J Gynecol Oncol. 2024 Mar;35(2):e21
https://doi.org/10.3802/jgo.2024.35.e21
pISSN 2005-0380·eISSN 2005-0399

JOURNAL OF GYNECOLOGIC ONCOLOGY

Original Article

LncRNA linc01194 promotes the progress of endometrial carcinoma by up-regulating SOX2 through binding to IGF2BP1

Zhenghao Huang, Fan Shen, Jingwen Chen, Bumin Xie, Xi Chen, Yang Zhao, Shuo Chen

Department of Obstetrics and Gynecology, Department of Gynecologic Oncology Research Office, Guangzhou Key Laboratory of Targeted Therapy for Gynecologic Oncology, Guangdong Provincial Key Laboratory of Major Obstetric Diseases, The Third Affiliated Hospital of Guangzhou Medical University, Guangzhou, China

Received: Dec 20, 2022
Revised: Aug 14, 2023
Accepted: Oct 26, 2023
Published online: Nov 21, 2023

Correspondence to
Yang Zhao
Department of Obstetrics and Gynecology, Department of Gynecologic Oncology Research Office, Guangzhou Key Laboratory of Targeted Therapy for Gynecologic Oncology, Guangdong Provincial Key Laboratory of Major Obstetric Diseases, The Third Affiliated Hospital of Guangzhou Medical University, No. 63 Duobao Road, Liwan District, Guangzhou, Guangdong 510150, P.R. China.
Email: yida.zhaoyang@163.com

Shuo Chen
Department of Obstetrics and Gynecology, Department of Gynecologic Oncology Research Office, Guangzhou Key Laboratory of Targeted Therapy for Gynecologic Oncology, Guangdong Provincial Key Laboratory of Major Obstetric Diseases, The Third Affiliated Hospital of Guangzhou Medical University, No. 63 Duobao Road, Liwan District, Guangzhou, Guangdong 510150, P.R. China.
Email: chenshuo077003@163.com

ABSTRACT

Objective: Endometrial carcinoma (EC) is one of the most common gynecological malignant tumors. Our study showed that long non-coding RNA (lncRNA) linc01194 plays an important role in EC. We explored the mechanism of lncRNA linc01194 in EC.
Methods: The expression of lncRNA linc01194 was detected in The Cancer Genome Atlas database and starBase database. The potential targeted protein of linc01194 was predicted through the starBase database. To determine the role of linc01194 in EC, we downregulated or upregulated the level of linc01194 in EC cell lines and analyzed the cell behaviors and the changes of its potential target proteins.
Results: The expression of linc01194 in EC tissues is higher than that in normal endometrial tissues. The knockdown of linc01194 inhibited the cell proliferation, invasion and migration and promoted the apoptosis of EC cells, while overexpression of linc01194 promoted cell proliferation, invasion and migration and inhibited the apoptosis of EC cells. The starBase database revealed that linc01194 could bind to insulin-like growth factor 2 binding protein 1 (IGF2BP1). Previous results showed that in EC, IGF2BP1 could promote the expression of sex-determining region Y-box 2 (SOX2) by promoting the stability of SOX2 mRNA. Our results showed that linc01194 regulate the expression of IGF2BP1 and SOX2.
Conclusion: Linc01194 can promote the expression of downstream protein SOX2 through binding to IGF2BP1, thus promoting the occurrence and development of EC.

Keywords: Endometrial Carcinoma; Linc01194; IGF2BP1; SOX2

Synopsis

Long non-coding RNA linc01194 was upregulated in endometrial carcinoma (EC). The expression level of linc01194 was related to the malignant behavior of EC cells. Linc01194 promote the expression of sex-determining region Y-box 2 through the binding to insulin-like growth factor 2 binding protein 1.

ORCID iDs
Zhenghao Huang
https://orcid.org/0000-0001-5848-2012
Fan Shen
https://orcid.org/0000-0002-8424-8403
Jingwen Chen
https://orcid.org/0000-0002-6924-1377
Bumin Xie
https://orcid.org/0000-0003-2554-6280
Xi Chen
https://orcid.org/0000-0001-9579-279X
Yang Zhao
https://orcid.org/0000-0003-3226-8127
Shuo Chen
https://orcid.org/0000-0002-5122-3656

Funding
This work was supported by the Natural Science Foundation of Guangdong Province (No. 2022A1515012293); the Project for Key Medicine Discipline Construction of Guangzhou Municipality, China (No. 2021-2023-17).

Conflict of Interest
No potential conflict of interest relevant to this article was reported.

Author Contributions
Conceptualization: S.F., X.B., Z.Y., C.S.; Data curation: H.Z., S.F.; Formal analysis: H.Z.; Funding acquisition: Z.Y.; Methodology: S.F., Z.Y., C.S.; Resources: Z.Y., C.S.; Software: C.J., X.B., C.X.; Supervision: S.F.; Validation: C.J.; Writing - original draft: H.Z.; Writing - review & editing: H.Z., S.F., C.J., X.B., C.X., Z.Y., C.S.

INTRODUCTION

Endometrial carcinoma (EC) ranks second in the incidence of female malignant tumors [1]. The metastatic and invasive capacity of EC is strong, which is the main factor affecting the long-term survival rate of EC patients [2]. Due to the increase in risk factors such as diabetes and obesity, the incidence of EC has gradually increased [3]. Notably, the incidence of EC will increase by 50% by 2040 [4]. Currently, surgery is the main therapeutic approach for EC treatment, with radiotherapy and chemotherapy applied as adjusted treatments when necessary. Although progress in EC treatment has been made, those with advanced EC have the poor prognosis [5]. A study conducted in 2018 showed that 80% of EC patients were diagnosed as well differentiated and moderately differentiated EC, with a good prognosis. Nevertheless, those with poorly differentiated EC have low survival rate [6]. Therefore, the discovery and development of effective early prognostic indicators are of considerable clinical significance.

Previous studies have shown that less than 2% of genes in the human genome encode proteins [7]. Non-coding RNAs (ncRNAs), as the name implies, are not involved in coding proteins. Increasingly more studies on ncRNAs have revealed that they are the key regulators in many cellular processes, such as transcription, post-transcriptional modification, cell growth, cell proliferation, apoptosis and metabolism [8].

As a type of RNAs without coding capability, long non-coding RNAs (lncRNAs), have a length of more than 200 nucleotides [9]. Accumulating studies had revealed that lncRNAs are related to several pathological and physiological processes, especially in cancer development [10,11]. The advances in RNA sequencing, gene microarray analysis, and high-throughput sequencing have enabled the recognition of the lncRNA potential as a tumor-suppressor or an oncogene gene that can regulate cell proliferation, migration, invasion, apoptosis, and immune response has been recognized [12]. Therefore, lncRNA can be used as a molecular marker for early diagnosis of tumors and a new target for tumor therapy [13]. In recent years, increasingly more studies have revealed that a large number of lncRNAs, such as lncRNA-ZXF1 [14], lncRNA DSCAM-AS1 [15], and lncRNA SNHG12 [16] and so on, have an abnormal expression in EC and are involved in its occurrence and development. The Cancer Genome Atlas (TCGA) database shows that linc01194 is upregulated in EC. We speculated that linc01194 may be involved in the development and occurrence of EC.

