International Journal of Biochemistry and Cell Biology 116 (2019) 105590

Contents lists available at ScienceDirect

International Journal of Biochemistry and Cell Biology

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

LINC00997, a novel long noncoding RNA, contributes to metastasis *via* regulation of S100A11 in kidney renal clear cell carcinoma

Yuan Chang[a], Na Li[b], Weitang Yuan[a], Guixian Wang[a], Jianguo Wen[c,*]

[a] Department of Colorectal Surgery, First Affiliated Hospital of Zhengzhou University, Zhengzhou, China
[b] Department of Prosthodontics, First Affiliated Hospital of Zhengzhou University, Zhengzhou, China
[c] Department of Urology, First Affiliated Hospital of Zhengzhou University, Zhengzhou, China

ARTICLE INFO

Keywords:
Kidney renal clear cell carcinoma
Long noncoding RNA
LINC00997
Metastasis

ABSTRACT

Long noncoding RNAs (lncRNAs) play an essential role in cancer development. However, the contribution of the lncRNA LINC00997 to kidney renal clear cell carcinoma (KIRC) has not been thoroughly elucidated to date. In this study, we examined the expression and biological effect of LINC00997 in KIRC development. We also investigated the potential mechanism underlying the observed effects. We found that LINC00997 is highly expressed in multiple carcinomas, being highest in stage IV KIRC in our RNA-Seq datasets. In addition, our data demonstrated that in KIRC patients, higher levels of LINC00997 are correlated with lower overall survival (OS) and disease-free survival (DFS) rates. In 18 cases of KIRC, we found that LINC00997 expression was greater in cancer tissues and metastases than in normal tissues. These results revealed that S100A11 is positively associated with LINC00997 in KIRC, which is positively correlated with metastasis-associated molecules VIM, MMP2 and MMP7. Our *in vitro* wound healing assay and Transwell tests demonstrated that interfering with either LINC00997 or S100A11 expression reduced migration of 786-O cells by inhibiting VIM, MMP2 and MMP7 expression. Importantly, we verified LINC00997 and STAT3 binding by RIP and determined that both LINC00997 and STAT3 bind to the S100A11 promoter, as shown by dual-luciferase reporter gene assay. In addition, inhibiting LINC00997 or STAT3 expression attenuated S100A11 levels. Consequently, the LINC00997-STAT3-S100A11 axis may promote the development of KIRC, and LINC00997 may represent a potential prognostic biomarker and therapeutic target for KIRC patients.

1. Introduction

Kidney renal clear cell carcinoma (KIRC) is the most common type of renal malignant tumor, accounting for 80%–90% of renal cell carcinomas (RCC). Disease incidence is approximately 209,000 new cases per year and 102,000 deaths per year worldwide (Gupta et al., 2008). In China, approximately 66,800 new cases and 13,860 deaths were estimated to have occurred in 2015 (Chen et al., 2016). Although the methods for tumor detection by ultrasound and computed tomography have improved, many renal masses remain asymptomatic until the late stages of disease. In fact, greater than 50% of RCCs are detected incidentally through investigation of various nonspecific symptoms and other abdominal diseases (Novara et al., 2011). Clinical data indicate that one-third of RCC patients already have metastases at diagnosis (Rini et al., 2009). Compared to other renal cell carcinomas, KIRCs have a high rate of recurrence and metastasis. Furthermore, the prognosis of patients with metastatic KIRC is notably poor with an average 5-year survival rate of less than 10% (Rini et al., 2009). Very few biomarkers have been identified for RCC compared to other cancers. Hence, detection and identification of new and sensitive biomarkers for predicting progression and prognosis and for discovery of new, targeted drugs for metastatic RCC are crucial.

Long noncoding RNAs (LncRNAs) are a subgroup of transcripts exceeding 200 nucleotides in length with limited protein coding capacity (Kapranov et al., 2007; Sun et al., 2018a). LncRNAs modulate gene expression at different levels, including epigenetic, transcriptional, and posttranscriptional processing (Huarte, 2015; Maxmen, 2013; Schmitt and Chang, 2013). Additionally, growing evidence has revealed that lncRNAs play an important role in various cancers by regulating oncogenic or tumor-suppressing effects and may represent a new class of cancer biomarkers and therapeutic targets (Yang et al., 2011; Hu et al., 2014; Kogo et al., 2011). The YAP1-induced lncRNA MALAT1 is highly expressed in colorectal cancer tissues and modulates epithelial-mesenchymal transition molecules, SLUG and TWIST. Elevated MALAT1

* Corresponding author.
E-mail address: fccchangy2@zzu.edu.cn (J. Wen).

https://doi.org/10.1016/j.biocel.2019.105590
Received 5 June 2019; Received in revised form 17 July 2019; Accepted 19 August 2019
Available online 20 August 2019
1357-2725/

expression is correlated with metastasis and poor prognosis (Sun et al., 2018b). LncRNA HOXA11-AS induces KLF2 mRNA degradation *via* interacting with STAU1, promoting metastasis of gastric cancer (Liu et al., 2017). Although studies have demonstrated that specific lncRNAs may play an essential role in tumor development and the metastatic process (Prensner et al., 2013), the precise roles of lncRNAs in KIRC remain largely unknown.

In this study, expression features of LINC00997 were analyzed in KIRC tissues, and LINC00997's biological function was investigated in KIRC cells. To further elucidate the biological effects of LINC00997, we assessed whether this lncRNA could represent a useful diagnostic and prognostic biomarker, as well as a potential therapeutic target, for KIRC patients.

