The mechanism of *LncRNA01977* in lung adenocarcinoma through the SDF-1/CXCR4 pathway

Xiao-Rong Lai[1#], Chang-Li Wang[2#], Fei-Zhang Qin[3]

[1]Department of Respiratory, The 2nd Affiliated Hospital of Chengdu Medical College, (Nuclear Industry 416 Hospital) Chengdu, China; [2]Department of Laboratory Pathology, Xijing 986 Hospital Department, Fourth Military Medical University, Xi'an, China; [3]Department of Pharmacology, Guangxi Medical University, Nanning, China

Contributions: (I) Conception and design: XR Lai, CL Wang; (II) Administrative support: FZ Qin; (III) Provision of study materials or patients: All authors; (IV) Collection and assembly of data: All authors; (V) Data analysis and interpretation: All authors; (VI) Manuscript writing: All authors; (VII) Final approval of manuscript: All authors.

[#]These authors contributed equally to this work.

Correspondence to: Fei-Zhang Qin. Department of Pharmacology, Guangxi Medical University, Shuangyong Road, No. 22, Nanning, China. Email: qinfeizhang8@sina.com.

Background: A significant correlation has been identified between lncRNA and tumor cell resistance, diagnosis, and prognosis. Although mRNA studies have dominated the field of non-coding RNA biology in tumorigenesis in recent years, long-chain non-coding RNA (the biological function) has also attracted increasing attention. However, the lncRNA associated with lung adenocarcinoma (LUAD) remains unexplored. This study used bioinformatics analysis to screen and identify *LncRNA019*77 as a key oncogenic driver of LUAD.

Methods: The experiment was divided into blank serum group (normal serum medium) and lung compound low, medium and high dose groups (5%, 10%, 15% and 15% lung compound drug serum medium, respectively). Transwell invasion ability of A549 cells was detected, and Western blot tested A549 cells SDF-1 specific receptor CXCR4, and CXCR4 gene expression in A549 cells were determined by reverse transcription–polymerase chain reaction (RT-PCR). In addition, western blotting, MTT proliferation test, colony formation test and apoptosis detection techniques were used to explore the mechanism of LncRNA01977's effects on LUAD.

Results: *In vitro* assays demonstrated that *LncRNA019*77 can significantly promote the progression of LUAD and that stromal cells in tumor microenvironment secrete chemokine CXCL12, also known as stromal derived factor-1 (SDF-1), and its receptor CXCR4 is low expressed in normal tissues and high expressed in LUAD tissues. Lung cancer patients with high expression of CXCR4 are more prone to metastasis.

Conclusions: *LncRNA019*77 can be used as a new prognostic indicator of LUAD, and can help patients to find more effective target treatment options for LUAD.

Keywords: Lung adenocarcinoma (LUAD); SDF-1/CXCR4; *LncRNA019*77; LncRNA; tumor marker

Introduction

Studies have reported that lung adenocarcinoma (LUAD) has one of the highest mortality rates among all malignancies worldwide (1,2). Therefore, continuing research into the mechanisms of the malignant progression of LUAD is crucial, in order to discover more effective therapeutic targets for LUAD patients and develop more effective treatment strategies.

In cancer, LncRNA is generally highly expressed and is associated in many aspects of cancer development. LncRNA can promote the drug resistance of lung cancer cells or regulate miRNA to promote the apoptosis of lung cancer cells (3). Furthermore, recent studies (4,5) have suggested that lncRNA dysregulation promotes malignant cancer gene progression. *MALAT* can pass through the *YAP1-MALAT-miR-126-5p* axis, which promotes angiogenesis and epithelial-stromal transformation in colorectal cancer. Moreover, Du *et al.* (6) reported that *lncRNA* inhibits miRNA (MicroRNAs)-mediated CDK6 and FN1 degradation via adsorption of a *microRNA let-*7. Kikushige *et al.* (7) found that *lncRNA* is leading to the lung through the induction of autophagy activation Patients with adenocarcinoma are drug-resistant. There is increasing evidence that LncRNA01977 plays an important role in cell differentiation and development (8). Transcriptional profiling studies have found that LncRNA01977 is dysregulated in a variety of complex human diseases, highlighting the potential of LncRNA01977 as a biomarker for cancer diagnosis and prognosis. TCGA database includes gene expression, protein expression, DNA methylation, etc., which provides a good research basis for searching for tumor specific biomarkers. *MiRNA* usually affects gene expression processes (9). Since its discovery, miRNA has been shown to be involved in multiple pathological processes, including oncogenic effects (10). And it provide effective therapeutic targets for patients with LUAD (11,12). Chemokines and their receptors are considered to play an important role in tumor metastasis (13-15). Sdf-1 and its receptor CXCR4 have been widely studied. CXCR4 is highly expressed in a variety of highly metastatic tumors. Sdf-1/CXCR4 axis can activate a variety of signaling pathways and participate in tumor invasion and metastasis, improve cell motility, migration and invasion, and promote matrix degradation, intercellular adhesion and angiogenesis, suggesting that precise intervention of SDF-1/CXCR4 axis can inhibit tumor metastasis. It has potential clinical application prospect.

In this study, we used bioinformatics to screen and analyze *LncRNA01977* with significant upregulation features in LUAD. we also evaluated tumor proliferation and invasion of *LncRNA01977*. Our results suggested that *LncRNA01977* could promote LUAD progression by targeting the *SDF-1/CXCR4* axis.

We present the following article in accordance with the MDAR reporting checklist (available at https://tcr.amegroups.com/article/view/10.21037/tcr-21-2903/rc).

Methods

Gene screening and identification

These data were processed by using the edge R package, the parameters were set to FDR (False Discovery Rate) <0.05 and the absolute value $\log_2$>1. In addition, the TCGA pan-cancer of GTEx dataset was acquired from Xena (http://xena.ucsc.edu/). Moreover, we also mapped all the probes to the human genome by using the SeqMap method (GRCh38) to re-annotate the probe set of the GPL22755. All probes were mapped to a gene group. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Cell line selection and culture

A549 cells were cultured in RPMI medium containing 10% FBS in an incubator with 5% CO_2 and 37 ℃. A549 cells at logarithmic growth stage were selected and divided into blank serum group (normal serum medium) and experimental group. In addition, other LUAD cell lines such as HCC-78, KTA-7 and PC-9 were also cited for validation and study.

Plasmid construction

The DNA vector enables the target gene to be expressed in cells to achieve our research objectives. The artificially modified plasmid is one of the most commonly used vectors and usually has the following characteristics: (I) one or more restriction endonuclease cutting points for easy insertion of the target gene; (II) with marker genes (resistance genes, fluorescent genes, etc.) for easy screening; (III) suitable size (generally less than 10 KB) for transfection. Targeted LncRNA01977 short hairpin RNA (shRNA) sequence is as follows: 1,5'-TGTTCCTAATTTGGACACTGG TTTA-3'; and 2,5'-AACCAGATACCATGGAAT-3'.