MATERIALS AND METHODS

1. Bioinformatics analysis

The expression levels of linc01194 in normal endometrial tissues and EC tissues was analyzed in the starBase database (https://starbase.sysu.edu.cn/) and the TCGA database (https://portal.gdc.cancer.gov/). Also, the downstream target protein of linc01194 was predicted in the starBase database. The relationship between insulin-like growth factor 2 binding protein 1 (IGF2BP1) and the overall survival rate of EC patients was revealed in the Kaplan-Meier Plotter database (http://kmplot.com/analysis/).

2. Endometrial specimens

We collected and analyzed the endometrial tissue of 127 gynecological patients (including 99 endometrial cancer tissues and 28 normal endometrial tissues) from the Third Affiliated Hospital of Guangzhou Medical University. All of them had not received preoperative

radiotherapy and chemotherapy. Two pathologists confirmed the endometrial specimens independently. Then these endometrial specimens were stored at −80°C until they were used for further studies. Obtained the informed consent of the subjects before obtaining the specimen. The experiment was approved by the Ethics Committee of the Third Affiliated Hospital of Guangzhou Medical University (No. 2020066). All endometrial specimens were treated in accordance with ethical and legal standards and treated anonymously.

3. Cell culture

The expression of linc01194 in 4 human EC cells lines (Ishikawa, HEC-1B, HEC-1A, and KLE), purchased from Jennio Biotech, was detected. Roswell Park Memorial Institute (RPMI) 1640 medium (HyClone Laboratories Inc., Logan, UT, USA) was utilized to culture Ishikawa cells, while Dulbecco's Modified Eagle Medium (DMEM; Thermo Fisher Scientific, Waltham, MA, USA) was utilized to culture HEC-1B cells, McCoy's 5A medium was utilized to culture HEC-1A cells and Ham's F-12 treatment medium was utilized to culture KLE cells. All culture media contained 10% fetal bovine serum (FBS) and penicillin/streptomycin. Cells were cultured in an incubator containing 5% CO_2 at 37°C. All cells were identified by short tandem repeat (STR) analysis and subjected to mycoplasma contamination tests.

4. Transfection of small interfering RNA (siRNA) and plasmid targeting linc01194

The siRNA targeting linc01194 (si-linc01194) and the negative control (si-NC) were synthesized by RiboBio Co., Ltd. (Guangzhou, China). The plasmid targeting linc01194 was used to upregulate the expression of linc01194. Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) was utilizes to transfect siRNA and plasmid. The concentration of si-linc01194 and control si-NC was 100 nM. The siRNA sequence targeting linc01194 was GCTTGATGGTGTAACTTCA. The si-NC sequence was provided by RiboBio Co., Ltd. The plasmid sequence targeting linc01194 was as listed in **Data 1**.

5. Cell proliferation detection with cell counting kit-8 (CCK-8)

We followed the instructions of CCK-8 kit (Yeasen, Shanghai, China) to detect cell proliferation. First, inoculated cells into a 96-well plate containing 100 μL of medium until they adhered to the wall. The concentration is 3,000 cells/well. The samples were divided into an experimental and a negative control group, which were transfected with si-linc01194 and si-NC, respectively. At 0, 24, 48 and 72 hours, add 10 uL CCK-8 solution to each well. Then, incubate for 2 hours in an incubator with 5% CO_2 at 37°C. The absorbance was then determined at 450 nm with a microplate reader.

6. Real-time quantitative reverse-transcription polymerase chain reaction (qRT-PCR)

Total RNA was extracted from EC cell lines with TRIzol reagent (TaKaRa, Shiga, Japan), and then Hifair® III 1st Strand cDNA Synthesis SuperMix was used for qPCR, and then total RNA reversed to cDNA. Next, Hieff® qPCR SYBR® Green Master Mix was utilized to amplify the target gene by real-time qPCR and measure its expression level. Further, 18sRNA and the U6 gene were acted as an internal reference gene. The related primer sequences were listed in the **Table 1**.

7. Wound-healing assay

Cells were inoculated in a 6-well plate containing 2 mL of medium containing 10% FBS and cultured until fusion into a monolayer was achieved. Make vertical scratches by a 200-μL pipette in the Petri dish, and then washed with phosphate-buffered saline (PBS) twice to

Table 1. Primer sequence

Gene	Sequence
Linc01194	F: GGATGACTATGGCTGGACG
	R: TGCGGTGAAAGTGGCTC
U6	F: CTCGCTTCGGCAGCACA
	R: AACGCTTCACGAATTTGCGT
18S	F: GAAACGGCTACCACATCC
	R: ACCAGACTTGCCCTCCA

three times, following by adding 2 mL of serum-free medium. At 0, 24 and 48 hours, took photo under a microscope, to compare the wound sizes in the experimental and negative control groups. The software ImageJ (National Institutes of Health, Bethesda, MD, USA) was utilized to calculate the cell migration rate. All experiment were repeated 3 times.

8. Cell invasion test

The diluted matrix binder was evenly placed in the small chamber of a 24-well plate Transwell chamber (Corning, Inc., Corning, NY, USA), following by incubation in an incubator containing 5% CO_2 at 37 °C for 4 hours. A total volume of 600 uL of medium containing 10% FBS, streptomycin and penicillin and the configured transfection liquid was added to the lower chamber. After 48 hours, small chamber was taken out and washed with PBS. Fix with 4% paraformaldehyde for 30 minutes, and dye with 0.1% crystal violet after washed with PBS. Remove cells on the surface of matrix binder. Cut it down and make it into a slide. Calculate cells in a field of vision.

9. Apoptosis - Flow cytometry analysis

The cells were cultured into a 6-well plate until they adhered to the wall. The experimental and the negative control groups were transfected with si-linc01194 and si-NC, respectively. Then, the supernatant was collected after 48 hours. After washing with PBS twice, the cells were digested with trypsin without ethylenediaminetetraacetic acid, collected, and centrifuged. The precipitate was washed with PBS twice, centrifuged, and retained. Following the instructions of the manufacturer of the Annexin V-fluorescein isothiocyanate (FITC)/propidium iodide (PI) double-staining reagent, flow cytometry was utilized to quantitatively analyze and compare the apoptosis between the experimental and the negative control groups.

