LncRNA LEF1-AS1 Promotes Metastasis of Prostatic Carcinoma via the Wnt/β-Catenin Pathway

Weiyuan Li
Tongren Hospital Shanghai Jiaotong University School of Medicine
Ganggang Yang
Tongren Hospital Shanghai Jiaotong University School of Medicine
Dengke Yang
Tongren Hospital Shanghai Jiaotong University School of Medicine
Dong Li
Tongren Hospital Shanghai Jiaotong University School of Medicine
Qian Sun (squrology@163.com)
Tongren Hospital Shanghai Jiaotong University School of Medicine https://orcid.org/0000-0001-7108-9127

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Abstract

Background: Long noncoding RNAs (lncRNAs), which are important functional regulators in cancer, have emerged as critical molecular regulators in various biological processes. However, the mechanisms by which LEF1-AS1 modulates Androgen-Independent Prostate Cancer (AIPC) development remain largely unknown.

Methods: The LEF1-AS1 expression level was detected in tumour tissues and adjacent normal tissues of AIPC patients by using next-generation sequencing technology and qRT-PCR. Cell proliferation, migration and invasion were assessed by colony formation, EDU assays and transwell assays, respectively. Xenograft assay was conducted to determine the effect of LEF1-AS1 on cell proliferation in vivo.

Results: LEF1-AS1 promoted the proliferation, migration, invasion and angiogenic ability of AIPC cells in vitro and in vivo. In this mechanism, LEF1-AS1 recruited the transcription factor C-myb to the promoter region of FZD2, which activated FZD2 transcription. Moreover, LEF1-AS1 functioned as a competing endogenous RNA (ceRNA) acting as a sponge for miR-328, which activated CD44.

Conclusion: Collectively, these data indicate that LEF1-AS1 is a tumour promoter in the development of AIPC and that it may contribute to the improvement of AIPC diagnosis and therapy.

Background

Prostate cancer (PCa) is the commonly diagnosed malignancy and the second-leading cause of cancer-related mortality in males in Western countries [1]. In its early stage, prostate cancer is androgen dependent; however, most prostate cancers can progress to androgen resistant status as time goes on [2]. It is well known that AIPC carcinogenesis is a complex biological process involving a variety of genomic variations and cellular events [3]. Therefore, further research regarding the oncogenic signalling mechanisms in AIPC progression is urgently needed.

Long noncoding RNAs (lncRNAs) are greater than 200 bases in length and play vital roles in multiple cellular functions, including cell proliferation, apoptosis, cellular differentiation, tumourigenesis, and metastasis [4–5]. Many lncRNAs have been reported to be involved in different types of cancers including AIPC [6]. For example, Chakravartyet al. demonstrated that the expression of lncRNA NEAT1 was significantly higher during AIPC progression, and promoted cancer cell proliferation by regulating the epigenetic signatures of target gene promoters [7]. Human lymphoid enhancer-binding factor 1 antisense RNA 1 (LEF1-AS1), a newly discovered lncRNA located on the plus strand of chromosome 4, was previously shown to be upregulated in glioblastoma (GBM) tissues and its dysregulation was postulated to correlate with poor overall survival in patients [8–9]. In addition, several studies also demonstrated that LEF1-AS1 promotes cell proliferation and invasion during multiple types of cancer, suggesting that LEF1-AS1 could function as an oncogene and might be a potential therapeutic target in the progress of malignancy [10–11]. Nevertheless, LEF1-AS1 has not been reported to be a regulatory gene associated

with AIPC progression. Hence, it is necessary to explore the detailed function and the underlying molecular mechanism of LEF1-AS1 in the regulation of AIPC progression.

The Wnt/β-catenin pathway, one of the vital mechanisms responsible for cell proliferation, cell polarity, migration and cell fate determination during embryonic development and maintaining tissue homeostasis, has been proven to be associated with the development of several pathologies, including cancer [12–13]. More and more evidence elucidates that the Wnt/β-catenin pathway participates in regulating many key process during cancer development, including promoting cell proliferation, maintaining cancer stem cells (CSCs) and increasing metastasis [14–15]. In the canonical Wnt/β-catenin signaling pathway, WNT ligands bind to corresponding frizzled receptors (FZD) and associated co-receptors in order to inhibit the downstream β-catenin complex degradation, resulting in β-catenin accumulating and nucleus translocating where it activates transcription through T-cell factor/lymphoid enhancer factor (TCF/LEF) [16]. In recent decades, accumulating researches demonstrated Wnt/β-catenin signaling pathway has been implicated in both normal prostate development and in PCa progression [17]. Multiple molecular could activate Wnt/β-Catenin signaling during PCa progression, such as PTEN/Akt, COX-2/PGE2, PDGF, and NF-κB pathways [18–20]. However, little is known regarding the effects of lncRNA LEF1-AS1 on the wnt/β-catenin pathway in AIPC. Therefore, understanding the underlying molecular mechanisms of wnt/β-catenin pathway would lead to more effective diagnosis and treatment in AIPC.

In this study, we report that LEF1-AS1 is upregulated in tumour tissues and that this lncRNA promotes the proliferation, migration, invasion and angiogenic abilities of AIPC cells as well as tumour growth in vivo. Furthermore, mechanistic investigations showed that LEF1-AS1 could initiate the activation of the Wnt/β-catenin pathway by upregulating the expression of Wnt/β-catenin pathway membrane receptor FZD2 and CD44. We speculated that LEF1-AS1 might function as an oncogene and aimed to detect the underlying molecular mechanisms in AIPC progression.

Methods And Materials

Patients and samples

AIPC samples were obtained from 45 patients who provided informed consent, which was in accordance with the ethical standards of the Tongren Hospital (Shanghai, China) Review Board. All samples were reviewed by a pathologist and were confirmed as AIPC based on a histopathological evaluation. No local or systemic treatments were administered in these patients before surgery.

Cell culture

The AIPC cell lines PC3 and DU145 were obtained from the American Type Culture Collection (Manassas, VA, USA). The normal prostatic cell line RWPE was purchased from Jennio Biotech Co(Guangzhou, China). PC3 and DU145 cells were cultured in DMEM (Gibco, Grand Island, NY, USA) supplemented with

10% heat-inactivated foetal bovine serum (FBS) (Gibco). RWPE cells were cultured in RPMI 1640 medium (Gibco) supplemented with 10% FBS. All cells were incubated at 37 °C in a 5% CO_2 humidified incubator.