2. Materials and methods

2.1. Tissue specimens and clinical data collection

This study was approved by the First Affiliated Hospital of Zhengzhou University. Written informed consent was obtained from all patients. Eighteen kidney renal clear cell carcinoma (KIRC) tissues and paired adjacent noncancerous tissues were collected from the First Affiliated Hospital of Zhengzhou University from June 2017 to August 2018. Inclusion criteria were as follows: no preoperative chemotherapy, radiotherapy, or targeted therapy; no other tumor types; and no autoimmune diseases. Specimens obtained during surgery were immediately snap frozen in liquid nitrogen and stored at −80 °C until RNA extraction. Clinical staging of specimens was based on NCCN guidelines.

2.2. Bioinformatics prediction

RNA-Seq datasets from GEPIA (http://gepia.cancer-pku.cn/) were utilized to analyze the expression, prognosis and association of LINC00997, S100A11, VIM, MMP2, and MMP7 with KIRC. LINC00997 is predicted to combine with the S100A11 promoter (Li et al., 2015). The S100A11 promoter has two potential binding sites with the STAT3 protein based on analysis using the JASPAR database (Mathelier et al., 2016). LINC00997 has the potential to combine with the STAT3 protein, as predicted by the LNCPRO database (Lu et al., 2013).

2.3. RNA extraction and reverse transcription

Total RNA was isolated from KIRC tissues, paired adjacent noncancerous tissues and KIRC cells using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) following the manufacturer's instructions. RNA quality was evaluated using a Nanodrop 2000 (Wilmington, DE, USA), and RNA integrity was assessed using agarose gel electrophoresis. An aliquot of 1 μg total RNA was reverse-transcribed into complementary DNA (cDNA) according to the manufacturer's protocol using a High-capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA, USA).

2.4. Quantitative real-time PCR

Quantitative real-time PCR (qRT-PCR) was performed using a Roche LightCycler® 480II system with TaqMan assays (Invitrogen, Carlsbad, CA, USA) (NNT-AS1 assay ID: Hs 04333390_s1; GAPDH Catalog number: 402869) and TaqMan® Universal Master Mix II without UNG (Invitrogen, Carlsbad, CA, USA) to detect NNT-AS1 expression. Transcript levels were normalized to GAPDH expression. qRT-PCR assays were performed in triplicate under the following conditions: (1) 95 °C for 10 min and (2) 40 cycles of 95 °C for 15 s and 60 °C for 1 min. Relative NNT-AS1 expression was calculated using the ΔCT (Ct lncRNA-Ct GAPDH) method.

2.5. Cell culture

Human KIRC 786-O cells were obtained from the American Type Culture Collection (USA). 786-O cells were cultured in RPMI-1640 medium containing 10% FBS (Gibco, Carlsbad, CA, USA) and were incubated at 37 °C under 5% CO_2.

2.6. siRNA transfection of KIRC cells

Silencer select small interference RNAs (siRNAs) specific to LINC00997, S100A11 and a control siRNA were obtained from Rio (Guangzhou, China). For silencing RNA in 786-O cells, LINC00997-specifc siRNAs (Invitrogen s53571), S100A11-specifc siRNAs (Invitrogen 102868) and siRNA control (Invitrogen 1585252) were transfected into 786-O cells. Transfection was performed using Lipofectamine 3000 (Invitrogen, Waltham, MA, USA) according to the manufacturer's instructions.

2.7. Migration assay

The migratory ability of cells was assessed using a wound healing assay created by a scrape using a 200 μl pipette tip. Swept cells were lightly washed with PBS, and serum-free medium was added to the 6-well plates. After 12 h and 72 h, the wound was observed and imaged to directly assess the level of cell migration.

To assess cell migration, Transwell chambers (Sigma-Aldrich Co. LLC., St. Louis, USA) were prepared with or without Matrigel. Then, blood serum medium (10% FBS) was added to the bottom chamber. 786-O cells transfected with si-LINC00997, si-S100A11 or negative control were digested for preparation of a cell suspension, which was added to upper chamber for 24 h incubation. Subsequently, residual cells in the upper chamber were gently wiped using a cotton bud. Cells were immobilized with 4% paraformaldehyde and stained using 1% crystal violet for 30 min. After washing three times with PBS, cells were imaged and counted using an IX71 inverted microscope (Olympus, Tokyo, Japan). Every operation was performed three times for statistical analysis.

2.8. RNA-binding protein immunoprecipitation assay

Two micrograms of cell extract were mixed with agarose beads, which had been precipitated with STAT3 antibodies (9139, CST, Boston, USA). Beads were washed briefly three times with GLB + lysis, and retrieved proteins were detected by Western blot. Coprecipitated RNAs were detected by RT-qPCR.

2.9. Luciferase assay

The S100A11 wild type promoter with potential LINC00997 and STAT3 binding sites or mutants for each site (for STAT3 binding) were generated and fused to the luciferase reporter vector psi-CHECK-2 (Promega, Madison, WI, USA). HEK293 cells were plated in 24-well plates and grown to 80% confluence. Cells were then cotransfected with luciferase plasmids and si-LINC00997 or si-STAT3 and respective negative controls. After transfection for 48 h, firefly and renilla luciferase activities were measured using a Dual-Luciferase Reporter Assay System (Promega).

2.10. Statistical analyses

All statistical analyses were performed using SPSS version 19.0 (SPSS, Chicago, IL, USA). All measurements were performed in triplicate. Significant differences between LINC00997 expression levels were determined using a two-sided Student's *t*-test. P-values less than 0.05 indicate a significant difference.

