Delta N}$-over express group was significantly lower than that in normal control group, whereas the average number of cells passing through the membrane in *ZNF148*^{FL}-over express group and *ZNF148*$^{\Delta N}$-siRNA group was significantly higher, indicating that *ZNF148*^{FL} could promote the invasiveness of the SW480 cells, and *ZNF148*$^{\Delta N}$ inhibit the invasiveness of SW480 cells.

3.5. Effect of *ZNF148*^{FL} and *ZNF148*$^{\Delta N}$ on cell migration

The migration distance of SW480 cells in each group was observed and compared, it was shown that the migration activity of SW480 cells in *ZNF148*^{FL}-siRNA group and *ZNF148*$^{\Delta N}$-over express group was evidently lower than that in normal control group, while the migration activity of SW480 cells in *ZNF148*^{FL}-over express group and *ZNF148*$^{\Delta N}$-siRNA group was considerably higher, indicating that *ZNF148*^{FL} could enhance the migration ability of SW480 cells, while *ZNF148*$^{\Delta N}$ can reduce the migration ability of SW480 cells (Fig. 5).

3.6. Effect of *ZNF148*^{FL} and *ZNF148*$^{\Delta N}$ on cell apoptosis

As compared with the normal control group, the apoptosis rate of SW480 cells was significantly higher in *ZNF148*^{FL}-siRNA group and *ZNF148*$^{\Delta N}$-over express group, while considerably lower in *ZNF148*^{FL}-

