Mathematical Biosciences and Engineering

MBE, 16 (6): 8109–8120.
DOI: 10.3934/mbe.2019408
Received: 20 February 2019
Accepted: 10 July 2019
Published: 06 September 2019

http://www.aimspress.com/journal/MBE

Research article

Effect and mechanism of long non-coding RNA ZEB2-AS1 in the occurrence and development of colon cancer

Xu Guo[1], Yuanming Jing[2], Haizhou Lou[3,*], Qiaonv Lou[4,*]

[1] Department of Anorectal Surgery, Dalian Municipal Central Hospital, Dalian, Liaoning 116033, China
[2] Department of General Surgery, Shaoxing People's Hospital (Shaoxing Hospital, Zhejiang University School of Medicine)
[3] Department of Medical Oncology, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou 310000, China
[4] Department of Orthopedics, The Sixth People's Hospital of Cixi, Ningbo 315300, China

* **Correspondence:** Email: lqn120470@163.com; louhz09@zju.edu.cn; Tel: +86-13867878493; +86-13588054124

Abstract: *Objective:* Clarify the expression changes, biological functions and related mechanisms of long non-coding RNA (lncRNA) ZEB2-AS1 in colon cancer tissues. *Methods:* The expression levels of ZEB2-AS1 in colon cancer tissues and adjacent tissues were detected by qRT-PCR and in situ hybridization methods. Cell biology experiments were performed to detect the proliferation, migration and apoptosis of colon cancer cells when the level of ZEB2-AS1 was overexpression or silencing. Then, Western blot was performed to analyze the effect of ZEB2-AS1 on the expression levels of β-catenin protein and related genes in the signal pathway. *Results:* We found that the expression level of ZEB2-AS1 in colon cancer tissues was significantly up-regulated compared with that in adjacent normal tissues. In colon cancer cell line of HCT8, overexpression of ZEB2-AS1 could promote cell proliferation and migration, while silencing ZEB2-AS1 would enhance cell apoptosis and inhibit proliferation. Study on the mechanism of ZEB2-AS1 showed that it could promote the expression of β-catenin, activate downstream genes to be transcribed and promote the occurrence and development of tumors. *Conclusion:* ZEB2-AS1 could promote colon cancer cell proliferation and inhibit apoptosis to promote the progression of colon cancer by upregulating the expression of β-catenin protein. ZEB2-AS1 may be a useful new target for treating colon cancer patients.

Keywords: long non-coding RNA; ZEB2-AS1; colon cancer; β-catenin protein

1. Introduction

Colon cancer (CC) is one of the most common cancers in the world, causing a large number of deaths each year [1]. So far, there has been no effective treatment for metastatic CC. As a result, CC remains a major risk to people's health. Gene expression disorders, including long non-coding RNA (lncRNA), are closely related to the occurrence and development of CC, leading to some changes in the biological characteristics of cancer cells, such as proliferation, migration, apoptosis and metabolism [2]. However, the underlying molecular mechanisms are still not fully understood, so we need the evidence urgently.

Long non-coding RNA (lncRNA), with the length over 200 nucleotides, is a new type of gene that has regulatory function but lacks protein-coding ability. Multiple studies have demonstrated the key role of lncRNA in a wide range of cellular processes, including X chromosome inactivation, splicing, imprinting, epigenetic control and gene transcription regulation [3–5]. In addition, the dissonant expression of lncRNA is also discovered in various human diseases, especially in cancer, including breast cancer, lung cancer, gastric cancer and colorectal cancer [6–9]. According to the latest study, lncRNA has been confirmed to be involved in human CC progression, metastasis and prognosis, and can be used as a new therapeutic target [10–12].

Lnc ZEB2-AS1 is closely related to the occurrence and development of many kinds of cancer [13–15]. In acute myeloid leukemia (AML), the overall survival rates (OS) and disease-free survival rates (DFS) of patients with high expression of ZEB2-AS1 are significantly lower than patients with low expression of ZEB2-AS1 patients and knock down ZEB2-AS1 can inhibit the migration and invasion of AML cells [16]. In gastric cancer, patient with high expression of ZEB2-AS1 have poor prognosis, while knockout of ZEB2-AS1 can inhibit the proliferation, invasion and metastasis of cancer cells and promote apoptosis [17]. Therefore, ZEB2-AS1 can be used as a potential therapeutic target and prognostic marker for cancer. However, the role of ZB2-AS1 in CC remains largely unknown. So, it is very necessary to study its effect and mechanism on the occurrence and development of colon cancer.

In this study, we found that ZEB2-AS1 expression was up-regulated in CC tissues compared with that in adjacent normal tissues. In colon cancer cell line of HCT8, overexpression of ZEB2-AS1 could promote cell proliferation and migration, while silencing ZEB2-AS1 would enhance cell apoptosis and inhibit proliferation. Study on the mechanism of ZEB2-AS1 showed that its overexpression could increase the expression of β-catenin proteins, which plays a role as intracellular signal transduction in Wnt signal pathway and could regulate cell proliferation, differentiation and apoptosis. Overall, our results reveal new functions of ZEB2-AS1 in CC and may provide new insights into the treatment of CC patients.

