Long non-coding RNA SPRY4-IT1 promotes development of hepatic cellular carcinoma by interacting with ERRα and predicts poor prognosis

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Hepatocellular carcinoma (HCC) has become one of the most common leading causes of cancer-related deaths worldwide. This study investigates the role of lncRNA, SPRY4-IT1 in the development of HCC. Quantitative real-time PCR (qRT-PCR) was performed and the results showed that SPRY4-IT1 expression was up-regulated in HCC tissues and high expression of SPRY4-IT1 was associated with poor 5-year overall survival in the HCC patient cohort. Clinicopathological analysis showed that the expression of SPRY4-IT1 was significantly correlated with TNM stage in HCC patients. In vitro CCK-8 assay, colony formation assay, cell invasion and migration assays demonstrated that knock-down of SPRY4-IT1 suppressed cell proliferation, colony formation, cell invasion and migration in HCC cells. Flow cytometric analysis showed that knock-down of SPRY4-IT1 induced cell cycle arrest at G0/G1 phase and induced apoptosis. In addition, knock-down of SPRY4-IT1 also suppressed the mRNA and protein expression of estrogen-related receptor α (ERRα). Similarly, knock-down of ERRα inhibited cell proliferation, colony formation, cell invasion and migration in HCC cells. More importantly, ERRα overexpression antagonized the effects of SPRY4-IT1 knock-down on cell proliferation, colony formation, cell invasion and migration in HCC cells. Taken together, our data highlights the pivotal role of SPRY4-IT1 in the tumorigenesis of HCC.
colorectal cancer, esophageal squamous cell carcinoma, glioma, and bladder cancer. However, the role of SPRY4-IT1 in other types of cancers remains unknown, particularly in HCC.

The estrogen-related receptor α (ERRα) belongs to the nuclear receptor family. Because there is no natural ligand of ERRα has been identified to date, ERRα is regarded as an "orphan" receptor. It regulates the target genes in a ligand-independent way. ERRα is found to involve in various physiological processes such as innate immunity, energy metabolism and osteoblast differentiation as well as bone formation. Recently, ERRα has been suggested to be associated with the development and progression of various types of cancers. Knock-down of ERRα inhibited the tumor development on uterine endometrial cancer; ERRα is a marker of tamoxifen response and survival in triple-negative breast cancer; ERRα was also found to coordinate colon cancer cell proliferation and tumorigenic capacity with energy metabolism; WNT11 expression is induced by ERRα and beta-catenin, and acts in an autocrine manner to increase cancer cell migration. However, whether ERRα plays a role in HCC development is still unclear.

In this study, we identified the up-regulation of SPRY4-IT1 in HCC tissues, which was associated with poor prognosis in HCC patients. We also found that high level of SPRY4-IT1 promoted proliferation, cell cycle progression and suppressed cell apoptosis of HCC. Furthermore, SPRY4-IT1 was found to function in the progression of HCC via interacting with ERRα.

### Materials and Methods

#### Human Tissues samples.

A total of 82 patients with HCC were included in this study. All the patients had undergone routine hepatic resection at the Fifth Affiliated hospital of Guangzhou Medical University from 2012 to 2015, and none of the patients had received chemotherapy or radiotherapy prior to surgical resection. This study was approved by the Ethics Committee of the Fifth Affiliated hospital of Guangzhou Medical University. All patients provided written informed consent for the use of tissue samples for clinical research. The histological diagnosis and differentiation of tumors were evaluated by pathologists according to the criteria of WHO classification system. The clinicopathological features are shown in Table 1. All the tissue samples were snap-frozen in liquid nitrogen immediately following surgical resection, and stored in −80°C for further experimentation.