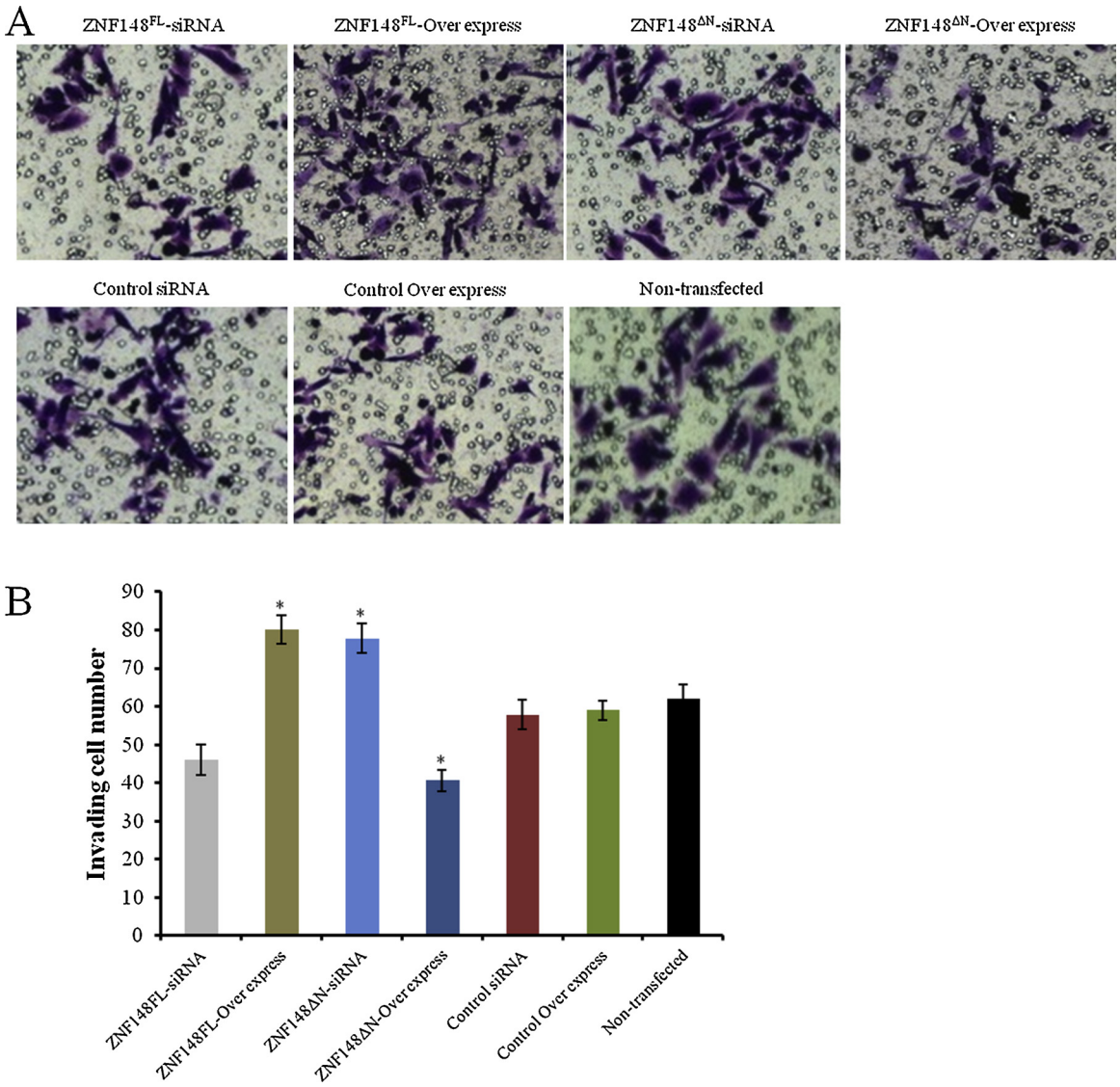

Fig. 4. Effect of two ZNF148 splicing isoforms on the invasion of CRC cells. (A) Representative microphotographs of invaded cells (200 ×). (B) Quantitative analysis of the invaded cells. Data were obtained in three independent experiments. * $P < 0.05$ *vs* the non-transfected group.

over express group and *ZNF148*$^{\Delta N}$-siRNA group. And the apoptotic rate of SW480 cells at 24 h, 48 h and 72 h in *ZNF148*^{FL}-siRNA group and *ZNF148*$^{\Delta N}$-over express group were significantly different from those in *ZNF148*^{FL}-over express group and *ZNF148*$^{\Delta N}$-siRNA group ($P < 0.05$). These results demonstrated that *ZNF148*$^{\Delta N}$ can promote the apoptosis of SW480 cells, while *ZNF148*^{FL} has the effect of inhibiting the apoptosis of SW480 cells (Fig. 6).

4. Discussion

ZNF148 is a zinc finger transcription factor, which is generally low in almost all mammalian cells, including colorectal mucosal epithelial cells. Previous studies have shown that *ZNF148* is involved in the regulation of cellular functions as well as cell growth and death [7]. *ZNF148* has two alternative splicing isoforms, which are functionally distinct [15]. In this study, in vitro experiments were performed to investigate the specific roles of two ZNF148 splicing isoforms on CRC cells and the underlying molecular mechanisms.

Law et al. [6] identified that *ZNF148*$^{\Delta N}$ was generated by different cleavage of the upstream promoter region of exon 4. *ZNF148*$^{\Delta N}$ lacks part of the transcriptional activation domain of the protein when compared with the full-length structure of *ZNF148*, and thus the mature form of ZNF148$^{\Delta N}$ proteins is smaller than ZNF148^{FL}. In the present study, our data showed that the total length of *ZNF148*^{FL} and *ZNF148*$^{\Delta N}$ is 2385 bp and 2004 bp, respectively; and *ZNF148*$^{\Delta N}$ protein was smaller than the size of *ZNF148*^{FL} in human CRC cells, thus confirmed the splicing isoforms of human *ZNF148*.

In the present study, our results showed that *ZNF148*^{FL} expressed at a high level in SW480 cells, and it can promote the proliferation, migration, and invasion of human CRC cells. And the proliferation, migration and invasion activity of SW480 cells were significantly reduced in response to siRNA-mediated depletion of *ZNF148*^{FL}. *ZNF148*$^{\Delta N}$, another splicing isoform of *ZNF148*, expressed at a lower level in SW480 cells. The two splicing isoforms of *ZNF148* play different roles in the development of CRC, and have mutually antagonistic effects on human CRC cells. Overexpression of *ZNF148*^{FL} could reduce the level of *ZNF148*$^{\Delta N}$, and promote the proliferation, migration, and invasion of human CRC cells. While, overexpression of *ZNF148*$^{\Delta N}$ can decrease *ZNF148*^{FL} expression, promote the apoptosis, and inhibits the proliferation, migration, and invasion of SW480 cells.