2. Materials and method

2.1. Patient samples

The 20 pairs of CC issues and adjacent normal tissues involved in our study were from our

hospital. Written consent was obtained from all patients to approve our use of these tissues in the study. The proposal was approved by the ethics committee of our hospital. All methods involving human patients were conducted in accordance with the relevant guidelines and regulations of the hospital. The inclusion criteria of patients are: (1) diagnosis of primary CRC; (2) no history of chemotherapy and radiotherapy; (3) confirmation of adenocarcinoma by histology.

2.2. In situ hybridization

CC and peritumor samples were fixed and paraffin-embedded. The 4 μm Paraffin sections with conventional dewaxing were sliced into heat treatment solution boiling above 98℃, and then immediately washed it with distilled water 3 times for each time 2 min. Pepsin was added for digestion for 6 min. The sections were successively placed in gradient ethanol to dehydrate for 1min each, and then dried. Added 10 μL probe and covered glass slide; Denaturation at 95℃, hybridization at 37℃ (>10 h). The next day, the sections were soaked in citric acid buffer to soak off the cover glass, and then the sections were placed in citric acid buffer at 75℃ and washed with distilled water for 3 times. The sections were kept out of light at 3%H_2O_2 for 10 min at room temperature and washed for 3 times, each time for 2 min. The sealant was added and incubated for 10min.Then the sealant was discarded. Rat anti-digoxin antibody, HRP antibody and DAB were added successively, incubated for 30 min respectively, and then washed with tap water. Cell nucleus was rapidly stained with hematoxylin, washed with tap water, dehydrated with gradient ethanol, transparent xylene, and sealed. The probe sequence was below: 5'-CTGTCGGAGTTGGAAAGGGAC-3'.

2.3. Cell line and cell culture

Human CC cell lines (HCT8, SW480, and HCT116) were obtained from ATCC and cultured in DMEM medium containing 10% fetal bovine serum (FBS, Invitrogen), 100 μg/ml penicillin and 100 U/ml streptomycin. Normal human colonic epithelial cell CCD 841 CoN (ATCC) was cultured in Eagle's Minimum Essential Medium containing 10% fetal bovine serum. All cells were cultured in an incubator containing 5% CO_2 at 37℃.

2.4. Cell transfection

ZEB2-AS1 was cloned into pCDNA3 plasmid. Then ZEB2-AS1 siRNA (5'-CAAAGGACACCTTTGGTTACCTGAA-3') was synthesized by invitrogen and cloned into pGPH1/Neo (GenePharma, Shanghai, China) as mentioned before [15]. PCDNA3-ZEB2-AS1 was transfected into HCT8 cells by Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) to make ZEB2-AS1 overexpression. Pgph1-sh ZEB2-AS1 siRNA or control plasmid was transfected into HCT8 cells by Lipofectamine 3000 and screened using neomycin (1000 μg/ ml) for 4 weeks

2.5. Real-time Quantitative PCR (qRT-PCR)

The total RNA of the above cells or CC tissues were extracted by TRIzol reagent. Reverse transcription was performed by the PrimeScript RT kit. The reverse transcription reaction was performed by Promega's RT kit. Quantitative PCR was performed by Takara's SYBR Premix Ex Taq

at the ABI 7500 amplifier. The conditions of PCR reaction were as follows: 94℃ for 4 mins. 94℃ for 40 s; 52℃ for 40 s; 72℃ for 40 s with a total of 40 cycles. PCR results were analyzed by 2-ΔΔCt method. GAPDH was taken as internal reference, and the PCR primers were designed and synthesized by Sangon Biotech (Shanghai) Co., Ltd. Details were shown in Table 1.

Table 1. Quantitative PCR primer sequence.

Gene name	Forward primer (5'-3')	Reverse primer (5'-3')
LncRNA ZEB2-AS1	CTGTCGGAGTTGGAAAGGGAC	GCGGAACTTCTAGCCTCTCTT
GAPDH	TCGACAGTCAGCCGCATCTT	GGCGCCCAATACGACCAAAT

2.6. Determination with cell count kit-8 (CCK-8)

CCK-8 assay was performed by (CK04-11) cell count kit-8 (Dojindo, CK04-11) to evaluate the effect of ZEB2-AS1 on HCT8 cell proliferation. HCT8 cells were cultured in a 96-well plate with a concentration of 3 × 103 cells/well. After 24, 48 and 72 hours of transfection, WST-8 solution (10 μL) was added to each well. Then, cells were cultured for another 2 hours at 37 ℃ with 5% CO_2 and the absorbance of each well at the wavelength of 450 nm was measured by the enzyme label instrument (Bio-Rad).

2.7. Flow cytometry analysis

Apoptosis of HCT8 cells was assessed by flow cytometry. Culture HCT8 cells in a 6-well plate with a concentration of 2 × 104 cells/wells and transfect with si-ZEB2-AS1. After 48 hours of cultivation, the cells were digested by trypsin (EDTA-free) and then collected and resuspended in PBS (4℃). After the cells were centrifuged with a rotation speed of 1000rpm and at 4℃ and the PBS was removed, the binding buffer (1×) was added to resuspend the cells, and then, annexin V-FITC of the annexin V-FITC apoptosis kit (Biovision, K101) and PI were added to stain away from light for 15 minutes. Flow cytometry instrument (BD Biosciences) was used to detect the cell apoptosis rate.

