Original Article

Elevated LOXL2 expression by LINC01347/miR-328-5p axis contributes to 5-FU chemotherapy resistance of colorectal cancer

Gui-Li Zheng\textsuperscript{1}, Yu-Lin Liu\textsuperscript{2}, Ze-Xuan Yan\textsuperscript{3}, Xiao-Ye Xie\textsuperscript{1}, Zhuo Xiang\textsuperscript{4}, Li Yin\textsuperscript{3}, Qing-Qing Wang\textsuperscript{2}, Dao-Chen Chong\textsuperscript{2}, Guo-Liang Xue\textsuperscript{1}, Li-Li Xu\textsuperscript{2}, Kai Zhou\textsuperscript{3}, Qiang Wang\textsuperscript{1,2}

\textsuperscript{1}Department of Oncology, 960\textsuperscript{th} Hospital of PLA, Jinan 250031, China; \textsuperscript{2}Clinical Laboratory, Navy 971\textsuperscript{st} Hospital of PLA, Qingdao 266071, China; \textsuperscript{3}Institute of Pathology and Southwest Cancer Center, Southwest Hospital, Army Medical University, Chongqing 400038, China; \textsuperscript{4}Department of Pharmacy, Navy 971\textsuperscript{st} Hospital of PLA, Qingdao 266071, China. \textsuperscript{*}Equal contributors.

Received July 8, 2020; Accepted October 22, 2020; Epub April 15, 2021; Published April 30, 2021

Abstract: Chemotherapy resistance after curative surgery is a major contributor to the mortality of colorectal cancer (CRC). Detailed mechanism studies of specific molecular alterations are critical to improving the available therapies for long-term disease administration. We explored the functional role of LINC01347 in chemotherapy resistance of CRC. Elevated LINC01347 expression was correlated with CRC disease progression during chemotherapy treatment. However, the functional role of LINC01347 and mechanism remained undefined. In this study, we demonstrated that elevated LINC01347 expression was correlated with late clinical stage and poor prognosis in CRC tumor tissues with TCGA data. Exogenous LINC01347 expression promoted cell proliferation and 5-FU resistance of CRC cells, while LINC01347 knockdown attenuated cell growth and 5-FU resistance in vitro and in vivo. Molecular analysis indicated that LINC01347 participated in the transcriptional regulation of LOXL2 by sponging miR-328-5p. LOXL2 knockdown impaired the LINC01347 overexpression induced 5-FU resistance in CRC cells. The clinical analysis supported miR-328-5p/LOXL2 as a candidate biomarker for chemotherapy resistance of CRC patients. Our study provided a molecular basis for the development of 5-FU based chemotherapy resistance in CRC by LINC01347/miR-328/LOXL2 axis. We identified LINC01347 as a prognostic biomarker and potential therapeutic target against 5-FU based chemotherapy resistance of CRC.

Keywords: Colorectal cancer, 5-FU, chemotherapy resistance, LINC01347, LOXL2, miR-328-5p

Introduction

5-Fluorouracil (5-FU) based chemotherapy is a predominant option for colorectal cancer (CRC), especially metastatic CRC [1]. However, it was still far from satisfactory in clinical outcome. Chemotherapy alone or in combination with other regimens showed limited efficiency for late-stage CRC [2]. More than half of the CRC patients suffered disease progression within 5 years, even cancer-related death. Further investigation was needed to identify molecules for chemotherapy resistance [3], which will develop a personalized and accurate treatment for CRC.

Recent studies supported long noncoding RNAs (lncRNAs) were involved in the gene expression regulation, although these transcripts could not translate into protein [4]. Some lncRNAs showed promoting or suppressing effects in malignant disease progression by genomic imprinting, gene transcription regulation and intranuclear transport [5]. High-throughput sequencing provides more information on lncRNAs which were involved in disease progression of CRC [6]. For instance, LncRNA HOTAIR and H19 showed significant prognostic value for colorectal cancer [7, 8]. Recent studies suggested some lncRNA transcripts worked as a ceRNA to competitively bind with miRNAs, resulting in the regulation of coding genes [9, 10]. Our recent study indicated LINC01347 overexpression was correlated with disease progression of CRC with unknown mechanisms. Thus, extensive molecular research on LINC-
01347 in CRC progression will provide us more evidence for disease diagnosis and prognosis.