10. RNA-binding protein immunoprecipitation (RIP)

Cells were washed with cold PBS twice to three times, scraped off, and transferred into a clean EP tube. Then the cell samples were centrifuged. The precipitate was retained, and RIPA buffer containing phenylmethylsulfonyl fluoride was added, followed by overnight separation at 4°C, and then centrifugation 12,000 rpm for 20 minutes at 4°C. Retain the supernatant, and take 20 uL as the input group. The remaining supernatant was divided into 2 equal portions, in one of which, we added 4 ug immunoglobulin G (IgG), while in the other, we added 4 ug IGF2BP1 antibody. The samples were then subjected to overnight shaking at 4°C. Next, 100 uL of magnetic bead suspension was added on the next day, followed by shaking at a constant speed of 4°C for 4 hours. Then, a magnetic rack was used to adsorb the magnetic beads and separate the reaction mixture. We further removed the supernatant, and utilizes PBS for 3-fold washing of the magnetic beads. TRIzol reagent was added to all samples for RNA extraction, followed by its reverse transcription into cDNA. And then compare the expression levels of linc01194 in the IgG and IGF2BP1 groups through qRT-PCR.

https://doi.org/10.3802/jgo.2024.35.e21

11. Protein nucleo-cytoplasmic separation experiment

We utilized the Cytoplasm and Nuclear Protein Extraction Kit (Beyotime Biotech Inc., Haimen, China) to extract the cytoplasmic and nuclear proteins of cells from the experimental and negative control groups following to the manufacturer's instructions.

12. Western blotting

IGF2BP1 antibody (Proteintech, Rosemont, IL, USA), SOX2 antibody (Proteintech), GAPDH antibody (Proteintech) and α-tubulin antibody (Proteintech) were used in our western blotting analyses. We determined the protein concentration with the bicinchoninic acid protein concentration determination kit (Beyotime Biotech Inc.), and then PBS and 5 × loading buffer were added for quantification of the protein concentration to 2 ug/uL. After denaturation at 100°C for 15 minutes, separate the protein with 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), and then transferred protein to a polyvinylidene difluoride membrane. Next, seal the membrane with 5% skimmed milk, and primary antibodies were added, and then incubation of the membrane overnight at 4°C. The membrane was washed with Tris buffered saline with Tween 20 (TBST) for 10 minutes 3 times, and added the secondary antibody (1:8000, Proteintech) and incubated the membrane for 2 hours at room temperature, on the next day. And then wash the membrane with TBST for 10 minutes 3 times. Analyze the protein bands through the hypersensitive ECL chemiluminescence kit (NcmECL UItra; Sliding Biotech, Shanghai, China). ImageJ software was utilized to protein quantification.

13. Statistical analysis

All data are presented as the mean ± standard error of mean (SEM). GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA) was applied. One-way analysis of variance and unpaired Student's t-test were utilized to analyze the difference. The p-value is less than 0.05 was considered statistically significant.

RESULTS

1. Linc01194 was overexpressed in EC

Our search in starBase database and the TCGA database revealed that the level of linc01194 in EC tissues is higher than that in normal endometrial tissues ($p<0.05$; **Fig. 1A and B**). To determine the level of linc01194 in EC, through qRT-PCR, we detected the expression of linc01194 in endometrial tissues of 127 patients from the Third Affiliated Hospital of Guangzhou Medical University (including 99 patients with EC and 28 patients without EC). The results indicated that the level of linc01194 in the endometrial tissues of patients with EC was higher than that in those without EC ($p<0.05$; **Fig. 1C**). Surprisingly, the level of linc01194 was higher in EC with lymphovascular invasion than that in EC without lymphovascular invasion ($p<0.05$; **Fig. 1D**). Subsequently, we detected the expression of linc01194 in EC cell lines (Ishikawa, KLE, HEC-1B, and HEC-1A cells). The results revealed that linc01194 had higher expression level in Ishikawa cells and KLE cells, while the expression level was lowest in HEC-1B ($p<0.05$; **Fig. 1E**). Therefore, we chose Ishikawa cells and KLE cells for the transfection of siRNA, and HEC-1B cells for the transfection of overexpression-plasmid.

2. The downregulation of linc01194 inhibits the malignant behaviors of EC cells

The expression of linc01194 decreased after the transfection of si-linc01194. To explore the effect between linc01194 and EC, we conducted CCK-8 assay, wound-healing assay, cell