| Characteristics       | Number of patients | Low  | High | P-value |
|-----------------------|--------------------|------|------|---------|
| Age (years)           |                    |      |      | 0.822   |
| <55                   | 30                 | 13   | 17   |         |
| ≥55                   | 52                 | 24   | 28   |         |
| Gender                |                    |      |      | 0.805   |
| Male                  | 60                 | 26   | 32   |         |
| Female                | 22                 | 9    | 13   |         |
| Serum AFP (ng/ml)     |                    |      |      | 0.246   |
| <20                   | 29                 | 16   | 13   |         |
| ≥20                   | 53                 | 21   | 32   |         |
| HBsAg                 |                    |      |      | 0.685   |
| Negative              | 6                  | 2    | 4    |         |
| Positive              | 76                 | 35   | 41   |         |
| Tumor size (cm)       |                    |      |      | 0.118   |
| <5                    | 32                 | 18   | 14   |         |
| ≥5                    | 50                 | 19   | 31   |         |
| Liver cirrhosis       |                    |      |      | 0.179   |
| Absence               | 19                 | 11   | 7    |         |
| Presence              | 63                 | 26   | 38   |         |
| Histological differentiation |               |      |      | 0.1737  |
| Well                  | 12                 | 7    | 5    |         |
| Moderate              | 32                 | 17   | 15   |         |
| Poor                  | 38                 | 13   | 25   |         |
| TNM stage             |                    |      |      | 0.03    |
| I + II                | 34                 | 22   | 12   |         |
| III + IV              | 48                 | 15   | 33   |         |
| Metastasis            |                    |      |      | 0.002   |
| No                    | 47                 | 28   | 19   |         |
| Yes                   | 35                 | 9    | 26   |         |

Table 1. Correlation between SPRY4-IT1 expression levels and clinicopathological characteristics in HCC patients. Low, low expression level of SPRY4-IT1; High, high expression level of SPRY4-IT1.
Cell culture. The normal liver cell line, HL7702 and the human liver cancer cell lines MHCC97L, MHCC97H, HepG2 and SMMC7721 were obtained from the Chinese Academy of Sciences (Shanghai, China). All the cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) with 10% fetal bovine serum (FBS, Thermo Scientific, Waltham, USA) in a humidified atmosphere at 37 °C with 5% CO₂.

Figure 1. Relative SPRY4-IT1 RNA expression and its association with overall survival of HCC patients. (A) Relative SPRY4-IT1 RNA expression was examined by qRT-PCR in 82 pairs of cancerous liver tissues and adjacent non-cancerous liver tissues from HCC patients, ***P < 0.001 (paired t-test). (B) Kaplan-Meier survival curve. Patients were divided into SPRY4-IT1-low (Low) and SPRY4-IT1-high (High) groups based on the median of relative SPRY4-IT1 RNA expression in HCC. *P < 0.05.

Figure 2. Knock-down of SPRY4-IT1 suppresses cell proliferation, colony formation, cell invasion and migration in HCC cells. (A) Relative SPRY4-IT1 expression levels in different liver cell lines measured by qRT-PCR. (B) Relative SPRY4-IT1 expression levels in scrambled siRNA (control) or SPRY4-IT1 siRNA (siRNA) transfected HepG2 and SMMC2117 cells were examined by qRT-PCR. (C) and (D) Cell viability in scrambled siRNA (control) or SPRY4-IT1 siRNA (siRNA) transfected HepG2 and SMMC2117 cells determined by CCK-8 assay at 0 h, 24 h, 48 h and 72 h time point. (E) Colony formation assay performed in HepG2 and SMMC2117 cells transfected with siRNA (control) or SPRY4-IT1 siRNA (siRNA). (F) Cell invasion and (G) migration assays performed in scrambled siRNA (control) or SPRY4-IT1 siRNA (siRNA) transfected HepG2 and SMMC2117 cells. Data are shown as mean ± SD, significant differences were marked as *P < 0.05, **P < 0.01, ***P < 0.001.
SiRNAs and vector construction. All the siRNAs (SPRY4-IT1 siRNA: CCCAGAATGTTGACAGCTGCTCTT or its respective scrambled siRNA; ERRα siRNA or its respective scrambled siRNA) were purchased from Genechem (Shanghai, China). The ERRα overexpression constructs or the empty vector were purchased from Genepharma (Shanghai, China). All the transfections were performed by using the Lipofetamine reagent (Invitrogen) according to the manufacturer's instructions.