Cell transfection

The LEF1-AS1 pcDNA3.3 vector and empty vector (pcDNA3.3) were based on the expression vector pcDNA3.3 (Invitrogen, USA). Small interfering RNA for LEF1-AS1, C-myb, FZD2 or CD44 (siLEF1-AS1, siC-myb) and scramble siRNA (si NC) were purchased or synthetized from RiboBio (Guangzhou, China). The cells were seeded into 6-well plates, and transfection was performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions.

RNA isolation and RT-PCR analyses

Total RNA was extracted from AIPC samples and cells using TRIzol reagent (Invitrogen), and cDNA was synthesised using the QuantiTect Reverse Transcription Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's protocol. qRT-PCR was then performed based on the instructions for SYBR Premix Ex Taq (Takara, Tokyo, Japan), and the expression levels were normalized to the level of GAPDH. Each experiment was repeated at least three times.

Chromatin immunoprecipitation (ChIP)

To verify the potential transcription factors binding to the LEF1-AS1 promoter, a ChIP assay was performed using the EZ-Magna ChIP kit (Millipore, Shanghai, China) according to the manufacturer's protocol. In brief, cells were fixed with 4% paraformaldehyde and incubated with glycine for 10 min to generate DNA–protein cross-links. Then, the cells were lysed with Cell Lysis Buffer and Nuclear Lysis Buffer and sonicated to generate chromatin fragments of 400–800 bp. The chromatin fragments were immunoprecipitated with an anti-C-myb antibody (Cell Signaling Technology, Danvers, MA, USA) or control IgG. The resulting DNA was purified for PCR analyses.

RNA binding protein immunoprecipitation (RIP) assay

RIP was performed according to the protocol for the EZ-Magna RIP kit (EMD Millipore, Billerica, MA, USA). Briefly, DU145 cells grown to 80–90% confluency were lysed in complete RIP lysis buffer, and 90% of 100 μL of whole cell extract was incubated with RIP buffer containing magnetic beads conjugated with 5 μg human anti-C-myb antibody (Abcam) or immunoglobulin G (IgG) control. Incubation with proteinase K with shaking was performed to digest the protein, and RNA was isolated by immunoprecipitation. Finally, the immunoprecipitated RNA was purified and analysed by qRT-PCR.

Immunoblotting analysis

Cells (5×10^6) were lysed for 20 min with lysis buffer (Beyotime Biotechnology) containing protease inhibitors (Roche, Indianapolis, IN, USA). Equal amounts of samples were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes. The membranes were blocked with 5% (wt/vol) skimmed milk in TBS plus Tween 20 at 4 °C overnight

before probing with antibodies. Membrane proteins were detected by an HRP-conjugated secondary antibody (1:1000, Santa Cruz Biotech, Santa Cruz, CA).

Colony formation assay

AIPC cells were collected, and 1000 cells were seeded in each well of a 6-well plate. After cell culture for 15 days, PC3 or DU145 cells were washed with PBS and fixed in methanol for 20 min. After washing three times with PBS, colonies were stained with 0.1% crystal violet for 20 min. Colonies were captured using a light microscope (Olympus, Tokyo, Japan). Clusters containing ≥ 30 cells were counted as a single colony.

EDU assays

The cells were cultured in 96-well plates at a density of 2×10^3 cells/well. Then, the EDU (5'-ethynyl-2'-deoxyuridine) incorporation assay was performed to evaluate cell proliferation using a KeyFluor488 Click-iT EDU kit (KeyGENBioTECH, Nanjing, China) according to the manufacturer's instructions. The percentage of Edu-positive cells was calculated after fluorescence microscopy analysis.

Cell migration and invasion assays

The cells were harvested, resuspended in serum-free media and placed into the upper chamber of a Transwell membrane filter (Corning, NY, USA) for the migration assays or in the upper chamber of a Transwell membrane filter coated with Matrigel (Corning) for the invasion assays. After 24 h of incubation, cells on the upper side were removed with a cotton swab. Evaluation of invasive capacity was performed by counting invading cells under a microscope (40 × 10). Five random fields of view were analysed for each chamber.

Immunohistochemical analysis

AIPC and adjacent tissues were fixed in 4% paraformaldehyde for paraffin embedding. Tissue specimens were cut into slices, and tissue slices were deparaffinized, rehydrated, and immersed in 3% hydrogen peroxide for 10 min to quench endogenous peroxidase activity. Cancer tissues were immunostained for FZD2 and CD44 (Abcam, San Francisco, CA, USA; 1:100 dilution). The signal was amplified and visualized with diaminobenzidine chromogen, followed by counterstaining with haematoxylin.

Tumourigenicity assays in nude mice

We used male nude mice (6 weeks of age) obtained from the Animal Facility of Shanghai Jiao Tong University School of Medicine; the mice were fed sterilized food and water. All of the animal experiments were approved by the responsible governmental animal ethics committee and complied with the ARRIVE guidelines. Thirty-day-old male nude mice were subcutaneously injected with 1×10^6 AIPC cells in 200 μL serum-free medium into the right flank. Once palpable tumours were observed (after approximately 4 weeks), the mice were sacrificed. The tumours were isolated and weighed.

Statistical analysis

The results are presented as the mean ± SD from three independent experiments performed in triplicate. The *P* values were calculated by using Student *t* test or one-way ANOVA. A *P* value of < 0.05 was considered to indicate a statistically significant result. Statistical analyses were performed using GraphPad Prism 6.0 statistical software (GraphPad Software Inc., La Jolla, California).

Results

LEF1-AS1 expression is upregulated in AIPC tissues and cell lines

To identify the lncRNA profile and to investigate the role of lncRNAs in the development of AIPC, we performed next-generation sequencing and compared lncRNA expression in AIPC patient samples with that in normal samples. We identified a series of abnormally expressed lncRNAs in AIPC and found that LEF1-AS1 was significantly increased in AIPC tissues (Fig. 1A). In terms of mRNAs, a total of 719 mRNAs (log2FC > 2, FDR < 0.1) were significantly differentially expressed (Fig. 1B-1C). To validate the findings from RNA sequencing, we analysed the expression of LEF1-AS1 in AIPC and adjacent tissues by qRT-PCR. The results confirmed the accuracy of the next-generation sequencing findings (Fig. 1D). Furthermore, the expression of LEF1-AS1 was increased in DU145 cells compared with PC3 and RWPE cells (Fig. 1E). These results suggested that LEF1-AS1 might function as an oncogene during AIPC progression. The result of subcellular fractionation showed that the LEF1-AS1 transcript was observed in the cytoplasm of AIPC cells, whereas minimal signal was observed in the nucleus (Fig. 1F), suggesting that LEF-AS1 might regulate gene expression at the transcriptional level.

