Long non-coding RNA LINC00115 contributes to the progression of colorectal cancer by targeting miR-489-3p via PI3K/AKT/mTOR pathway

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Abstract

Background

Long noncoding RNAs (lncRNAs) are tumor-related regulators and have been found to be involved in the underlying molecular mechanisms of colorectal cancer (CRC). However, the role of lncRNA LINC00115 during CRC progression is not entirely elucidated.

Methods

The expression of LINC00115 was analyzed in paired CRC tissue samples and its clinical significance was evaluated. The biological effects on CRC cells proliferation, apoptosis, migration, invasion and PI3K/AKT/mTOR signaling were assessed by Cell Counting Kit-8 assay, Transwell assay, flow cytometry analysis and Western blot, respectively. The regulatory relationship between LINC00115 and miR-489-3p was determined by dual-luciferase reporter assays.

Results

LINC00115 was significantly overexpressed in CRC and its overexpression predicted poor outcome of the patients. Downregulation of LINC00115 markedly inhibited CRC cell proliferation, increased cell apoptosis, and suppressed cell migration and invasion. Moreover, downregulation of LINC00115 led to the inactivation of PI3K/AKT/mTOR signaling. Bioinformatics analysis identified miR-489-3p as a candidate target of LINC00115. Furthermore, we revealed an inverse correlation between LINC00115 and miR-489-3p in CRC tissues. miR-489-3p might directly target LINC00115 and downregulation of miR-489-3p could rescue the biological effects induced by the absence of LINC0015.

Conclusion

LINC00115 serves as an excellent oncogene of CRC metastasis, the deeper understanding of LINC00115/miR-489-3p axis might provide potential therapeutic targets for CRC metastasis.

Background

As an aggressive and metastatic disease, colorectal cancer (CRC) ranks the fifth most common cancer globally and the third most common cause of cancer associated mortality [1]. In a nearly report by Chen et al, CRC mounts to the second leading cause of cancer mortality and its incidence still keeps rising in China[2]. Although mounting improvements in surgical and chemotherapy progress have been achieved during the past decades, the survival and prognosis are still unsatisfactory because majority of the patients with advanced CRC accompanied by emergent chemoresistance, cancer recurrence or distant metastasis[3, 4]. Furthermore, the underlying molecular mechanisms of CRC carcinogenesis and
progression has not yet been fully understood. In recent years, research has reported that CRC develops through the accumulation of genetic mutants and epigenetic modifications[5–7]. Thus, it is extremely necessary to develop a better understanding of development and progression of CRC to identify the novel improved therapeutic strategies for CRC patients.

Noncoding RNAs (ncRNAs), including microRNAs (miRNAs) and long ncRNAs (lncRNAs), have been recently verified to be involved in the regulation of protein-coding genes in both physiological and in pathological conditions[8–10]. In the study of carcinogenesis and progression of some miRNAs are reported to play a crucial role in the modulation of cell function such as cell growth, invasion, autophagy, and apoptosis[11, 12]. For example, miR-708 is reported to be one of the lowest expressed miRNAs in CRC tissues and could suppress cell proliferation, induce apoptosis, and reduce metastasis by directly targeting ZEN1. Just like the extensive participation of miRNAs in cancer, lncRNAs, which are transcripts usually longer than 200 nucleotides with limited protein-coding capability have attracted much more attention due to their unique role during cancer development and progression. Several lncRNAs such as metastasis-associated lung adenocarcinoma transcript 1 (MALAT1), HOX transcript antisense RNA (HOXTAIR), TTN antisense RNA 1 (TTN-AS1), and small nucleolar RNA host gene 6 (SNHG6) and long intergenic non-protein coding RNA 1503 (LINC001503) have been elucidated recently to be involved in ceRNA network and regulate gene expression in several cancers, including CRC[13–18]. For instance, IncRNA growth arrest-specific 5 (GAS5) upregulated the expression of phosphatase and tensin homologue (PTEN) by functioning as a ceRNA of miR-222-3p, thus inhibiting CRC cell migration and invasion and promoting cell autophagy[19]. It is certain that the interplay between lncRNAs and miRNAs exerts their potential roles in CRC carcinogenesis and progression and is critical the regulatory network of CRC.