Quantitative real-time PCR (qRT-PCR). Total RNA from tissue samples or cells were extracted using Trizol reagent (Invitrogen, Carlsbad, USA), and the RNA was reversely transcribed to cDNA by using PrimeScript RT-polymerase (Takara, Dalian, China). Real-time PCR was performed using the Applied Biosystems 700 Sequence Detection System (Applied Biosystems, Foster City, USA). GAPDH was used as an internal control. The relative expressions of SPRY4-IT1 and ERRα were normalized to GAPDH expression levels by using the 2-ΔΔCt method. The primers for SPRY4-IT1 are: forward, 5′-AGCCACATAAATTCAGCAGA-3′; reverse, 5′-CGATGTAGTAGGATTCCTTT-3′; the primers for ERRα are: forward, 5′-CAGGAAAGTGAATCCCAG-3′; reverse, 5′-CTTGGACAGCAATAATACATT-3′; the primers for GAPDH are: forward, 5′-GTGGGAGTCAACGGATTTGG-3′; reverse, 5′-AAAAGCAGCCCTGGTGACC-3′.

Western blotting assay. The HepG2 and SMMC7721 cells were lysed with denaturing SDS-PAGE sample buffer using standard methods. Protein lysates were separated on a 10% SDS-PAGE gel and transferred to the nitrocellulose membranes. The membranes were blocked with 5% skimmed non-fat milk for 1 h at room temperature, and then the membrane were incubated with rabbit polyclonal anti-ERRα antibody (Abcam, Cambridge, USA) at 4°C overnight. After primary antibody incubation, the membranes were then incubated with HRP-conjugated anti-IgG at room temperature for 2 h. Signal was detected by an ECL system (Amersham Pharmacia, Piscataway, USA).

CCK-8 assay. Twenty-four hours after transfected with SPRY4-IT1 siRNA, or its respective scrambled siRNA; ERRα siRNA or its respective scrambled siRNA; or after co-transfected with scrambled siRNA + empty vector, SPRY4-IT1 siRNA + empty vector, or SPRY4-IT1 siRNA + ERRα overexpressing vector, cells were seeded in the 96-well plate at a concentration of 5 × 10^3 cells, and further cultured for 24 h, 48 h and 72 h accordingly. Cell viability was determined by a Cell Counting Kit-8 (Beyotime, Shanghhai, China) according to the manufacturer's instructions.

Figure 3. Knock-down of SPRY4-IT1 induces cell cycle arrest and apoptosis. (A) Cell cycle prolife in scrambled siRNA (control) or SPRY4-IT1 siRNA (siRNA) transfected HepG2 and SMMC2117 cells examined by flow cytometry with propidium iodide staining. (B) Cell apoptosis in scrambled siRNA (control) or SPRY4-IT1 siRNA (siRNA) transfected HepG2 and SMMC2117 cells examined by flow cytometry. Data are shown as mean ± SD, significant differences were marked as *P < 0.05.
instructions. The cell viability index was detected at the wavelength of 450 nm using the Elx800 reader (Bio-Tek Instruments, Winooski, USA).

**Colony formation assay.** Twenty-four hours after transfection (see CCK-8 assay section), cells were seeded in six-well plates containing DMEM medium supplemented with 10% FBS. After 24 h, the medium was replaced with new medium containing G418. After further culturing for 14 days, cells were fixed with methanol and stained with 0.1% crystal violet. Visible colonies were manually counted.

**Invasion and migration assays.** Cell invasion and migration was evaluated using Transwell assay. For cell migration assay, twenty-four hours after transfection (see CCK-8 assay section), cells at a concentration of $5 \times 10^5$ were seeded in the top chamber with the non-coated membrane transwell ($8 \mu$m pore size inserts, BD Biosciences, San Jose, USA). For the cell invasion assay, matrigel (BD Biosciences) was polymerized in the transwell inserts for 45 min at 37°C. For both assays, cells were plated in the top chamber in medium without FBS, while the lower chamber was filled with 10% FBS and EGF (Sigma, St Louis, USA). Cells were further incubated for 24 h and the cells that did not migrate or invade through the pores were removed by a cotton swab. Cells on the lower surface of the membrane were stained with crystal violet and counted under a microscope.