Cell proliferation test

In this study, a 3-(4,5-dimethylthiazole-2-group)-2,5-diphenyl tetrazole (MTT) determination was used to assess cell viability. Cell viability was determined according to the CCK-8 system (Dojindo, Japan). Cells were seeded in 96-well plates (1,104 cells per well). Cells were incubated in 37 ℃ dark, adding 10 µL CCK-8 solution at 0, 24, 48, 72 h, and incubated for another 4 h. The absorbance of each well was then assessed at 450 nm, using a microplate reader (Tecan, Switzerland).

Cells and tissue specimens

Human LUAD cell lines HCC-78, KTA-7 and PC-9, and human bronchial epithelial cell lines A549 were all purchased from the Cell Bank of Typical Culture Conservation Committee, Chinese Academy of Sciences. All cell lines were cultured in DMEM medium (Dulbecco's Modified Eagle Medium, sigma, USA) containing 10% fetal bovine serum (Hyclone, General Life Sciences, USA), plus another 100 U/mL Streptomycin and 100 U/mL of penicillin (Thermo Fisher Scientific, USA). The culture conditions were obtained at 37 ℃ of 5% CO_2. Cells used in this study were all passed through 2 to 4 passages after resuscitation.

Cell culture

Human lung adenocarcinoma cells A549 were cultured using RPMI 1640 medium and Ham's F-12K medium with fetal calf serum with volume fraction of 0.1, and human normal lung bronchial epithelial cells BEAS-2B using DMEM medium with volume fraction of 0.1, both in a 5% CO_2, 37 ℃ incubator. A549 group: conventional culture without any treatment, A549/NC group: transfer to overexpression control plasmid, A549/*LncRNA01977* group: transfer to *LncRNA01977* overexpression plasmid.

Transwell trial

Two small interfering RNA species (small interfering RNA, siRNA, Oligobio, China) targeting *LncRNA01977* were designed and synthesized, and the negative scrambled control sequence siRNA (si-NC) was set. The TAF15 overexpression plasmid vector was constructed based on the pcDNA3.1 plasmid (Invitrogen, USA). Cells were seeded in six-well plates with a density of 1.8×10^5/well and placed at 37 ℃ culture conditions were used for 24 h. Subsequently, cells were transfected with the corresponding vector for DMEM containing 10% FBS without antibiotics for 48 h, and cells were collected for the relevant experiments.

Construction of SDF-1 lung adenocarcinoma cells

The wild-type *LncRNA01977* construct and *SDF-1* mimics were co-transfected into LUAD cells. After 24 h of incubation, the transfected cells were seeded in 96-well plates. 48 h after transfection, lung adenocarcinoma cells were detected using the dual luciferase reporter gene assay system (Promega).

Real-time PCR analysis

At 24 h after transfection, total RNA from cells in each group was extracted by TRIzol and transcribed by polymerase-chain reaction after reverse transcription. The primer sequence is as follows: *LncRNA01977* upstream primer: 5-CAGTGG ACTTGGTGACGCTGTATG-3, downstream primer: 5-CGCCTCGCCTAG TCTGGTC TC-3; GAPDH upstream primer: 5-GTTGGAGGT CGGAGTC AACGG-3, the downstream primer: 5-GAG GGATCTCGCTCCTGGAGGA-3.GAPDH was used as an internal reference in 3 independently repeated experiments.

Western blotting analysis

Total protein from transfected cells in each group were extracted and used for SDS-PAGE gel electrophoresis. Primary antibodies were prepared in 5% blocking buffer according to the dilution of the corresponding antibodies. 4 ℃ was incubated overnight and washed at room temperature and incubated with secondary antibodies for 1 h. After washing, they were visualized using an ECL chemiluminescent solution-actin was used as an internal reference for 3 independent repeated experiments.

Weighted gene co-expression network analysis

To obtain more accurate results, 1,000 permutation tests were performed. The Z-summary score was used to determine which modules are low-saving modules, with a Z-score <10 indicating that the module was weakly preserved.

Statistical analysis

All experiments in this study were repeated at least three times, and the experimental data were expressed as the mean ± standard deviation (SD). In addition, we performed statistical analysis using GraphPad Prism 8 (Graphpad, USA).

Results

LncRNA01977 predicts a poor prognosis in patients with LUAD

We performed different analyses in the TCGA and GSE155478 datasets, respectively based on the limma

package (*Figure 1A,1B*). High expression level of *LncRNA01977* had higher IC50 (Inhibitory Concentration) values for DOX compared with the other subgroups (*Figure 1C-1F*).

LncRNA01977 Inhibit the development of LUAD

Decreased number of colony formations (*Figure 2A-2C*). Cytotoxicity assays indicated that downregulation of LncRNA01977 reduced the DOX sensitivity of LUAD cells, with reduced IC50 values compared to the controls (*Figure 2D,2E*). The effect of LncRNA01977 on cell proliferation was assessed by EdU cell staining assay and CCK-8 cell proliferation assay. After inhibition of LncRNA01977 expression in A549, Calu-3, the number of EdU stained cells was significantly reduced, while the increase of EdU stained cells increased significantly after LncRNA01977 overexpression (P<0.05) (*Figure 2F,2G*).

Overexpression of LncRNA01977

We reached a similar conclusion in the CCK-8 trial. Upon inhibition of LncRNA01977, cell proliferation decreased and increased after overexpression of LncRNA01977 (*Figure 3A-3D*). Based on the knockdown results, *LncRNA01977* overexpression increased HCC-78, as did the IC50 values of DOX in KTA-7 and PC-9 cells (*Figure 3E*). LncRNA01977 overexpression group showed stronger DOX resistance (*Figure 3F-3H*).

LncRNA01977 and SDF-1 correlation analysis

Cell grade analysis revealed that assays to investigate whether *LncRNA01977* and SDF-1 were present in the RISC, and its main component is Argonaute2 (AGO2) (*Figure 4A,4B*). Furthermore, transfection of the *SDF-1* mimic reversed *LncRNA01977* overexpression, while the transfection of *LncRNA01977 SDF-1* expression was inhibited by the overexpression vector (*Figure 4C-4E*). To further investigate the relationship between *LncRNA01977* and *SDF-1* (*Figure 4F,4G*).

LncRNA01977 inhibits CXCR4 expression level

In order to analysis of correlation of *SDF-1*, we transfected the *SDF-1* simulants into *HCC-78*, *KTA-7* and *PC-9* cells. We found that LncRNA01977 promotes the growth and proliferation of lung adenocarcinoma cells through the SDF-1 pathway, and chemical resistance to DOX in *HCC-78*, *KTA-7* and *PC-9* 10 cells (*Figure 5A-5C*). Target Scan Human 7.2 (http://www.targetscan.org/vert_72/) was used to speculate that *LncRNA01977* expression could partially rescue this effect (*Figure 5D,5E*).