In our study, we firstly detected the expression patterns of LINC00115 in CRC and found that LINC00115 was significantly upregulated in CRC. We also found that knockdown of LINC00115 significantly inhibited CRC cell proliferation, induced cell apoptosis and suppressed metastatic capacity. Bioinformatics analysis revealed a potential interaction between LINC00115 and miR-489-3p. Therefore, we explored the expression profiles of miR-489-3p in CRC tissues and verified its relationship with LINC00115, revealing a promising LINC00115/miR-489-3p axis that contributes to the progression of CRC.

**Materials And Methods**

**Tissue samples**

The CRC and normal tissue samples were selected from 100 CRC patients who were treated with radical surgery in Henan Oncology Hospital between 2012 to 2014. In addition, all patients signed the informed consent voluntarily, and their tissue samples had been confirmed by at least three experienced pathologists. Moreover, this study had been approved by the medical ethics committee of Henan Oncology Hospital approved the study.

**Cell culture**
Five CRC cancer cell lines (LoVo, HT-29, Caco-2, SW620 and SW480) and normal epithelial cell lines (FHC) were obtained from the ATCC (Shanghai, P. R. China). In addition, the DMEN medium (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Gibco, Australia origin) were used for cell incubation. Moreover, these cells were cultured in the incubator with 37°C and 5% CO₂.

Cell transfection

The LINC00115-specific small interfering RNAs (siRNAs) were applied to transfect the CRC cell lines (including HT-29 and LoVo) with Lipofectamine 3000 Transfection Reagent (Thermo Fisher, USA). In addition, the miR-489-3p inhibitors or relative negative control sequence (miR-487-3p NC) were further transfected the LINC00115-1 down-regulated CRC cells were further transfected with Lipofectamine 3000 Transfection Reagent. Moreover, the complete sequences of siRNA are listed in Table I.

RT-qPCR assay

According to the manufacturer's protocol, the total RNA of tissue samples or CRC cell lines were all extracted with the Trizol reagent (Takara, Dalian, P. R. China). Then, the Prime Script RT Master Mix (Takara) were used to reversed transcribe 500 ng RNA into cDNA, followed by the cDNA were amplified with SYBR-Green PCR kit (Roche, Basel, Switzerland). The expression levels of targets genes were analyzed with $2^{-\Delta\Delta Ct}$ method. The complete sequences of primes are showed in Table 1.

| Name             | Sequence (5'-3')                                      |
|------------------|------------------------------------------------------|
| si-LINC00115-1   | AAG GAA UUU GGG AAU UAG GCU                         |
|                  | CCU AAU UCC CAA AUU CCU UAG                         |
| si-LINC00115-2   | UUG GAA AUC AGA AAA CCA CUA                         |
|                  | GUG GUU UUC UGA UUU CCA AAU                         |
| LINC00115 forward| TGG CTT GTC TTC CAT CGT CC                           |
| LINC00115 reverse| GCA CGA GGG TTG TTA CAG GA                          |
| miR-489-3p forward| AGG GGG TGA CAT CAC ATA TAC                         |
| miR-489-3p reverse| GAG AGG AGA GGA AGA GGG AA                          |
| GAPDH forward    | CCT TCC GTG TCC CCA CT                               |
| GAPDH reverse    | GCC TGC TTC ACC ACC TTC                              |

Cell counting kit 8 (CCK-8) assay

Firstly, the treated cells were collected and planted in 96-well plates. After incubation for 24, 48, 72 and 96 h, diluted CCK-8 solution was added into each well for reaction respectively. Subsequently, OD value
was collected by a microplate reader (Molecular Devices, Sunnyvale, CA, USA) at 450 nm.