**Flow cytometry.** Twenty-four hours after transfection with SPTRY4-IT1 siRNA, cells were harvested by trypsinization. After the double staining with FITC-Annexin V and propidium iodide, cell apoptosis detection was performed by using the FITC Annexin V Apoptosis Detection Kit (BD Biosciences) according to the manufacturer’s instructions. The cell apoptosis was analyzed with a flow cytometer (FACScan; BD Biosciences) equipped with a Cell Quest Software (BD Biosciences). For the cell cycle analysis, cells were stained with

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**Figure 4.** Knock-down of ERα suppresses cell proliferation, colony formation, cell invasion and migration in HCC cells. (A) The mRNA and (B) protein expression of ERα in scrambled siRNA (control) or SPTRY4-IT1 siRNA (siRNA) transfected HepG2 cells examined by qRT-PCR and Western blotting. (C) The mRNA and (D) protein expression of ERα in scrambled siRNA (control) or EERα siRNA (siRNA) transfected HepG2 cells examined by qRT-PCR and Western blotting. (E) Cell viability in scrambled siRNA (control) or ERα siRNA (siRNA) transfected HepG2 cells determined by CCK-8 assay at 48 h time point. (F) Colony formation assay performed in scrambled siRNA (control) or EERα siRNA (siRNA) transfected HepG2 cells. (G) Cell invasion and (H) migration assays performed in scrambled siRNA (control) or EERα siRNA (siRNA) transfected HepG2 cells. Data are shown as mean ± SD, significant differences were marked as *P < 0.05, **P < 0.01.
propidium oxide by using the Cycle TEST PLUS DNA Reagent Kit (BD Biosciences) following the manufacturer's instructions and analyzed by FACScan.

**Statistical analysis.** GraphPad Prism software was used for data analysis. All the data are shown as mean ± SD, and data were analyzed by t-test (for comparing two groups) or by one-way ANOVA followed by Turkey’s multiple comparison tests (for comparing more than two groups). Results were considered statistically significant at \( P < 0.05 \).

**Results**

**SPRY4-IT1 mRNA is up-regulated in HCC tissues and is correlated with poor prognosis in HCC patients.** We evaluated the RNA levels of SPRY4-IT1 in 82 pairs of human primary HCC tissues and the respective adjacent normal non-cancerous liver tissues using qRT-PCR. We found that the expression of SPRY4-IT1 in HCC tissues were significantly higher than that examined in the respective adjacent normal non-cancerous liver tissues (Fig. 1A, \( P < 0.001 \)). Clinical analysis demonstrated that SPRY4-IT1 RNA levels were correlated with TNM stage and metastasis (Table 1, \( P < 0.05 \)). However, we have not observed significant correlation between SPRY4-IT1 expression levels and other clinical characteristics such as age, gender, serum AFP level, HBsAg status, tumor size, liver cirrhosis and histological differentiation (Table 1). To understand the prognostic role of SPRY4-IT1 in HCC, we examined the correlation between SPRY4-IT1 expression and HCC patients’ overall survival. The results showed that high level of SPRY4-IT1 was significantly correlated with poor 5-year overall survival rate in HCC patients (Fig. 1B, \( P < 0.05 \)).
SPRY4-IT1 knock-down inhibits cell proliferation, colony formation, cell invasion and migration in HCC cell lines. The expression of SPRY4-IT1 was further examined in vitro in one normal liver cell line (HL7702) and four HCC cell lines (MHCC97H, HCCLM6, HepG2 and SMMC7721) by qRT-PCR. We found that the relative expression of SPRY4-IT1 in HCC cell lines were higher than that in normal liver cell line, with the highest expression in HepG2 and second highest in SMMC7721 cell lines (Fig. 2A, P < 0.05). In the following study, HepG2 and SMMC7721 cell lines were chosen for further functional investigation in vitro. We transfected the siRNA targeting SPRY4-IT1 to knock-down SPRY4-IT1 in HepG2 and SMMC7721 cell lines. The SPRY4-IT1 siRNA transfection in HepG2 and SMMC7721 cells significantly reduced the expression levels of SPRY4-IT1 (Fig. 2B, P < 0.05). To examine the role of SPRY1-IT1 knock-down in cell proliferation, CCK-8 assay was performed at 0h, 24h, 48h and 72h after SPRY1-IT1 transfection. In comparison with scrambled siRNA (Control) transfected HepG2 and SMMC7721 cells at 48 or 72h, HepG2 and SMMC7721 cells transfected with SPRY4-IT1 siRNA had significantly lower proliferative ability (Fig. 2C and D, P < 0.05). To further investigate the effect of SPRY4-IT1 knock-down on cell growth, we performed colony formation assay. The results showed that the number of colonies in SPRY4-IT1 transfected HepG2 and SMMC7721 cells were significantly lower than that in scrambled siRNA transfected cells (Fig. 2E, P < 0.05). To examine the effect of SPRY4-IT1 knock-down on cell invasive and migratory abilities, we performed the cell invasion and cell migration assays. As shown in Fig. 2F and G, the number of invaded and migrated cells in SPRY4-IT1 transfected HepG2 and SMMC7721 cells were significantly reduced when compared to scrambled siRNA transfected cells (Fig. 2F and G, P < 0.05).