2.8. Migration assay

Migration assay was carried out in the improved 24-well (8 μm) Boyden lab (BD Biosciences, San Jose, CA, USA). After transfection for 24 hours, 3 × 105 cells were added to the upper chamber containing serum-free DMEM were added to the upper chamber and the DMEM containing 20% FBS was added to the lower chamber as a chemical attractant. After incubation at 37℃ for 24 hours, cotton swab was used to gently remove unfiltered cells. Then the cells were fixed in paraformaldehyde for 30 minutes. Air-dry the chamber properly and stain the cells with 0.1% crystal violet for 15 minutes, then cleaned the cells by using PBS for 3 times. Select five proper vision fields under a microscope at a magnification of 400 × o count the transmembrane cells in each treatment group. Three independent experiments were conducted in each group.

2.9. Western blot

Western blot was performed to detect the expression of β-catenin, the key molecule of Wnt

pathway after the protein isolation of cells in different groups. The Western blot method was described as follows: cells of each group were washed with clod PBS for three times and lysed to protein on ice with whole protein lysate for 10 minutes. Then, the protein was quantitated by BCA protein assay kit and separated by SDS-PAGE gel electrophoresis after adding appropriate concentrated SDS-PAGE loading buffer and transferred to PVDF membrane. The PVDF membrane was blocked with 5% skimmed milk and then primary antibodies including anti-β-catenin rabbit monoclonal antibody (CST, D10A8, 1:1000) and GAPDH rabbit monoclonal antibody (CST, 14C10, 1:1000) were added to incubate overnight at 4℃. After finishing the incubation of primary antibodies, wash the membrane for three times and add the horseradish peroxidase-labeled secondary antibody goat-anti-mouse IgG (H+L) (CST, 14709S) to incubate at room temperature for 2 hours. Proteins were examined by chemiluminescence and analyzed the quantitation of the obtained protein bands with Image J software.

2.10. Statistical analysis

All data were represented in the form of mean value ± standard deviation (means ± SD), and non-paired t-test (two-tail) and two-way ANOVA were used to compare the differences between two groups and among multiple groups, respectively. $P < 0.05$ is considered to have statistical significance. All statistical methods were analyzed by GraphPad Prism 6.0 and SPSS 18.0 statistical software.

3. Results

3.1. The expression and subcellular location of LncRNA ZEB2-AS1 in colon cancer tissues

To understand the role of ZEB2-AS1 in CC, we analyzed its expression pattern. We examined the expression levels of ZEB2-AS1 mRNA in 20 pairs of tumor tissues and adjacent normal tissues by RT-qPCR and found that the expression of ZEB2-AS1 was significantly upregulated in tumor tissues ($p < 0.05$) (Figure 1A). We then located ZEB2-AS1 expression using in-situ RNA hybridization and found that ZEB2-AS1 was expressed mainly in CC cell nucleus (Figure 1B).

3.2. LncRNA ZEB2-AS1 expression significantly increased in colon cancer cells

Compared with normal colonic epithelial cell CCD 841 CoN, ZEB2-AS1 expression was significantly upregulated in CC cell lines, such as HCT8, SW480 and HCT116 cells (Figure 2, $p < 0.05$), especially in HCT8 cells ($p < 0.01$), which was used in subsequent experiments.

3.3. ZEB2-AS1 overexpression promotes cell proliferation and migration

To determine the mechanism of ZEB2-AS1 regulating CC cells, we constructed ZEB2-AS1 overexpression plasmid by cloning the full length of ZEB2-AS1 into a PCDNA3 vector. We first confirmed that ZEB2-AS1 was overexpressed in HCT8 cells (Figure 3A). Then we compared changes in cell proliferation, apoptosis and migration of cell strains in overexpressed ZEB2-AS1 with those in control group. The cell growth curve detected by CCK-8 showed that the

overexpression of ZEB2-AS1 in HCT8 cells promoted the cell growth (Figure 3B). Meanwhile, the results of flow cytometry showed that overexpression of ZEB2-AS1 resulted in significantly decreased apoptosis rate ($p < 0.05$) (Figure 3C). In addition, enhanced ZEB2-AS1 expression promoted the migration of HCT8 cells, as shown in the results of Transwell assay (Figure 3D). In conclusion, ZEB2-AS1 overexpression promotes proliferation and migration of CC cells.

