Biomedicine & Pharmacotherapy 108 (2018) 338–346

Contents lists available at ScienceDirect

Biomedicine & Pharmacotherapy

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

LncRNA THOR increases the stemness of gastric cancer cells via enhancing SOX9 mRNA stability

Hu Song[a,b], Yixin Xu[a,b], Linseng Shi[a,b], Teng Xu[a,b], Ruizhi Fan[a,b], Meng Cao[a,b], Wei Xu[a,b], Jun Song[a,b,*]

[a] Department of General Surgery, the Affiliated Hospital of Xuzhou Medical University, 99 West Huaihai Road, Xuzhou, Jiangsu, 221002, PR China
[b] Institute of Digestive Disease, Xuzhou Medical University, 84 West Huaihai Road, Xuzhou, Jiangsu, 221002, PR China

ARTICLE INFO

Keywords:
LncRNA
THOR
SOX9 3'UTR
Stemness
Gastric cancer

ABSTRACT

This work aims to explore the roles and mechanisms of long non coding RNA (lncRNA) THOR in regulating the stemness of gastric cancer cells. RNA-sequencing combined with quantitative real-time PCR (qRT-PCR) indicated that lncRNA THOR level was significantly upregulated in gastric cancer tissues compared with that in normal adjacent tissues. Knockdown of THOR attenuated the stemnness of gastric cancer cells, evident by the decrease of stemness markers expression and capacity of cells spheroid formation. Further RNA-sequencing combined with qRT-PCR and western blot analysis demonstrated that expression of transcriptional factor SOX9 was remarkably decreased in gastric cancer cells with THOR stable knockdown. Additionally, RNA immunoprecipitation (RIP) combined with luciferase reporter assay revealed that THOR directly bound to SOX9 3′ untranslated region (3′UTR), but not its 5'UTR or coding area. Notably, overexpression of SOX9 rescued THOR knockdown-mediated inhibition on the stemness of gastric cancer cells. Thus, our results suggest that THOR could potentiate the stemness of gastric cancer cells via directly binding to SOX9 3′UTR.

1. Introduction

Gastric cancer has high incidence and mortality, and due to the early atypical clinical symptoms and screening early gastric cancer is seriously insufficient, the majority of patients are diagnosed with end-stage disease [1]. Traditional chemotherapy has been the main treatment of advanced gastric cancer, however, single chemotherapy is still very limited and has reached the bottleneck [2]. With the research development of tumor molecular biology, molecular targeted therapy provides new clues for gastric cancer. Therefore, it is very important to find novel targets and dig the mechanisms for gastric cancer progression.

Cancer stem cells (CSCs) are a small group of cells within the tumor bulk with the capacity of self-renewal and infinite proliferation, this characteristic maintains the vitality of tumor cell population. And the migration ability of CSCs contributes to the metastasis of tumor cells. Additionally, CSCs can be dormant for a long time, and hold high expression level off drug-resistant molecules and thus are resistant to the chemotherapy. These traits of CSCs lead to tumor progression, recurrence or chemoresistance [3]. Thus, targeting CSCs has been shown as an effective method to inhibit cancer progression [4]. Transcriptional factor SOX9 has been identified to enhance the stemness of glioma cells and temozolomide resistance [5], and maintain cells pluripotency [6]. Additionally, SOX9 regulates cancer stem-like properties and metastatic potential of single-walled carbon nanotube-exposed cells [7]. Notably, recent studies have revealed SOX9 as a potential target for suppressing CSCs progression, like melatonin inhibits osteosarcoma stem cells by suppressing SOX9-mediated signaling [8]. KDM6A promotes chondrogenic differentiation of periodontal ligament stem cells by demethylation of SOX9 [9]. And KLF15 regulates *in vitro* chondrogenic differentiation of human mesenchymal stem cells by targeting SOX9 [10]. Nevertheless, it remains a mystery whether there are additional vital regulators of SOX9 during CSCs progression, such as long non-coding RNAs (lncRNAs).