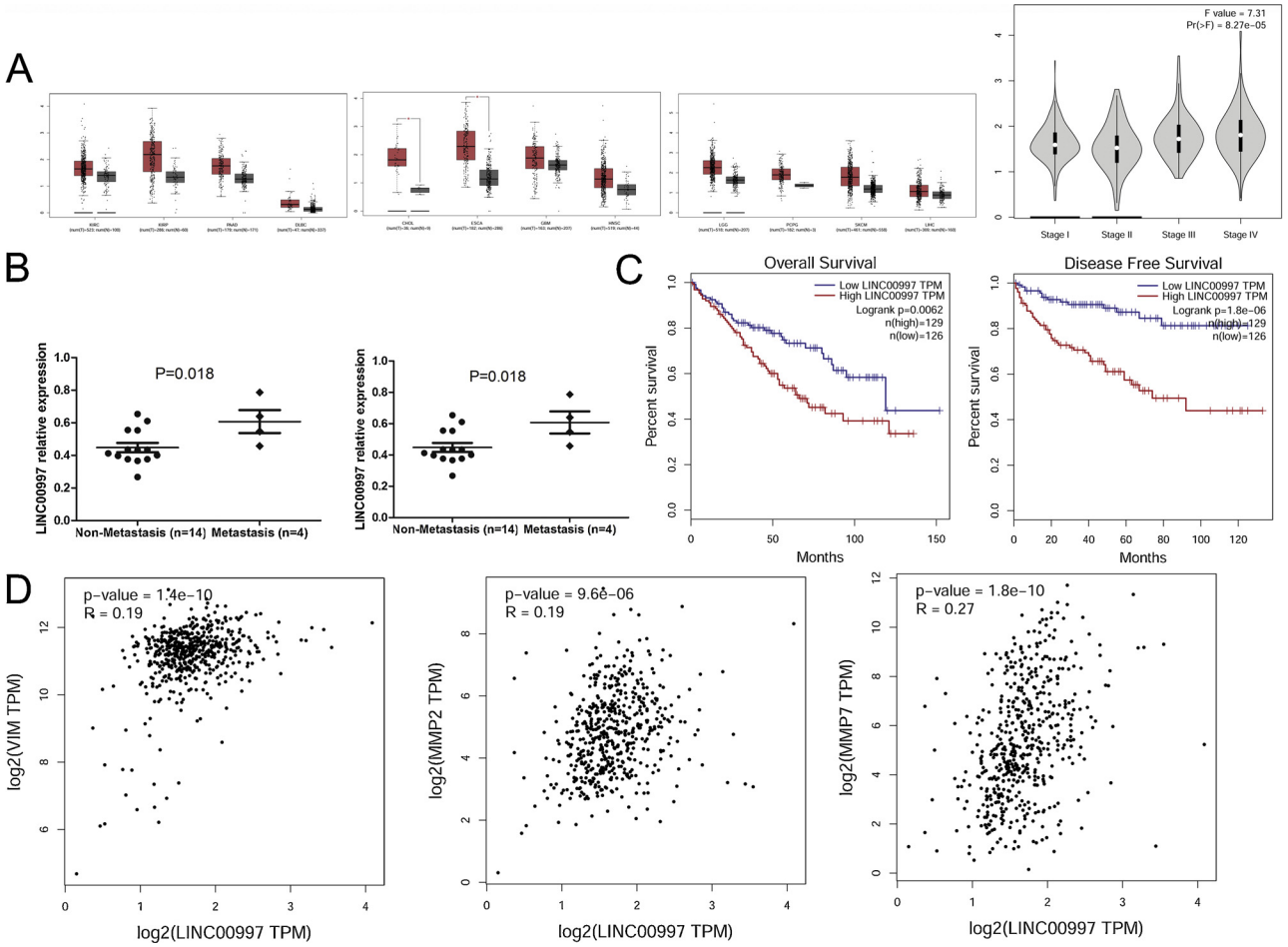

Fig. 1. High expression of LINC00997 predicts poor prognosis and is correlated with metastasis-associated molecules in KIRC. A) LINC00997 was highly expressed in multiple cancers compared to respective normal tissues and is closely correlated with KIRC metastasis, as shown by analysis of the GEPIA RNA-Seq datasets. B). In 18 cases of KIRC, LINC00997 was more highly expressed in tumors compared to normal adjacent normal tissues ($P < 0.05$). In addition, LINC00997 is associated with metastasis. C) LINC00997 prognostic value in KIRC patients was analyzed *via* the GEPIA RNA-Seq datasets. OS and DFS rates in KIRC patients with high LINC00997 expression were lower than those tumors with low expression (both $P < 0.01$). D) LINC00997 expression is closely correlated with metastasis-associated molecules VIM, MMP2 and MMP7 (all $P < 0.01$).

3. Results

3.1. LINC00997 is correlated with poor prognosis and metastasis-associated molecules in KIRC

To explore the expression characteristics and prognostic value of LINC00997 in KIRC, the GEPIA RNA-Seq datasets were explored. Results demonstrated that LINC00997 is highly expressed in multiple cancers compared to normal tissues, including in KIRC, kidney renal papillary cell carcinoma (KIRP), prostate adenocarcinoma (PAAD), lymphoid neoplasm diffuse large B-cell lymphoma (DLBC), cholangio carcinoma (CHOL), esophageal carcinoma (ESCA), glioblastoma multiforme (GBM), head and neck squamous cell carcinoma (HNSC), brain lower grade glioma (LGG), pheochromocytoma and paraganglioma (PCPG), skin cutaneous melanoma (SKCM) and liver hepatocellular carcinoma (LIHC). LINC00997 expression in KIRC metastases was higher than in nonmetastatic tumors (Fig. 1A). We collected 18 cases of KIRC at our hospital and found that LINC00997 was detected at higher expression levels in tumors compared to adjacent normal tissues ($P < 0.05$). Meanwhile, LINC00997 was also associated with metastasis ($P < 0.05$) (Fig. 1B). LINC00997 prognostic value in KIRC patients was analyzed *via* GEPIA RNA-Seq datasets. Overall survival (OS) and disease-free survival (DFS) rates in highly expressed LINC00997 KIRC patients were decreased compared to patients with low LINC00997 expression (both $P < 0.01$) (Fig. 1C). Importantly, LINC00997 expression was closely correlated with metastasis-associated molecules VIM, MMP2 and MMP7 (all $P < 0.001$) (Fig. 1D). Consequently, LINC00997 may play an oncogene role, and its high expression could predict poor prognosis in KIRC.