SPRY4-IT1 knock-down induces cell cycle arrest and cell apoptosis. The cell cycle and cell apoptosis in HCC cell lines were analyzed by flow cytometry. Compared with the scrambled siRNA transfected HepG2 and SMMC7721 cells, SPRY4-IT1 transfected cell lines had higher proportion of cell population at G0/G1 phase with lower proportion of cell population at S+G2/M phases (Fig. 3A, P < 0.05). In addition, SPRY4-IT1 transfection in HepG2 and SMMC7721 cells significantly increased the cell apoptotic rate when compared with scrambled siRNA transfection in these cell lines (Fig. 3B, P < 0.05).

SPRY4-IT1 knock-down suppresses ERRα expression, and ERRα knock-down suppressed cell proliferation, colony formation, cell invasion and cell migration. To investigate if SPRY4-IT1 had an interaction with ERα, we performed qRT-PCR and Western blotting to examine the RNA and protein expression of ERα in SPRY4-IT1 siRNA or scrambled siRNA transfected HCC cell line. As shown in Fig. 4A and B, SPRY4-IT1 siRNA transfection HepG2 cells significantly suppressed the mRNA and protein expression levels of ERα (Fig. 4A and B). Furthermore, we examined the role of ERα in HCC cell proliferation, colony formation, cell invasion and migration. ERα inhibitory transfection significantly suppressed the mRNA and protein expression of ERα in HepG2 cells (Fig. 4C and D, P < 0.05). The CCK-8 results showed that, HepG2 cells transfected with ERα siRNA had significantly suppressed proliferative ability in comparison with scrambled siRNA (Control) transfected HepG2 cells at 48h (Fig. 4E, P < 0.05). To further investigate the effect of ERα knock-down on cell growth, colony formation assay was also performed. The results showed that the number of colonies in ERα siRNA transfected HepG2 cells were reduced compared with scrambled siRNA transfected cells (Fig. 4F, P < 0.05). To examine the effect of ERα knock-down on cell invasive and migratory abilities, cell invasion assay and cell migration assay were performed, and the results demonstrated that the number of invaded and migrated cells in ERα transfected HepG2 cells were significantly lower than that in scrambled siRNA transfected cells (Fig. 4G and H, P < 0.05).

ERRα overexpression antagonized the effects of SPRY4-IT1 knock-down on cell proliferation, colony formation, cell invasion and migration in HCC cell lines. To further confirm the interaction between SPRY4-IT1 and ERα, HepG2 cells were transfected with scrambled siRNA+ empty vector, SPRY4-IT1 siRNA+ empty vector, or SPRY4-IT1 siRNA+ ERRα overexpressing vector. After transfection, CCK-8, colony formation, cell invasion and migration assays were performed. In consistent with previous results, co-transfection with SPRY4-IT1 siRNA and empty vector in HepG2 cells significantly suppressed cell proliferation, colony formation, cell invasion and migration in comparison with co-transfection with scrambled siRNA+ empty vector (Fig. 5, P < 0.05). Further, co-transfection with SPRY4-IT1 siRNA + ERRα overexpressing vector in HepG2 cells increased the cell proliferation, colony formation, cell invasion and migration in comparison with co-transfection with SPRY4-IT1 siRNA + empty vector (Fig. 5, P < 0.05).