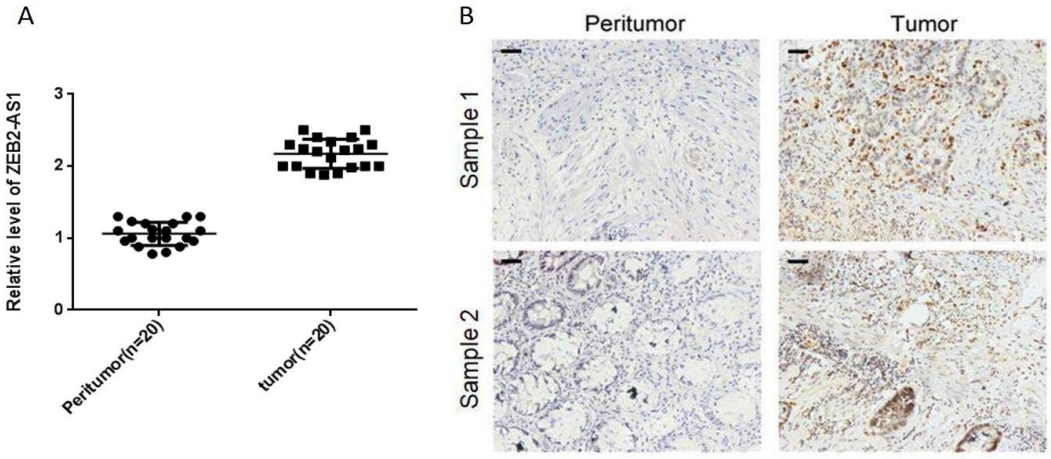

Figure 1. LncRNA ZEB2-AS1 expression significantly increased in colon cancer tissues. (A) The expression of ZEB2-AS1 in 20 couples of tumors and adjacent normal tissues were examined by RT-qPCR finding that ZEB2-AS1 expression was up-regulated in tumor tissues. (B) In situ hybridization revealed more ZEB2-AS1 in colon cancer tissue than in adjacent normal tissue.

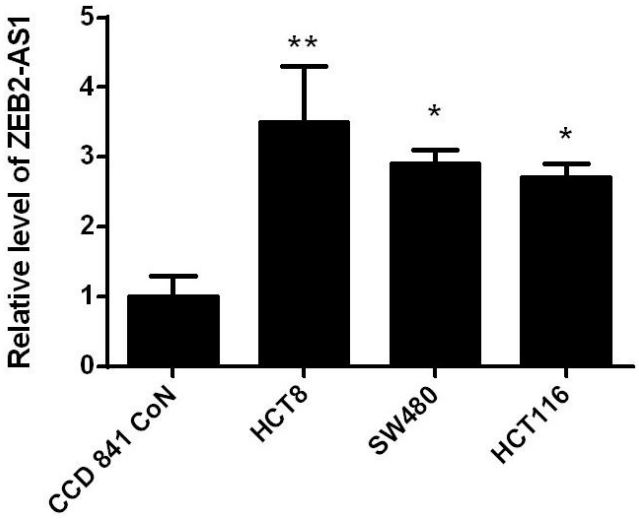

Figure 2. LncRNA ZEB2-AS1 expression significantly increased in colon cancer cells. Compared with normal colonic epithelial cell CCD 841 CoN, ZEB2-AS1 expression was higher in colon cancer cell lines such as HCT8, SW480 and HCT116 cells. Note: "*" represents the comparison with CCD 841 CoN, $p < 0.05$; "**" represents the comparison with CCD 841 CoN, $p < 0.01$.