In addition, our study also showed that the proliferation, migration, and invasion of SW480 cells in control over-express group and control siRNA group were lower than that in the normal control group, and the apoptosis rate was higher than that in the normal control group, which

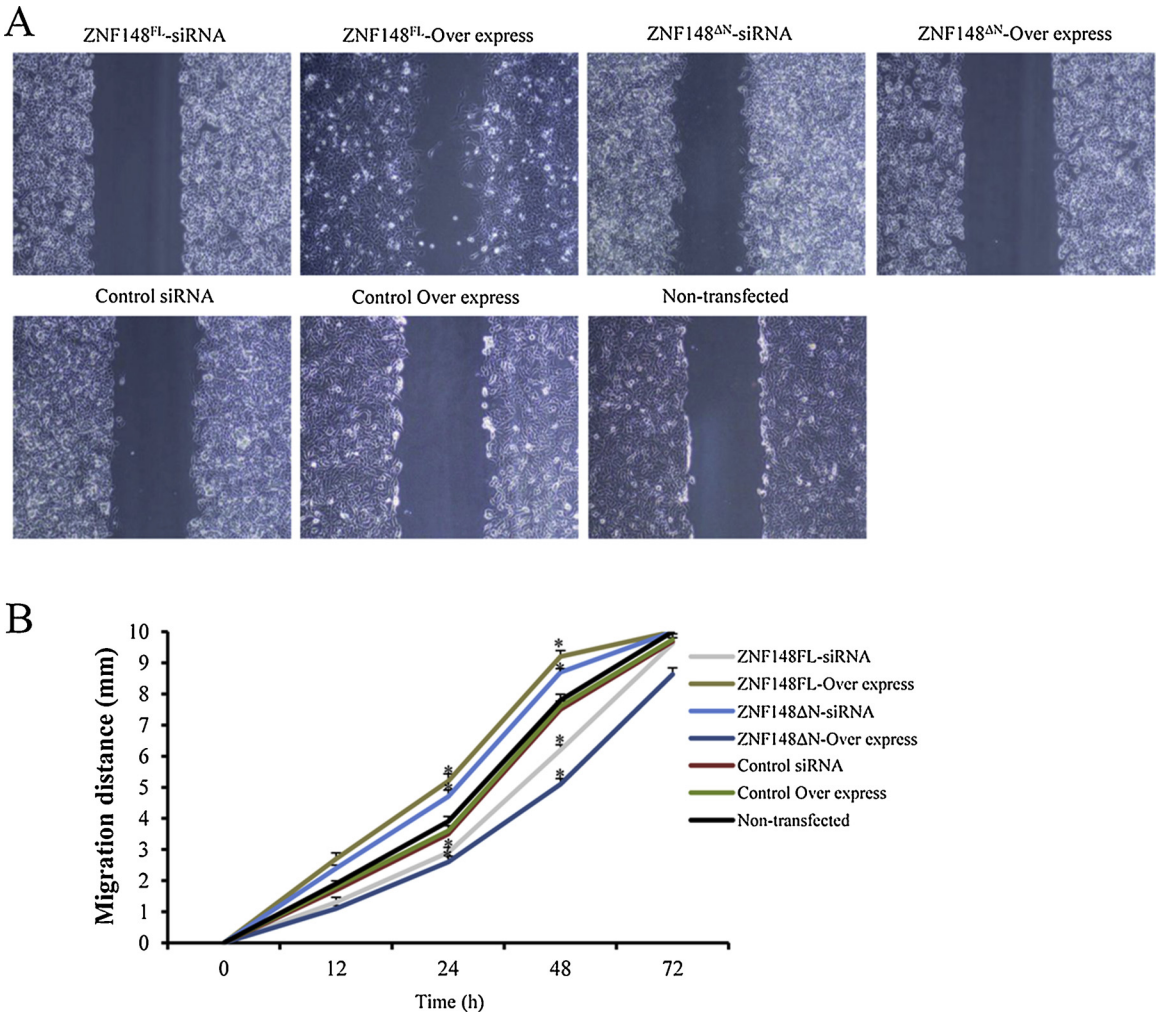

Fig. 5. Effect of two ZNF148 splicing isoforms on the migration of CRC cells. * $P < 0.05$ *vs* the normal control group. (A) Representative microphotographs of wound healing (40×). (B) Quantitative analysis of the migration distance. Data were obtained in three independent experiments. * $P < 0.05$ *vs* the non-transfected group.

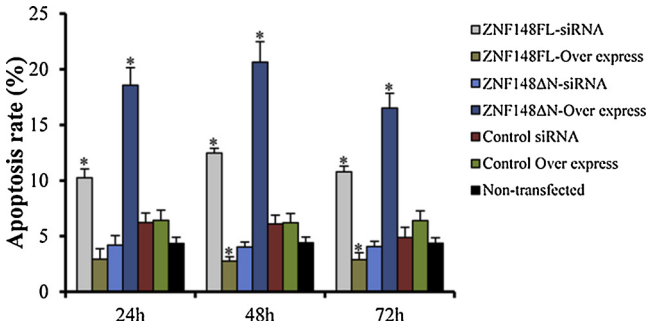

Fig. 6. Effect of two ZNF148 splicing isoforms on the apoptosis of CRC cells. Data were obtained in three independent experiments. * $P < 0.05$ *vs* the non-transfected group.

was considered to be a certain degree of toxic effect of Lipofectamine 2000 transfection reagent on cells. Furthermore, the apoptosis rate of SW480 cells in *ZNF148*$^{\Delta N}$-over express group at 72 h was lower than that at 24 h and 48 h after transfection, whereas the apoptosis rate at 48 h after transfection was higher than at 24 h, probably due to the peak time of *ZNF148*$^{\Delta N}$ expression was within 72 h. In concordance with the result of apoptosis, the expression level of *ZNF148*$^{\Delta N}$ protein at 72 h was significantly lower than that at 24 and 48 h after transient transfection.