**Transwell assay**

The Transwell chambers (0.8. µm; Corning, NY, USA) with or without Matrigel coating (Corning) were used to evaluate the cell migration and invasion abilities. Firstly, the treated cells were collected and placed in the top chamber, while 500 µl DMEN medium with 20% FBS were added in the low chamber. Subsequently, after removing the non-invading and non-migrating cells with cotton-tipped swabs, the other cells were fixed and stained with 0.1% crystal violet. Finally, the invaded and migrated cells were imaged with a light microscope.

**Flow cytometric analysis**

For cell apoptosis rate detection, cells were treated with a cell apoptosis detection kit (Keygen, Nanjing, P. R. China) and were evaluated by a FACSCanto II flow cytometer (San Jose, CA, USA) according to the manufacturer’s instructions.

**Dual-luciferase reporter assays**

Cells were transfected with pmirGLO-LINC00115-MUT or pmirGLO-LINC00115-WT plasmid, and then co-transfected with miR-489-3p mimics or miR-489-3p NC. Then, the relative luciferase activity was detected and evaluated by the dual-luciferase reporter assay system (Promega, Madison, WI) according to the manufacturer’s instructions.

**Western blotting**

RIPA Lysis buffer (Beyotime, Shanghai, P. R. China) were applied to extract the total proteins. Subsequently, concentration of the total proteins was detected and evaluated via the BCA protein Assays Kit (Beyotime). Then, equal quantities of proteins were separated on 10% SDS-PAGE gel, and then transferred into polyvinylidene fluoride (PVDF) membranes. Subsequently, the membranes were incubated with 10% BSA, and then with primary antibodies (PI3K, p-PI3K, AKT, p-AKT, mTOR, p-mTOR and GADPH), and then incubated with secondary antibodies. Finally, GeneSnap using SynGene systems was performed to evaluate the protein bands.

**Statistical analysis**

All data were showed as mean ± standard deviation (SD). Then, GraphPad Prism v7.0 software and IBM SPSS 20.0 software were applied for data evaluation in this study. All experiments were performed at least three times. Furthermore, data difference between two groups were evaluated by Chi-square test or student’s t test. When \( P < 0.05 \), significant differences were considered.

**Results**

**LINC00115 is upregulated in CRC**
Expression pattern of LINC00115 in CRC tissues was detected using RT-qPCR assay, in which LINC00115 was founded to be dramatically upregulated in CRC tissue samples (Fig. 1a, \( P \leq 0.001 \)). Meanwhile, in this study cohort, cases with LINC00115 overexpression accounted for 76% (76/100) of CRC patients (Fig. 1b). Subsequently, the tissue samples cohort was divided into low expression group and high expression group according to the median of relative LINC00115 expression. Further statistical analysis revealed that expression of LINC00115 was highly associated with T stage (\( P = 0.000 \)), N stage (\( P = 0.000 \)) and TNM stage (\( P = 0.000 \)) (Table 2). In addition, we performed multivariate analysis with Cox regression model to evaluate the prognostic correlation between LINC00115 expression and clinicopathological characteristics of patients with CRC. The results showed that TNM stage (\( P = 0.000 \)) and LINC00115 expression (\( P = 0.000 \)) were highly related with relapse-free survival time of CRC patients, while multivariate analysis showed that TNM stage (95%CI 2.308–22.954, \( P = 0.001 \)) and LINC00115 expression (95%CI 1.203–5.108, \( P = 0.014 \)) might be the independent risk factors for CRC patients in relapse-free survival (Table 3). Taken together, our results indicated that LINC00115 was upregulated in CRC tissues, and its expression was closely associated with pathogenesis and outcome of CRC patients.
Table 2
Correlation between LINC00115 expression and clinicopathological characteristics of patients with colorectal cancer (n = 100).