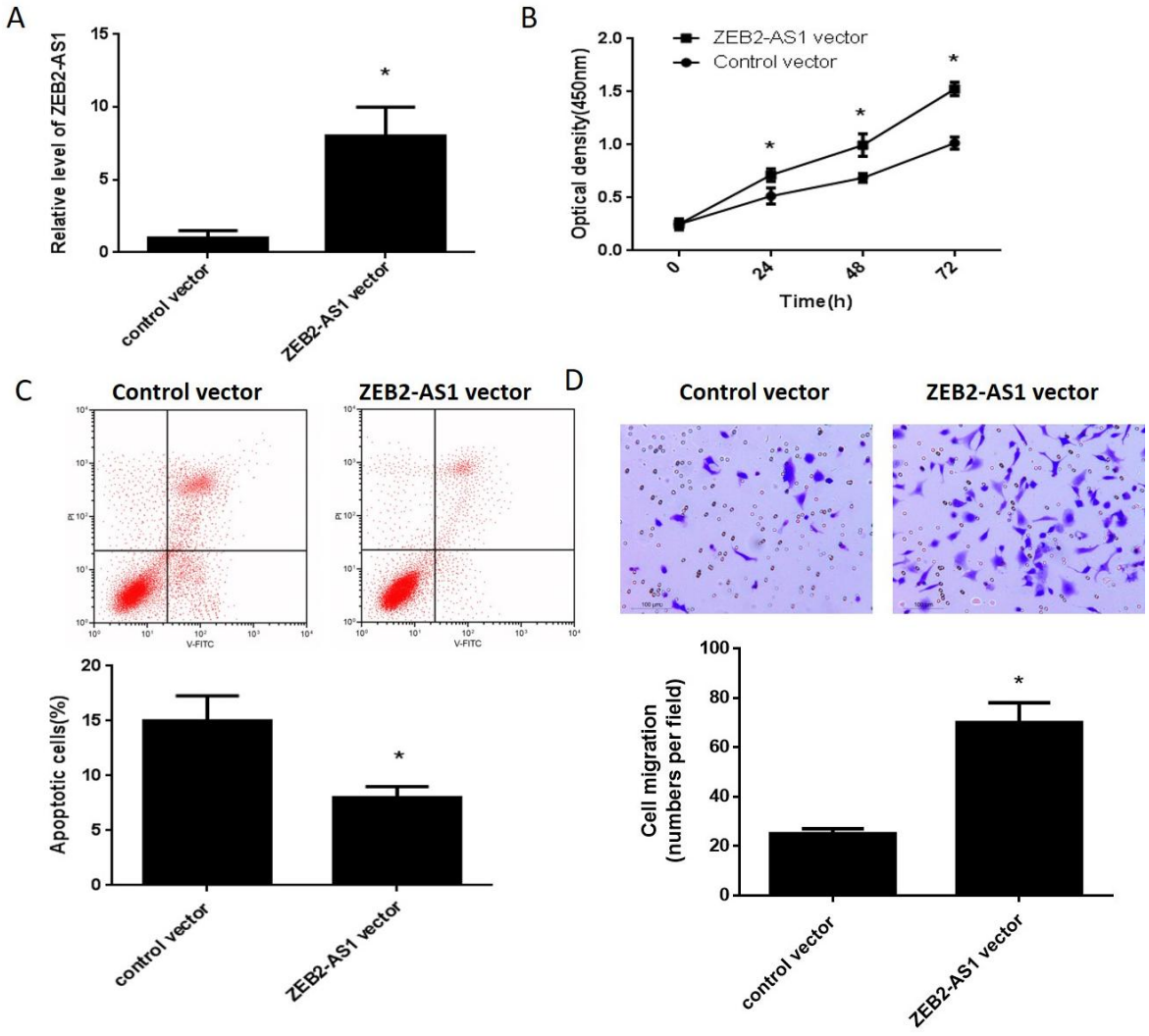

Figure 3. ZEB2-AS1 overexpression promoted cell proliferation and migration. **(**A) Quantitative PCR was used to detect the relative level of ZEB2-AS1; (B) CCK-8 was used to detect cell proliferation; (C) Flow cytometry was used to detect cell apoptosis; (D) Transwell was used to examine cell migration. Note: "*" represents the comparison with control group, $p < 0.05$. ZEB2-AS1 interfered with and inhibited cell proliferation and migration.

3.4. Silencing ZEB2-AS1 inhibits cell proliferation and migration

To determine the mechanism of how ZEB2-AS1 regulating the biological behaviors of CC cells, we constructed stably expressed cell strain through the transfection of ZEB2-AS1 siRNA. Firstly, we verified the efficiency of three siRNA interference. The results found that transfection siRNA# 1, 3 of CC cells ZEB2 -AS1 expression quantity was significantly lower, including the most obvious downward trend of siRNA#1 group ($P < 0.05$, Figure 4A), while the expression level in siRNA# 2 group had no significant change compared with the si - NC group ($P > 0.05$). So, we chose siRNA#1 group named si-ZEB2-AS1 to perform the following experiments. CCK-8 results proved that the

interference of ZEB2-AS1 expression in HCT8 cells inhibited the cell growth (Figure 4B). Consistent with the above results, interference of ZEB2-AS1 expression resulted in increased cell apoptosis rate (Figure 4C). In addition, the low expression of ZEB2-AS1 inhibited HCT8 cell migration, as shown in the results of Transwell assay (Figure 4D).