LncRNA01977 promotes LUAD progression by targeting the SDF-1/CXCR4 axis

Chemokines can recruit specific leukocytes to the inflammatory region. If sustained high expression of chemokines has the potential to cause extensive tissue damage to increase the probability of carcinogenesis. On the one hand, tumors can autonomously secrete or paracrine SDF-1 and express its receptor CXCR4 to stimulate tumor growth, on the other hand, it can also promote tumor growth by promoting angiogenesis (*Figure 6A-6F*).

Discussion

The chemokine SDF-1, also known as CXCL12 PBSF and its specific receptor, CXCR4, are widely expressed in various tissues and organs (16-20). SDF-1/CXCR4 signaling pathway have significant influence in the multiple tumors as well as metastasis, which may function through the MAPK or AKT pathway (21). The SDF-1/CXCR4 biological axis refers to the coupled molecule with close relationship to cell signal transduction and the interaction of CDF and its specific receptor CXCR4 (22-24). Cell migration lies in the high affinity and absolute specificity of CXCR4 for its ligand SDF, namely, CXCR4 is the only receptor for SDF. Studies show that the impact of the SDF-1/CXCR4 biological axis on tumors is multifaceted and playing an important role in the occurrence, development and metastasis of multiple tumors (25-30).

Lung cancer is the highest incidence and fatality malignancy worldwide (31). Despite the certain success of conventional therapies and targeted therapies, the 5-year overall survival rate of lung cancer is still only 10% to 15% (32-35). Recent immune-related targeted therapies such as cytotoxic T lymphocyte-associated antigens 4, the programmed