LEF1-AS1 promoted AIPC cell proliferation, migration and invasion in vitro and in vivo

We investigated the role of LEF1-AS1 in AIPC cell proliferation using a colony formation assay and an Edu assay. The results suggested that the overexpression of LEF1-AS1 could promote the proliferation of PC3 cells, while knockdown of LEF1-AS1 could inhibit the proliferation of DU145 cells (Fig. 3A). Next, we evaluated AIPC cell migration and invasion using Transwell-based assays. As shown in Fig. 3B, cell migration and invasion were up-regulated in the presence of LEF1-AS1, while the opposite result was observed when LEF1-AS1 was inhibited. It is known that new blood vessels are essential to promote tumour development. Therefore, we utilized the endothelial tube formation assay to assess the ability of LEF1-AS1 to affect the development of biological systems in AIPC cells. The results demonstrated that the neovascularization rate was especially increased after transfection with LEF1-AS1 overexpression plasmid and decreased after transfection with siLEF1-AS1 compared with the corresponding negative controls (Fig. 3C). To further determine the role of LEF1-AS1 during AIPC progression in vivo, we conducted a subcutaneous tumour formation experiment in nude mice. After 4 weeks, tumour weight and volume were dramatically increased in the LEF1-AS1 overexpression group. In contrast, tumour growth, indicated by weight and volume, was significantly decreased in the siLEF1-AS1 group (Fig. 3D).

Collectively, these data strongly demonstrated that LEF1-AS1 aggravated cell migration, invasion, and vasculogenic mimicry and suppressed cell proliferation in vivo during AIPC progression.

LEF1-AS1 initiated the activation of the Wnt/β-catenin pathway via frizzled class receptor 2 (FZD2)

Gene Ontology (GO) analysis and KEGG pathway enrichment analysis showed that the Wnt/β-catenin signalling pathway was one of the most enriched pathways within the set of upregulated mRNAs, suggesting that it may be involved in the pathogenesis and development of AIPC (Fig. 3A-4B). To elucidate the functions of the molecular mechanisms induced by LEF1-AS1, the activity of the Wnt/β-catenin signalling pathway was detected in the AIPC cell lines. Compared with that of the normal cell line RWPE, the activity of the Wnt/β-catenin signalling pathway was significantly higher in PC3 and DU145 cells (Fig. 4A). Next, we examined the levels of GSK3β, p-GSK3β, β-catenin, MMP-7 and c-myc protein in AIPC cells after LEF1-AS1 plasmid or siLEF1-AS1 transfection. The results showed that significantly higher expression levels of p-GSK3β(ser9), β-catenin, MMP-7 and c-myc were observed in PC3 cells transfected with LEF1-AS1 overexpression plasmid. Correspondingly, in the DU145 cell lines, the expression level of Wnt/β-catenin pathway proteins was decreased in cells transfected with the siLEF1-AS1 (Fig. 4B). We next evaluated how LEF1-AS1 modulated the Wnt/β-catenin signalling pathway in AIPC progression. Using the starBase 3.0 database, we found that multiple genes positively correlated with LEF1-AS1 in AIPC, including frizzled class receptor 2 (Fig. 4C). Moreover, as shown in Fig. 4D, a significantly higher expression level of FZD2 was observed in cells transfected with the LEF1-AS1 plasmid. To further dissect the molecular mechanism of FZD2 regulation by LEF1-AS1, we analysed the sequences of LEF1-AS1 and the FZD2 promoter (+ 500 to -2000 bp) using the LongTarget tool. A LEF1-AS1-FZD2 binding pattern was predicted as shown in Fig. 4E. Luciferase assays demonstrated that LEF1-AS1 with a deletion of this binding region (LEF1-AS1 del) failed to bind the FZD2 promoter as LEF1-AS1 wt did. To explore how LEF1-AS1 regulates the expression of FZD2, we proposed that LEF1-AS1 might affect FZD2 transcription via alteration of transcription factors. We predicted the interaction of LEF1-AS1 and C-myb by using an *in silico* analysis of regulatory RNA elements. RIP analysis further demonstrated that LEF1-AS1 RNA could be pulled down by anti-C-myb antibody in AIPC cells, indicating the physical binding of LEF1-AS1 with C-myb (Fig. 4F). Finally, a ChIP assay confirmed the occupancy of C-myb in the FZD2 promoter. AIPC cells were subjected to ChIP with anti-C-myb antibody, followed by RT-PCR measurements with specific probes targeting the FZD2 promoter or -5 kb upstream (control). The results indicated that the occupancy of C-myb in the FZD2 promoter region was significantly increased compared to that of the − 5 kb upstream/control (Fig. 4G). The corresponding western blot assays with anti-FZD2 antibody showed similar FZD2 attenuation upon C-myb interference (Fig. 4H). Taken together, these data suggest that LEF1-AS1 enhances FZD2 transcription via recruitment of C-myb to the FZD2 promoter region.

LEF1-AS1 increased CD44 via function through the ceRNA sponging pattern of miR-328

To further study the mechanism of LEF1-AS1 during Wnt/β-catenin pathway activation, we used the RegRNA database and found that LEF1-AS1 was a potential targets of 3 microRNAs (Fig. 5A). Next, we confirmed binding interactions between miR-328 with LEF1-AS1 by demonstrating a reduction in luciferase activity (Fig. 5B). Furthermore, multiple mRNAs were predicted by TargetScan and PITA; the regulatory networks of LEF1-AS1/miR-328 axis are shown in Fig. 5A. Among them, the CD44 gene was predicted as a downstream target gene of the LEF1-AS1/miR-328 axis. Interestingly, an early study reported that miR-328 promotes cell proliferation by targeting CD44 in MCF-7 cells. Therefore, we detected whether or not LEF1-AS1 regulates CD44 expression via sponging miR-328. As shown in Fig. 5C-5D, CD44 expression was significantly increased by LEF1-AS1 or miR-328 inhibitor transfection. In contrast, CD44 expression was suppressed by LEF1-AS1 siRNAs or miR-328 mimic. In addition, a dual-luciferase reporter assay was used to test whether CD44 is a direct target of miR-328. The results showed that luciferase activity was reduced in AIPC cells that were co-transfected with miR-328 and CD44-WT but was not reduced in cells containing CD44-Mut (Fig. 5E). The ceRNA binding pattern between LEF1-AS1/CD44 and miR-328 was further validated by RNA immunoprecipitation. As shown in Fig. 5F, RNA was significantly more enriched in the Ago2-IP fractions of AIPC cells compared with the IgG-IP group ($P < 0.001$), indicating a direct targeted relationship between LEF1-AS1 and miR-328. Therefore, in agreement with recent findings, the transcriptional increase in LEF1-AS1 expression seemed to be an important mechanism contributing to the activation of the Wnt/β-catenin pathway in AIPC cells by upregulating CD44.