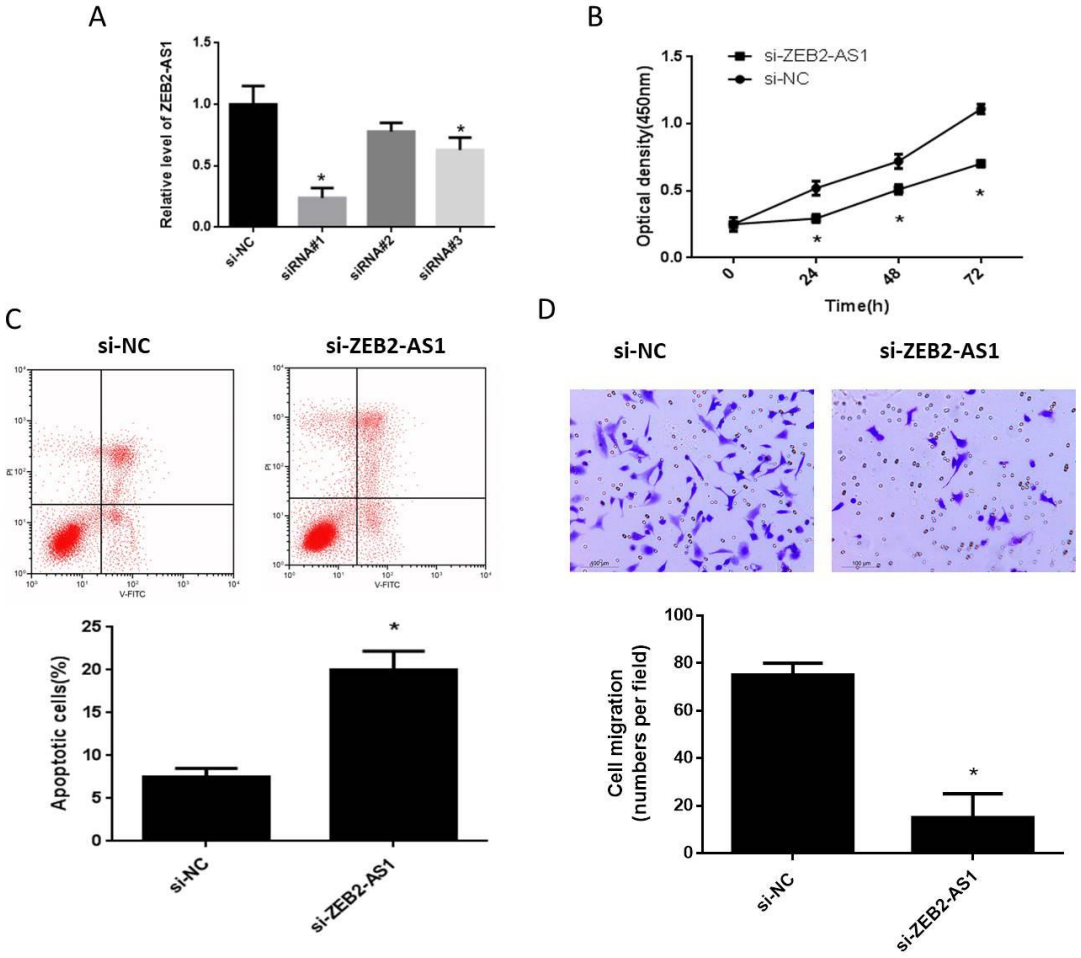

Figure 4. ZEB2-AS1 interfered with and inhibited cell proliferation and migration. (A) Measurement of 3 siRNA interference efficiency; (B) CCK-8 was used to detect the cell proliferation; (C) Flow cytometry was used to detect the cell apoptosis; (D) Transwell was used to detect the cell migration. Note: "*" represents the comparison with control group, $p < 0.05$. migration.

3.5. The effect of silencing ZEB2-AS1 on the expression of β-catenin protein

In order to explore the mechanism of ZEB2-AS1 regulating CC cells, we analyzed the expression of β-catenin protein, c-myc and Cyclin D1 when ZEB2-AS1 was overexpressed or silenced in CC cell HCT8. Then we found that the expressions of all these three kinds of proteins were significantly decreased in HCT8 cell in the si-ZEB2-AS1 group ($p < 0.05$) (Figure 5), which indicated that ZEB2-AS1 could prevent degradation of β-catenin proteins in CC cell samples, activate the transcription of downstream target genes (c-myc, CyclinD1) and promote cell

proliferation. The results showed that ZEB2-AS1 could promote proliferation and migration of cancer cells by activating Wnt/β-catenin signaling pathway.

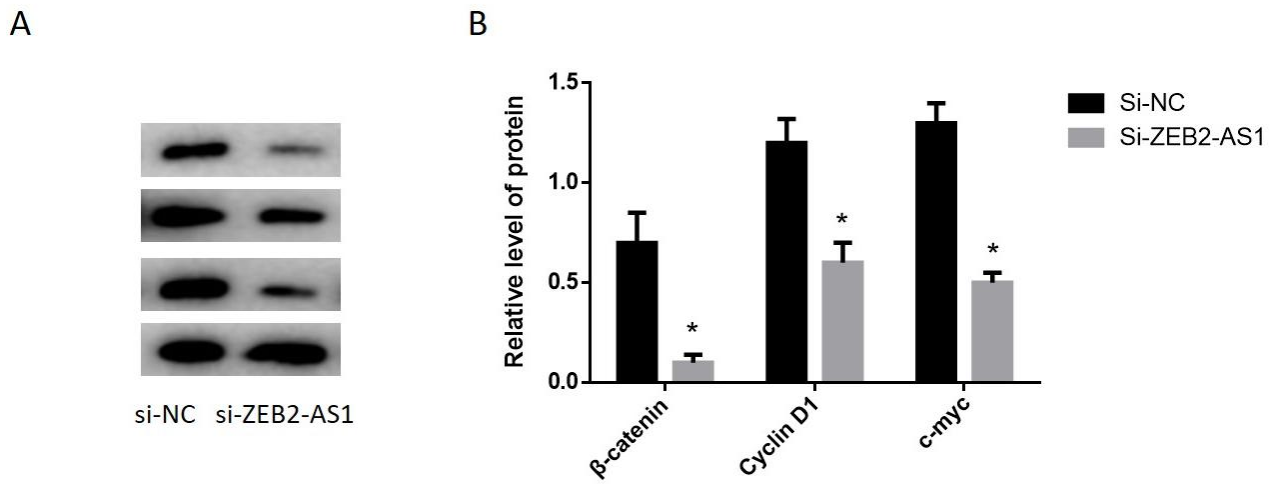

Figure 5. ZEB2-AS1 activated Wnt/β-catenin protein signaling in HCT8 cell. (A). ZEB2-AS1 knock down decreased the expression of β-catenin, c-myc and Cyclin D1 proteins; (B). Histogram of relative level of protein. Note: "*" represents the comparison with si-NC group, $p < 0.05$.