Discussion
LncRNAs have become increasingly recognized as important regulators of gene expressions, as LncRNAs can regulate protein-coding genes at epigenetic, transcriptional, and post-transcriptional levels. Recent studies have shown that a lot of LncRNAs are frequently aberrantly expressed in various types of cancers, and the dysregulation of LncRNAs have been suggested to be associated with tumor pathogenesis and metastasis. They are also useful in tumor diagnosis and prognosis. Therefore, it is necessary to understand the molecular mechanisms of LncRNAs in cancer development and progression.

In this study, we detected the up-regulation of SPRY4-IT1 RNA in the HCC tissues when compared to adjacent non-cancerous liver tissues. Furthermore, the expression of SPRY4-IT1 was significantly correlated with TNM stage and tumor metastatic status. This was consistent with a previous study which also showed that SPRY4-IT1 was up-regulated in HCC tissues and was correlated with tumor differentiation, tumor size, and TNM stage. Furthermore, we performed the loss-of-function experiments to look into the in vitro molecular mechanisms of SPRY4-IT1 in HCC development. The in vitro results showed that knock-down of SPRY4-IT1 suppressed the cell proliferation, colony formation, cell invasion and migration in HCC cell lines, and the results were in agreement with previous studies showing that knock-down of SPRY4-IT1 suppressed the cell proliferation, colony...
formation, cell invasion and migration in several types of cancers including colorectal cancer, esophageal squa-
mal cell carcinoma, prostate cancer, glioma, gastric cancer, bladder cancer and breast cancer.17–20. Thus, our
results may suggest the oncogenic role of SPRY4-IT1 in the pathogenesis of HCC. In addition, the flow cytometry
was performed to investigate the mechanistic role of SPRY4-IT4 in cell cycle and cell apoptosis. We found that
knock-down of SPRY4-IT1 induced G2/G1 cell cycle arrest and also increased the apoptotic rate of HCC cell
lines. Similarly, in other types of cancers such as esophageal squamous cell carcinoma, breast cancer, lung cancer,
melanoma, knock-down of SPRY4-IT1 also induced G2/G1 cell cycle arrest and cell apoptosis.18,31–38 Therefore,
these results indicate that knock-down of SPRY4-IT1 inhibited HCC progression by inducing cell cycle arrest as
well as cell apoptosis.

The roles of ERRα in cancer development have been revealed in recent studies. In breast cancer, ERRα has
been extensively studied, in which dysregulation of ERRα not only contributes to the progression of breast can-
cer, but also closely associated with the chemo-resistance of breast cancers.34. In addition, ERRα was also found
to play an important role in prostate cancer. ERRα augments HIF-1 signaling by directly interacting with HIF-1α
in normoxic and hypoxic prostate cancer cells.35 However, the role of ERRα in HCC is still unclear. The elevated
levels of ERRα are associated with the increased cell proliferation and migration in breast cancer and prostate
cancer cells.36–37 In the present study, we demonstrated that knock-down of ERRα significantly inhibited cell
proliferation, colony formation, cell invasion and migration. Moreover, we examined whether SPRY4-IT1 had
an interaction with ERRα, and the transfection study showed that SPRY4-IT1 knock-down also suppressed the
expression of ERRα in HCC cells. More importantly, ERRα overexpression antagonized the effects of SPRY4-IT1
knock-down on HCC cell progression. These results may suggest that SPRY4-IT1 regulated HCC progression via
interacting with ERRα. However, our results only showed the preliminary findings about the interaction between
SPRY4-IT1 and ERRα. In order to find out more detailed molecular mechanisms underlying these interactions,
more experiments should be performed in the future studies.

In conclusion, the present study showed that SPRY4-IT1 was up-regulated in HCC tissues and was associated
with poor prognosis in HCC patients. Knock-down of SPRY4-IT1 inhibited cell proliferation, colony formation,
cell invasion and migration at least via interacting with ERRα. These results suggest that SPRY4-IT1 may serve as
a novel target for the management of HCC or as a biomarker for HCC diagnosis or prognosis, though the detailed
molecular mechanisms of SPRY4-IT1 on HCC development may require further investigation.