Recent studies indicated the critical role of the lysyl oxidase family of proteins in fibrotic diseases and malignant progression. Among them, lysyl oxidase-like protein 2 (LOXL2) contributes to cancer progression [11, 12], which was correlated with its function in the extracellular matrix (ECM) [13, 14] and epithelial-mesenchymal transition (EMT) [15]. Recent evidence pointed out that EMT also showed significant roles in chemotherapy resistance [16, 17]. Further investigation was needed for the underlying role of LOXL2 and related gene regulation in chemotherapy resistance of CRC. In the present study, elevated LINC01347 was identified in CRC progression. We sought to assess the functional roles of LINC01347 and the modification mechanism of LOXL2 in CRC.

Materials and methods

TCGA analysis

The RNA-seq data were downloaded from TCGA Data Portal [18]. Totally of 455 tumor tissues and 41 matched normal samples were collected for analysis.

Cell culture

Totally three CRC cell lines (SW480, HCT116, and LoVo) and the 293T cells were used in this study. The cells were cultured in Dulbecco’s Modified Eagle Medium (Hyclone, Logan, UT) with 10% fetal bovine serum (Gibco, NY) in a 5% CO₂ humidified atmosphere at 37°C. The cells were passaged every three days within a totally continuous ten generations.

Cell transfection

Ectopic expression of LINC01347 plasmids and siRNAs were synthesized in RiboBio (Guangzhou, China). Cells were transfected with LINC01347 plasmids or mutant ones with Lipofectamine 2000 (Invitrogen, Green Island, CA) following the manufacturer’s instructions. The shRNA targeting LINC01347 was designed with a sequence of 5'-UUUUCAGCUACCCUUCUCAGGGAAAGGUGAAGAAAAAU-3'; shRNA targeting LOXL2 with a sequence of 5'-GCCACAUAGGUGUCCUCUCAUU-3'. The microRNA mimics, inhibitor, and negative control were purchased from RiboBio.

Quantitative RT-PCR (RT-qPCR)

The RT-qPCR assays were performed as previously described [19]. Primer sequences used in each PCR set were designed as follows: LINC01347: Forward 5'-TACCACATCTGTGTGATGG-3', Reverse 5'-TACCACATCTGTGTGATTG-3'; LOXL2: Forward 5'-AGGACATTCCGATTCCAGGCC-3', Reverse 5'-CTTCCTCTCAGGGAAGCAAC-3'; miR-328-5p: Forward 5'-GGGGGCAGGAGG-GC-3' and Reverse 5'-GTCGTATCCAGTGCGAGGG-TCCGAGTATTGCAGACTGACCAGAAC-3'. GAPDH and small nuclear RNA U6 was an inner control for gene or miRNA expression.

Cell proliferation assays

Cell Counting Kit-8 (CCK-8, Beyotime Inst Biotech, China) was used for the analysis of cell proliferation as previous reports [3]. In brief, 2000 cancer cells were seeded in a well of 96-well flat-bottomed plate, and cultured with indicated treatment (30 μM 5-FU). The light absorbance at 450 nm was measured with Multiskan Spectrum 1500 (Thermo Scientific, PA) to calculate relative cell proliferation after the indicated time's treatment.

Xenograft tumor formation

Subcutaneous xenografts were planted as a previous report [3]. Totally 12 nude mice (6 weeks old with an average weight of 20 g) were purchased from Shanghai SLAC Laboratory Animal Co. (Shanghai, China). Indicated cells (1 *10⁶) were subcutaneously injected in the right flanks of nude mice. Then the mice were treated with 5-Fu (20 mg/kg, i.p. every day) or PBS. Tumor volume was calculated at the indicated time with the formula: volume = length × width² × 1/2. The animal experiments were approved by the Institutional Animal Care and Use Committee of Army Medical University, following the Guide for the Care and Use of Laboratory Animals.

RNA pull-down assays

HCT116 cells were transfected with 20 nmol/L wild-type or mutant miR-328-5p mimics. Streptavidin-coated magnetic beads (Life Technologies, Baltimore, MD) were added to collect biotin-coupled RNA complex. Then RT-qPCR assays were performed to assess the abundance of LINC01347.
Luciferase reporter assays

Luciferase reporter assays were carried out by transfection of 500 ng of the reporter constructs in specified cell lines with indicated vectors. The assays were carried out using Dual-Luciferase Reporter Assay kit (Promega, Madison, WI) according to the manufacturer’s instructions.