https://doi.org/10.3802/jgo.2024.35.e21

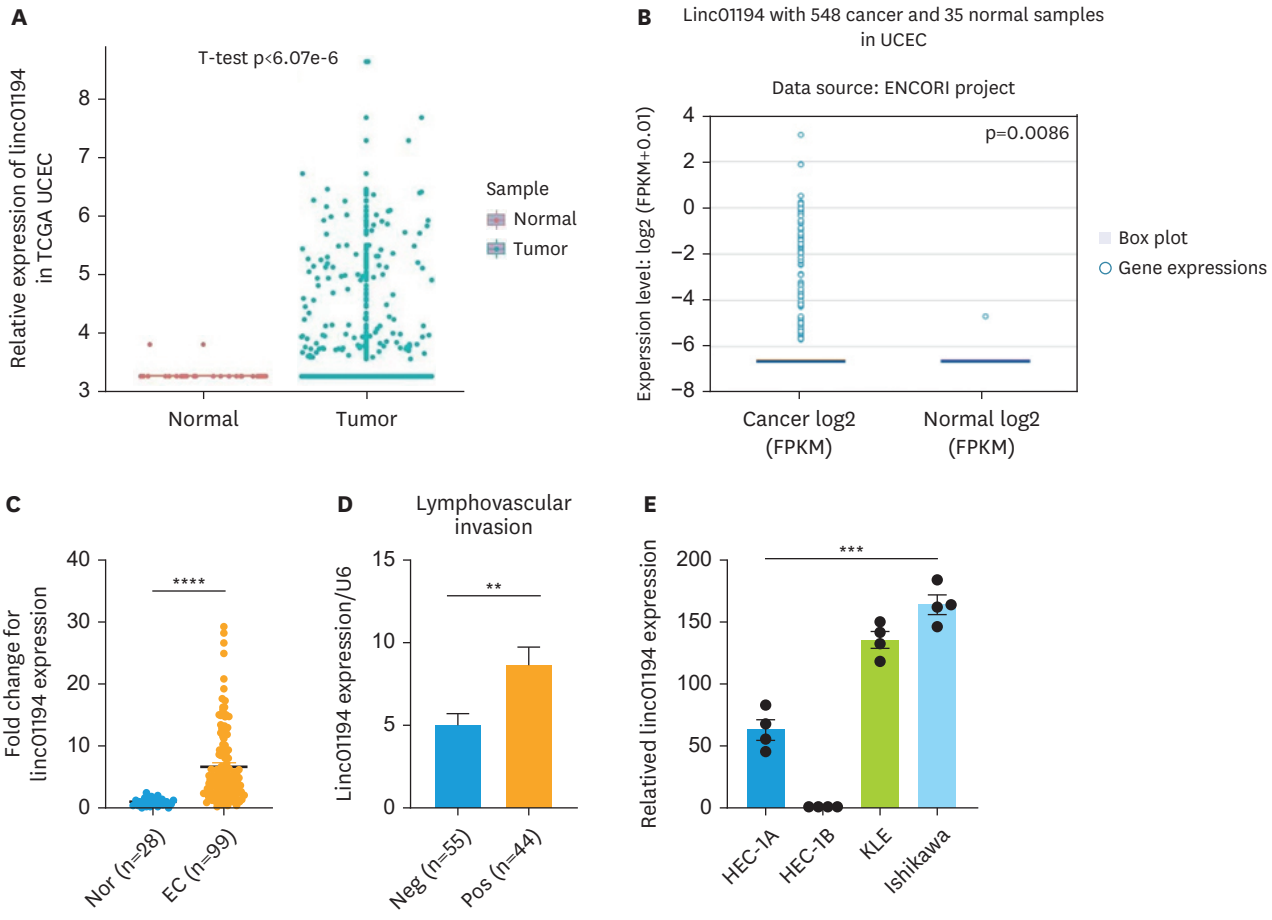

Fig. 1. Linc01194 was overexpressed in EC.
(A) Data from TCGA database showed that linc01194 was overexpressed in EC. (B) Data from starBase database showed that linc01194 was overexpressed in EC. (C) qRT-PCR showed that the level of linc01194 in EC tissue (n=99) was higher than normal endometrial tissue (n=28). (D) The expression level of linc01194 in EC with lymphatic vessel invasion (n=44) was higher than that in EC without lymphatic vessel invasion (n=55). (E) The expression of linc01194 in four EC cell lines. The above data are presented as the mean±standard error of mean.
EC, endometrial carcinoma; qRT-PCR, quantitative reverse-transcription polymerase chain reaction; TCGA, The Cancer Genome Atlas.
*p≤0.05; **p≤0.01; ***p≤0.001; ****p<0.0001.

invasion assay and apoptosis experiments. We found that the low level of linc01194 was related to the decrease of cell proliferation, cell migration, cell invasion, and the increase of apoptosis (p<0.05; **Figs. 2** and **3**).

3. The upregulation of linc01194 promotes the malignant behavior of EC cells

After transfection with plasmid targeting linc01194, the expression of linc01194 was increased (p<0.05; **Fig. 4A**), and cell proliferation (p<0.05; **Fig. 4B**), cell migration (p<0.05; **Fig. 4C**) and cell invasion (p<0.05; **Fig. 4D**) were promoted, while cell apoptosis was inhibited (p<0.05; **Fig. 4E**).

4. Linc01194 regulates the expression of IGF2BP1 and SOX2 in EC

To identify the downstream target protein that binds to linc01194, we used the starBase database to predict the target protein of linc01194. The data obtained suggested that IGF2BP1 may be the downstream target protein of linc01194 (**Fig. 5A**). The relationship between the levels of IGF2BP1 and the overall survival rate of EC patients was searched in Kaplan-Meier Plotter database, which showed that high levels of IGF2BP1 was related to poor survival

https://doi.org/10.3802/jgo.2024.35.e21

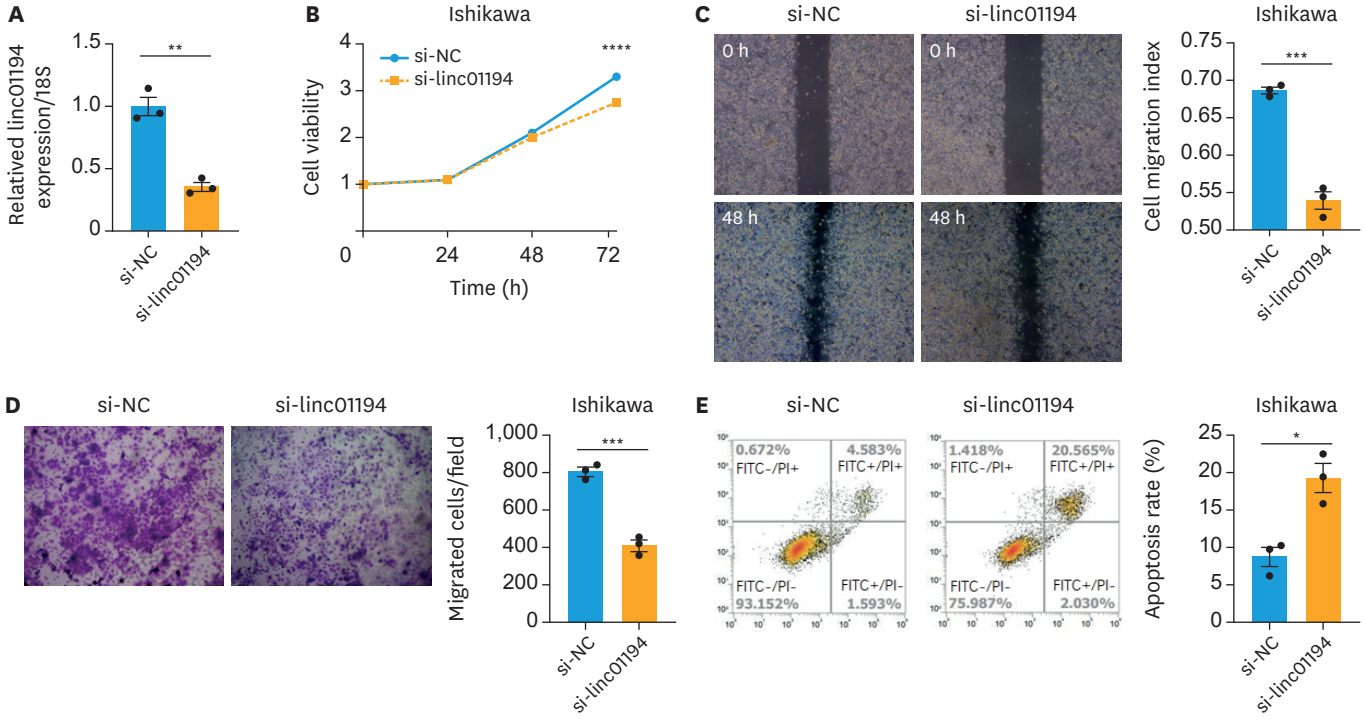

Fig. 2. In Ishikawa cells, the knockdown of linc01194 inhibited the cell malignant behavior.
(A) The expression level of linc01194 was decreased after the transfection of si-linc01194. (B) The knockdown of linc01194 inhibited cell viability of Ishikawa cells. (C) The knockdown of linc01194 inhibited cell migration of Ishikawa cells. (D) The knockdown of linc01194 inhibited cell invasion of Ishikawa cells. (E) The knockdown of linc01194 promoted cell apoptosis of Ishikawa cells.
The above data are presented as the mean±standard error of mean.
si-linc01194, siRNA targeting linc01194; si-NC, siRNA targeting negative control.
*p≤0.05; **p≤0.01; ***p≤0.001; ****p<0.0001.

rate of EC patients (p<0.05; **Fig. 5B**). Additionally, we established that linc01194 binds to IGF2BP1 through RIP experiments (p<0.05; **Fig. 5C**). Moreover, western blots experiment indicated that the knockdown of linc01194 decreased the level of IGF2BP1 in the cytoplasm of EC cells (p<0.05; **Fig. 5D**). A related study found that the functions of IGF2BPs are exerted mainly in the cytoplasm [17]. Meanwhile, we found that the knockdown of linc01194 reduced the expression level of SOX2 (p<0.05; **Fig. 5E**). Conversely, the upregulation of linc01194 increased IGF2BP1 expression in the cytoplasm (p<0.05; **Fig. 5F**). Similarly, the upregulation of linc01194 promoted the expression of SOX2 (p<0.05; **Fig. 5G**).