| Variables            | LINC00115 expression | P |
|----------------------|----------------------|---|
|                      | High | Low |    |
| Gender               |      |     |    |
| Male                 | 32   | 25  | 0.157 |
| Female               | 18   | 25  |    |
| Age (years)          |      |     |    |
| < 60                 | 33   | 32  | 0.834 |
| ≥ 60                 | 17   | 18  |    |
| Histologic grade     |      |     |    |
| Well                 | 19   | 20  | 0.296 |
| Moderate             | 18   | 23  |    |
| Poor                 | 13   | 7   |    |
| T stage              |      |     |    |
| T1 + T2              | 9    | 36  | 0.000 |
| T3 + T4              | 41   | 14  |    |
| N stage              |      |     |    |
| N0                   | 20   | 42  | 0.000 |
| N1-N2                | 30   | 8   |    |
| TNM stage            |      |     |    |
| I + II               | 17   | 40  | 0.000 |
| III + IV             | 33   | 10  |    |
| CEA levels           |      |     |    |
| Normal               | 32   | 26  | 0.224 |
| Elevated             | 18   | 24  |    |
| CA199 levels         |      |     |    |
| Normal               | 38   | 30  | 0.086 |
| Elevated             | 12   | 20  |    |
Table 3

Univariate and multivariate Cox regression analysis of LINC00115 for predicting relapse-free survival of patients with colorectal cancer (n = 100).

| Variables                          | HR    | 95% CI          | P#   |
|------------------------------------|-------|-----------------|------|
| **Univariate analysis**            |       |                 |      |
| Gender (Male vs. Female)           | 1.240 | 0.726–2.116     | 0.431|
| Age (years) (≥ 60 vs. <60)         | 0.866 | 0.504–1.487     | 0.602|
| Histologic grade (Well, Moderate, Poor) | -     | -               | 0.225|
| Histologic grade (Poor vs. Well)   | 1.661 | 0.933–2.957     | 0.085|
| Histologic grade (Moderate vs. Well) | 1.265 | 0.615–2.603     | 0.523|
| T stage (T3 + T4 vs. T1 + T2)      | 2.524 | 1.458–4.372     | 0.001|
| N stage (N1-N2 vs. N0)             | 5.685 | 3.264–9.902     | 0.000|
| TNM stage (III + IV vs. I + II)    | 7.302 | 4.073–13.091    | 0.000|
| CEA levels (Elevated vs. Normal)   | 0.829 | 0.488–1.407     | 0.487|
| CA199 levels (Elevated vs. Normal) | 0.713 | 0.395–1.285     | 0.261|
| LINC00115 expression (High vs. Low) | 3.927 | 2.152–7.167     | 0.000|
| **Multivariate analysis**          |       |                 |      |
| T stage (T3 + T4 vs. T1 + T2)      | 0.680 | 0.305–1.518     | 0.347|
| N stage (N1-N2 vs. N0)             | 0.893 | 0.335–2.376     | 0.820|
| TNM stage (III + IV vs. I + II)    | 7.278 | 2.308–22.954    | 0.001|
| LINC00115 expression (High vs. Low) | 2.479 | 1.203–5.108     | 0.014|

# P < 0.05 indicates statistically significant.

Downregulation of LINC00115 inhibits cell growth in CRC cells

As shown in RT-qPCR results, LINC00115 is also significantly upregulated in CRC cell lines (LoVo, HT-29, Caco-2, SW620 and SW480 cells), when compared with the normal cell (FHC) (Fig. 1d). and LoVo and HT-29 cells showed a higher expression level. Then, we constructed LINC00115 knockdown LoVo and HT-29 cells via transfection of two independent siRNA sequences. The results revealed that compared to a negative control group (si-Control), the selected siRNAs could significantly downregulate LINC00115 expression in LoVo and HT-29 cells (referred to si-LINC00115-1 group and si-LINC001156-2 group), while si-LINC00115-1 group showed higher inhibition efficiency (Fig. 2a, b). CCK-8 assay revealed that silencing
of LINC00115 could significantly suppress the proliferative ability of LoVo and HT-29 cells (Fig. 2c, d). Consequently, our data revealed that LINC00115 was closely implicated in the CRC cell growth.