4. Discussion

Colon cancer is one of the most common malignant tumors in the world, which severely threatens health and lives of patients. There is an urgent need to develop new and effective treatments for colon cancer, and before that we need to better understand the underlying mechanisms of colon cancer development and progression. Studies have showed that when the expression of ZEB2-AS1 is up-regulated, it will play a role as a carcinogenic non-coding RNA in breast cancer [18], non-small cell lung cancer [13] and so on. In our study, we first identified ZEB2-AS1 as a key factor in the proliferation and migration of colon cancer cells. We found that ZEB2-AS1 expression was upregulated in CC tissues compared with adjacent normal tissues. The up-regulation of ZEB2-AS1 expression promoted cell proliferation and migration, while its down-regulation inhibited cell proliferation and promoted apoptosis. This was consistent with its effect on the development of triple negative breast cancer [19] that was ZEB2-AS1 could promote proliferation, metastasis and epithelial mesenchymal transformation (EMT) of triple negative breast cancer. However, in terms of mechanism research, this report showed thatZEB2-AS1 promoted EMT and the development of triple-negative breast cancer by activating the PI3K/Akt/GSK3 β/ZEB2 signaling pathway and positively regulating ZEB2 expression. However, in the study, we demonstrated that overexpression of ZEB2-AS1 could stabilize the β-catenin proteins and prevent its degradation. β-catenin proteins played a role as intracellular signal transduction in Wnt signal pathway and could regulate cell proliferation, differentiation and apoptosis. This was consistent with the mechanism of ZEB2-AS1 in gastric cancer. It had been reported [20] that in gastric cancer, ZEB2-AS1 promoted the occurrence of gastric cancer by activating the Wnt/β-catenin pathway, and inhibited the expression of active

β-catenin, c-myc and cyclin D1 proteins in gastric cancer cells by knockdown ZEB2-AS1. ZEB2-AS1 could be used as a potential biomarker for the diagnosis of colon cancer. And this novel ZEB2-AS1/ Wnt/β-catenin protein axis could be used to develop new strategies for treating colon cancer patients.

More and more evidence has shown that lncRNAs play an important role in oncogenesis and cancer progression [6–9]. They could act as tumor suppressors or oncogenes through various mechanisms, including epigenetic regulation, transcriptional regulation and post-translational regulation. LncRNA is reported can be involved in the regulation of tumor cell proliferation, apoptosis, migration and invasion [21]. Our findings suggest that lncRNA ZEB2-AS1 is highly expressed in colon cancer and regulates cell proliferation and migration. The overexpression of ZEB2-AS1 could promote the occurrence and development of tumors.

Wnt/β-catenin signaling pathway is an evolutionarily conserved pathway involved in embryonic development, tissue homeostasis and a host of human diseases. For example, abnormal activation of the Wnt/β-catenin signaling pathway usually leads to colon cancer [22]. Many researches have shown that adding Wnt/β-catenin signaling pathway inhibitors can inhibit tumor growth in colon cancer and some inhibitors have the potential to treat colon cancer [23]. The regulation of the activity of β-catenin in cancer is considered as a therapeutic opportunity. However, how to regulate the Wnt/β-catenin signaling pathway accurately remains to be defined. Recent reports showed that KDM3 could regulate Wnt target gene transcription by epigenetic modifications to control human colorectal cancer stem cells [24]. Another study showed that lnc-β-Catm could regulate the stability of the β-catenin protein and maintained liver CSC self-renewal [25]. However, knowledge about the regulation of β-catenin protein stabilization in colon cancer is limited. In this report, we found that ZEB2-AS1 interacted with β-catenin proteins and prevented their degradation, resulting in the presence of more activated β-catenin proteins in the nucleus.

5. Conclusion

In summary, our study showed that ZEB2-AS1 was highly expressed in colon cancer and might promote cell proliferation and migration by stabilizing the β-catenin proteins, which suggested that ZEB2-AS1 could be a novel biomarker for colon cancer and a potential therapeutic target for treating colon cancer.

Conflict of interest

The authors declare that they have no conflict of interest.

Funding

This study was supported by the Zhejiang Natural Sciences Foundation Grant (LY14H160018) and Ningbo Science and Technology Grant (CN2018027).