DISCUSSION

Accumulating evidence on lncRNA has indicated that they are key regulators in cancer pathways. They affect signal pathways and regulate gene expression at the epigenetic level. Also, they can interact with chromatin, and proteins to affect RNA splicing, and transcriptional and post-transcriptional levels, thereby regulating several cellular biological processes [18]. Slack et al [19] summarized the relevant mechanisms of action of lncRNAs. First, they can act by directly binding to protein complexes. For example, they direct chromatin modification complexes to target gene promoters, which affects the processes of transcriptional inhibition or activation. Additionally, they combined with transcription factors, which have extensive downstream effects on the cell transcription process. Furthermore, they directly interact with RNA-binding proteins (RBPs), which regulates

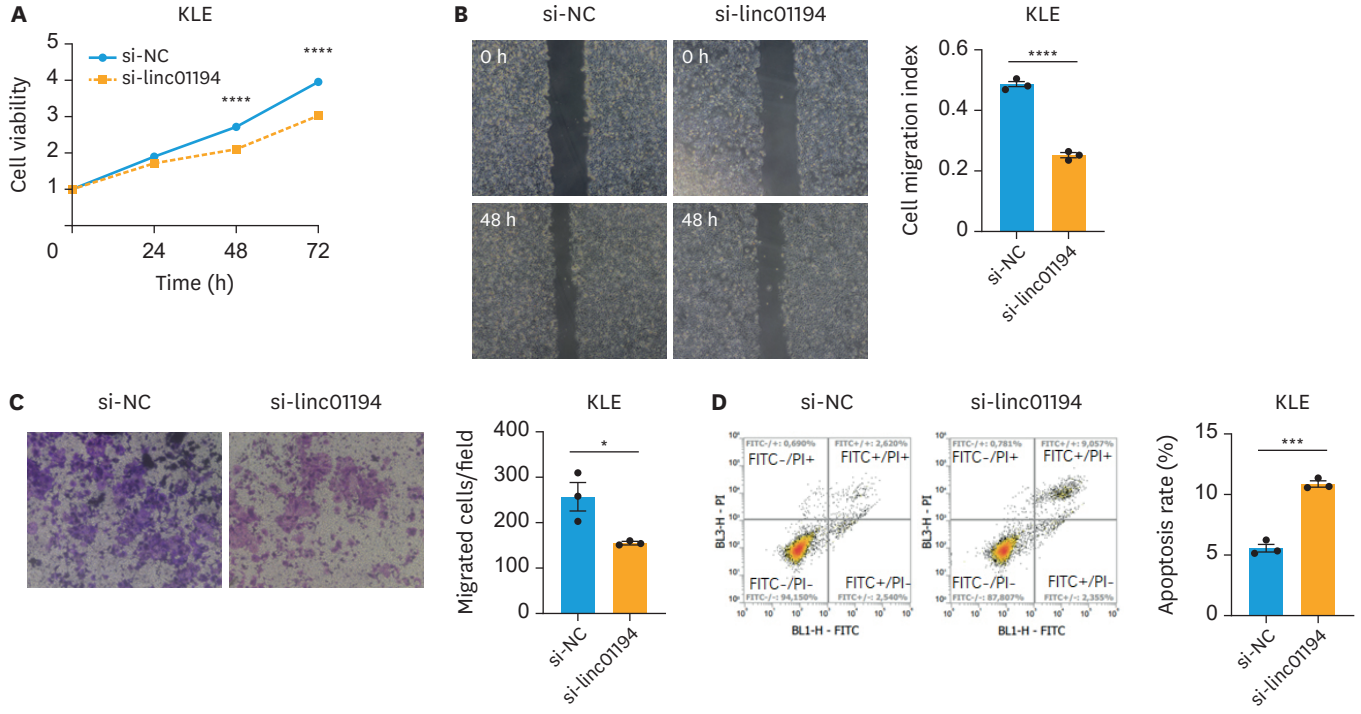

Fig. 3. In KLE cells, the downregulation of linc01194 inhibited the cell malignant behavior.
(A) The knockdown of linc01194 inhibited cell viability of KLE cells. (B) The knockdown of linc01194 inhibited cell migration of KLE cells. (C) The knockdown of linc01194 inhibited cell invasion of KLE cells. (D) The knockdown of linc01194 promoted cell apoptosis of KLE cells.
The above data are presented as the mean±standard error of mean.
si-linc01194, siRNA targeting linc01194; si-NC, siRNA targeting negative control.
*p≤0.05; **p≤0.01; ***p≤0.001; ****p<0.0001.

mRNA processing and stability and serve as a scaffold for regulatory molecules found in nuclear speckles and paraspeckles. Second, lncRNAs also bind to nucleic acids to regulate their molecular mechanism of action. Besides, lncRNA as a competitive endogenous RNA (ceRNA) or a "sponge" for miRNA, regulated gene expression.

Data in TCGA database revealed that the expression levels of linc01194 were higher in EC. In our study, we confirmed that linc01194 was overexpressed in EC by analyzing the endometrial tissues of 127 patients. Relevant research shows that linc01194 expression level in hepatocellular carcinoma [20], PCa [21], lung cancer [22,23], laryngeal squamous cell carcinoma [24], and colorectal cancer [25] is increased, which promotes their occurrence and development. However, linc01194 expression in EC has not been studied.