Western blotting

Western blotting was performed as described elsewhere [19]. The antibodies against LOXL2 (ab96233, Abcam, Cambridge, MA), GAPDH (#5174, Cell Signaling Technology, Danvers, MA) were used in the immunoblotting assays.

Amplex Red Assay

Cells were cultured with indicated treatment and collected the cell culture media to measure the LOX family enzyme activity. The Amplex Red protocol (Thermo) and 2 mM benzylamine (Sigma) were used for the analysis following the manufacturer’s instructions.

Patients and specimens

Our study collected 452 consecutive CRC specimens in Navy 971 Hospital of PLA and 960th Hospital of PLA during the period from 2010 to August 2017. Surgery information and first-line chemotherapy regiments were obtained from the medical records. The treatment response and follow-up information were assessed as our previous report [3]. Tumor recurrence, metastasis or cancer-related death within three years was regarded as chemotherapy resistance. IHC staining for LOXL2 were performed with Ventana Discovery XT automated staining system (Ventana Medical Systems, Inc., Tucson, AZ) following the manufacturer’s instructions. Our study was reviewed and approved by the Navy 971 Hospital of PLA and 960th Hospital of PLA. All conducted experiments were strictly followed the ethical standards and the Declaration of Helsinki and guidelines.

Statistical analysis

The statistical analysis was performed with SPSS Statistics software v. 24 (SPSS Inc., Chicago, IL). Student’s t-test was used for the measurement data and the determination of p-values. Receiver operating characteristic (ROC) analysis was performed for the prognostic value. Kaplan-Meier survival analysis with log-rank statistics was performed to evaluate the survival estimation. Data are presented as mean ± SEM. And p-values < 0.05 were considered as statistically significant.

Results

Elevated LINC01347 expression indicates disease progression of CRC

We screened for the premising IncRNA in CRC disease progression with TCGA Data Portal, and found elevated LINC01347 expression in late-stage CRCs than early-stage CRCs (n = 192 vs. 262, res) (Figure 1A). Further analysis was performed with all paired tissues similar results, which also indicated higher LINC01347 expression in tumor tissues than corresponding normal ones (P = 0.005, Figure 1B). ROC analysis was performed for the prognostic value of LINC01347, which showed the area under the curve was 0.618, with a standard error of 0.035 (95% confidence interval 0.55-0.69, P < 0.001, Figure 1C). Kaplan-Meier analysis showed significantly shorter periods of overall survival (OS) and disease-free survival (DFS) for the patients with high LINC01347 expression (P = 0.010 and 0.013, respectively. Figure 1D, 1E). The results suggested that elevated LINC01347 expression participated in the disease-progression of CRC, which was a promising biomarker for the chemotherapy resistance for CRC patients.

LINC01347 promotes cell proliferation and 5-FU resistance of CRC cells

The expression levels of LINC01347 were examined in three CRC cell lines (SW480, HCT116 and LoVo). Another human embryonic stem cell line (HUES-17) was also used as a control. The RT-qPCR assays showed that significantly lower level of LINC01347 expression in SW480, HCT116 cells than HUES-17 cells (P < 0.01), while comparable LINC01347 levels was observed in LoVo cells (Figure 2A). Gain-of-function assays were performed to assess the role of LINC01347 in HCT116 and LoVo cells. The expression levels of LINC01347 were confirmed with RT-qPCR assays (Figure 2B). Exogenous expression of LINC01347 maintained
LINC01347 promotes 5-FU resistance

**Figure 1.** Expression of LINC01347 in CRC tissues and its prognostic value. A. LINC01347 was higher expressed in stage III-IV CRC tissues than stage I-II ones in the TCGA RNA-seq data (P = 0.003). *, t-test P < 0.01. B. LINC01347 showed significantly increased levels in CRC tissues in comparison to adjacent normal tissues in 41 paired patients. P = 0.005. C. ROC analysis was performed for the prognostic value of LINC01347 in CRC patients. The area under the curve was 0.618, standard error was 0.035, 95% confidence interval (0.55-0.69), P < 0.001. D, E. Positive LINC01347 expression patients showed poor overall survival and disease-free survival estimation in the TCGA database (n = 350).