**Downregulation of LINC00115 induces cell apoptosis in CRC cells**

Since CRC cell apoptotic status is highly associated with their proliferative ability, we further detected the apoptosis rates of CRC cells using flow cytometry. Compared with those in the control group, LoVo cells in si-LINC00115-1 group and si-LINC00115-2 group exhibited increased apoptotic rate, including early, late and total proportions (Fig. 3a). Similar results were observed in HT-29 cells, downregulation of LINC00115 by siRNAs induce more early, late and total cell apoptosis (Fig. 3b). Therefore, our data suggested that LINC00115 could induce CRC cell apoptosis *in vitro*.

**Downregulation of LINC00115 inhibits metastasis in CRC cells**

Regarding that patients with LINC00115 dysregulation were always accompanied with lymph node metastasis, we speculated that LINC00115 might play a pivotal role in CRC cell metastasis. As shown in Transwell assay, silencing of LINC00115 significantly inhibited cell migration and invasion abilities of LoVo cells (Fig. 4a). Consistently, HT-29 cells in si-LINC00115-1 and si-LINC00115-2 groups of showed a less migrated or invaded count, when compared with those in control group, (Fig. 4b). Hence, our data indicated that downregulation of LINC00115 inhibits metastasis in CRC cells.

**Downregulation of LINC00115 regulates PI3K/AKT/mTOR pathway in CRC cells**

To clarify how LINC00115 regulate proliferation and metastasis of CRC cells, we performed western blotting and found that silence of LINC00115 in LoVo cells could significantly reduce the expression levels of p-mTOR p-PI3K and p-AKT proteins (Fig. 5a). In line with this observation, reduction of p-mTOR p-PI3K and p-AKT proteins were also observed in HT-29 cells with si-LINC00115-1 or si-LINC00115-2 transfection (Fig. 5b). Therefore, we could conclude that downregulation of LINC00115 might suppress metastatic and proliferative abilities depending on PI3K/AKT/mTOR pathway in CRC.

**MiR-489-3p is a direct downstream target of LINC00115 in CRC**

Using a publicly available bioinformatic algorithms (Starbase.2), we identified miR-489-3p might be a downstream target of LINC00115. To further address the relationship between the miR-489-3p and LINC00115 in CRC progression, we firstly detected the expression pattern of miR-489-3p in CRC samples. RT-qPCR results showed that compared with the normal tissue, miR-489-3p was significantly
downregulated in CRC tumor samples (Fig. 6a, P<0.05), and the downregulation of miR-489-3p accounted for 70% (70/100) of CRC tissue samples (Fig. 6b). Interestingly, we also found that the expression levels of miR-489-3p was negatively correlated with those of LINC00115 (Fig. 6c). Meanwhile, RT-qPCR results also showed that compared with the normal cell (FHC), miR-489-3p is significantly downregulated in CRC cell lines (Fig. 6d), and LoVo and HT-29 cells showed a lower expression (Fig. 6d). To clarify the underlining association, we subsequently predicted their potential binding site, which was shown in Fig. 6e. Further luciferase reporter assay indicated that when treated with miR-489-3p mimics, the wild-type cells showed lower luciferase activity (Fig. 6f). Therefore, these results consistently revealed that miR-489-3p might be a direct target of LINC00115 in CRC.