LEF1-AS1 aggravated cell proliferation and migration partly dependent on its regulation on FZD2 and CD44 in human prostate cancer cells

After confirming that LEF1-AS1 regulates FZD2 and CD44 in AIPC cells, it is necessary to further determine whether the oncogenic functions of LEF1-AS1 is dependent on its modulation of FZD2/CD44. Colony formation, transwell assays and tube formation assays were carried out to validate that knockdown of FZD2 or CD44 that could partly inhibit the tumor promoting effect caused by LEF1-AS1 overexpression. The results demonstrated that co-transfected with LEF1-AS1 and siFZD2/siCD44 partly reversed the oncogenic roles caused by LEF1-AS1 overexpression (Fig. 6A-6C). In addition, the expression of β-catenin and downstream targets (MMP-7 and c-myc) was also decreased after siFZD2/siCD44 transfection in LEF1-AS1 overexpression cells compared with control group (Fig. 6D-E). Taken together, as shown in Fig. 6F, our findings demonstrated that LEF1-AS1 could enhance FZD2 transcription via recruitment of c-myb to its promoter region and increase CD44 mRNA levels via miR-328 sponge, resulting in the activation of Wnt/β-catenin pathway.

Discussion

Prostate carcinoma is a common male malignancy and a major cause of cancer-associated mortality worldwide [21]. In recent decades, knowledge regarding the molecular changes that underlie PCa has increased, which has greatly increased interest in the discovery of new molecular markers for diagnosis and treatment [22]. In the present study, our results clarified that LEF1-AS1 may serve a role as an oncogene in AIPC by aggravating cancer cell proliferation, migration and vascularization. The underlying mechanism of LEF1-AS1 in AIPC might be through activating Wnt/β-catenin signalling.

Increasing efforts have contributed to elucidating the molecular and cellular mechanisms underlying the progression of cancer, among which lncRNAs have attracted increasing attention [23]. Recently, an increasing number of studies have elucidated that long non-coding RNA LEF1-AS1 plays critical roles in cellular proliferation, apoptosis, differentiation, and invasion during the progression of multiple types of cancer [24]. Zhang et al. reported that LEF1-AS1 knockdown inhibited cell survival, proliferation and migration, whereas enhanced cell apoptosis and induced G0/G1 cell cycle arrest in oral squamous cell carcinoma, hinting a cancerigenic role of LEF1-AS1 in tumour progression [25]. Furthermore, LEF1-AS1 also has been proven to be positively correlated with lymph node metastasis and advanced stage in ovarian cancer and Enhanced expression of LEF1-AS1 may predict a poor prognosis [26]. Nevertheless, the role of LEF1-AS1 and the underlying mechanism in AIPC progression remain unclear. In the present study, we performed next-generation sequencing to investigate lncRNA expression profiles and identified LEF1-AS1 as a significantly upregulated lncRNA in advanced AIPC. Afterwards, we detected the LEF1-AS1 expression level in AIPC by using RT-qPCR. The results showed that LEF1-AS1 was significantly increased during AIPC progression, which was consistent with the sequencing results. Moreover, our results showed that LEF1-AS1 promoted the proliferation, migration, invasion and vasculogenic mimicry of AIPC cells both in vitro and in vivo. These data suggest that LEF1-AS1 acts as an oncogene during AIPC progression.

Recent studies have demonstrated that Wnt/β-catenin and the downstream TCF/LEF complex, as key regulators of mitogenesis and tumourigenicity, participate in regulating cell transformation, cell growth and cell cycle progression [27–28]. In view of the frequent activation of Wnt signaling pathway in tumour progression, FZDs have an apparent role in modulating this pathway as they serve as upstream regulators of the Wnt signaling pathway. In general, up-regulation of FZDs lead to the activation of Wnt/β-catenin pathway and, to a lesser extent, the non-canonical pathway [29–30]. In the present study, we confirmed that LEF1-AS1 acts in both the cytoplasm and nucleus. In the nucleus, LEF1-AS1 binds to the promoter region of FZD2. Interestingly, LEF1-AS1 also recruits the transcription factor C-myb in this region. Our results confirmed that LEF1-AS1 enhanced FZD2 transcription via recruitment of C-myb to its promoter region.

In the cytoplasm, LEF1-AS1 may mainly serve as a sponge for regulatory miRNAs. For example, a previous study reported that lncRNA LEF1-AS1 functions as a ceRNA by regulating the expression of miR-544a in lung cancer [11]. In this study, we demonstrated that LEF1-AS1 functions as a ceRNA by sponging miR-328, which leads to CD44 activation. CD44, as a multifunctional transmembrane adhesion glycoprotein, plays an important role in signal transduction. Recent evidence shows that the expression

of CD44 is mainly regulated by specific signaling networks, transcriptional factors, and epigenetic mechanisms [31]. Moreover, CD44 was shown to target the Wnt/β-catenin signaling pathway in tumour progression [32–33]. In this study, for the first time, we demonstrated that LEF1-AS1 acted as a powerful regulator in AIPC and regulated the activation of Wnt/β-catenin and the downstream pathway by functioning as a ceRNA to increase the expression of CD44.

Conclusion

In summary, as shown in Fig. 6F, we determined that LEF1-AS1 was significantly increased during AIPC progression. Furthermore, a mechanistic study showed that LEF1-AS1 functioned as a ceRNA and served as a regulator in the activation of the Wnt/β-catenin pathway via FZD2 and CD44. Our results provide new insight into the mechanism that links the function of LEF1-AS1 with AIPC and suggest that LEF1-AS1 may serve as a novel potential target for the improvement of AIPC therapy.

Declarations

Acknowledgements

Not applicable.

Authors’ contributions

Weiyuan Li and Ganggang Yang designed the research and wrote the manuscript; Weiyuan Li and Dengke Yang performed experiments and analyzed data; Dong Li collected and analyzed data; Qian Sun revised the manuscript and approved the final submission. All authors discussed the results and reviewed the manuscript.