the cell survival of HCT116 cell lines with 5-FU treatment according to CCK-8 assays, while decreased in LINC01347 knockdown LoVo cells (Figure 2C, 2D). Flowcytometry analysis indicated a positive correlation between LINC01347 levels and cell apoptosis in transfected cells with 5-FU treatment (Figure 2E). Subcutaneous xenografts were plated with HCT116 cells with stable LINC01347 overexpression. Then we treated the nude mice with 5-FU. Increased xenografts growth was observed in those of ectopic LINC01347 expressing HCT116 cells, which showed significantly larger volume than the control group (Figure 2F). Our data suggested that LINC01347 expression promoted cell proliferation and invasion of CRC cells.

*miR-328-5p binds to and suppresses LINC01347 expression*

To further explore the molecular mechanism of LINC01347 in CRC progression, we analyzed the candidate involved miRNAs with DIANA online tools. The results suggested that miR-328-5p and miR-26a/b-5p were the candidate miRNAs for LINC01347 transcriptional regulation (Figure 3A). We analyzed the correlation between LINC01347 and the candidate miRNAs with TCGA data. We found only miR-328-5p
LINC01347 promotes 5-FU resistance

A. Relative LINC01347 expression

B. Relative LINC01347 expression

C. Relative cell growth

D. Relative cell growth

E. Cell apoptosis (%)

F. Tumor volume (mm$^3$)
showed a significant reverse correlation with LINC01347 (Figure 3B). Then we performed RNA pull-down assays with miR-328-5p-wt or binding site mutant mimics. The results showed that higher levels of LINC01347 in miR-650-wt than mutant ones (Figure 3C). Moreover, luciferase vector with wild-type LINC01347 or binding site mutant ones were prepared (Figure 3D). Dual-luciferase assays indicated that only the co-transfection of wild-type LINC01347 and miR-328-5p mimics showed significant decrease in luciferase activities (Figure 3E). The RT-qPCR assays showed decreased levels of LINC01347 in miR-328-5p overexpressing HCT116 cells. The transfection of miR-328-5p together with LINC01347 in HCT116 cells also indicated decreased levels of LINC01347 (Figure 3F). These results supported that miR-328-5p bound to and suppressed LINC01347 expression in CRC cells. Furthermore, HCT116 cells were transfected with mutant LINC01347 plasmids to validate the role of LINC01347 in cell phenotype. Exogenous expression of mutant LINC01347 showed lower cell survival of HCT116 cells with 5-FU treatment than the wild type (Figure 3G). Flowcytometry analysis also indicated mutant LINC01347 expression showed higher percentage of cell apoptosis in HCT116 cells with 5-FU treatment than the wild-type ones (Figure 3H). Our results supported the crucial role of LINC01347 and miR-328-5p in 5-FU resistance.

**LINC01347 competed with miR-328-5p to regulate LOXL2 expression**

We further analyzed the mRNA expression profile of LINC01347 overexpressing cells. LINC01347 overexpression significantly increased LOXL2 expression, while LINC01347 knockdown inhibited the expression of LOXL2 by RT-qPCR analysis (Figure 4A). Elevated protein levels of LOXL2 in LINC01347 overexpressing cells were confirmed with Western blot assays (Figure 4B). Furthermore, the conditioned media from HCT116-LINC01347 cells demonstrated an increased active LOXL2 enzymatic function by Amplex Red assays (Figure 4C). Then we analyzed the role of miR-328-5p in the regulation of LOXL2 expression. Bioinformatic analysis predicted a binding site in 3’UTR of LOXL2 (Figure 4D). Wild type and mutant 3’UTR of LOXL2 luciferase vectors were prepared (Figure 4D). And luciferase activity results indicated that miR-328-5p inhibited the transcriptional activity of wild-type of LOXL2-3’UTR, whereas miR-328-5p failed to inhibit mutant ones (Figure 4E). Then we transfected HCT116 cells with LINC01347 and miR-328-5p together or with empty control. The RT-qPCR assays were performed to measure the expression levels of LOXL2. The results showed that miR-328-5p transfection inhibited the LOXL2 expression, which was induced by LINC01347 overexpression (Figure 4F). Then we examined the 5-FU resistance ability of transfected cells, which showed miR-328-5p inhibited cell proliferation in LINC01347 expressing cells with 5-FU treatment (Figure 4G). Moreover, flowcytometry assays also indicated decreased cell apoptosis when the LINC01347 overexpression cells were co-transfected with miR-328-5p (Figure 4H).