death receptor 1 (PD-1), and the programmed death receptor Antibody-targeted therapy with body-ligand 1 (PD-L1) has shown good efficacy in many malignancies and also plays a role in the treatment of advanced NSCLC, suggesting that immune-related targeted therapy may have a potential role in lung cancer therapy (36-38). *LncRNA01977* is a long chain non-coding RNA of 200 nucleotides with
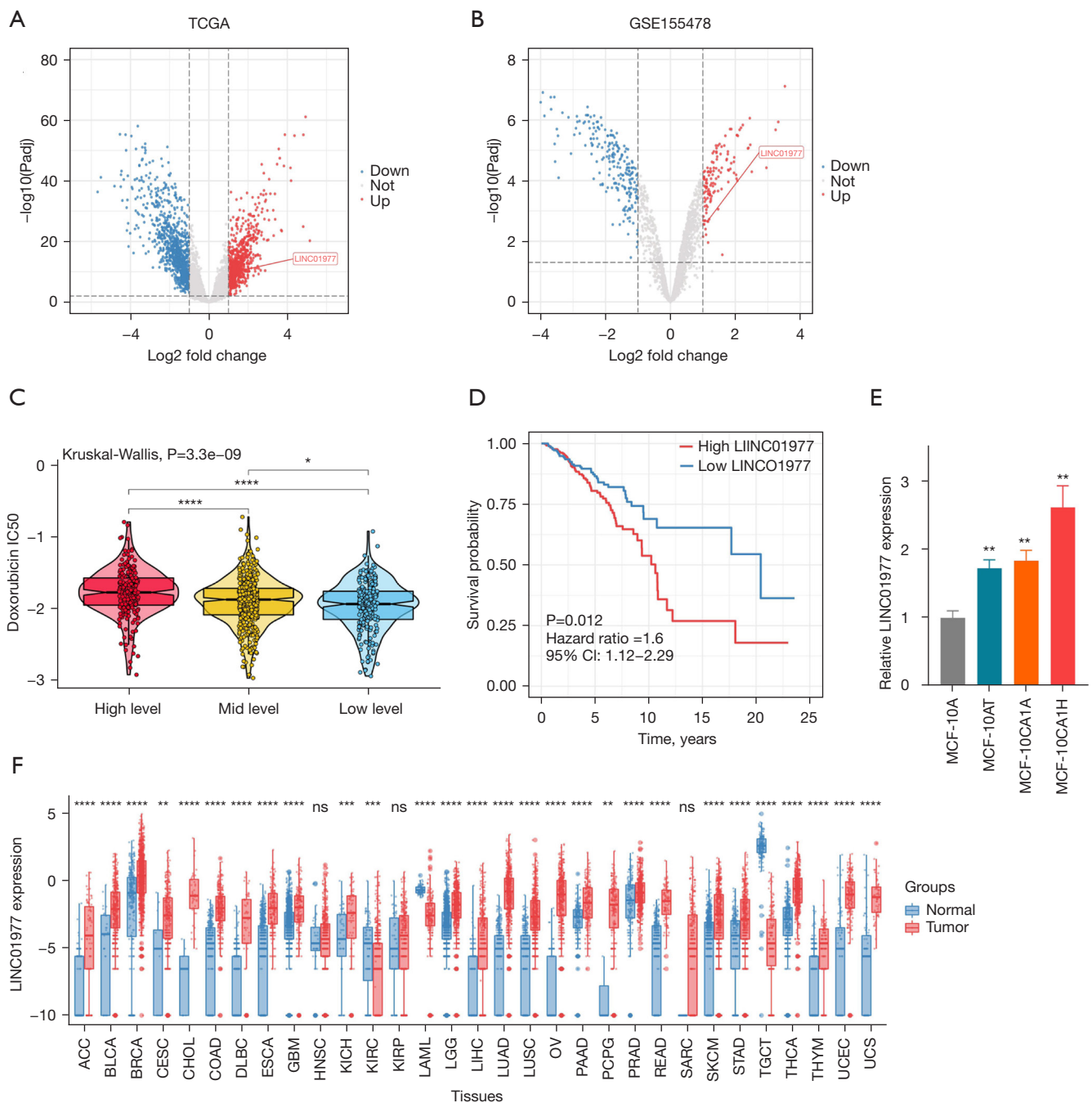
Figure 1 *LncRNA01977* is an oncogenic driver and predicts poor prognosis in patients with LUAD. (A) Paired normal samples of 112 tumors obtained from TCGA lung adenocarcinoma data; (B) different analysis of DOX-sensitive and DOX-resistant cell lines from GSE155478. (C) DOX IC50 estimated drug response in TCGA LUAD patients based on GDSC drug response data, with three subgroups representing the *LncRNA01977* expression levels. (D) TCGA LUAD patients Kaplan-Meier survival analysis. (E) Expression profile of *LncRNA01977* in LUAD progression cell line models. (F) Case of the expression of *LncRNA01977* in the pan-cancer dataset. LUAD, lung adenocarcinoma. *, P<0.05, **, P<0.01, ***, P<0.001, ****, P<0.0001; ns, meaningless; Student's *t*-test. LUAD, lung adenocarcinoma; DOX, Docetaxel; TCGA, The Cancer Genome Atlas; GDSC, Genomics of Drug Sensitivity in Cancer.
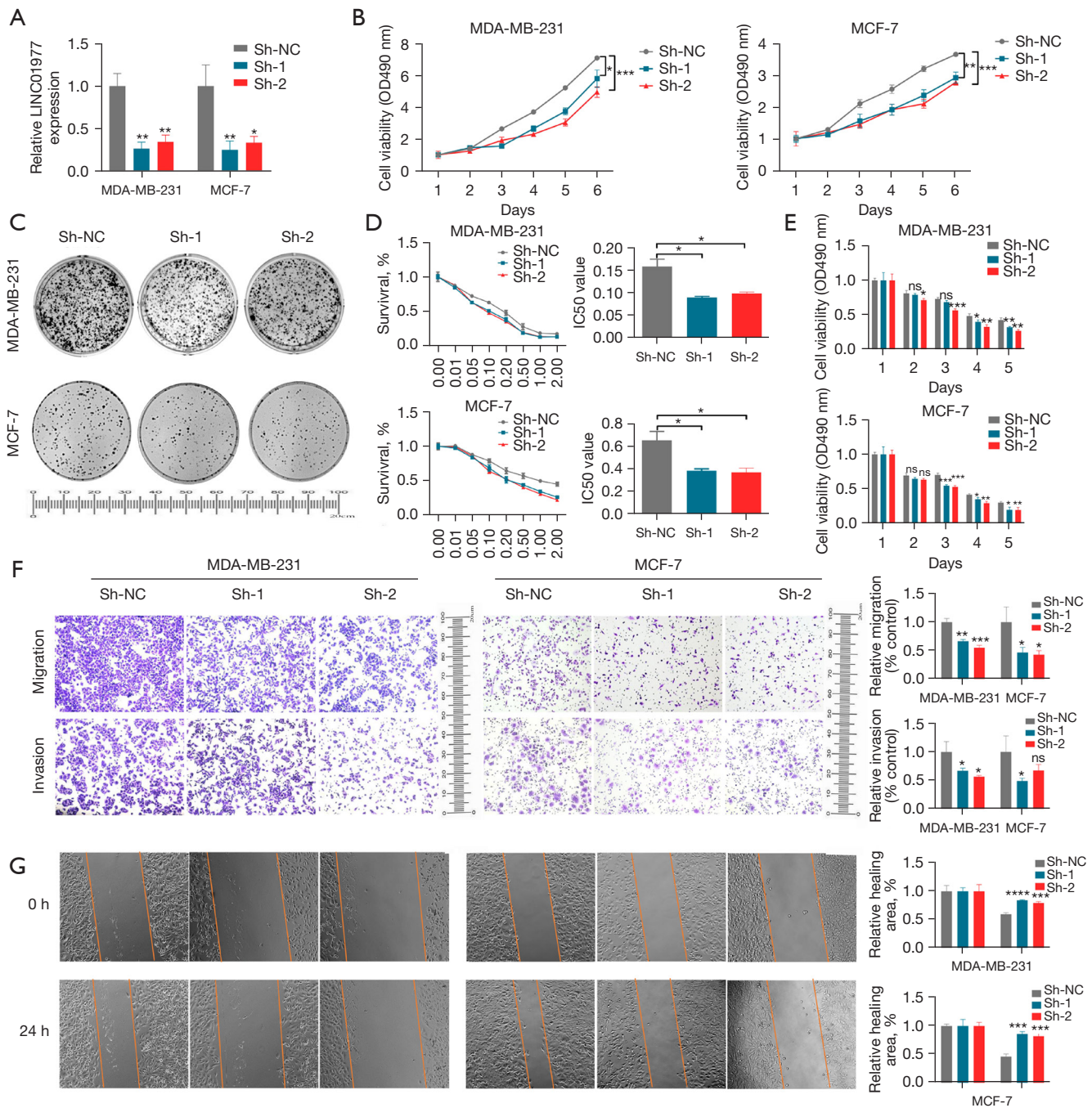
Figure 2 *LncRNA01977* knockdown decreased the proliferation, metastasis, and chemochemical resistance to DOX in LUAD cells. (A) The efficiency of shRNA knockdown was assessed by real-time PCR in *HCC-78*, *KTA-7* and *PC-9* cells. (B) The MTT assay was used to assess the cell viability of HCC-78, KTA-7 and PC-9 transfected with a control