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Availability of data and materials

The analyzed data sets generated during the present study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The present study was approved by the ethical review committee of the Tongren Hospital of Shanghai jiaotong University.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

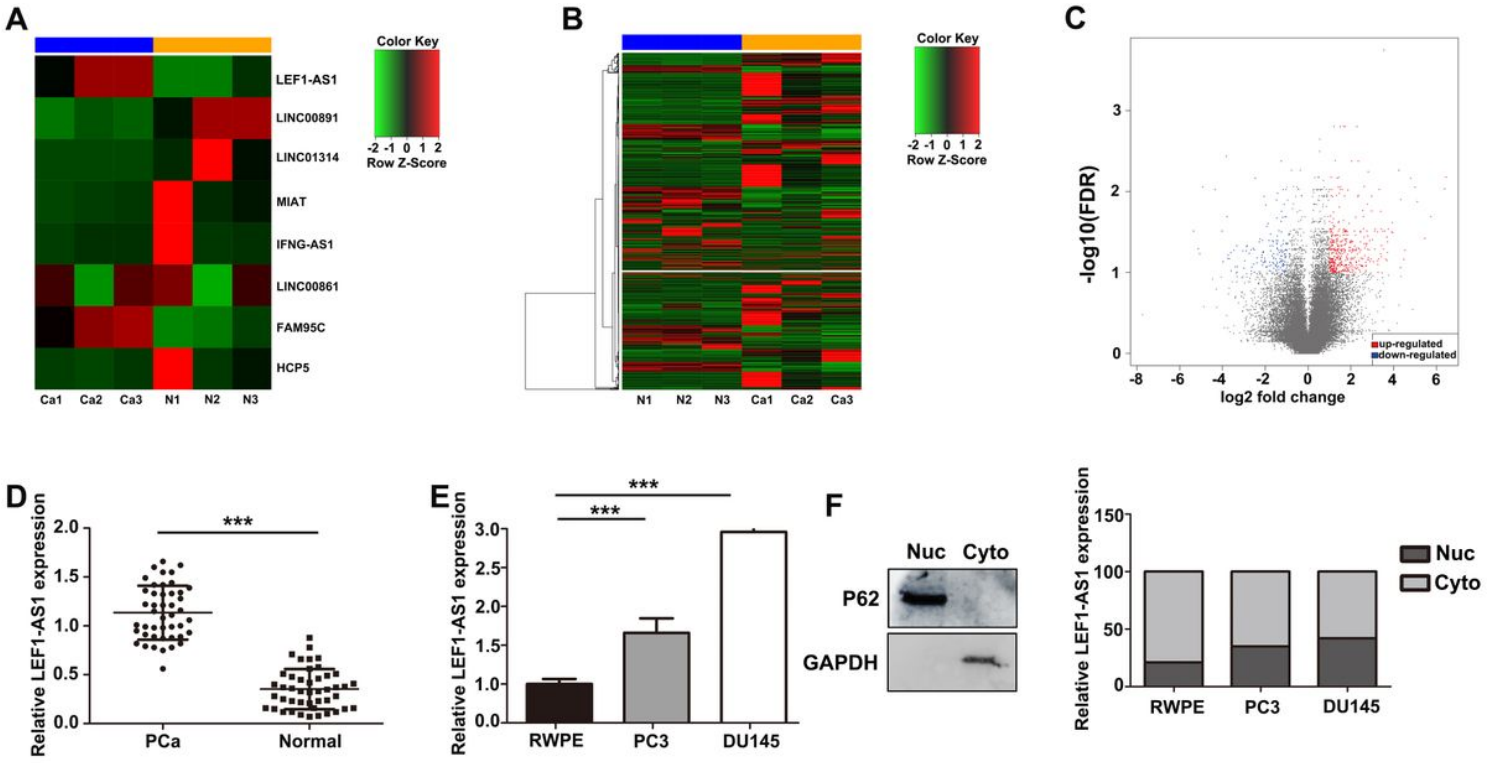

Figure 1

LEF1-AS1 was significantly increased in AIPC tissues compared with adjacent tissues. (A-C) The heat maps and volcano plot show the different expression lncRNAs and mRNAs in AIPC tissues. (D-E) LEF1-AS1 expression level was measured by qRT-PCR in clinical AIPC tissues and AIPC cells. (F) Subcellular fractionation assay was used to determine the localization of LEF1-AS1 in AIPC cells.