**LOXL2 knockdown inhibits LINC01347 induced 5-FU resistance of colorectal cancer cells**

We next assessed the functional relevance of LOXL2 in CRC cell survival in 5-FU treatment. A positive correlation was observed between LINC01347 and LOXL2 expression in TCGA data (Figure 5A). Moreover, significantly increased LOXL2 levels were observed in miR-328 expressing cells than negative ones (Figure 5B). Then LOXL2 knockdown was performed in LINC01347 expressing HCT116 cells, which was confirmed with Western blot assays (Figure 5C). Inhibited cell proliferation and viability were observed in LOXL2 knockdown cells com-

**Figure 2.** LINC01347 promotes cell proliferation and 5-FU resistance in vitro and in vivo. A. The RT-qPCR assays were performed for the expression levels of LINC01347 in CRC cell lines. GAPDH was used as a control. B. Cell transfection was performed with HCT116 and LoVo cells. The LINC01347 expression levels were confirmed with the RT-qPCR assays. C, D. The effects of LINC01347 in cell proliferation were assessed by CCK-8 assays. Transfected HCT116 and LoVo cells were treated with 5-FU. E. The percentage of cell apoptosis was evaluated in LINC01347 overexpressing or knockdown CRC cells and corresponding control cells with 5-FU treatment. Bar graphs represent cell apoptosis percentage (mean ± SD). F. HCT116 cells with stable LINC01347 overexpression were planted in nude mice for subcutaneous tumors (n = 6). Tumor volume was measured every three days. Photographs of the tumors excised 21 days after plantation. Bar, 1 cm. All in vitro results were obtained from three independent experiments. *, t-test P < 0.01.
**Figure 3.** miR-328-5p binds to and suppresses LINC01347 expression. A. The candidate miRNAs to competitively bind with LINC01347 were predicted with Online tools. Three candidate miRNAs were selected for further analysis. B. The correlation of LINC01347 with miR-328-5p in CRC tissues was analyzed with TCGA data, in which miR-328-5p showed a negative correlation with LINC01347. C. HCT116 cells were transfected with miR-328 or miR-mut. The RNA pull-down assays indicated abundant LINC01347 levels were observed in wild-type miR-328 mimics than mutant ones with RT-qPCR analysis. D. LINC01347 luciferase reporters with indicated binding sites were co-transfected with miR-328-5p mimics or negative control (NC) in HCT116 cells. E. The luciferase activity was compared between groups, which was plotted as the mean ± SEM. F. miR-328-5p were co-transfected with LINC01347 in HCT116 cells as indicated. The expression of mutant LINC01347 in HCT116 cell proliferation were assessed by CCK-8 assays, which were treated with 5-FU. H. The percentage of cell apoptosis was evaluated in mutant LINC01347 infected HCT116 cells and corresponding control cells with 5-FU treatment. Bar graphs represent cell apoptosis percentage (mean ± SD). All experiments were conducted independently at least three times. *, t-test P < 0.01.
LINC01347 promotes 5-FU resistance

Figure 4. LINC01347 competed with miR-328-5p to regulate LOXL2 expression. A. Exogenous LINC01347 expression or knockdown was performed with HCT116 and LoVo cells. Box plot depicting LOXL2 mRNA levels in transfected cells.
LINC01347 promotes 5-FU resistance