References
1. Stacy, S. et al. Patterns of consultation and treatment of patients with hepatocellular carcinoma presenting to a large academic
medical center in the US. Journal of gastrointestinal surgery: official journal of the Society for Surgery of the Alimentary Tract
17(9), 1600–1608 (2013).
2. Colecchia, A. et al. Prognostic factors for hepatocellular carcinoma recurrence. World journal of gastroenterology 20(20),
5935–5950 (2014).
3. Lee, S. C., Tan, H. T. & Chung, M. C. Prognostic biomarkers for prediction of recurrence of hepatocellular carcinoma: current status
and future prospects. World journal of gastroenterology 20(12), 3112–3124 (2014).
4. Beermann, J., Piccoli, M. T., Viereck, J. & Thum, T. Non-coding RNAs in Development and Disease: Background, Mechanisms, and
Therapeutic Approaches. Physiological reviews 96(4), 1297–1325 (2016).
5. Schmitz, S. U., Grote, P. & Herrmann, B. G. Mechanisms of long noncoding RNA function in development and disease. Cellular and
molecular life sciences: CMLS 73(13), 2491–2509 (2016).
6. Sullenger, B. A. & Nair, S. From the RNA world to the clinic. Science 352(6292), 1417–1420 (2016).
7. Wang, L. et al. Long non-coding RNA TUG1 promotes colorectal cancer metastasis via EMT pathway. Oncotarget (2016).
8. Qiu, J. J. & Yan, J. B. Long non-coding RNA LINC01296 is a potential prognostic biomarker in patients with colorectal cancer.
Tumour biology: the journal of the International Society for Oncodevelopmental Biology and Medicine 36(9), 7175–7183 (2015).
9. McCleland, M. L. et al. CCAT1 is an enhancer-templated RNA that predicts BET sensitivity in colorectal cancer. The Journal of
clinical investigation 126(2), 639–652 (2015).
10. Fang, J., Sun, C. C. & Gong, C. Long noncoding RNA XIST acts as an oncogene in non-small cell lung cancer by epigenetically
repressing KLF2 expression. Biochemical and biophysical research communications 478(2), 811–817 (2016).
11. Wu, Y. et al. Downregulation of the long noncoding RNA GAS5–AS1 contributes to tumor metastasis in non-small cell lung cancer.
Scientific reports 6, 31093 (2016).
12. Wang, R. et al. MiR-326 regulates cell proliferation and migration in lung cancer by targeting phox2a and is regulated by HOTAIR.
American journal of cancer research 6(2), 173–186 (2016).
13. Liu, M., Sun, W., Liu, Y. & Dong, X. The role of LncRNA MALAT1 in bone metastasis in patients with non-small cell lung cancer.
Oncology reports 36(3), 1679–1685 (2016).
14. Yu, J. et al. The long noncoding RNAs PVT1 and uc002mbe.2 in sera provide a new supplementary method for hepatocellular
carcinoma diagnosis. Medicine 95(31), e4346 (2016).
15. Li, S. P. et al. LncRNA HULC enhances epithelial-mesenchymal transition to promote tumorigenesis and metastasis of hepatocellular
carcinoma via the miR-200a-3p/ZEB1 signaling pathway. Oncotarget (2016).
16. Li, H. et al. LncRNA HOTAIR promotes human liver cancer stem cell malignant growth through downregulation of SETD2.
Oncotarget 6(29), 27847–27864 (2015).
17. Cao, D., Ding, Q., Yu, W., Gao, M. & Wang, Y. Long noncoding RNA SPRY4-IT1 promotes malignant development of colorectal
cancer by targeting epithelial-mesenchymal transition. Oncotargets and therapy 9, 5417–5425 (2016).
18. Xue-Liang, J., Qicong, D., Bi, Y., Bao, W. & Wang-Yue, W. Upregulated long noncoding RNA SPRY4-IT1 contributes to increased cell
viability by activating zinc finger 703 expression in esophageal squamous cell carcinoma. Indian journal of cancer 52(Suppl 3),
E164–167 (2015).
19. Zhao, X. L., Zhao, Z. H., Xu, W. C., Hou, J. Q. & Du, X. Y. Increased expression of SPRY4-IT1 predicts poor prognosis and promotes
tumor growth and metastasis in bladder cancer. International journal of clinical and experimental pathology 8(2), 1594–1600 (2015).
20. Zhou, Y., Wang, D. L. & Pang, Q. Long noncoding RNA SPRY4-IT1 is a prognostic factor for poor overall survival and has an