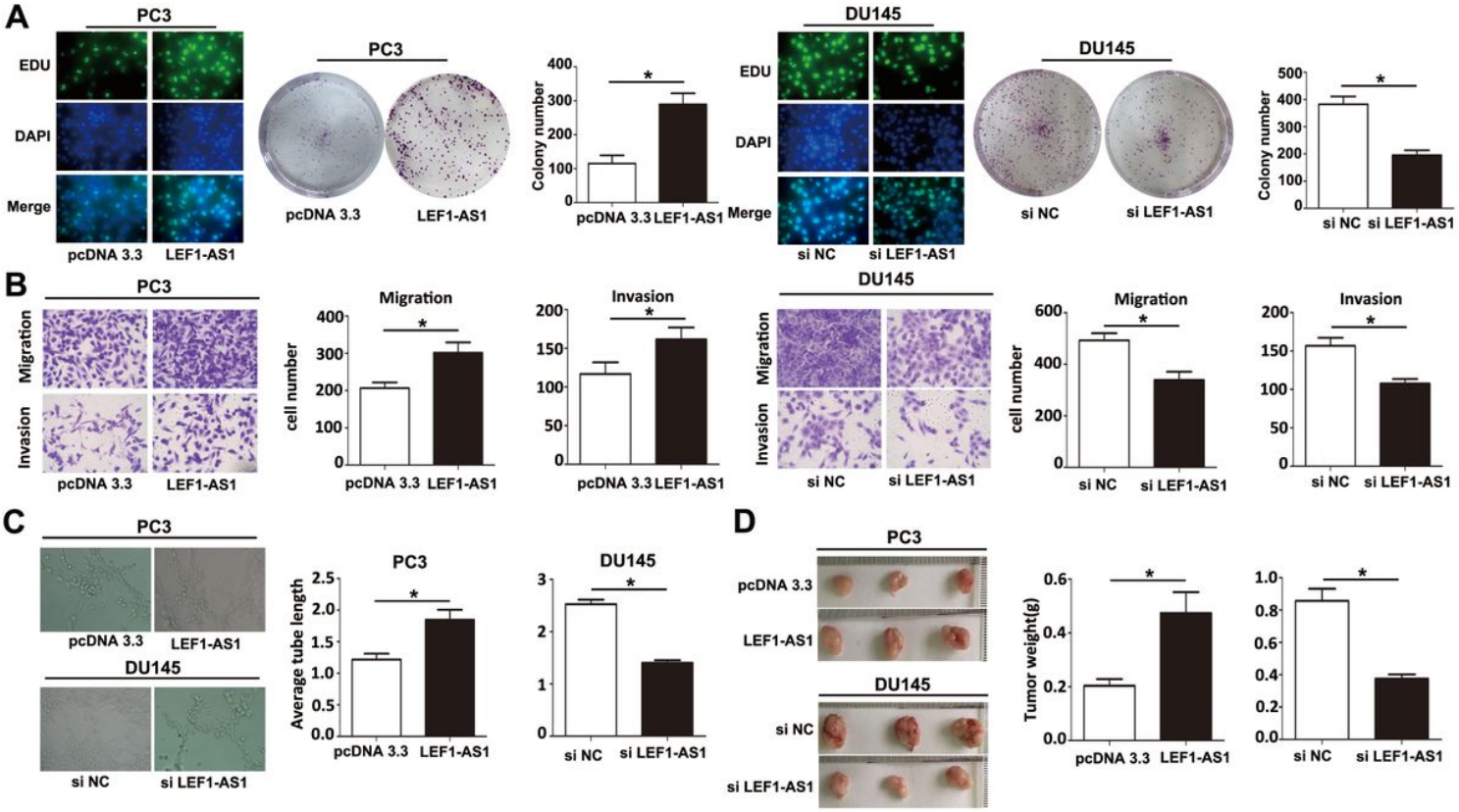

Figure 2

LEF1-AS1 promoted AIPC cell proliferation, migration and invasion in vitro and in vivo. (A) The proliferative ability of AIPC cells was measured by colony formation assay and EDU assay. (B) LEF1-AS1 promoted the migratory and invasive ability of AIPC cells. (C) The effect of LEF1-AS1 on AIPC vascularisation was measured by tube formation assays. (D) Subcutaneous tumour formation experiments were used to measure cell proliferation ability in vivo.

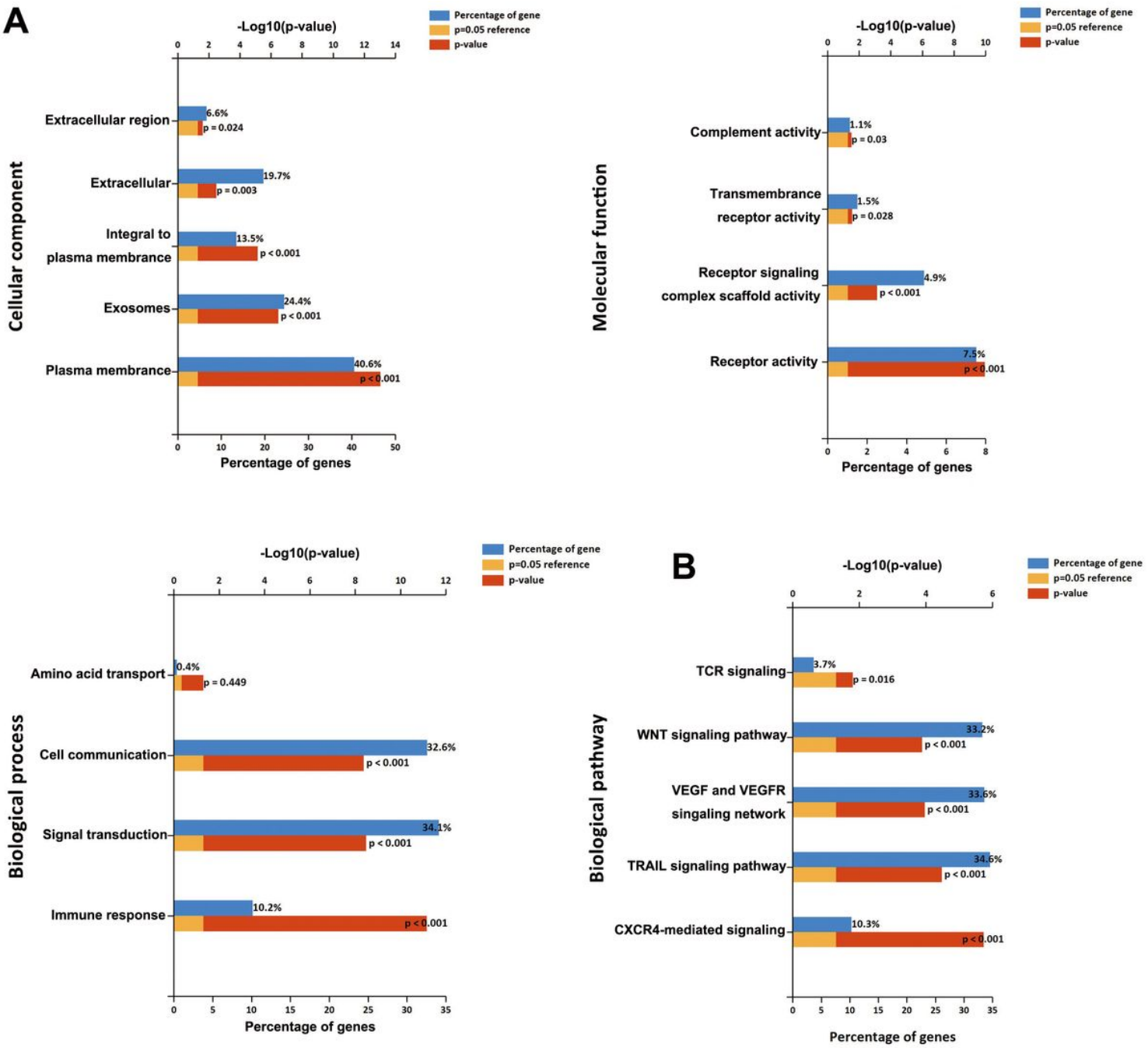

Figure 3

(A-B) Enriched GO terms and KEGG pathways for the upregulated genes in AIPC tissues.