Inhibition of miR-489-3p rescue cell proliferation abilities CRC cells induced by depletion of LINC00115

Firstly, we discovered that compared to si-Control group, si-LINC00115-1 and si-LINC00115-2 groups showed higher expression of miR-489-3p, while si-LINC00115-1 group showed highest expression of miR-489-3p (Fig. 7a,b). To further confirm the potential role of miR-489-3p in regulating CRC progression, we downregulated the expression of miR-489-3p in si-LINC00115-1 LoVo and HT-29 cells by transfecting miR-489-3p specific inhibitors (Fig. 7a, b). CCK-8 assay showed that inhibition of miR-489-3p could increase cell proliferation ability in LINC00115-knockdown LoVo and HT-29 cells (Fig. 7c, d). Moreover, flow cytometric analysis showed that downregulation of miR-489-3p could significantly reduce cell apoptosis rate, when compared with miR-489-3p NC group, (Fig. 7e, f). Therefore, these results consistently indicated that downregulation of LINC00115 might suppress CRC cell growth by targeting miR-489-3p.

Inhibition of miR-489-3p rescue cell metastatic abilities and PI3K/AKT/mTOR signaling pathway in CRC cells induced by depletion of LINC00115

The results of Transwell assays showed that both in LoVo (Fig. 8a) and HT-29 cells (Fig. 8b), anti-cell metastatic abilities induced by the depletion of LINC00115 could be reversed by miR-489-3p inhibitor. Furthermore, after the depletion of miR-489-3p, the expression levels of p-mTOR, p-AKT and p-PI3K proteins in si-LINC00115-1 LoVo and HT-29 cells were significantly increased relative to those in miR-489-3p NC group (Fig. 8c, d). In summary, these results consistently indicated that downregulation of LINC00115 could suppress metastatic abilities and regulated PI3K/AKT/mTOR signaling pathway in CRC.

Discussion

Mounting studies have highlighted the critical roles of lncRNAs in CRC carcinogenesis and cancer cells metastasis. They could serve as a multifaceted regulator in a broad range of cancer gene modulation in transcriptional, post-transcriptional and epigenetic levels. Here, we firstly reported lncRNA LINC00115 as a novel cancer-promoted regulator in CRC. LINC00115 was originally reported in lung cancer as a potential
prognostic biomarker[20]. In another study, LINC00115 was identified to be a critical regulator of glioma stem-like cell tumorigenicity[21]. In our study, qRT-PCR assay was conducted to estimate the expression levels of LINC00115 in 100 paired CRC tissues and adjacent normal tissues. The results showed a significantly increased LINC00115 expression in CRC tissues compared to adjacent normal tissues. We also observed that upregulation of LINC00115 was strongly related to advanced TNM stage, larger tumor size, lymphatic metastasis of CRC patients, suggesting that LINC00115 could be a prognostic factor of CRC.

There is increasing evidence that a high lncRNA expression is significantly correlated with unfavorable CRC prognosis. The elevated expressions of lncRNA TTN-AS1, XIST, TUG1 and SNHG12 indicate poorer prognoses of CRC patients[17, 22–24]. Our multivariate survival study proved that LINC00115 are independent prognostic factors for poor relapse-survival in CRC patients. Importantly, functional assays further identified that knockdown of LINC00115 could inhibited cell proliferation, migration and invasion and facilitated cell apoptosis in vitro. These findings indicated that LINC00115 was associated with the carcinogenesis and progression of CRC, but the exact regulatory mechanism is needed to illuminated.

Increasing amount of multiscale omics data showed that IncRNAs exert their biological effects by working together with different molecules, such as miRNAs, mRNAs and proteins. For instance, IncRNAs might interact with miRNAs by binding them onto theirs 3′-UTR region and therefore making themselves acting as a miRNA sponge. Currently, the functional pattern of LINC00115 in CRC cells remains to be established. Hence, we searched several databases and found that miR-489-3p was a target gene for LINC00115. Previous research has shown that miR-489-3p was sponged by LINC01446 and targeted TPT1 to regulate glioblastoma progression[25]. In osteosarcoma, miR-489-3p significantly suppressed cell invasion and metastasis both in vitro and in vivo[26]. In the present study, we firstly proposed that miR-489-3p might be a target of LINC00115. Through the luciferase reporter gene assay, we confirmed that LINC00115 could directly bind to miR-489-3p. We also found a negative correlation between LINC00115 and miR-489-3p expressions in CRC tissues. Therefore, we predicted that LINC00115 acts as a ceRNA to sequester miR-489-3p, but further experiment is needed.