or knockdown plasmid. (C) The colony formation assay showed the effect of *LncRNA01977* knockdown on LUAD cell proliferation. (D) DOX IC50 in the control plasmid or *HCC-78*, *KTA-7* and *PC-9* transfected with the knockdown plasmid. (E) The cytotoxicity assay was used to test the inhibition of DOX resistance caused by *LncRNA01977* knockdown in *HCC-78*, *KTA-7* and *PC-9* cells. DOX concentration: 0.1 M in HCC-78, KTA-7 and PC-9 cells, 0.5 M in MCF-7 cells. (F) Cell migration and invasion assay [Cell HE staining]. (G) Cell scratch assay (*, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001). LUAD, lung adenocarcinoma; DOX, Docetaxel; TCGA, The Cancer Genome Atlas; PCR, polymerase chain reaction; MTT, Methyl tetrazolium; HE, hematoxylin-Eosin; NC, normal contras; ns, meaningless. (Observational method: High power microscope ×45).
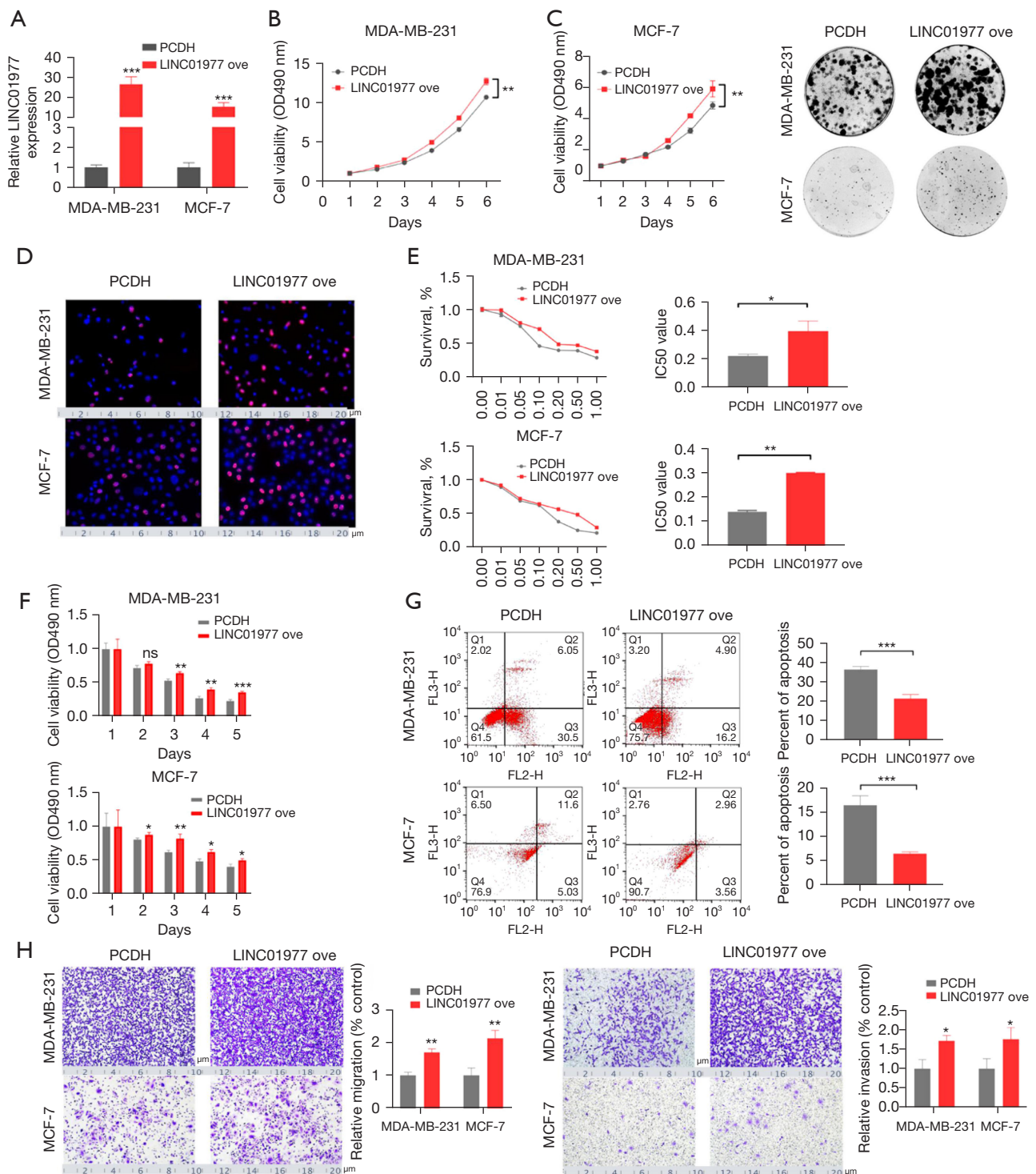
Figure 3 *LncRNA01977* overexpression promotes proliferation, metastasis and chemoresistance to DOX in LUAD and carcinoma cells. (A) The efficiency of *LncRNA01977* expression was assessed by real-time PCR in *HCC-78*, *KTA-7* and *PC-9* cells. (B) MTT assay. (C) Colony formation assay. (D) EDU assay. (E) IC50. (F) Cytotoxicity assay. (G) Apoptosis assay (*, P<0.05; **, P<0.01; ***, P<0.001). (H) Transwell assay (Staining method: Cell HE staining). MTT, Methyl tetrazolium; PCDH: Protocadherin; PCR, Polymerase Chain Reaction; EDU, Ethynyl-2'-deoxyuridine; HE, Hematoxylin-eosin; ns, meaningless; LUAD, lung adenocarcinoma; DOX, Docetaxel; TCGA, The Cancer Genome Atlas.
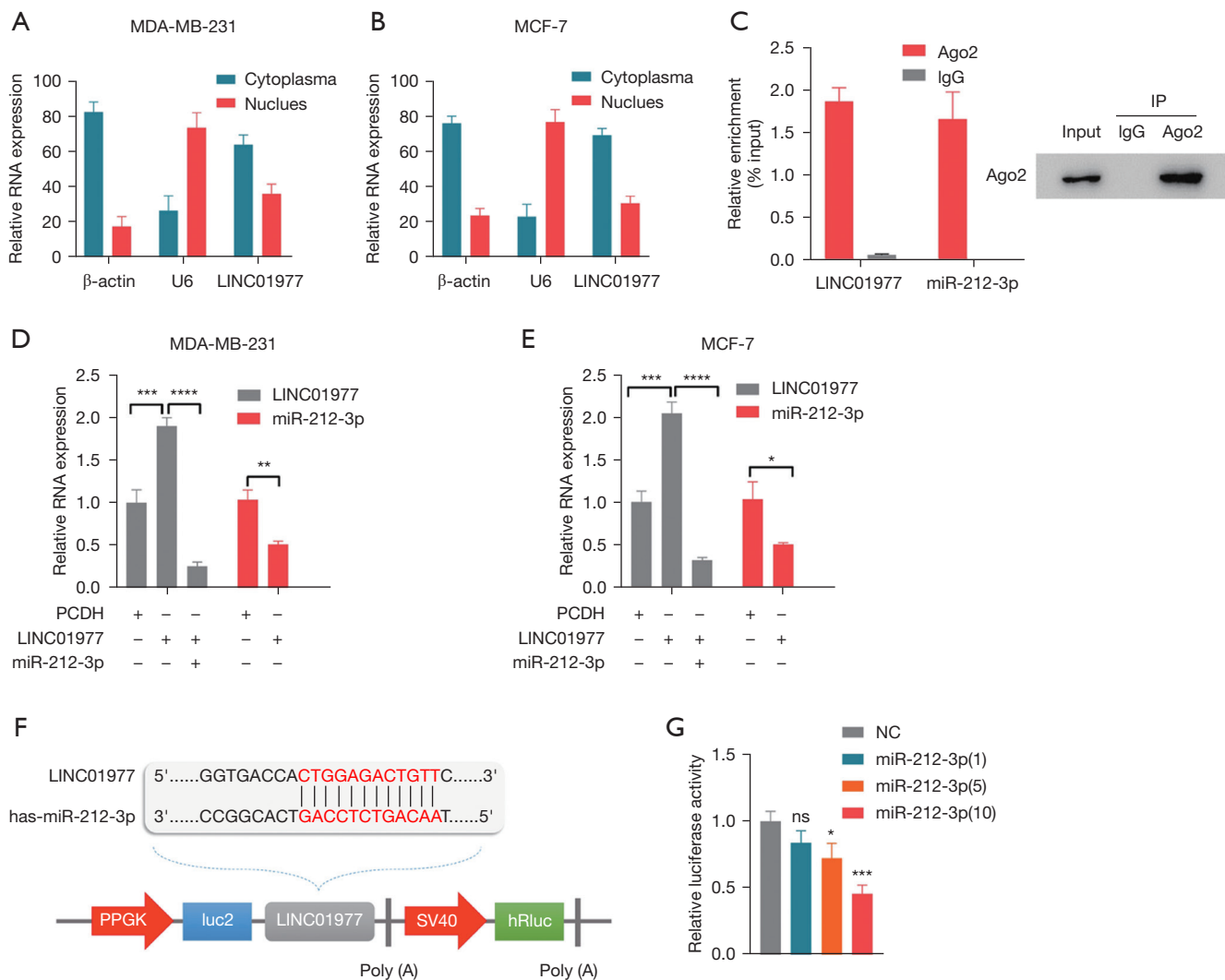
Transl Cancer Res 2022;11(3):475-487 | https://dx.doi.org/10.21037/tcr-21-2903