References

1. Y. Yin, J. Zhong, S. W. Li, et al., TRIM11, a direct target of miR-24-3p, promotes cell

proliferation and inhibits apoptosis in colon cancer, *Oncotarget*, **7** (2016), 86755–86765.
2. K. Liu, H. Yao, S. Lei, et al., The miR-124-p63 feedback loop modulates colorectal cancer growth, *Oncotarget*, **8** (2017), 29101–29115.
3. M. Yue, J. L. Charles Richard and Y. Ogawa, Dynamic interplay and function of multiple noncoding genes governing X chromosome inactivation, *Biochim. Biophys. Acta*, **1859** (2016), 112–120.
4. A. A. Butler, W. M. Webb and F. D. Lubin, Regulatory RNAs and control of epigenetic mechanisms: expectations for cognition and cognitive dysfunction, *Epigenomics*, **8** (2016), 135–151.
5. C. Kanduri, Long noncoding RNAs: Lessons from genomic imprinting, *Biochim. Biophys. Acta*, **1859** (2016), 102–111.
6. C. Tordonato, P. P. Di Fiore and F. Nicassio, The role of non-coding RNAs in the regulation of stem cells and progenitors in the normal mammary gland and in breast tumors, *Front Genet.*, **6** (2015), 72.
7. J. R. Prensner, M. K. Iyer, O. A. Balbin, et al., Transcriptome sequencing across a prostate cancer cohort identifies PCAT-1, an unannotated lincRNA implicated in disease progression, *Nat. Biotechnol.*, **29** (2011), 742–749.
8. W. Li, J. Zheng, J. Deng, et al., Increased levels of the long intergenic non-protein coding RNA POU3F3 promote DNA methylation in esophageal squamous cell carcinoma cells, *Gastroenterology*, **146** (2014), 1714–1726.e1715.
9. R. Chen, G. Wang, Y. Zheng, et al., Long non-coding RNAs in osteosarcoma, *Oncotarget*, **8** (2017), 20462–20475.
10. Z. Yang, X. Li, Y. Yang, et al., Long noncoding RNAs in the progression, metastasis, and prognosis of osteosarcoma, *Cell Death Dis.*, **7** (2016), e2389.
11. K. P. Zhu, X. L. Ma and C. L. Zhang, LncRNA ODRUL Contributes to Osteosarcoma Progression through the miR-3182/MMP2 Axis, *Mol. Ther.*, **25** (2017), 2383–2393.
12. B. Wang, Y. Su, Q. Yang, et al., Overexpression of Long Non-Coding RNA HOTAIR Promotes Tumor Growth and Metastasis in Human Osteosarcoma, *Mol. Cells*, **38** (2015), 432–440.
13. Y. Guo, Y. Hu, M. Hu, et al., Long non-coding RNA ZEB2-AS1 promotes proliferation and inhibits apoptosis in human lung cancer cells, *Oncol. Lett.*, **15** (2018), 5220–5226.
14. H. Gao, N. Gong, Z. Ma, et al., LncRNA ZEB2-AS1 promotes pancreatic cancer cell growth and invasion through regulating the miR-204/HMGB1 axis, *Int. J. Biol. Macromol.*, **116** (2018), 545–551.
15. P. Diao, H. Ge, Y. Song, et al., Overexpression of ZEB2-AS1 promotes epithelial-to-mesenchymal transition and metastasis by stabilizing ZEB2 mRNA in head neck squamous cell carcinoma, *J. Cell. Mol. Med.*, **23** (2019), 4269–4280.
16. X. Shi, J. Li, L. Ma, et al., Overexpression of ZEB2-AS1 lncRNA is associated with poor clinical outcomes in acute myeloid leukemia, *Oncol. Lett.*, **17** (2019), 4935–4947.
17. C. Xu, H. Cui, H. Li, et al., Long non-coding RNA ZEB2-AS1 expression is associated with disease progression and predicts outcome in gastric cancer patients, *J. Buon.*, **24** (2019), 663–671.
18. S. Xu, D. Kong, Q. Chen, et al., Oncogenic long noncoding RNA landscape in breast cancer, *Mol. Cancer*, **16** (2017), 129.
19. G. Zhang, H. Li, R. Sun, et al., Long non-coding RNA ZEB2-AS1 promotes the proliferation,

metastasis and epithelial mesenchymal transition in triple-negative breast cancer by epigenetically activating ZEB2, *J. Cell. Mol. Med.*, **23** (2019), 3271–3279.
20. F. Wang, W. Zhu, R. Yang, et al., LncRNA ZEB2-AS1 contributes to the tumorigenesis of gastric cancer via activating the Wnt/beta-catenin pathway, *Mol. Cell. Biochem.*, **456** (2019), 73–83.
21. C. He, W. Yang, J. Yang, et al., Long noncoding RNA MEG3 negatively regulates proliferation and angiogenesis in vascular endothelial cells, *DNA Cell Biol.*, **36** (2017), 475-481.
22. L. E. Dow, K. P. O'Rourke, J. Simon, et al., APC restoration promotes cellular differentiation and reestablishes crypt homeostasis in colorectal cancer, *Cell*, **161** (2015), 1539–1552.
23. J. N. Anastas and R. T. Moon, WNT signalling pathways as therapeutic targets in cancer, *Nat. Rev. Cancer*, **13** (2013), 11–26.
24. J. Li, B. Yu, P. Deng, et al., KDM3 epigenetically controls tumorigenic potentials of human colorectal cancer stem cells through Wnt/beta-catenin signalling, *Nat. Commun.*, **8** (2017), 15146.
25. P. Zhu, Y. Wang, G. Huang, et al., lnc-beta-Catm elicits EZH2-dependent beta-catenin stabilization and sustains liver CSC self-renewal, *Nat. Struct. Mol. Biol.*, **23** (2016), 631–639.

AIMS Press