3.2. LINC00997 is closely associated with the oncogene S100A11

Through analyzing the GEPIA RNA-Seq datasets, we found that LINC00997 was closely correlated with S100A11 in KIRC ($P < 0.001$) (Fig. 2A). To examine the role of S100A11 in KIRC, we further analyzed the GEPIA RNA-Seq datasets. S100A11 was highly expressed in multiple cancers compared to their respective normal tissues, including KIRC, kidney renal papillary cell carcinoma (KIRP), uterine corpus endometrial carcinoma (UCEC), uterine carcinosarcoma (UCS), bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), colon adenocarcinoma (COAD), rectum adenocarcinoma (READ), stomach adenocarcinoma (STAD), thyroid carcinoma (THCA), glioblastoma multiforme (GBM), brain lower grade glioma (LGG), liver hepatocellular carcinoma (LIHC), lung squamous cell carcinoma (LUSC), ovarian serous cystadenocarcinoma (OV) and testicular germ cell tumors (TGCT). Similar to LINC00997, S100A11 expression in KIRC metastases was higher than in non-metastatic tumors (Fig. 2B). In the 18 cases of KIRC from our hospital, S100A11 was expressed at significantly higher

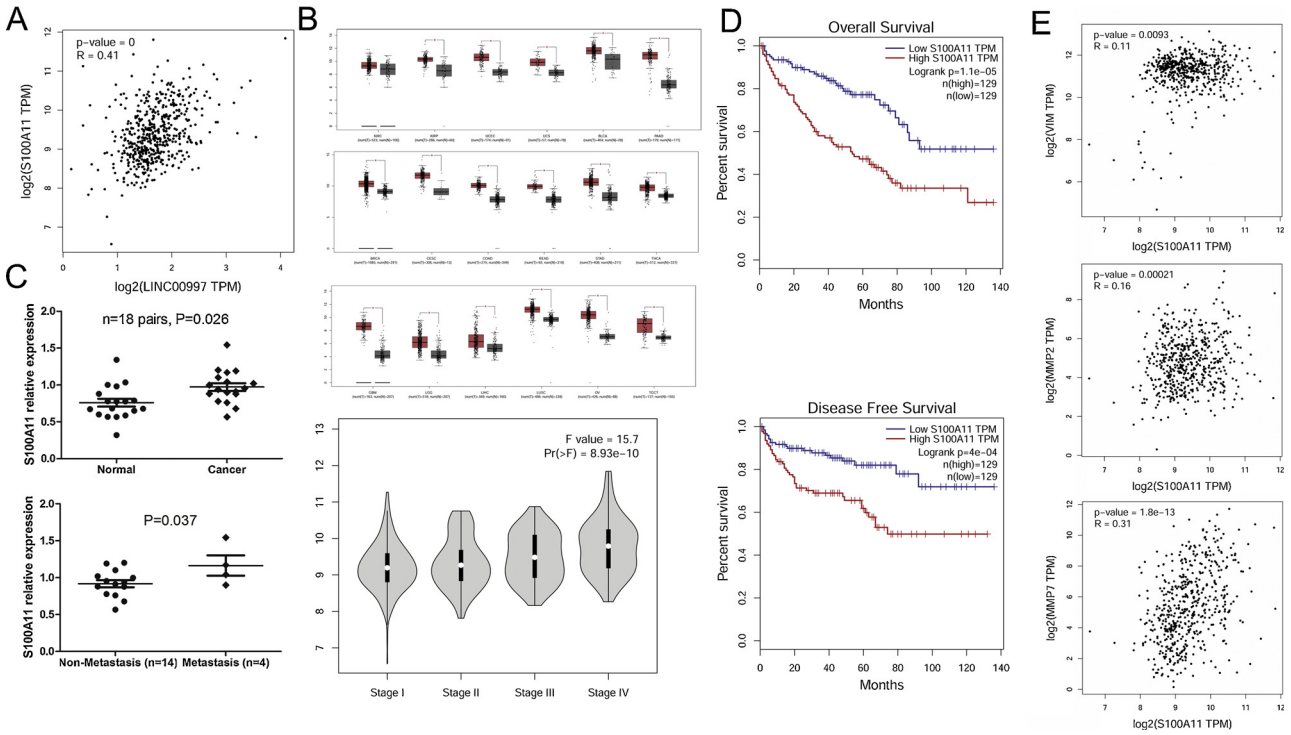

Fig. 2. LINC00997 is closely associated with the oncogene S100A11 in KIRC. A) LINC00997 is closely correlated with S100A11 in KIRC shown by analysis of the GEPIA RNA-Seq datasets ($P < 0.01$). B) S100A11 was highly expressed in multiple cancers compared to their respective normal tissues and was positively correlated with metastasis in GEPIA RNA-Seq datasets. C) In 18 cases of KIRC, S100A11 was exhibited higher expression in tumors and metastasis cases compared to adjacent normal tissues (both $P < 0.05$). D) S100A11 prognostic value in KIRC patients was analyzed *via* GEPIA RNA-Seq datasets. OS and DFS rates in tumors with high expression of S100A11 were lower than those of tumors with low expression (both $P < 0.01$). E) S100A11 is closely correlated with metastasis-associated molecules VIM, MMP2 and MMP7 in GEPIA (RNA-Seq datasets all $P < 0.01$).