Figure 4 *LncRNA01977* acts as a molecular sponge by binding to SDF-1. (A,B) Subcellular localization of *LncRNA01977*, Actin: cytoplasmic positive control; U6: nucleus positive control. (C) Left panel: real-time PCR analysis of *LncRNA01977* and *SDF-1* enriched with AGO2 protein in HCC-78, KTA-7 and PC-9 cells. Right panel: protein blotting was used to confirm the specific immunoprecipitation of Ago2. (D,E) Real-time PCR confirmed the interactions between *LncRNA01977* and *SDF-1* in *HCC-78*, *KTA-7* and *PC-9* cells. (F) A schematic representation of the wild-type *LncRNA01977* luciferase reporter vector. (G) Double-luciferase gene activity detection. *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001; +, positive; –, negative; ns, meaningless. LUAD, lung adenocarcinoma; DOX, Docetaxel; TCGA, The Cancer Genome Atlas; NC, normal contras; PCR, polymerase chain reaction.

no or low translational potential, regulates gene expression at epigenetic, transcriptional and posttranscriptional levels and is widely involved in the organism pathophysiological process (39). For example, *LncRNA01977* can act as a target for a transcription factor or RNA polymerase, disrupting their binding to target gene promoters, thereby promoting

or repressing gene expression, which contain immune-related genes (40). Currently, the immune-related lncRNA has been less frequently reported in lung adenocarcinoma. The bioinformatics analysis of the immune-related lncRNA and its relationship with the prognosis of lung adenocarcinoma patients is expected to provide a
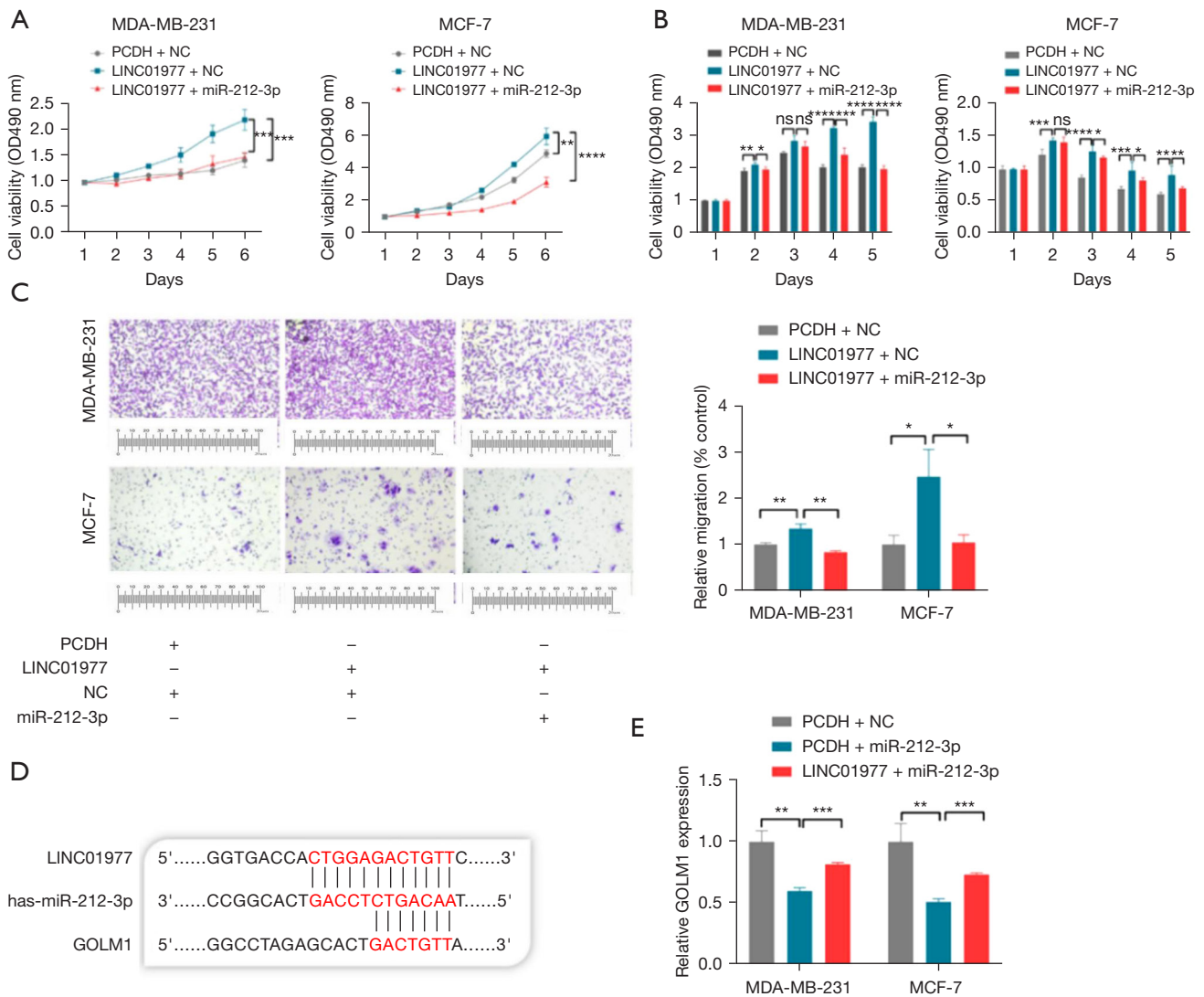
Figure 5 *LncRNA019*77 inhibits CXCR4 expression through the RNA mechanism. (A) *LncRNA019*77 promotion of cell proliferation was inhibited by *SDF-1* expression. (B) Inhibition of DOX resistance by *SDF-1* expression. (C) Promotion of SDF-1 expression suppresses the effect of *LncRNA019*77 on cell migration(Staining method: Cell HE staining). (D) Predicted *CXCR4* binding sites. (E) Real-time PCR identification of *miR212-3p* and *CXCR4* expression, and *LncRNA019*77 expression promotes *CXCR4* expression. *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$; ****, $P<0.0001$; +, positive; –, negative; ns, meaningless. LUAD, lung adenocarcinoma; DOX, Docetaxel; TCGA, The Cancer Genome Atlas; HE, hematoxylin-eosin; PCR, polymerase chain reaction.

reference for the prognosis judgment and treatment of lung adenocarcinoma (41). The SDF-1/CXCR4 biological axis plays an important role in the dissemination and organ-specific metastasis of a wide variety of tumors. It is suggested that intervention of SDF-1/CXCR4 biological axis can be used to treat tumors (42-44).