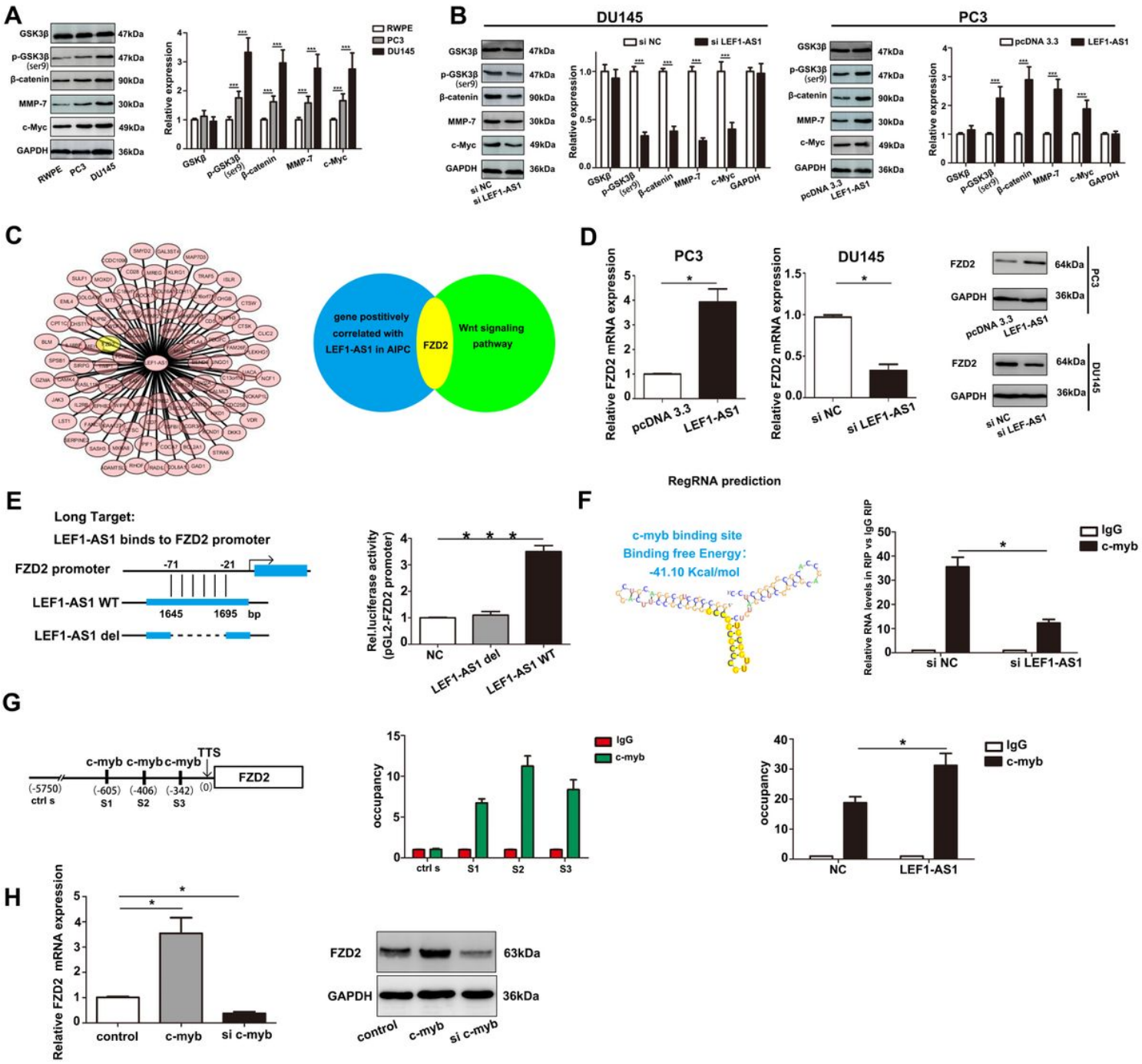

Figure 4

LEF1-AS1 promoted the activation of the Wnt/β-catenin pathway via FZD2. (A) The expression of Wnt/β-catenin pathway-related proteins was measured by western blot. (B) The expression of β-catenin and downstream proteins was measured in AIPC cells treated with siLEF1-AS1 or LEF1-AS1 plasmid by western blot. (C) The expression of FZD2 was positively correlated with that of LEF1-AS1 in AIPC as shown by using the starBase 3.0 database. (D) The expression of FZD2 was measured in AIPC cells treated with siLEF1-AS1 or the LEF1-AS1 plasmid by qRT-PCR and western blot. (E) Schematic graph of the deletion of the LEF1-AS1 binding site in the FZD2 promoter (predicted by LongTarget). (F) RegRNA in silico predicted the secondary structure of LEF1-AS1 interacting with the c-myb transcription factor

(protein), employing a calculated binding free energy of -41.1 Kcal/mol. (G) Multiple binding sites of the transcription factor c-myb are predicted in the FZD2 promoter region (JASPAR database). The results of the ChIP assay confirmed the occupancy of C-myb in the FZD2 promoter. (H) The expression of FZD2 was measured by qRT-PCR and western blot assays.