levels in tumors compared to adjacent normal tissues ($P < 0.05$). In addition, S100A11 was associated with metastasis ($P < 0.05$) (Fig. 2C). Furthermore, through analyzing the GEPIA RNA-Seq datasets, OS and DFS rates in highly expressed S100A11 KIRC patients were lower than in tumors with low S100A11 expression (both $P < 0.01$) (Fig. 2D). S100A11 expression was closely correlated with VIM, MMP2 and MMP7 expression (all $P < 0.01$) (Fig. 2E). Therefore, LINC00997 is closely correlated with S100A11, which has been reported to regulate metastasis in multiple cancers, and plays an essential role in KIRC metastasis.

3.3. LINC00997 and S100A11 regulate migration and expression of VIM, MMP2 and MMP7

As LINC00997 and S100A11 were both highly expressed in KIRC and predicted a poor prognosis, we next examined their biological effects. 786-O cells were transfected with si-LINC00997, si-S100A11 or negative controls. Transfected cells were cultured for subsequent arrays. A wound healing assay was performed to evaluate migration ability of 786-O cells. Our results showed that inhibiting LINC00997 or S100A11 decreased migration in 786-O cells (Fig. 3A). Transwell assays were also performed, demonstrating that interfering with LINC00997 or S100A11 expression inhibits migration of 786-O cells (both $P < 0.05$) (Fig. 3B). Western blotting was performed and revealed that interfering with LINC00997 or S100A11 expression inhibits metastasis-associated molecular expression of VIM, MMP2 and MMP7 (all $P < 0.05$) (Fig. 3C). Taken together, these findings indicate that LINC00997 and S100A11 regulate cancer cell migration in KIRC.

3.4. LINC00997-STAT3 regulates S100A11 expression

Finally, we assessed the relationship between LINC00997 and S100A11. Our bioinformatics prediction demonstrated that LINC00997 might combine with the S100A11 promoter (Li et al., 2015). The S100A11 promoter has two potential binding sites for the STAT3 protein suggested by analysis of the JASPAR database (Mathelier et al., 2016). LINC00997 also has the potential of combining with STAT3 protein, as suggested by analyzing the LNCPRO database (Lu et al., 2013). Therefore, we hypothesized that LINC00997 combines with the STAT3 transcription factor to regulate S100A11 expression, promoting KIRC metastasis. To test our hypothesis, we constructed luciferase plasmids of the S100A11 promoter, S100A11-pro-luc (S100A11-pro wt), S100A11-pro-1 mut (1264-1274: CTTTTTAGAAT to GCAAAGTC TGC) and S100A11-pro-2 mut (335-345: TTTACAGAAAA to GCCTGC ATTCG) for the following assays. To confirm that LINC00997 combines with the S100A11 promoter, the plasmid and vector, along with si-LINC00997 and negative control, were transfected into HEK293 cells. Results from dual-luciferase reporter gene assays showed that silencing LINC00997 significantly decreases luciferase values of S100A11-pro transfected cells compared to negative control cells (Fig. 4A). To identify combined activity of STAT3 with the S100A11 promoter, we transfected these luciferase plasmids, si-STAT3 and their controls into HEK293 cells. Dual-luciferase reporter gene assays were performed, and results demonstrated that si-STAT3 significantly inhibits luciferase values in both S100A11-pro wt and S100A11-pro-2 mut cells (both $P < 0.05$) but not in S100A11-pro-1 mut cells ($P > 0.05$, not significant) (Fig. 4B). Lastly, the interaction between LINC00997 and STAT3 was assessed through RIP assays in 786-O cells, which demonstrated that LINC00997 combines with the STAT3 protein (Fig. 4C). Importantly, to confirm the gene regulating expression ability of LINC00997 and STAT3, si-LINC00997, si-STAT3 and their controls were transfected into 786-O cells. Western blotting results revealed that interfering with LINC00997 or STAT3 expression inhibited S100A11 expression compared to controls in 786-O cells (both $P < 0.05$)

