Abstract. The effect of long non-coding RNA (lncRNA) THOR on proliferation and migration of colon cancer cells was investigated. Lentiviral vector expressing lncRNA THOR shRNA was used to establish colon cancer SW620 lncRNA THOR knockdown cell line (experimental group), and at the same time, a control vector cell line (control group) was established by empty vector virus. Proliferation ability of the two groups was analyzed by CCK8 and EdU methods. Migration ability of the cells was analyzed by Transwell method. Xenograft tumor method was used to analyze the in vivo proliferation ability of the two groups of cells. mRNA levels of lncRNA THOR target genes were analyzed by reverse transcription-quantitative PCR (RT-qPCR). Compared with control cells, the cell proliferation ability of the experimental group was significantly decreased (P<0.05). Compared with the control group, the cell migration ability of the experimental group was significantly decreased (P<0.05). The tumor growth rate of the experimental group in the mice was significantly lower than that of the control group (P<0.05). Compared with the control group, mRNA levels of lncRNA THOR target genes IGF2BP1, SOX9 and c-myc in the experimental group were significantly down-regulated (P<0.05). The results indicated that lncRNA THOR knockdown can significantly downregulate the expression of genes involved in tumor proliferation and migration, promote tumor cell proliferation and migration, indicating that lncRNA THOR plays an important role in colon cancer.

Introduction

Colon cancer is one of the common types of nausea and digestive tract tumors, which has become a disease that seriously affects human health (1). The molecular mechanism of colon cancer development and progression is still unclear.

Materials and methods

General information. Thirty female Balb/c nude mice weighing 20 g were purchased from Shanghai SLAC Laboratory Animal Co., Ltd. The mice (6 per cage) were kept in SPF animal room at 25°C with a 12 h light/12 h dark cycle and free access to food and water and humidity was 50%. Colon cancer cell line SW620 was purchased from ATCC. CCK8 cell proliferation assay kit and BeyoClick™ EdU-488 cell proliferation assay kit were purchased from Biyuntian Biotechnology Co., Ltd. Transwell kit was purchased from Corning. MTT assay kit and RNA extraction kit were purchased from Sigma-Aldrich; Merck KGaA. RNA extraction kit (RC101), reverse transcription kit (R323-01), quantitative PCR detection kit (Q431-02) were purchased from Nanjing Nuoweizan Biological Co., Ltd. Control shRNA lentivirus, and Lenti-THOR-shRNA lentivirus were obtained from Shandong Weizhen Biological Co., Ltd. The study was approved by the Ethics Committee of Jinan Central Hospital Affiliated to Shandong University (Jinan, China).
Colon cancer cell line SW620 [SW-620] (ATCC® CCL-227™) was cultured in DMEM medium (10% FBS) twice to restore cell status. After digestion with 0.25% trypsin, the cell concentration was adjusted to 1x10^5 cells/ml and inoculated in 12-well plate with 1 ml per well. After 12 h of cell adherence, the control shRNA lentivirus and Lenti-THOR-shRNA lentivirus were added respectively, and the cell-to-virus titer ratio was 1:100. Culture medium was replaced by fresh medium at 24 h after transfection, followed by cell culture for additional 4 h. Then cells were treated with 1 µg/ml puromycin for 24 h, and viable cells were observed under the microscope (ZEISS) to observe GFP fluorescence. When GFP in all cells is expressed, stable cell line is successfully constructed. Lenti-THOR-shRNA stable expression cell line was used as the experimental group, and control shRNA stable expression cell line was used as the control group.

Preparation of a reverse transcription-quantitative PCR (RT-qPCR) system. The same number of living cells were collected both from the control and experimental groups. Total RNA was extracted using an RNA extraction kit, and then the RNA was reverse-transcribed into cDNA using a reverse transcription kit to serve as the template for RT-qPCR. The temperature protocol for reverse transcription was 37˚C for 30 min, 85˚C for 15 sec and 4˚C for storage. cDNA template was diluted 1:100 before use. PCR reaction systems included: 2X SYBR-Green premix 10 µl, template 8.8 µl, upstream primers 0.6 µl and downstream primers 0.6 µl. PCR reaction conditions were: 95˚C for 30 sec, followed by 40 cycles of 95˚C for 10 sec and 60˚C for 30 sec. Sequences of primers used in PCR reactions are shown in Table I. β-actin was the reference gene. The method of quantification was 2-ΔΔCq (11).

Cell proliferation analysis (CCK8 and EDU methods). Control and experimental cells were trypsinized at a cell density of 5x10^5 cells/ml, and seeded in 96-well plates with 100 µl per well. Cells were cultured for 6 h at 37˚C in a 5% CO₂ incubator. After that, 10 µl of CCK solution was added 12, 24, 36, 48 and 72 h later. After that, cells were cultivated for additional 3 h. Finally, OD values at 450 nm were measured using a microplate reader (BioTek) to reflect cell proliferation.

Cells of the control and experimental groups were digested and inoculated into a 12-well plate. After the cells were completely adhered, the EdU was diluted to 50 µM with a cell culture medium, and 100 µl was added to each well. Cells were incubated for 2 h in a 37˚C and 5% CO₂ incubator. After that, cells were washed three times with PBS, and then fixed with 4% paraformaldehyde at room temperature for 20 min. After washing three times with PBS, nuclei were stained with DAPI. After washing three times with PBS, cells were observed under a microscope.