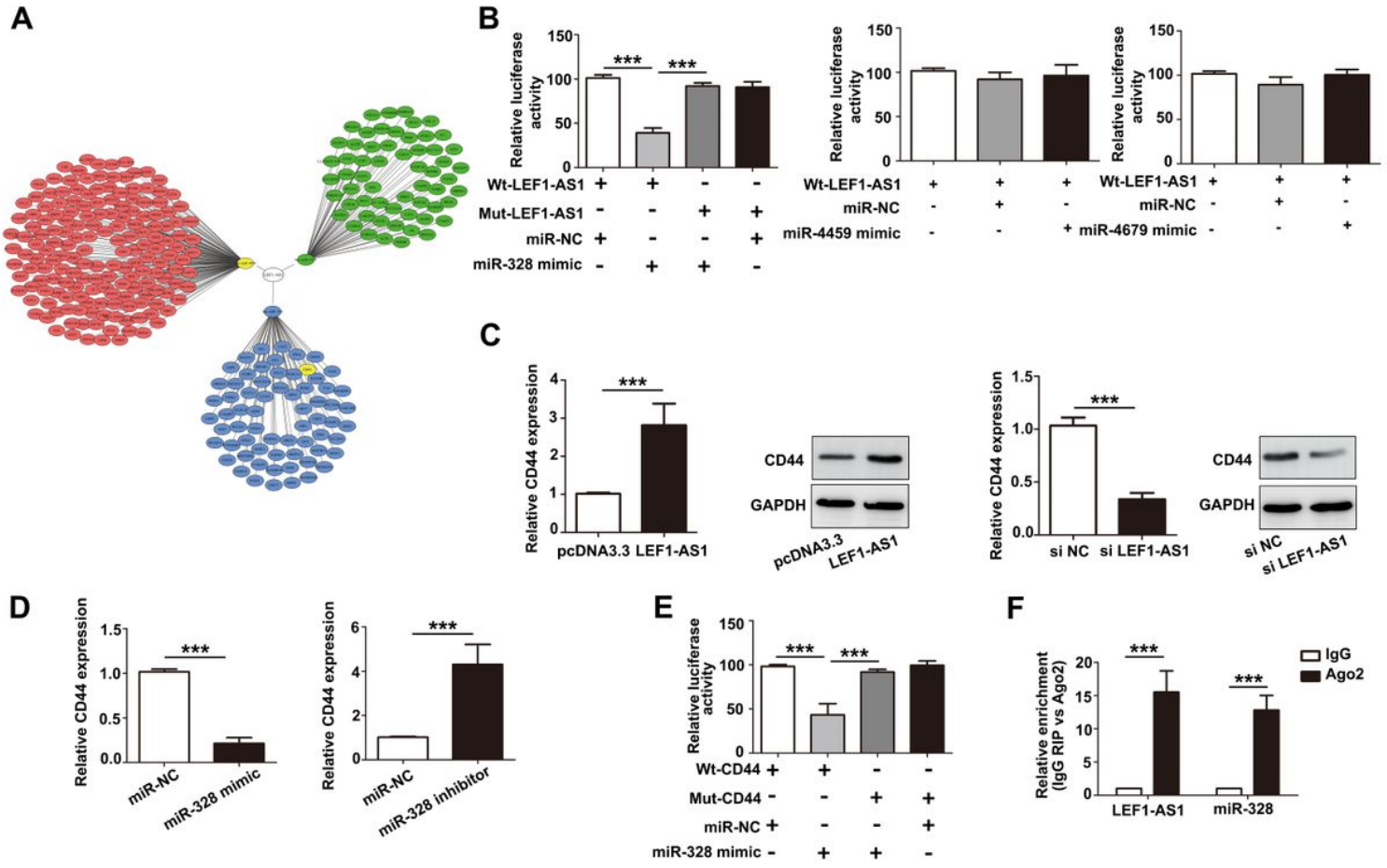

Figure 5

LEF1-AS1 promoted the activation of the Wnt/β-catenin pathway via CD44. (A) Using the starBase database, we found that both LEF1-AS1 and CD44 were potential targets of miR-328. (B) A dual luciferase reporter assay was used to test whether LEF1-AS1 is the target of miR-328. (C) The expression of CD44 was measured by qRT-PCR and western blot after transfection. (D) The effect of miR-328 on CD44 was measured by qRT-PCR and western blot. (E) The luciferase activity of cells co-transfected with miR-328 mimic and luciferase reporters containing CD44-Wt or CD44-Mut transcript were shown. (F) The result of RIP experiment showed that LEF1-AS1 was enriched in Ago2-contaning miRNAs compared with IgG; miR-328 was also detected in the same precipitate.

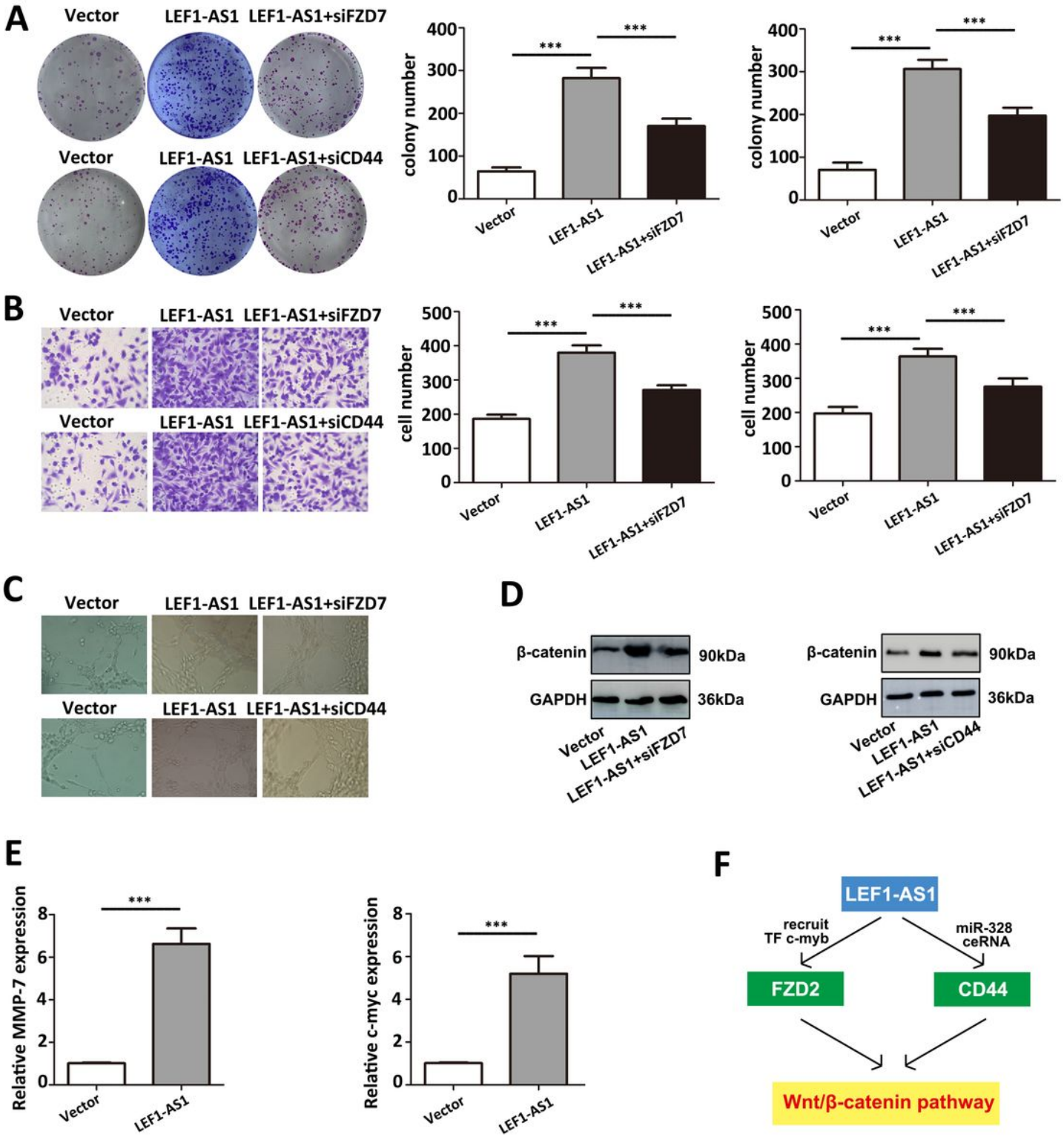

Figure 6

LEF1-AS1 promoted AIPC aggressiveness by targeting FZD2 and CD44. (A-C) Colony formation (a), transwell (b) and tube formation assays (c) were used to detect the effect of LEF1-AS1 on AIPC cells after FZD2 or CD44 knockdown. (D) The protein level of β-catenin was measured by western blot after transfection. (E) The mRNA levels of both MMP-7 and c-myc were detected by qRT-PCR. (F) Mechanism for the regulatory function of LEF1-AS1 in AIPC progression.

Supplementary Files

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