oncogenic role in glioma. European review for medical and pharmacological sciences 20(14), 3035–3039 (2016).
21. Horard, B. & Vanacker, J. M. Estrogen receptor-related receptors: orphan receptors desperately seeking a ligand. Journal of molecular
dermatoclinical oncology 31(3), 349–357 (2003).
22. Carnesecchi, J. & Vanacker, J. M. Estrogen-Related Receptors and the control of bone cell fate. Molecular and cellular endocrinology
432, 37–43 (2016).
23. Sonoda, J. et al. Nuclear receptor ERR alpha and coactivator PGC-1 beta are effectors of IFN-gamma-induced host defense. *Genes & development* **21**(15), 1909–1920 (2007).
24. Yuk, J. M. et al. Orphan Nuclear Receptor ERRalpha Controls Macrophage Metabolic Signaling and A20 Expression to Negatively Regulate TLR-Induced Inflammation. *Immunity* **43**(1), 80–91 (2015).
25. Villena, J. A. & Krali, A. ERRalpha: a metabolic function for the oldest orphan. *Trends in endocrinology and metabolism: TEM* **19**(8), 269–276 (2008).
26. Matsushima, H. et al. Anti-tumor effect of estrogen-related receptor alpha knockdown on uterine endometrial cancer. *Oncotarget* (2016).
27. Manna, S. et al. ERRalpha Is a Marker of Tamoxifen Response and Survival in Triple-Negative Breast Cancer. *Clinical cancer research: an official journal of the American Association for Cancer Research* **22**(6), 1421–1431 (2016).
28. Bernatchez, G. et al. ERRalpha metabolic nuclear receptor controls growth of colon cancer cells. *Carcinogenesis* **34**(10), 2253–2261 (2013).
29. Dwyer, M. A. et al. WNT11 expression is induced by estrogen-related receptor alpha and beta-catenin and acts in an autocrine manner to increase cancer cell migration. *Cancer research* **70**(22), 9298–9308 (2010).
30. Jing, W. et al. Potential diagnostic value of IncRNA SPRY4-IT1 in hepatocellular carcinoma. *Oncology reports* **36**(2), 1085–1092 (2016).
31. Shi, Y. et al. The long noncoding RNA SPRY4-IT1 increases the proliferation of human breast cancer cells by upregulating ZNF703 expression. *Molecular cancer* **14**, 51 (2015).
32. Mazar, J. et al. The functional characterization of long noncoding RNA SPRY4-IT1 in human melanoma cells. *Oncotarget* **5**(19), 8959–8969 (2014).
33. Sun, M. et al. EZH2-mediated epigenetic suppression of long noncoding RNA SPRY4-IT1 promotes NSCLC cell proliferation and metastasis by affecting the epithelial-mesenchymal transition. *Cell death & disease* **5**, e1298 (2014).
34. Deblouw, G. & Giguere, V. Oestrogen-related receptors in breast cancer: control of cellular metabolism and beyond. *Nature reviews Cancer* **13**(1), 27–36 (2013).
35. Zou, C. et al. ERRalpha augments HIF-1 signalling by directly interacting with HIF-1alpha in normoxic and hypoxic prostate cancer cells. *The Journal of pathology* **233**(1), 61–73 (2014).
36. Wu, D., Cheung, A., Wang, Y., Yu, S. & Chan, F. L. The emerging roles of orphan nuclear receptors in prostate cancer. *Biochimica et biophysica acta* **1866**(1), 23–36 (2016).
37. Han, L., Liu, B., Jiang, L., Liu, J. & Han, S. MicroRNA-497 downregulation contributes to cell proliferation, migration, and invasion of estrogen receptor alpha negative breast cancer by targeting estrogen-related receptor alpha. *Tumour biology: the journal of the International Society for Oncodevelopmental Biology and Medicine* 2016.

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**Author Contributions**

Guifang Yu designed and performed some of the experiments and wrote the manuscripts, Jieheng Lin and Min Liang collected HCC tissues, Chengcheng Liu and Kailian Hou cultured cells and performed functional assays, and Boyun Shi performed microbiology experiments.

**Additional Information**

**Competing Interests:** The authors declare that they have no competing interests.

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