Numerous studies collectively indicated the importance of intricate crosstalk existing between IncRNAs and PI3K/AKT/mTOR signaling[27, 28]. mTOR frequently exerts as an oncogenic signaling cascade in human malignancies[29]. It is a serine/threonine protein kinase belonging to PI3K-related kinase family and forms the catalytic subunit of two kinds of protein complexes including mTOR complex 1 (mTORC1) and 2 (mTORC2)[30]. While mTORC1 controls gene transcription and protein translation in growth-related processed, mTORC2 promotes cell proliferation and survival. Accumulating studies have revealed that PI3K/AKT/mTOR acted as a key driver of cellular growth, adhesion, migration and survival in human carcinogenesis, including CRC, in which activation of PI3K/AKT/mTOR signaling supports cancer cell growth, metastasis, and drug-resistance[31, 32]. PI3K/AKT/mTOR signaling also serves as an integration mediator in the crosstalk of oncogenic signaling pathways[31]. Phosphorylation of PI3K and AKT is the key step for their activation. Once phosphorylation, PI3K activates and phosphorylates its downstream AKT and mTOR to cause a cascade reaction. In recent study, miR-489-3p was shown to interact with
PI3K/AKT/mTOR signaling in various cancers, such as glioma, breast cancer and melanoma[33–35]. Interestingly, it was found that the phosphorylated levels of PI3K (p-PI3K), AKT (p-AKT), and mTOR (p-mTOR) were decreased after LINC00115 depletion, while their total protein levels remained unchanged, suggesting that LINC00115 positively regulates the PI3K/AKT/mTOR pathway. On finding on the implication of LINC00115 in mediating cell proliferation and migration in CRC via modulating PI3K/AKT/mTOR pathway exemplified the idea that anti-LINC00115 compound or agent that consequently targeting PI3K/AKT/mTOR pathway might serve as a novel therapeutic tactics for the treatment of CRC.

In conclusion, our study has delineated the unique role of LINC00115 in CRC and specifically described underlying molecular mechanisms. We confirmed that LINC00115 is upregulated in CRC and function as an independent predictor of progression-free survival, leading to tumor progression and aggressiveness. Importantly, we are the first to demonstrate that the LINC00115/miR-489-3p axis is markedly linked to CRC cell proliferation, migration, and invasion, which was achieved by interacting with PI3K/Akt/mTOR signaling pathway. The findings from our study have opened up a new understanding of CRC and identified a promising biomarker and target for enhancing anti-CRC therapy.

Declarations

Ethics approval and consent to participate

This study had been approved by the medical ethics committee of Henan Oncology Hospital approved the study. all patients signed the informed consent voluntarily.

Consent for publication

Not applicable.

Availability of data and materials

The datasets and data used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors report no conflict of interest in this work.

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None.
Authors' contributions

Conceived and designed the experiments: WF, JZ. Performed the experiments: WF, BL, DX, KC. Analyzed the data: WF, BL, JW. Contributed reagents/materials/tools: HZ, YL, DL. Wrote the paper: WF, JZ. All authors read and approved the final manuscript.