LncRNAs are a kind of non-coding RNA with 200 nt – 1×10^5 bp length and no obvious reading framework [11]. LncRNAs have been previously regarded as "junk DNA" for their no functions, however, recent studies have shown the ectopic expression of lncRNAs in cancers, such as in liver cancer [12], neuroblastoma [13], lung cancer [14] and breast cancer [15]. Importantly, the critical roles of lncRNAs have been

* Corresponding author at: Department of General Surgery, the Affiliated Hospital of Xuzhou Medical University, 99 West Huaihai Road, Xuzhou, Jiangsu, 221002, PR China.
E-mail address: junj_song@163.com (J. Song).

https://doi.org/10.1016/j.biopha.2018.09.057
Received 14 August 2018; Received in revised form 31 August 2018; Accepted 11 September 2018

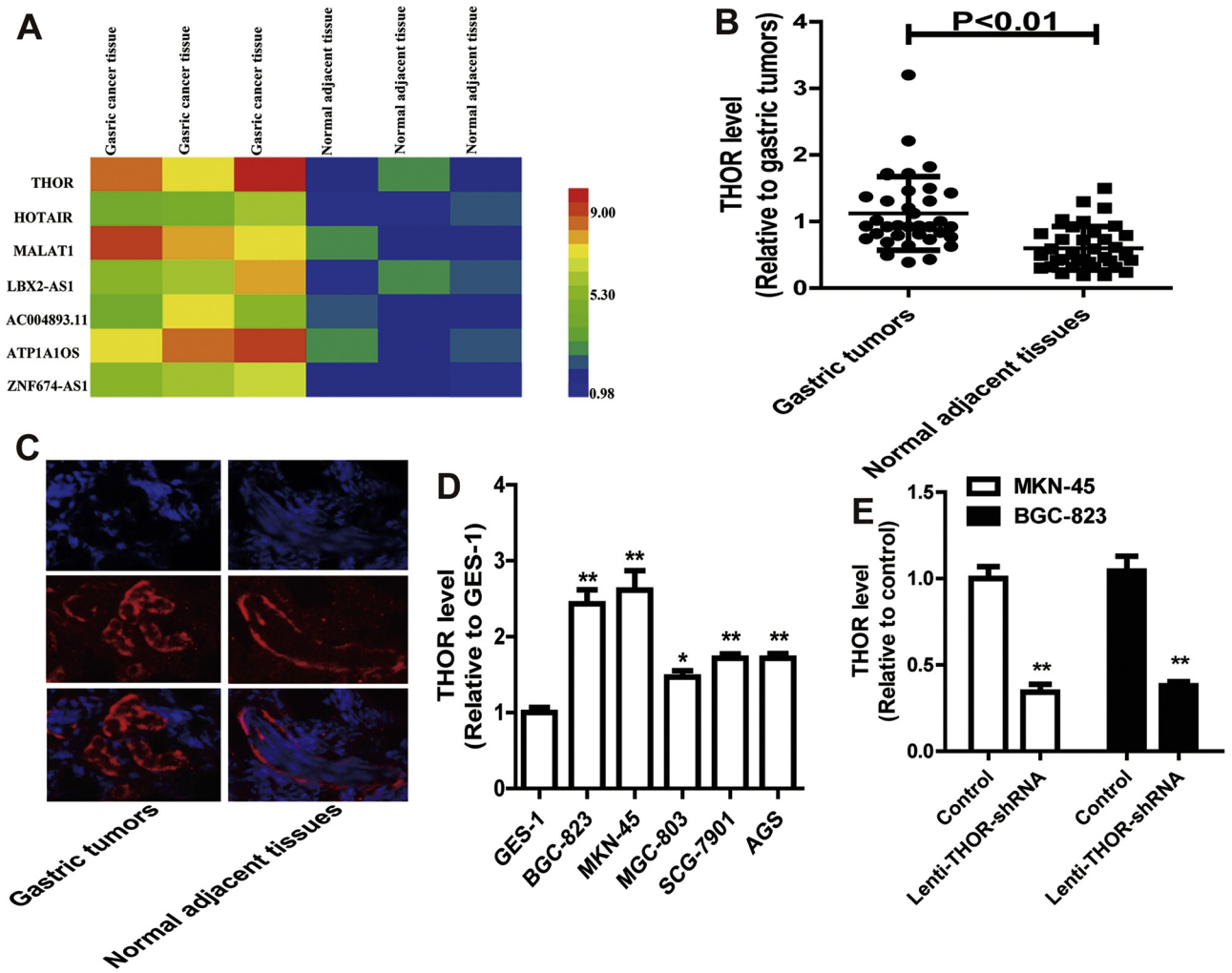

Fig. 1. Expression of lncRNA THOR is significantly increased in gastric cancer tissues and cells. (**A**) Expression of represented lncRNAs in gastric cancer tissues and normal adjacent tissues via RNA-sequencing assay, shown as heatmap. (**B**) THOR level was examined in gastric cancer tissues and normal tissues via qRT-PCR. (**C**) RNA-FISH was performed to analyze the expression of THOR in paraffin-embedded gastric cancer and normal adjacent tissues. THOR expression was higher in gastric cancer tissues compared to adjacent normal tissues. (**D**) THOR level was determined in gastric cancer cells and normal gastric epithelial cells. (**E**) Knockdown efficiency of Lenti-THOR-shRNA was confirmed in MKN45 and BGC-823 cells. Data were presented as mean ± s.d ; *P < 0.05, **P < 0.01 vs. normal adjacent tissues or GES-1 or control.

demonstrated in cancers. Such as Chou. et al indicated that lncRNA MALAT1 promotes breast cancer cells metastasis via regulating CDC24 expression [16], and lncRNA uc.134 represses hepatocellular carcinoma progression by inhibiting CUL4 A-mediated ubiquitination of LATS1 [17]. Importantly, lncRNAs could exert its functions via acting as guidance or co-activators or co-repressors, like Wu et al. showed that lncRNA HOTAIR promotes tumor cell invasion and metastasis by recruiting EZH2 