cells was assessed by RT-qPCR. GAPDH was used as a control. B. The expression levels of LOXL2 were evaluated with Western blotting. C. Amplex Red assays was performed to measure LOXL2 enzymatic activity in the conditioned medium of the transfected cells. D. The predicted binding site of LOXL2-3’UTR and miR-328-5p was shown. Wild-type or mutant LOXL2-3’UTR luciferase reporters were prepared for luciferase activity in HCT116 cells. E. Luciferase activity was analyzed in transfected cells, which showed miR-328-5p inhibited the transcriptional activity of wild-type of LOXL2-3’UTR. F. HCT116 cells were transfected with miR-328-5p and LINC01347 as indicated. Relative LOXL2 mRNA expression was analyzed by RT-qPCR assays. miR+LINC, miR-328-5p+ LINC01347 together transfection. G. Transfected cells were cultured for 3 days. Cell viability was detected with CCK-8 assays. H. The apoptotic activity was analyzed in transfected cells, which showed miR-328-5p inhibited the transcriptional activity of wild-type or mutant LOXL2-3’UTR luciferase reporters were prepared for luciferase activity in HCT116 cells. E. Luciferase activity was analyzed in transfected cells, which showed miR-328-5p inhibited the transcriptional activity of wild-type of LOXL2-3’UTR. F. HCT116 cells were transfected with miR-328-5p and LINC01347 as indicated. Relative LOXL2 mRNA expression was analyzed by RT-qPCR assays. miR+LINC, miR-328-5p+ LINC01347 together transfection. G. Transfected cells were cultured for 3 days. Cell viability was detected with CCK-8 assays. H. The apoptotic cell percentage of the transfected cells were analyzed. The data are presented as mean ± SEM of at least three independent experiments. *, t-test P < 0.01.

pared to control cells with 5-FU treatment (Figure 5D). Moreover, increased cell apoptosis was observed in LOXL2 knockdown cells with 5-FU treatment (Figure 5E). We next evaluated the effects of LOXL2 knockdown on tumor growth of HCT116-LINC01347 cells in vivo. [The xenografts from LOXL2 knockdown cells showed a significant decrease in tumor size and growth with 5-FU treatment (Figure 5F). These results indicated that LOXL2 participated in the 5-FU chemotherapy resistance, which was induced by LINC01347 in CRC.

MiR-328-5p/LOXL2 predicts poor chemotherapy sensitivity of colorectal cancer

We further evaluated the clinical significance of miR-328-5p/LOXL2 in a cohort of 273 CRC cases. The expression levels of miR-328-5p/LOXL2 were determined by RT-qPCR and IHC assays, respectively. IHC staining of LOXL2 indicated a heterogeneity location, which showed elevated expression levels in the invasive front (Figure 6A). Totally 42.12% of tumor specimens were classified into LOXL2 positive group (n = 115). Positive expression of miR-328-5p was observed in 17.95% of tumor specimens (n = 49). And 95 cases were determined as the miR-328-5p/LOXL2+ group. A higher percentage of LOXL2 positive specimens were observed in miR-328-5p negative ones than the others (38.84% vs. 10.20%, P < 0.001, Figure 6B). Among the patients, 5-FU-based first-line chemotherapy regimens were applied in a totally 233 patients. Chemotherapy resistance was observed in 45.06% (105/233) patients within 2.5 years after chemotherapy. Increased number of chemotherapy-resistant cases were observed in miR-328-5p/LOXL2+ group than others (67.39% vs. 30.71%, P < 0.001, Figure 6C). Moreover, the patients resistant to chemotherapy showed a higher percentage of miR-328-5p/LOXL2+ status (59.05% vs. 23.81%, P < 0.001, Figure 6D). Further Kaplan-Meier analysis was performed for the prognostic value of miR-328-5p/LOXL2+ with TCGA data, which indicated significant worse prognosis in OS and DFS in miR-328-5p/LOXL2+ group than other patients (P = 0.007 and P < 0.001, respectively. Figure 6E and 6F). Our results collectively supported the miR-328-5p/LOXL2+ status as a candidate biomarker for 5-FU based chemotherapy resistance in CRC patients.

Discussion

Previous studies indicated some IncRNAs showed significant promoting or suppressing effects on the disease progression of CRC, such as HOTAIR, H19 and ZFAS1 [7, 8, 20]. Here, we initially assessed the clinical relation of LINC01347 in CRC with TCGA data. Our results revealed that late-stage CRC tumors showed higher LINC01347 expression than early-stage tumors or adjacent normal tissues. More importantly, high LINC01347 expression predicted poor prognosis according to Kaplan-Meier analysis, which was a candidate detrimental factor for CRC patients. Our analysis implied that LINC01347 promoted CRC progression. Further in vitro and in vivo assays also confirmed the hypothesis. LINC01347 overexpression increased CRC cell growth and invasion ability, while the knockdown of LINC01347 inhibited tumor growth and chemotherapy resistance. These results supported that LINC01347 could be a therapeutic target for inhibiting CRC progression. However, a larger patient population is needed for clinical significance in future validation.