Cell migration analysis (Transwell method). The control and experimental cells were trypsinized to prepare a single cell suspension, and the cell density was adjusted to 5x10^5 cells/ml, and inoculated into the Transwell upper chamber, 100 µl per well. The lower chamber was filled with fresh cell culture medium. Cells were incubated in a 37˚C and 5% CO₂ incubator for 24 h, and were fixed with paraformaldehyde. Then the upper chamber membrane was fixed with 1% crystal violet (MXB Biotechnologies) at room temperature for 5 min. Migrating cells were observed under an optical microscope, and 20 visual fields were selected for both the experimental and control groups to calculate the average number of migrating cells.

Xenograft tumor. After cells of the control and experimental groups were trypsinized, cell density was adjusted to 1x10^8/ml. Thirty Balb/c nude mice were randomly divided into the control and experimental groups. Tumor cells (100 µl) were injected into the fat pad of each mouse, and mice were raised in SPF-level animal house. Tumor formation and growth in the mice were observed. Tumor length (L) and width (W) were...
measured. Tumor volume was calculated by the following formula: \( V = \frac{1}{2} L \times W^2 \).

Statistical analysis. All data were analyzed by SPSS 17.0 statistical software (SPSS, Inc.). Measurement data were expressed as mean ± standard deviation and compared by t-test. \( P<0.05 \) indicated a difference with statistical significance.

Results

Comparison of lncRNA THOR levels in the control and experimental groups. The lncRNA THOR knockdown stable cell line was established by lentiviral-mediated THOR-shRNA. The expression level of lncRNA THOR in the control group (1.21±0.21) was significantly higher than that in the experimental group (0.28±0.10), and the difference was statistically significant (\( P<0.05 \)) (Fig. 1).

Effect of lncRNA THOR on cell proliferation. CCK8 analysis showed that compared with the control cells, cell proliferation ability of the experimental group was significantly reduced (\( P<0.05 \)) (Fig. 2A). EdU staining analysis showed that the cell proliferation ability of the experimental group was significantly decreased compared with that of the control group (\( P<0.05 \)) (Fig. 2B).

Effect of lncRNA THOR on cell migration. Compared with the number of migrating cells in the control group (91.23±11.44), the number of migrating cells in the experimental group (49.32±8.14) decreased significantly (\( P<0.05 \)) (Fig. 3).

Effect of lncRNA THOR on tumor growth. Control and experimental group cells were inoculated into nude mice, and cell proliferation rate in vivo was analyzed. Compared with the control cells, growth rate of tumors in the experimental group was significantly reduced (\( P<0.05 \)) (Fig. 4).

Effect of lncRNA THOR knockdown on its target genes. Effect of lncRNA THOR knockdown on its target genes at mRNA level was further analyzed by RT-qPCR. Compared with
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In the control group, the mRNA levels of IGF2BP1, SOX9 and c-myc in the experimental group were significantly reduced (P<0.05) (Fig. 5).

Discussion

Colon cancer, as the third most common type of malignant tumor in the world, seriously affects human health (12). Despite the efforts made in cancer diagnosis, many cancer patients are still diagnosed at advanced stages. Therefore, novel early diagnosis biomarkers are needed (13). Studies have shown that lncRNAs participate in many biological processes by regulating gene expression (14). It has been reported that lncRNA CCTA1, ATB and HOTAIR participate in the regulation of cell behavior of colon cancer by mediating the downstream signaling pathways (15,16). Because of the important role of lncRNA in tumorigenesis, lncRNAs have been used as a novel tumor marker and tumor therapeutic target. This study analyzed the role of lncRNA THOR in the pathogenesis of colon cancer.

At cellular level, lncRNA THOR knockdown led to significantly inhibited proliferation and migration of colon cancer SW620 cells. At animal level, lncRNA THOR knockdown mediated the significantly inhibited growth of tumors in mice. It indicated that lncRNA THOR plays an important role in the development of colon cancer.

Sox9 plays an important role in early embryonic development, cell fate determination and differentiation of tissues and organs, sex determination, occurrence and development of nervous system and cartilage (17). The high expression of Sox9 is related to the size of the tumors, TNM stage, lymph node metastasis and differentiation of colorectal cancer patients (18). lncRNA THOR maintains the stability and activity of IGF2BP1 through conservative interaction with IGF2BP1 mRNA (7). The results showed that knockdown of lncRNA THOR resulted in significant downregulation of the target genes SOX9 and IGF2BP1 mRNA, suggesting that lncRNA THOR has important significance in stabilizing SOX9 and IGF2BP1 mRNA. C-myc, as an oncogene, plays an important role in tumorigenesis (19). This study also found that knockdown of lncRNA THOR in colon cancer cells resulted in a significant decrease in c-myc mRNA level, which further demonstrated that lncRNA THOR, was involved in the occurrence and progress of colon cancer genes.

Collectively, lncRNA THOR, as an oncogene, promotes the proliferation and migration of colon cancer cells, which can be used as a therapeutic target for colon cancer treatment.

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Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors’ contributions

YL and XY drafted the paper and performed PCR. XY and LW were responsible for CCK8 assay and Transwell method. All the authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of Jinan Central Hospital Affiliated to Shandong University (Jinan, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.
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