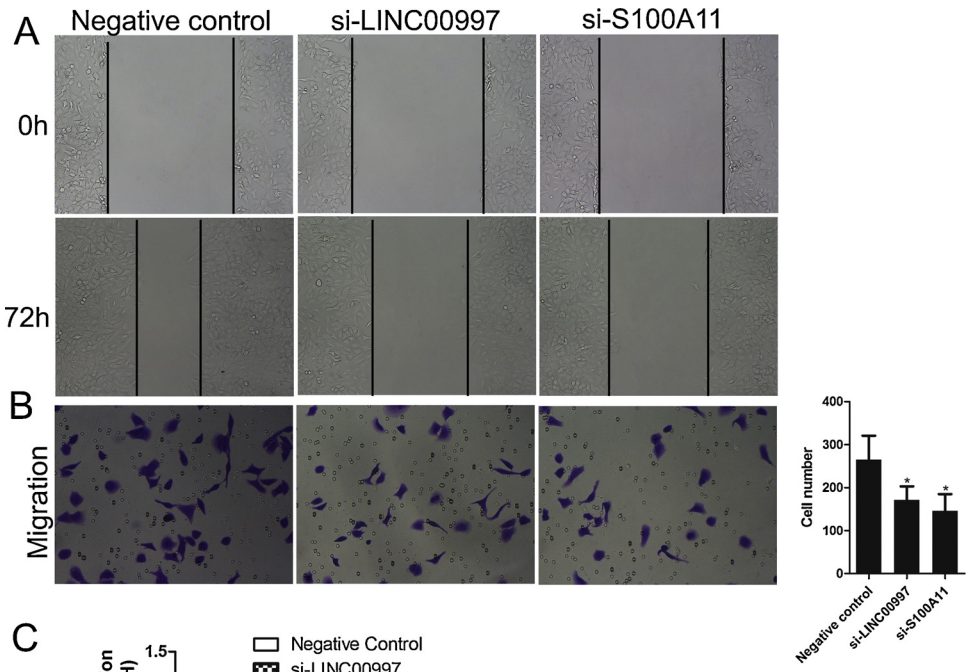

Fig. 3. LINC00997 and S100A11 regulate migration and expression of VIM, MMP2 and MMP7. A) Si-LINC00997, si-S100A11 or negative control was transfected into 786-O cells. Transfected cells were cultured for subsequent arrays. A wound healing assay was performed to evaluate migration of 786-O cells. Results showed that inhibiting LINC00997 or S100A11 decreased migration of 786-O cells. B) Transwell test was also performed, showing that interfering with LINC00997 or S100A11 expression inhibits migration of 786-O cells (*P < 0.05). C) Western blotting was performed and showed that interfering with LINC00997 or S100A11 expression inhibited metastasis-associated molecular expression VIM, MMP2 and MMP7 (*P < 0.05). Three independent experiments were performed.

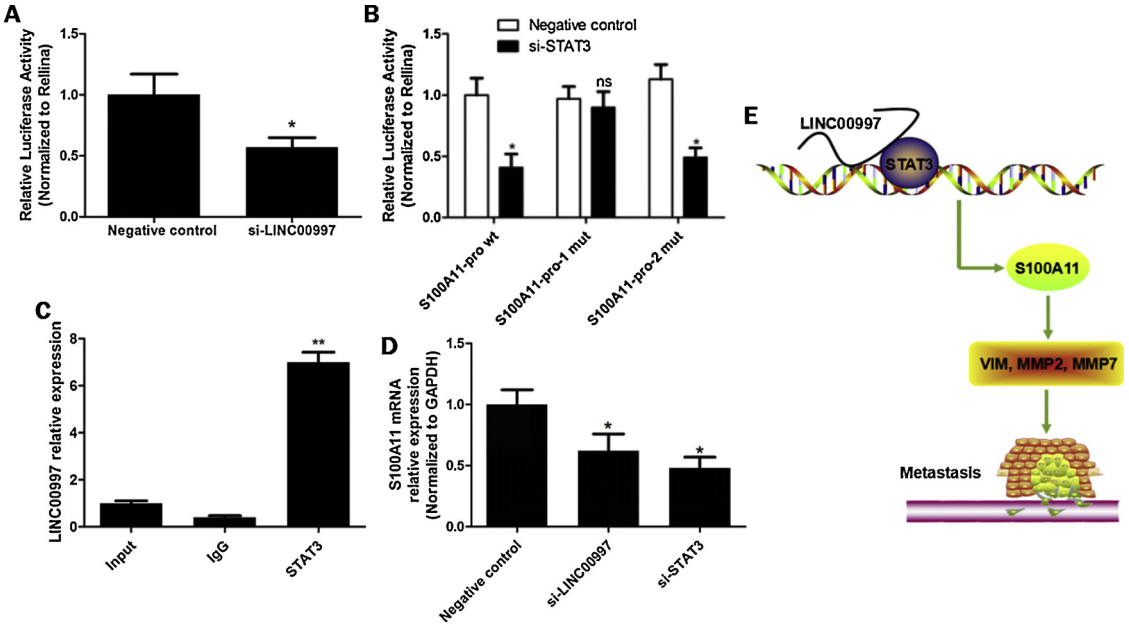

Fig. 4. LINC00997-STAT3 regulates S100A11 expression. To verify binding of LINC00997 to the S100A11 promoter, a luciferase plasmid with the S100A11 promoter, S100A11-pro-luc (S100A11-pro wt), was constructed. A) The plasmid and vector along with si-LINC00997 and negative control were transfected into HEK293 cells. Dual-luciferase reporter gene assay showed that LINC00997 silencing decreases luciferase values in S100A11-pro transfected cells compared to negative control cells (*P < 0.05). B) To identify binding activity of STAT3 to the S100A11 promoter, the luciferase mutation plasmid of the S100A11 promoter, S100A11-pro-1 mut and S100A11-pro-2 mut were constructed. These luciferase plasmids, si-STAT3 and their controls were transfected into HEK293 cells. Dual-luciferase reporter gene assays were performed and showed that si-STAT3 inhibited luciferase values in S100A11-pro wt cells and S100A11-pro-2 mut cells (*P < 0.05) but not in S100A11-pro-1 mut cells (ns, not significant). C) The interaction between LINC00997 and STAT3 was verified through RIP assays in 786-O cells. Results demonstrated that LINC00997 combines with STAT3 protein (**P < 0.01). D). Interfering with LINC00997 or STAT3 expression inhibits S100A11 expression in 786-O cells (*P < 0.05). E) Schematic representation of a model depicting the major molecular mechanisms of the LINC00997-STAT3-S100A11 axis in KIRC. Three independent experiments were performed.

(Fig. 4D). These results suggest that the LINC00997-STAT3-S100A11 axis regulates metastasis in KIRC (Fig. 4E).

4. Discussion

Although surgical treatment has been reported to cure early stage and local KIRC, for metastatic KIRC, patients face a worse prognosis with limited therapeutic options (Novara et al., 2011). Therefore, biomarkers are urgently needed for early diagnosis and evaluation of disease progression in KIRC patients. In the present study, we detected a novel lncRNA, LINC00997, which is significantly overexpressed in metastatic KIRC. Importantly, we found that LINC00997 promotes KIRC metastasis by inducing S100A11 transcription and increasing metastasis-associated molecules VIM, MMP2 and MMP7. These results reveal that LINC00997 plays an essential role in KIRC progression.