Recent studies supported the critical role of ceRNA in IncRNAs induced malignancy [9, 21]. The crosstalk between IncRNA and microRNA contributes to the disease progression of malignant tumors [22, 23]. To clarify the molecular mechanisms of LINC01347 in CRC progression, we analyzed the candidate competing miRNAs and identified the crosstalk with miR-328-5p. Previous studies indicated that miR-
LINC01347 promotes 5-FU resistance

A

LOXL2 expression
LINC01347 expression

$p=0.036$

B

LOXL2 mRNA expression

C

HCT116-LINC01347

NT
shLOXL2

LOXL2 (87 kD)
GAPDH (36 kD)

D

Relative cell proliferation

E

HCT116-LINC01347

NT
shLOXL2

F

HCT116-LINC01347

NT
shLOXL2

Cell apoptosis (%)

Tumor volume (mm³)

Am J Cancer Res 2021;11(4):1572-1585
LINC01347 promotes 5-FU resistance

Figure 5. LOXL2 knockdown inhibits LINC01347 induced colorectal cell proliferation and 5-FU resistance. A. The correlation of LINC01347 with LOXL2 in CRC tissues was analyzed with TCGA data, which showed a significantly positive correlation. B. Increased LOXL2 levels were observed in miR-328 negative CRC tissues compared to positive ones in the TCGA RNA-seq data (P = 0.029). C. LOXL2 knockdown was performed in LINC01347 overexpressing HCT116 cells. LOXL2 protein levels were evaluated with Western blotting. GAPDH was used as a control. D. Cell proliferation was assessed with CCK-8 assays, which showed significant inhibition in cell proliferation with LOXL2 knockdown. E. The cell apoptosis percentage was evaluated in LOXL2 knockdown CRC cells and control cells with 5-FU treatment. F. HCT116-LINC01347 cells with LOXL2 knockdown were planted in nude mice for subcutaneous tumors (n = 6). Tumor volume was measured every three days. Subcutaneous tumor pictures were presented (LOXL2 knockdown and control cells). NT, scramble control cells. Scale bar = 10 mm. #, t-test P < 0.05. *, t-test P < 0.01.

Figure 6. MiR-328-5p/LOXL2 predicts poor chemotherapy sensitivity of colorectal cancer. (A) IHC staining of LOXL2 was performed with CRC specimens. Representative images were provided with positive and negative expression.
LINC01347 promotes 5-FU resistance

In summary, our data supported a model in which LINC01347 enhances LOXL2 expression through competing with miR-328-5p. Gain of function assays indicated that LINC01347 expression was involved in cell viability and chemoresistance of CRC. Evaluation of LINC-01347 levels provided important prognostic information for CRC patients. These findings provide a molecular basis for the promotion of chemoresistant cancer phenotype by LINC-01347/miR-328/LOXL2 axis.

Acknowledgements

This research was supported by grants from National Natural Science Foundation of China (81972793, 81803400, 81602730, 8150-2283), Shinan District Science and Technology Plan, Qingdao (2016-3-020-YY and 2018-4-026-YY), Shandong province Medical Health Science and Technology Project (2018WS447), Qingdao Science and Technology Program for Benefiting People Special Project 2019 (19-6-1-25-nsh), and Qingdao Outstanding Health Professional Development Fund.

Disclosure of conflict of interest

None.

Abbreviations

CRC, colorectal cancer; 5-FU, 5-Fluorouracil; lncRNAs, long noncoding RNAs; LOXL2, Lysyl oxidase-like 2; RT-qPCR, Quantitative RT-PCR; ECM, extracellular matrix; EMT, epithelial-mesenchymal transition; CCK-8, Cell Counting Kit-8; FAK, focal adhesion kinase.

Address correspondence to: Qiang Wang, Department of Oncology, 960th Hospital of PLA, Jinan 250031, China. E-mail: wangqiang401@gmail.com

References

LINC01347 promotes 5-FU resistance


[22] Ji D, Qiao M, Yao Y, Li M, Chen H, Dong Q, Jia J, Cui X, Li Z, Xia J and Gu J. Serum-based microRNA signature predicts relapse and thera-
LINC01347 promotes 5-FU resistance