In conclusion, LncRNA01977 plays a very important regulatory role in the occurrence and development of lung adenocarcinoma, and its maladjustment will promote the rapid growth and proliferation of lung adenocarcinoma cells, and has a certain correlation with the metastasis of tumor cells. In addition, LncRNA01977 also has a proportional
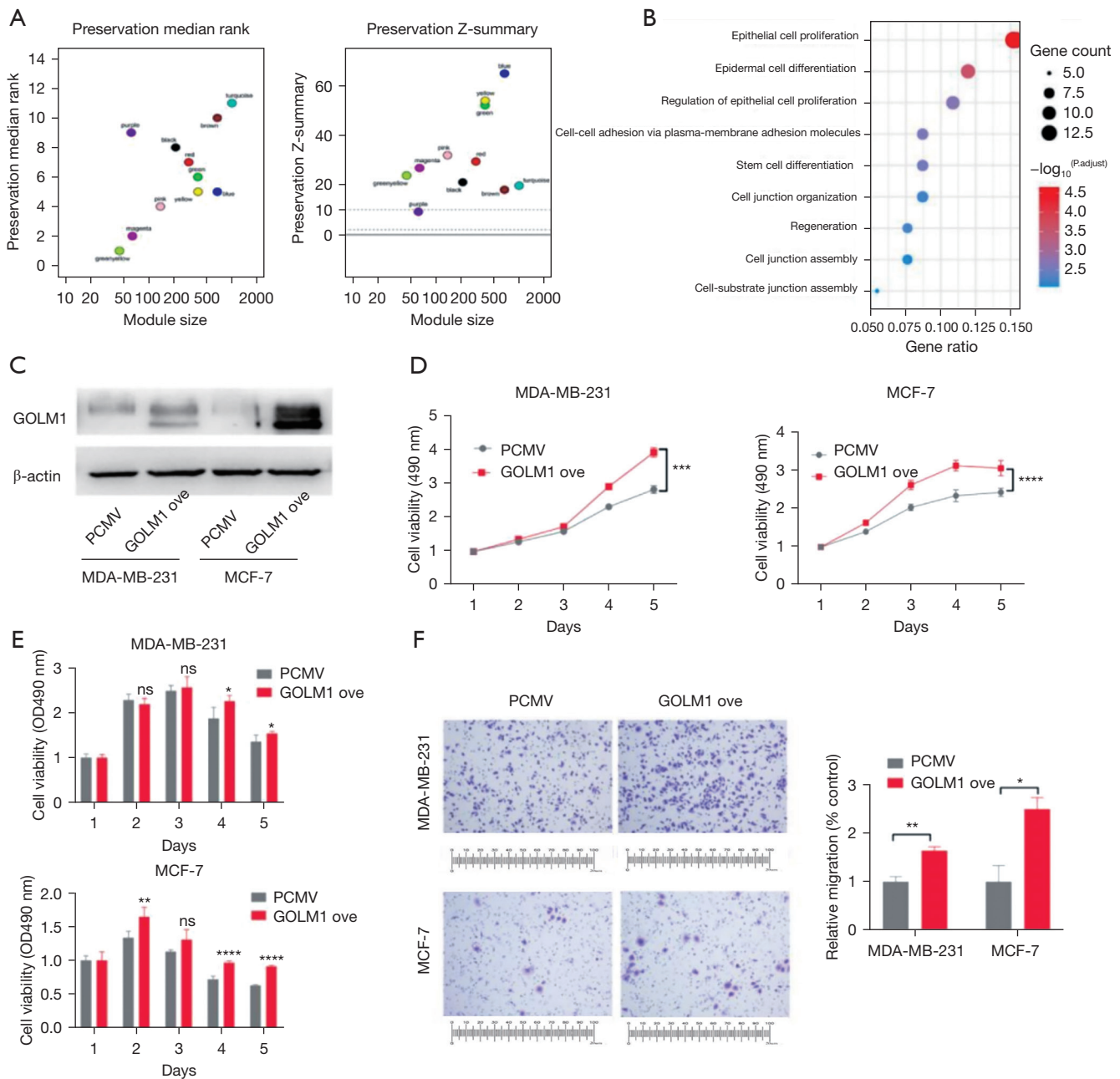
Figure 6 *LncRNA01977* promotes LUAD progression and chemoresistance to DOX by targeting the *SDF-1/CXCR4* axis. (A) WGCNA, the module preservation analysis in each module is represented by its color code and name. The left panel shows the save median level. The right figure shows the saved Z-summary value. A Z-summary value <10 represents the low-retention module. (B) Gene ontology analysis. (C) The expression efficiency of *CXCR4* was verified by the protein blotting assay. (D) MTT assay. (E) Cytotoxicity Assay. (F) Cell migration array (staining method: Cell HE staining). *, P<0.05; **, P<0.01; ***, P<0.001; ****, P<0.0001; ns, meaningless; Student's *t*-test; PCMV, number of a plasmid. LUAD, lung adenocarcinoma. DOX, Docetaxel; TCGA, The Cancer Genome Atlas; MTT, Methyl tetrazolium; HE, hematoxylin-eosin; WGCNA, weighted gene co-expression network analysis.

relationship with docetaxel resistance in patients with lung adenocarcinoma, and may play its biological effect by targeting sdF-1/CXCR4 axis. which broadens our understanding of post-transcriptional regulatory mechanisms, and helps to provide new prognostic indicators and therapeutic targets for patients with LUAD.

Acknowledgments

Footnote

Reporting Checklist: The authors have completed the MDAR reporting checklist. Available at https://tcr.amegroups.com/article/view/10.21037/tcr-21-2903/rc

Data Sharing Statement: Available at https://tcr.amegroups.com/article/view/10.21037/tcr-21-2903/dss

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tcr.amegroups.com/article/view/10.21037/tcr-21-2903/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

References

1. Das M, Zhu C, Kuchroo VK. Tim-3 and its role in regulating anti-tumor immunity. Immunol Rev 2017;276:97-111.
2. Datar I, Sanmamed MF, Wang J, et al. Expression Analysis and Significance of PD-1, LAG-3, and TIM-3 in Human Non-Small Cell Lung Cancer Using Spatially Resolved and Multiparametric Single-Cell Analysis. Clin Cancer Res 2019;25:4663-73.
3. Acharya N, Sabatos-Peyton C, Anderson AC. Tim-3 finds its place in the cancer immunotherapy landscape. J Immunother Cancer 2020;8:e000911.
4. Liu F, Liu Y, Chen Z. Tim-3 expression and its role in hepatocellular carcinoma. J Hematol Oncol 2018;11:126.
5. Tang R, Rangachari M, Kuchroo VK. Tim-3: A co-receptor with diverse roles in T cell exhaustion and tolerance. Semin Immunol 2019;42:101302.
6. Du W, Yang M, Turner A, et al. TIM-3 as a Target for Cancer Immunotherapy and Mechanisms of Action. Int J Mol Sci 2017;18:645.
7. Kikushige Y, Miyamoto T, Yuda J, et al. A TIM-3/Gal-9 Autocrine Stimulatory Loop Drives Self-Renewal of Human Myeloid Leukemia Stem Cells and Leukemic Progression. Cell Stem Cell 2015;17:341-52.
8. Wang Y, Zhao E, Zhang Z, et al. Association between Tim 3 and Gal 9 expression and gastric cancer prognosis. Oncol Rep 2018;40:2115-26.
9. Li YM, Li Y, Yan L, et al. Assessment of serum Tim-3 and Gal-9 levels in predicting the risk of infection after kidney transplantation. Int Immunopharmacol 2019;75:105803.
10. Song L, Wang Y, Sui Y, et al. High Interleukin-37 (IL-37) Expression and Increased Mucin-Domain Containing-3 (TIM-3) on Peripheral T Cells in Patients with Rheumatoid Arthritis. Med Sci Monit 2018;24:5660-7.
11. Zhang R, Li H, Bai L, et al. Association between T-Cell Immunoglobulin and Mucin Domain 3 (TIM-3) Genetic Polymorphisms and Susceptibility to Autoimmune Diseases. Immunol Invest 2019;48:563-76.
12. Gayden T, Sepulveda FE, Khuong-Quang DA, et al. Germline HAVCR2 mutations altering TIM-3 characterize subcutaneous panniculitis-like T cell lymphomas with hemophagocytic lymphohistiocytic syndrome. Nat Genet 2018;50:1650-7.
13. Ma X, Bi E, Lu Y, et al. Cholesterol Induces CD8+ T Cell Exhaustion in the Tumor Microenvironment. Cell Metab 2019;30:143-156.e5.
14. Oweida A, Hararah MK, Phan A, et al. Resistance to Radiotherapy and PD-L1 Blockade Is Mediated by TIM-3 Upregulation and Regulatory T-Cell Infiltration. Clin

Cancer Res 2018;24:5368-80.