The two splicing isoforms of *ZNF148* increase the genetic diversity, not only participate in the occurrence and development of cancer but also regulate the function of other tissues. *ZNF148* can induce p53-dependent cell growth arrest and apoptosis directly or indirectly [16]. In butyrate-induced CRC cell growth arrest and apoptosis, *ZNF148* could form a complex with P300, the amino terminus of *ZNF148* contains an acidic domain that is required for p300-mediated induction [8]. However, the residue was deficient in *ZNF148*$^{\Delta N}$, and the lacking of the domain structure resulted in the destroyed stability of the internal environment [17]. Generating a homozygous mouse model in which only the *ZNF148*$^{\Delta N}$ form was expressed, revealed that loss of the p300-interacting domain results in delayed growth and a shortened lifespan [6,9].

In addition, the incidence of colitis in mice was significantly higher than that in wild-type mice. Therefore, *ZNF148*^{FL} can also prevent the occurrence of colitis to a certain extent [18]. In the *ZNF148*$^{\Delta N}$ knockout mouse model, defects in the development of male germ cells and dysfunction were occurred. The development of male germ cells depends on the mediation of p53, and *ZNF148*$^{\Delta N}$ has a DNA-binding domain structure that interacts with p53. Therefore, the N-terminal p300 binding structure of *ZNF148*^{FL} is not only important for the growth and development, but also play an important role in maintaining intestinal function [10].

In the invasion and metastasis of human CRC, there is a functional antagonism between *ZNF148*$^{\Delta N}$ and *ZNF148*^{FL}. The full-length isoform *ZNF148*^{FL} promotes the EMT transformation through binding to the transcription factor p300 and modulating the Wnt signaling pathway

[19]. While, the N-terminally truncated isoform *ZNF148*$^{\Delta N}$, inhibits the upregulation of Wnt signaling by *ZNF148*^{FL}, and thus inhibits the invasion and metastasis of CRC.