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Figures
Figure 1

LINC00115 is upregulated in CRC. (A) LINC00115 was significantly upregulated in CRC tissues samples relative to normal tissues evaluated by RT-qPCR assay. (B) LINC00115 is overexpressed in 76% (76/100) cases of CRC patients. (C) The relationship between the expression of LINC00115 and relapse-free survival time. (D) LINC00115 was upregulated in CRC cell lines (LoVo, HT-29, Caco-2, SW620 and SW480) relative to the normal cells (FHC). *** P < 0.001.
Figure 2

Knockdown of LINC00115 suppresses cell growth in CRC cells. (A&B) Downregulation efficiency of LINC00115 by a siRNA-transfected method in LoVo cells and HT-29 cells respectively. (C&D) Cell growth ability of LINC00115-downregulated LoVo cells and HT-29 cells detected by CCK-8 assay. *** P < 0.001.
Figure 3

Downregulation of LINC00115 induces more apoptosis in CRC cells. (A) Cell apoptotic images of LoVo cells and transfected with LINC00115-specific siRNAs (up), and the relative statistical analysis (down). (B) Cell apoptotic images of HT-29 cells transfected with LINC00115-specific siRNAs (up), and the relative statistical analysis (down). *** P < 0.001.
Figure 4

Downregulation of LINC00115 suppresses migration and invasion abilities of CRC cells. (A) Knockdown of LINC00115 dramatically reduced the number of invaded and migrated LoVo cells detected by Transwell assay. (B) Knockdown of LINC00115 dramatically reduced the number of invaded and migrated HT-29 cells detected by Transwell assay. *** P < 0.001.
Downregulation of LINC00115 regulates PI3K/AKT/mTOR pathway in CRC cells. (A) After transfected with LINC00115-specific siRNAs, the expression levels of PI3K, AKT, mTOR, p-PI3K, p-AKT and p-mTOR in LoVo cells (up), and the relative gray value (down). (B) After transfected with LINC00115-specific siRNAs, the expression levels of PI3K, AKT, mTOR, p-PI3K, p-AKT and p-mTOR in HT-29 cells (up), and the relative gray value (down).
the expression levels of PI3K, AKT, mTOR, p-PI3K, p-AKT and p-mTOR in HT-29 cells (up), and the relative gray value (down). *** P < 0.001.

Figure 6

MiR-489-3p might be the downstream of LINC00115. (A) MiR-489-3p was significantly upregulated in CRC tissues relative to adjacent normal tissues detected by RT-qPCR. (B) MiR-489-3p is down-regulated in 70% (70/100) CRC patients. (C) Correlation between LINC00115 and miR-489-3p in 100 cases of CRC tissue samples. (D) The expression of miR-489-3p in CRC cell lines and the normal cells. (E) The predicted 3’UTR binding regions of LINC00115 on miR-489-3p. (F) Relative luciferase activity in 293T cells after co-transfection with pmirGLO-LINC00115-WT or pmirGLO-LINC00115-MUT, along with miR-489-3p specific mimics or NC. *** P < 0.001.
Figure 7

Inhibition of miR-489-3p rescue anti-cell proliferation abilities CRC cells induced by depletion of LINC00115 in CRC cells. (A&B) The expression of miR-489-3p in LINC00115-downregulated LoVo and HT-29 cells, after transfection with miR-489-3p negative control (NC) or miR-489-3p inhibitors. (C&D) Cell proliferation ability of LINC00115-downregulated LoVo and HT-29 cells, with or without depletion of miR-489-3p, detected by CCK-8 assay. (E&F) Cell apoptotic rates of LINC00115-downregulated LoVo and HT-29 cells, with or without depletion of miR-489-3p, detected by flow cytometry. *** P < 0.001.
Inhibition of miR-489-3p reverse the anti-cell metastasis abilities and PI3K/AKT/mTOR pathway induced by depletion of LINC00115 in CRC cells. (A&B) Knockdown of LINC00115 dramatically reduced the number of invaded and migrated LoVo and HT-29 cells detected by Transwell assay. (C&D) The expression levels of PI3K, AKT, mTOR, p-PI3K, p-AKT and p-mTOR on LoVo and HT-29 cells (up), and the relative gray value (down). *** P < 0.001.