LncRNAs are crucial functional regulators involved in cancer diagnosis, prognosis and therapeutics. Multiple sources of evidence have reported that abnormal lncRNA expression contributes to the occurrence and development of various cancers, including renal cell carcinoma, by acting as oncogenes or tumor suppressors. LncRNA HOTTIP promotes renal cell carcinoma progression through regulation of the miR-615/IGF-2 pathway (Wang et al., 2018), lncRNA SARCC suppresses renal cell carcinoma progression by altering androgen receptor (AR)/miRNA-143-3p signaling (Zhai et al., 2017), and therapeutic regulation of HOTAIR by miR-203 overexpression may provide an opportunity to regulate renal cell carcinoma growth and metastasis (Dasgupta et al., 2018). In addition, 11 lncRNAs are reportedly abnormally expressed in KIRC tissues (Blondeau et al., 2015). In this study, we detected LINC00997, which was not included in the above reported lncRNAs, demonstrating that LINC00997 is a novel lncRNA that is highly expressed in KIRC tissues compared to normal tissues.

STAT3, a transcriptional activator and oncogene under normal physiological conditions, is involved in cancer cell proliferation, invasion, and migration (Yu et al., 2014). In addition, STAT3 promotes cancer development by altering expression of other genes in cancer cells (Yuan et al., 2015). It has been reported that STAT3-induced lncRNA HAGLROS overexpression contributes to the malignant progression of gastric cancer cells *via* mTOR signal-mediated inhibition of autophagy. In this study, we verified that LINC00997 combines with the STAT3 transcription factor to regulate S100A11 expression, promoting KIRC metastasis.

To the best of our knowledge, our study is the first to reveal the biological effects of LINC00997 and to confirm a close association between LINC00997 and KIRC prognosis. Additionally, we demonstrated that LINC00997 is closely correlated with metastasis-associated molecules VIM, MMP2 and MMP7. Using bioinformatics analysis, we found that LINC00997 might regulate metastasis-associated molecules VIM, MMP2 and MMP7 *via* regulation of S100A11 in KIRC. To identify the mechanism whereby this occurs, we demonstrated that LINC00997 and STAT3 binding occurs by RIP and that both LINC00997 and STAT3 bind the S100A11 promoter. Importantly, inhibiting LINC00997 or STAT3 resulted in reduced S100A11 expression. Taken together, our results suggest that the LINC00997-STAT3-S100A11 axis plays an essential role in KIRC progression and potentially contributes to KIRC diagnosis and therapy.

In summary, LINC00997 plays a critical role in promoting KIRC metastasis by combining with STAT3, increasing S100A11 expression and elevating levels of metastasis-associated molecules VIM, MMP2 and MMP7. Based on these findings, LINC00997 may represent a potential prognostic biomarker, a therapeutic target, and a predictor for clinical outcomes in KIRC patients.

Declaration of Competing Interest

The authors have declared that no competing interests exist.

Acknowledgements

Prof. Jianguo Wen designed and conducted this study. Dr. Yuan Chang and Dr. Na Li performed all experiments and wrote the manuscript. Prof. Weitang Yuan and Prof. Guixian Wang provided suggestions and revised this manuscript.

References

Blondeau, J.J., Deng, M., Syring, I., Schrodter, S., Schmidt, D., Perner, S., Muller, S.C., Ellinger, J., 2015. Identification of novel long non-coding RNAs in clear cell renal cell carcinoma. Clin. Epigenetics 7, 10. https://doi.org/10.1186/s13148-015-0047-7.

Chen, W., Zheng, R., Baade, P.D., Zhang, S., Zeng, H., Bray, F., Jemal, A., Yu, X.Q., He, J., 2016. Cancer statistics in China, 2015. CA Cancer J. Clin. 66, 115–132. https://doi.org/10.3322/caac.21338.

Dasgupta, P., Kulkarni, P., Majid, S., Varahram, S., Hashimoto, Y., Bhat, N.S., Shiina, M., Deng, G., Saini, S., Tabatabai, Z.L., 2018. MicroRNA-203 inhibits long noncoding RNA HOTAIR and regulates tumorigenesis through epithelial-to-mesenchymal transition pathway in renal cell carcinoma. Mol. Cancer Ther. 17, 1061–1069. https://doi.org/10.1158/1535-7163.MCT-17-0925.

Gupta, K., Miller, J.D., Li, J.Z., Russell, M.W., Charbonneau, C., 2008. Epidemiologic and socioeconomic burden of metastatic renal cell carcinoma (mRCC): a literature review. Cancer Treat. Rev. 34, 193–205. https://doi.org/10.1016/j.ctrv.2007.12.001.

Hu, Y., Wang, J., Qian, J., Kong, X., Tang, J., Wang, Y., Chen, H., Hong, J., Zou, W., Chen, Y., 2014. Long noncoding RNA GAPLINC regulates CD44-dependent cell invasiveness and associates with poor prognosis of gastric cancer. Cancer Res. 74, 6890–6902. https://doi.org/10.1158/0008-5472.CAN-14-0686.

Huarte, M., 2015. The emerging role of lncRNAs in cancer. Nat. Med. 21, 1253–1261. https://doi.org/10.1038/nm.3981.