and repressing E-cadherin in oral squamous cell carcinoma [18]. Additionally, lncRNA LncSHGL recruits hnRNPA1 to suppress hepatic gluconeogenesis and lipogenesis [11]. LncRNA THOR was firstly identified by Hosono, Y. et al in 2017, and shown as a conserved cancer/testis lncRNA [19]. Further study has shown that lncRNA THOR could promote human osteosarcoma cell growth *in vitro* and *in vivo* [20]. Recent study has indicated that lncRNA THOR promotes human renal cell carcinoma cell growth [21], and attenuates cisplatin sensitivity of nasopharyngeal carcinoma cells via enhancing cells stemness [22]. However, the roles and mechanisms of THOR in gastric cancer progression are still unclear.

In the present study, we tried to identify lncRNAs with ectopic expression in gastric cancer, and found that lncRNA THOR was significantly increased in gastric cancer tissues. Further *in vitro* experiments showed that knockdown of THOR attenuated the stemness of gastric cancer cells. Mechanistically, we revealed that THOR directly bound to the 3′ untranslated region (3′UTR) of stemness marker SOX9, which was necessary for THOR-mediated effects. Our results firstly identified THOR/SOX9 regulatory axis in regulating the stemness of gastric cancer cells, this could be a novel target for gastric cancer.

2. Material and methods

2.1. Clinical samples and cells culture

Thirty-six pairs of gastric cancer and normal adjacent tissue samples were collected from the Affiliated Hospital of Xuzhou Medical University. Written informed consent from all patients and approval of the Hospital Ethic Review Committees were obtained. Gastric cancer cell lines AGS, BGC-823, MKN-45, MGC-803, SCG-7901 and normal gastric epithelial cell line GES-1 were purchased from the Chinese Academy of Sciences Cell Bank. SCG7901-DDP (SCG7901 cisplatin resistant cells) were purchased from (KeyGEN BioTECH, Nanjing, China). All of the cell lines were cultured at 37 °C under humidified atmosphere with 5% CO_2, and cultured in 1640 medium (Gibco, USA) supplemented with 10% FBS (Fetal Bovine Serum) (Gibco), 80 U/ml penicillin and 0.08 mg/ml streptomycin.