To clarify the function of linc01194 in EC, we silenced linc01194 in EC cells. We found that the cell proliferation, migration and invasion of EC cells were inhibited, whereas apoptosis was promoted. On the contrary, when we upregulated the level of linc01194, cell proliferation, migration and invasion of EC cells were promoted, while apoptosis was inhibited. LncRNA can exert these effects by binding to a target protein. Therefore, through the starbase database, we predicted that linc01194 might bind to IGF2BP1. The results of RIP and WB experiments showed that linc01194 could combine with IGF2BP1, and the expression level of linc01194 is positively correlated with the expression level of IGF2BP1 in the cytoplasm. In addition, an earlier study showed that the activities of IGF2BPs are exerted mainly in the cytoplasm [17].

https://doi.org/10.3802/jgo.2024.35.e21

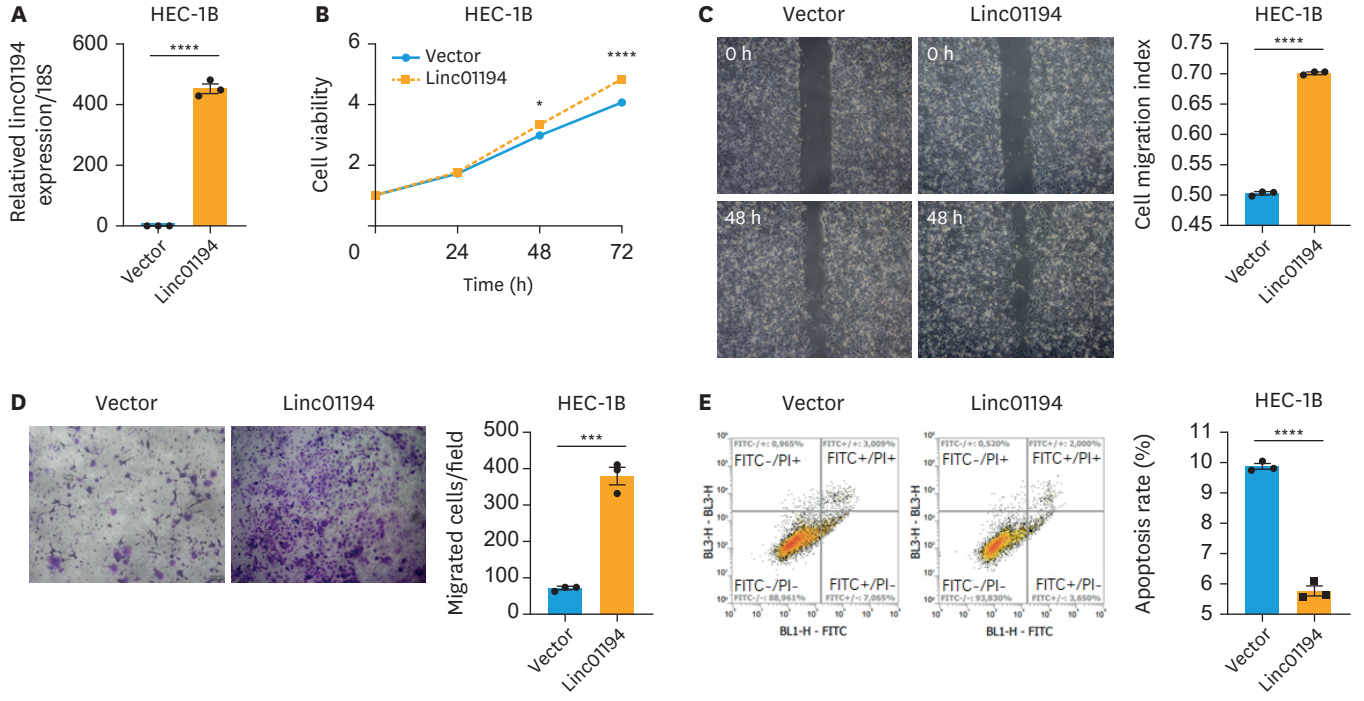

Fig. 4. In HEC-1B cells, the upregulation of linc01194 promoted the cell malignant behavior.
(A) The expression level of linc01194 was increased after the transfection of plasmid targeting linc01194. (B) The upregulation of linc01194 promoted cell viability of HEC-1B cells. (C) The upregulation of linc01194 promote cell migration of HEC-1B cells. (D) The upregulation of linc01194 promote cell invasion of HEC-1B cells. (E) The upregulation of linc01194 inhibited cell apoptosis of HEC-1B cells.
The above data are presented as the mean±standard error of mean.
FITC, fluorescein isothiocyanate; PI, propidium iodide.
*p≤0.05; **p≤0.01; ***p≤0.001; ****p<0.0001.

The insulin-like growth factor (IGF) system is related to organism development and cell function maintenance. This system includes IGF family ligands, binding proteins and receptors [26]. IGF2BPs are members of this system, which are highly expressed in human cancer tissues. Meanwhile, a high level of IGF2BPs is associated with a poor prognosis in cancer patients [27]. IGF2BPs are involved in cell growth, stem cell maintenance and differentiation during cell development [28]. IGF2BP1 is a member of the IGF2BPs. IGF2BPs consists of 6 typical RNA-binding domains, including 4 K homology (KH) domains and 2 RNA recognition motif (RRM) domains. Of them, KH-1/2 is known to stabilize IGF2BP-RNA complexes. IGF2BPs preferentially recognize mRNAs modified by m6A and promote an m6A-dependent mode, thereby affecting gene expression. In tumors, IGF2BPs may play a carcinogenic role by stabilizing m6A-modified mRNAs [17].

In their study, Xue et al. [29] established that IGF2BP1 enhanced the stability of the sex-determining Y-box 2 (SOX2) mRNA in EC in an m6A-dependent manner, thereby promoting the expression of SOX2. On the other hand, SOX2 is a key transcription factor of embryonic development and the maintenance stem cell characteristics [30]. As a carcinogenic transcription factor, SOX2 is related to cell stemness in tumors [31]. In cancer, SOX2 promotes cell proliferation, inhibits apoptosis, and regulates cell invasion, migration and metastasis [32]. In addition, SOX2 was upregulated in many human cancers, such as colorectal cancer [33], breast cancer [34], esophageal cancer [35], glioblastoma [36], lung cancer [37], hepatocellular carcinoma [38], and to promote the occurrence and progression of tumors.