15. Tan S, Xu Y, Wang Z, et al. Tim-3 Hampers Tumor Surveillance of Liver-Resident and Conventional NK Cells by Disrupting PI3K Signaling. Cancer Res 2020;80:1130-42.

16. Tomkowicz B, Walsh E, Cotty A, et al. TIM-3 Suppresses Anti-CD3/CD28-Induced TCR Activation and IL-2 Expression through the NFAT Signaling Pathway. PLoS One 2015;10:e0140694.

17. Dao TN, Utturkar S, Atallah Lanman N, et al. TIM-3 Expression Is Downregulated on Human NK Cells in Response to Cancer Targets in Synergy with Activation. Cancers (Basel) 2020;12:2417.

18. Phong BL, Avery L, Sumpter TL, et al. Tim-3 enhances FcεRI-proximal signaling to modulate mast cell activation. J Exp Med 2015;212:2289-304.

19. Gao X, Zhu Y, Li G, et al. TIM-3 expression characterizes regulatory T cells in tumor tissues and is associated with lung cancer progression. PLoS One 2012;7:e30676.

20. Yan W, Liu X, Ma H, et al. Tim-3 fosters HCC development by enhancing TGF-β-mediated alternative activation of macrophages. Gut 2015;64:1593-604.

21. Khalaf S, Toor SM, Murshed K, et al. Differential expression of TIM-3 in circulation and tumor microenvironment of colorectal cancer patients. Clin Immunol 2020;215:108429.

22. Zhang L, Tian S, Zhao M, et al. SUV39H1-DNMT3A-mediated epigenetic regulation of Tim-3 and galectin-9 in the cervical cancer. Cancer Cell Int 2020;20:325.

23. Xu Y, Zhang H, Huang Y, et al. Role of TIM-3 in ovarian cancer. Clin Transl Oncol 2017;19:1079-83.

24. Dama P, Tang M, Fulton N, et al. Gal9/Tim-3 expression level is higher in AML patients who fail chemotherapy. J Immunother Cancer 2019;7:175.

25. Shariati S, Ghods A, Zohouri M, et al. Significance of TIM-3 expression by CD4+ and CD8+ T lymphocytes in tumor-draining lymph nodes from patients with breast cancer. Mol Immunol 2020;128:47-54.

26. Li X, Wang B, Gu L, et al. Tim-3 expression predicts the abnormal innate immune status and poor prognosis of glioma patients. Clin Chim Acta 2018;476:178-84.

27. Cheng G, Li M, Wu J, et al. Expression of Tim-3 in gastric cancer tissue and its relationship with prognosis. Int J Clin Exp Pathol 2015;8:9452-7.

28. Zelba H, Bedke J, Hennenlotter J, et al. PD-1 and LAG-3 Dominate Checkpoint Receptor-Mediated T-cell Inhibition in Renal Cell Carcinoma. Cancer Immunol Res

2019;7:1891-9.

29. Xu YY, Wang SC, Lin YK, et al. Tim-3 and PD-1 regulate CD8+ T cell function to maintain early pregnancy in mice. J Reprod Dev 2017;63:289-94.

30. Kawaguchi N, Zhang TT, Nakanishi T. Involvement of CXCR4 in Normal and Abnormal Development. Cells 2019;8:185.

31. Bianchi ME, Mezzapelle R. The Chemokine Receptor CXCR4 in Cell Proliferation and Tissue Regeneration. Front Immunol 2020;11:2109.

32. Zhou Y, Cao HB, Li WJ, et al. The CXCL12 (SDF-1)/CXCR4 chemokine axis: Oncogenic properties, molecular targeting, and synthetic and natural product CXCR4 inhibitors for cancer therapy. Chin J Nat Med 2018;16:801-10.

33. Treon SP, Cao Y, Xu L, et al. Somatic mutations in MYD88 and CXCR4 are determinants of clinical presentation and overall survival in Waldenstrom macroglobulinemia. Blood 2014;123:2791-6.

34. Zhu Q, Luo R, Gu J, et al. High CXCR4 Expression Predicts a Poor Prognosis in Resected Lung Adenosquamous Carcinoma. J Cancer 2020;11:810-8.

35. Tahirovic YA, Pelly S, Jecs E, et al. Small molecule and peptide-based CXCR4 modulators as therapeutic agents. A patent review for the period from 2010 to 2018. Expert Opin Ther Pat 2020;30:87-101.

36. Martin M, Mayer IA, Walenkamp AME, et al. At the Bedside: Profiling and treating patients with CXCR4-expressing cancers. J Leukoc Biol 2021;109:953-67.

37. Jaffar J, Griffiths K, Oveissi S, et al. CXCR4+ cells are increased in lung tissue of patients with idiopathic pulmonary fibrosis. Respir Res 2020;21:221.

38. Mitchell B, Mahalingam M. The CXCR4/CXCL12 axis in cutaneous malignancies with an emphasis on melanoma. Histol Histopathol 2014;29:1539-46.

39. Liu N, Wan Q, Cheng Z, et al. Radionuclide-Labeled Peptides for Imaging and Treatment of CXCR4-Overexpressing Malignant Tumors. Curr Top Med Chem 2019;19:17-32.

40. Shi J, Wei Y, Xia J, et al. CXCL12-CXCR4 contributes to the implication of bone marrow in cancer metastasis. Future Oncol 2014;10:749-59.

41. Yang X, Lu Q, Xu Y, et al. Clinicopathologic significance of CXCR4 expressions in patients with esophageal squamous cell carcinoma. Pathol Res Pract 2020;216:152787.

42. Zhu Y, Tang L, Zhao S, et al. CXCR4-mediated

osteosarcoma growth and pulmonary metastasis is suppressed by MicroRNA-613. Cancer Sci 2018;109:2412-22.

43. Burger JA, Peled A. CXCR4 antagonists: targeting the microenvironment in leukemia and other cancers. Leukemia 2009;23:43-52.

44. Katsumoto K, Kume S. The role of CXCL12-CXCR4 signaling pathway in pancreatic development. Theranostics 2013;3:11-7.

(English Language Editor: A. Kassem)