Kapranov, P., Cheng, J., Dike, S., Nix, D.A., Duttagupta, R., Willingham, A.T., Stadler, P.F., Hertel, J., Hackermüller, J., Hofacker, I.L., 2007. RNA maps reveal new RNA classes and a possible function for pervasive transcription. Science 316, 1484–1488. https://doi.org/10.1126/science.1138341.

Kogo, R., Shimamura, T., Mimori, K., Kawahara, K., Imoto, S., Sudo, T., Tanaka, F., Shibata, K., Suzuki, A., Komune, S., 2011. Long non-coding RNA HOTAIR regulates Polycomb-dependent chromatin modification and is associated with poor prognosis in colorectal cancers. Cancer Res. 71, 6320–6326. https://doi.org/10.1158/0008-5472.CAN-11-1021.

Li, J., Ma, W., Zeng, P., Wang, J., Geng, B., Yang, J., Cui, Q., 2015. LncTar: a tool for predicting the RNA targets of long noncoding RNAs. Brief Bioinform. 16, 806–812. https://doi.org/10.1093/bib/bbu048.

Liu, Z., Chen, Z., Fan, R., Jiang, B., Chen, X., Chen, Q., Nie, F., Lu, K., Sun, M., 2017. Over-expressed long noncoding RNA HOXA11-AS promotes cell cycle progression and metastasis in gastric cancer. Mol. Cancer 16, 82. https://doi.org/10.1186/s12943-017-0651-6.

Lu, Q., Ren, S., Lu, M., Zhang, Y., Zhu, D., Zhang, X., Li, T., 2013. Computational prediction of associations between long non-coding RNAs and proteins. BMC Genomics 14, 651. https://doi.org/10.1186/1471-2164-14-651.

Mathelier, A., Fornes, O., Arenillas, D.J., Chen, C.-y., Denay, G., Lee, J., Shi, W., Shyr, C., Tan, G., Worsley-Hunt, R., 2016. JASPAR 2016: a major expansion and update of the open-access database of transcription factor binding profiles. Nucleic Acids Res. 44, D110–115. https://doi.org/10.1093/nar/gkv1176.

Maxmen, A., 2013. RNA: the genome's rising stars. Nature 496, 127–129.

Novara, G., Ficarra, V., Antonelli, A., Artibani, W., Bertini, R., Carini, M., Cunico, S.C., Imbimbo, C., Longo, N., Martignoni, G., 2011. Validation of the 2009 TNM version in a large multi-institutional cohort of patients treated for renal cell carcinoma: are further improvements needed? Eur. Urol. 58, 588. https://doi.org/10.1016/j.eururo.2010.07.006.

Prensner, J.R., Iyer, M.K., Sahu, A., Asangani, I.A., Cao, Q., Patel, L., Vergara, I.A., Davicioni, E., Erho, N., Ghadessi, M., 2013. The long noncoding RNA SChLAP1 promotes aggressive prostate cancer and antagonizes the SWI/SNF complex. Nat. Genet. 45, 1392–1398. https://doi.org/10.1038/ng.2771.

Rini, B.I., Campbell, S.C., Escudier, B., 2009. Renal cell carcinoma. Lancet 373, 1119–1132. https://doi.org/10.1016/S0140-6736(09)60229-4.

Schmitt, A.M., Chang, H.Y., 2013. Gene regulation: long RNAs wire up cancer growth. Nature 500, 536–537. https://doi.org/10.1038/nature12548.

Sun, Z., Yang, S., Zhou, Q., Wang, G., Song, J., Li, Z., Zhang, Z., Xu, J., Xia, K., Chang, Y., 2018a. Emerging role of exosome-derived long non-coding RNAs in tumor microenvironment. Mol. Cancer 17, 82. https://doi.org/10.1186/s12943-018-0831-z.

Sun, Z., Ou, C., Liu, J., Chen, C., Zhou, Q., Yang, S., Li, G., Wang, G., Song, J., Li, Z., 2018b. YAP1-induced MALAT1 promotes epithelial–mesenchymal transition and angiogenesis by sponging miR-126-5p in colorectal cancer. Oncogene. https://doi.org/10.1038/s41388-018-0628-y. in press.

Wang, Q., Wu, G., Zhang, Z., Tang, Q., Zheng, W., Chen, X., Chen, F., Li, Q., Che, X., 2018. Long non-coding RNA HOTTIP promotes renal cell carcinoma progression through the regulation of the miR-615/IGF-2 pathway. Int. J. Oncol. 53, 2278–2288. https://doi.org/10.3892/ijo.2018.4539.

Yang, F., Zhang, L., Huo, Xs., Yuan, Jh., Xu, D., Yuan, Sx., Zhu, N., Zhou, Wp., Yang, Gs., Wang, Yz., 2011. Long noncoding RNA high expression in hepatocellular carcinoma facilitates tumor growth through enhancer of zeste homolog 2 in humans. Hepatology 54, 1679–1689. https://doi.org/10.1002/hep.24563.

Yu, H., Lee, H., Herrmann, A., Buettner, R., Jove, R., 2014. Revisiting STAT3 signaling in cancer: new and unexpected biological functions. Nat. Rev. Cancer 14, 736. https://doi.org/10.1038/nrc3818.

Yuan, J., Zhang, F., Niu, R., 2015. Multiple regulation pathways and pivotal biological functions of STAT3 in cancer. Sci. Rep. 5, 17663. https://doi.org/10.1038/srep17663.

Zhai, W., Sun, Y., Guo, C., Hu, G., Wang, M., Zheng, J., Lin, W., Huang, Q., Li, G., Zheng, J., 2017. LncRNA-SARCC suppresses renal cell carcinoma (RCC) progression via altering the androgen receptor (AR)/miRNA-143-3p signals. Cell Death Differ. 24, 1502. https://doi.org/10.1038/cdd.2017.74.