https://doi.org/10.3802/jgo.2024.35.e21

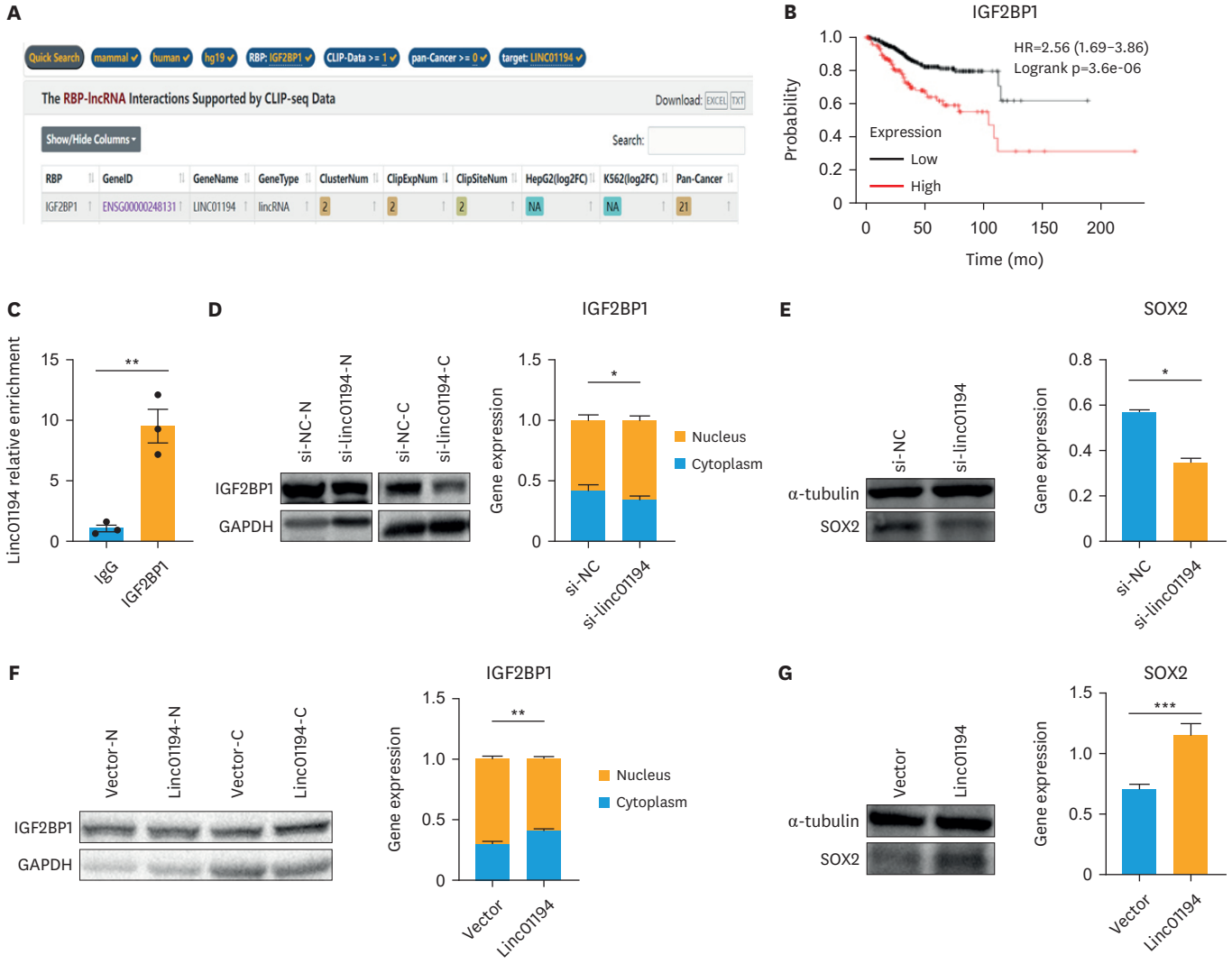

Fig. 5. Linc01194 regulated the expression of SOX2 through binding to IGF2BP1.
(A) The starBase database predicted that IGF2BP1 might be the target protein of linc01194. (B) High levels of IGF2BP1 was associated with lower overall survival rate of EC patients. (C) RIP assay indicated that linc01194 could bind to IGF2BP1. (D) Knockdown of linc01194 decreased the expression level of IGF2BP1 in the cytoplasm of EC cells. (E) Knockdown of linc01194 reduced the expression level of SOX2. (F) Upregulation of linc01194 increased the expression level of IGF2BP1 in the cytoplasm. (G) Upregulation of linc01194 increased the expression level of SOX2.
The above data are presented as the mean±standard error of mean.
CLIP-seq, cross-linking and immunoprecipitation; EC, endometrial carcinoma; GAPDH, glyceraldehyde phosphate dehydrogenase; HR, hazard ratio; IGF2BP1, insulin-like growth factor 2 binding protein 1; IgG, immunoglobulin G; lncRNA, long non-coding RNA; RBP, RNA-binding protein; RIP, RNA-binding protein immunoprecipitation; si-linc01194, siRNA targeting linc01194; si-NC, siRNA targeting negative control; SOX2, sex-determining region Y-box 2.
*p≤0.05; **p≤0.01; ***p≤0.001; ****p<0.0001.

In our study, we found that the knockdown of linc01194 downregulated the expression of IGF2BP1 and SOX2. Combined with the research results of Xue et al. [29], our present findings suggest that linc01194 may promote the expression of SOX2 in an m6A-dependent manner through the interaction with IGF2BP1, promoting the progress of EC.

Our study demonstrated for the first time that linc01194 can promote the expression of downstream protein SOX2 through binding to IGF2BP1, thus promoting the occurrence and development of EC, which may provide a new approach for the diagnosis and treatment of EC. However, further research on the mechanism and function of linc01194 is needed,

https://doi.org/10.3802/jgo.2024.35.e21

including the design of diagnostic kits, research on specific inhibitors, and, if possible, preclinical transformation research based on organoids.

SUPPLEMENTARY MATERIAL

Data 1

The plasmid sequence targeting linc01194.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin 2020;70:7-30. PUBMED | CROSSREF
2. Lamothe S, Ramia de Cap M. Survival analysis and treatment effects in patients with endometrial cancer and POLE mutations. Cancer 2021;127:4306-7. PUBMED | CROSSREF
3. Chen W, Xia C, Zheng R, Zhou M, Lin C, Zeng H, et al. Disparities by province, age, and sex in site-specific cancer burden attributable to 23 potentially modifiable risk factors in China: a comparative risk assessment. Lancet Glob Health 2019;7:e257-69. PUBMED | CROSSREF
4. Zhang S, Gong TT, Liu FH, Jiang YT, Sun H, Ma XX, et al. Global, regional, and national burden of endometrial cancer, 1990-2017: results from the Global Burden of Disease Study, 2017. Front Oncol 2019;9:1440. PUBMED | CROSSREF
5. Gentry-Maharaj A, Karpinskyj C. Current and future approaches to screening for endometrial cancer. Best Pract Res Clin Obstet Gynaecol 2020;65:79-97. PUBMED | CROSSREF
6. Remmerie M, Janssens V. Targeted therapies in type II endometrial cancers: too little, but not too late. Int J Mol Sci 2018;19:2380. PUBMED | CROSSREF
7. Djebali S, Davis CA, Merkel A, Dobin A, Lassmann T, Mortazavi A, et al. Landscape of transcription in human cells. Nature 2012;489:101-8. PUBMED | CROSSREF
8. Wang J, Zhu S, Meng N, He Y, Lu R, Yan GR. ncRNA-encoded peptides or proteins and cancer. Mol Ther 2019;27:1718-25. PUBMED | CROSSREF
9. Goodall GJ, Wickramasinghe VO. RNA in cancer. Nat Rev Cancer 2021;21:22-36. PUBMED | CROSSREF
10. Geisler S, Coller J. RNA in unexpected places: long non-coding RNA functions in diverse cellular contexts. Nat Rev Mol Cell Biol 2013;14:699-712. PUBMED | CROSSREF
11. Prensner JR, Chinnaiyan AM. The emergence of lncRNAs in cancer biology. Cancer Discov 2011;1:391-407. PUBMED | CROSSREF
12. Lorenzi L, Avila Cobos F, Decock A, Everaert C, Helsmoortel H, Lefever S, et al. Long noncoding RNA expression profiling in cancer: challenges and opportunities. Genes Chromosomes Cancer 2019;58:191-9. PUBMED | CROSSREF
13. Matsui M, Corey DR. Non-coding RNAs as drug targets. Nat Rev Drug Discov 2017;16:167-79. PUBMED | CROSSREF
14. Kong D, Hou Y, Li W, Ma X, Jiang J. LncRNA-ZXF1 stabilizes P21 expression in endometrioid endometrial carcinoma by inhibiting ubiquitination-mediated degradation and regulating the miR-378a-3p/PCDHA3 axis. Mol Oncol 2022;16:813-29. PUBMED | CROSSREF
15. Li L, Chen P, Huang B, Cai P. lncRNA DSCAM-AS1 facilitates the progression of endometrial cancer via miR-136-5p. Oncol Lett 2021;22:825. PUBMED | CROSSREF
16. Cai P, Li G, Wu M, Zhang B, Bai H. ZIC2 upregulates lncRNA SNHG12 expression to promote endometrial cancer cell proliferation and migration by activating the Notch signaling pathway. Mol Med Rep 2021;24:632. PUBMED | CROSSREF
17. Bell JL, Wächter K, Mühleck B, Pazaitis N, Köhn M, Lederer M, et al. Insulin-like growth factor 2 mRNA-binding proteins (IGF2BPs): post-transcriptional drivers of cancer progression? Cell Mol Life Sci 2013;70:2657-75. PUBMED | CROSSREF
18. Chen Z, Lei T, Chen X, Gu J, Huang J, Lu B, et al. Long non-coding RNA in lung cancer. Clin Chim Acta 2020;504:190-200. PUBMED | CROSSREF
19. Slack FJ, Chinnaiyan AM. The role of non-coding RNAs in oncology. Cell 2019;179:1033-55. PUBMED | CROSSREF