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Conflict of interest statement

All authors state that they have no conflict of interest to disclose.

References

[1] V.V. Lao, W.M. Grady, Epigenetics and colorectal cancer, Nat. Rev. Gastroenterol. Hepatol. 8 (12) (2011) 686.
[2] R.A. Liddle, R.L. Jirtle, Epigenetic silencing of genes in human colon cancer, Gastroenterology 131 (3) (2006) 960–962.
[3] E.R. Fearon, Molecular genetics of colorectal cancer, Annu. Rev. Pathol. Mech. Dis. 6 (2011) 479–507.
[4] H. Clevers, K.M. Loh, R. Nusse, An integral program for tissue renewal and regeneration: Wnt signaling and stem cell control, Science 346 (6205) (2014) 1248012.
[5] L. Vermeulen, et al., Wnt activity defines colon cancer stem cells and is regulated by the microenvironment, Nat. Cell Biol. 12 (5) (2010) 468.
[6] D.J. Law, et al., An isoform of ZBP-89 predisposes the colon to colitis, Nucleic Acids Res. 34 (5) (2006) 1342–1350.
[7] A.G. Vaiopoulos, K.C. Athanasoula, A.G. Papavassiliou, Epigenetic modifications in colorectal cancer: molecular insights and therapeutic challenges, Biochim. Biophys. Acta (BBA)—Mol. Basis Dis. 1842 (7) (2014) 971–980.
[8] L. Bai, J.L. Merchant, Transcription factor ZBP-89 cooperates with histone acetyltransferase p300 during butyrate activation of p21 waf1 transcription in human cells, J. Biol. Chem. 275 (39) (2000) 30725–30733.
[9] A.G. Schepers, et al., Lineage tracing reveals Lgr5+ stem cell activity in mouse intestinal adenomas, Science 337 (6095) (2012) 730–735.
[10] S. Shen, et al., rMATS: robust and flexible detection of differential alternative splicing from replicate RNA-Seq data, Proc. Natl. Acad. Sci. U. S. A. 111 (51) (2014) E5593–E5601.
[11] S.J. Stevens, et al., Truncating de novo mutations in the Krüppel-type zinc-finger gene ZNF148 in patients with corpus callosum defects, developmental delay, short stature, and dysmorphisms, Genome Med. 8 (1) (2016) 131.
[12] X.H. Gao, et al., Expression of ZNF148 in different developing stages of colorectal cancer and its prognostic value: a large Chinese study based on tissue microarray, Cancer 119 (12) (2013) 2212–2222.
[13] C.Z. Zhang, G.G. Chen, P.B. Lai, Transcription factor ZBP-89 in cancer growth and apoptosis, Biochim. Biophys. Acta (BBA)—Rev. Cancer 1806 (1) (2010) 36–41.
[14] C.G. Ye, et al., ZBP-89 reduces histone deacetylase 3 by degrading IkappaB in the presence of Pin1, J. Transl. Med. 13 (1) (2015) 23.
[15] S. Hahn, H. Hermeking, ZNF281/ZBP-99: a new player in epithelial–mesenchymal transition, stemness, and cancer, J. Mol. Med. 92 (6) (2014) 571–581.
[16] J.A. Miller, et al., Transcriptional landscape of the prenatal human brain, Nature 508 (7495) (2014) 199.
[17] S.H. Lelieveld, et al., Meta-analysis of 2,104 trios provides support for 10 new genes for intellectual disability, Nat. Neurosci. 19 (9) (2016) 1194.
[18] D.J. Law, E.M. Labut, J.L. Merchant, Intestinal overexpression of ZNF148 suppresses APC Min/+ neoplasia, Mamm. Genome 17 (10) (2006) 999–1004.
[19] B.E. Essien, S. Sundaresan, R. Ocadizruiz, A. Chavis, A.C. Tsao, A.J. Tessier, Transcription factor zbp-89 drives a feedforward loop of β-catenin expression in colorectal cancer, Cancer Research 76 (23) (2016) 6877–6887.