20. Liu Y, Liu J, Cui J, Zhong R, Sun G. Role of lncRNA LINC01194 in hepatocellular carcinoma via the miR-655-3p/SMAD family member 5 axis. Bioengineered 2022;13:1115-25. PUBMED | CROSSREF
21. Song HR, Guo XB, Duan Y, Meng HY, Wang ZY. PAX5-induced upregulation of LINC01194 exerts oncogenic properties by regulating GOLPH3 expression via miR-486-5p in prostate cancer. Eur Rev Med Pharmacol Sci 2021;25:2528-41. PUBMED | CROSSREF
22. Meng F, Zhou Y, Dong B, Dong A, Zhang J. Long non-coding RNA LINC01194 promotes the proliferation, migration and invasion of lung adenocarcinoma cells by targeting miR-641/SETD7 axis. Cancer Cell Int 2020;20:588. PUBMED | CROSSREF
23. Xing Z, Zhang Z, Gao Y, Zhang X, Kong X, Zhang J, et al. The lncRNA LINC01194/miR-486-5p axis facilitates malignancy in non-small cell lung cancer via regulating CDK4. Onco Targets Ther 2020;13:3151-63. PUBMED | CROSSREF
24. Liu DM, Yang H, Yuan ZN, Yang XG, Pei R, He HJ. Long noncoding RNA LINC01194 enhances the malignancy of laryngeal squamous cell carcinoma by sponging miR-655 to increase SOX18 expression. Biochem Biophys Res Commun 2020;529:148-55. PUBMED | CROSSREF
25. Wang X, Liu Z, Tong H, Peng H, Xian Z, Li L, et al. Linc01194 acts as an oncogene in colorectal carcinoma and is associated with poor survival outcome. Cancer Manag Res 2019;11:2349-62. PUBMED | CROSSREF
26. LeRoith D, Roberts CT Jr. The insulin-like growth factor system and cancer. Cancer Lett 2003;195:127-37. PUBMED | CROSSREF
27. Bell JL, Turlapati R, Liu T, Schulte JH, Hüttelmaier S. IGF2BP1 harbors prognostic significance by gene gain and diverse expression in neuroblastoma. J Clin Oncol 2015;33:1285-93. PUBMED | CROSSREF
28. Ramesh-Kumar D, Guil S. The IGF2BP family of RNA binding proteins links epitranscriptomics to cancer. Semin Cancer Biol 2022;86:18-31. PUBMED | CROSSREF
29. Xue T, Liu X, Zhang M, e Q, Liu S, Zou M, et al. PADI2-catalyzed MEK1 citrullination activates ERK1/2 and promotes IGF2BP1-mediated SOX2 mRNA stability in endometrial cancer. Adv Sci (Weinh) 2021;8:2002831. PUBMED | CROSSREF
30. Wegner M. All purpose Sox: the many roles of Sox proteins in gene expression. Int J Biochem Cell Biol 2010;42:381-90. PUBMED | CROSSREF
31. Bareiss PM, Paczulla A, Wang H, Schairer R, Wiehr S, Kohlhofer U, et al. SOX2 expression associates with stem cell state in human ovarian carcinoma. Cancer Res 2013;73:5544-55. PUBMED | CROSSREF
32. Weina K, Utikal J. SOX2 and cancer: current research and its implications in the clinic. Clin Transl Med 2014;3:19. PUBMED | CROSSREF
33. Zhu Y, Huang S, Chen S, Chen J, Wang Z, Wang Y, et al. SOX2 promotes chemoresistance, cancer stem cells properties, and epithelial-mesenchymal transition by β-catenin and Beclin1/autophagy signaling in colorectal cancer. Cell Death Dis 2021;12:449. PUBMED | CROSSREF
34. Yu JM, Sun W, Wang ZH, Liang X, Hua F, Li K, et al. TRIB3 supports breast cancer stemness by suppressing FOXO1 degradation and enhancing SOX2 transcription. Nat Commun 2019;10:5720. PUBMED | CROSSREF
35. Wang Z, Kang L, Zhang H, Huang Y, Fang L, Li M, et al. AKT drives SOX2 overexpression and cancer cell stemness in esophageal cancer by protecting SOX2 from UBR5-mediated degradation. Oncogene 2019;38:5250-64. PUBMED | CROSSREF
36. Zhang LH, Yin YH, Chen HZ, Feng SY, Liu JL, Chen L, et al. TRIM24 promotes stemness and invasiveness of glioblastoma cells via activating Sox2 expression. Neuro-oncol 2020;22:1797-808. PUBMED | CROSSREF
37. Wang K, Ji W, Yu Y, Li Z, Niu X, Xia W, et al. FGFR1-ERK1/2-SOX2 axis promotes cell proliferation, epithelial-mesenchymal transition, and metastasis in FGFR1-amplified lung cancer. Oncogene 2018;37:5340-54. PUBMED | CROSSREF
38. Hosseini-Khah Z, Babaei MR, Tehrani M, Cucchiarini M, Madry H, Ajami A, et al. SOX2 and Bcl-2 as a novel prognostic value in hepatocellular carcinoma progression. Curr Oncol 2021;28:3015-29. PUBMED | CROSSREF