

LINC 01436 is Overexpressed in Colorectal Cancer and Promotes Cancer Cell Proliferation by Suppressing the Maturation of Tumor Suppressive miR-466

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Abstract

LINC 01436 (lncRNA) promotes lung and gastric two types of cancers. However, it is unclear that whether this lncRNA also participate in colorectal cancer (CRC). This study was therefore carried out to analyze the role of LINC 01436 in CRC. Expression of LINC 01436 in CRC patient tissues was analyzed by RT-qPCR and follow-up study was performed for prognostic analysis. Correlation between LINC 01436 and mature miR-466 or miR-466 precursor was analyzed by linear regression. Mature miR-466 and miR-466 precursor expression in CRC cells with the overexpression of LINC 01436 was studied by performing RT-qPCR. The proliferation of CRC cells was subjected to CCK-8 assay analysis. LINC 01436 was upregulated in CRC and predicted poor survival. LINC 01436 and mature miR-466 were inversely correlated, but LINC 01436 and miR-466 precursor were not correlated. In CRC cells, LINC 01436 mediated the downregulation of mature miR-466, but not miR-466 precursor. Cell proliferation analysis showed that LINC 01436 overexpression rescued cell proliferation reduced by miR-466. LINC 01436 is overexpressed in CRC and it may promote cancer cell proliferation by suppressing the maturation of miR-466.

Introduction

Colorectal cancer (CRC), also refers to colon cancer, bowel cancer, or rectal cancer, is a type of malignancy originates from rectum or colon [1, 2]. With the increased lifespan and changes in people's lifestyle, the burden of CRC is expected to be increased by 60% by 2030 worldwide [3]. It is estimated that more than 90% of patients with localized CRC can survive 5 years after the initial diagnosis [4]. CRC is rarely diagnosed at early stages and 5-year survival rate of metastatic CRC is below 15% [5, 6]. Therefore, novel therapeutic approaches are needed.

It has been well established that, physical inactivity, obesity, smoking and alcohol abuse are the major risk factors for CRC [7, 8]. Besides that, molecular factors also contribute to the development and progression of CRC[9]. In effect, understanding the functionality of these factors provides novel insights to the development of targeted anti-CRC approaches [10, 11]. However, the development of targeted therapy is limited by the lacking of promising targets. Although lncRNAs are no protein-coding RNAs, they regulate cancers by affecting gene expression [12]. LINC 01436 promotes lung and gastric two types of cancers [13, 14]. It is hardly known that whether this lncRNA also participate in CRC. We performed preliminary deep sequencing analysis and observed the altered expression of LINC 01436 and its inverse correlation with mature miR-466, a cancer-related miRNA [15]. This study aimed to explore the potential interaction between LINC 01436 and miR-466 in CRC.

Materials And Methods

CRC patients and follow-up

This study was approved by People's Hospital of Baoan District Ethics Committee. From June 2013 to May 2015, a total of 62 CRC patients (36 males and 26 females) were enrolled. Age of the patients

ranged from 46 years to 68 years (57.1 ± 5.6 year). CRC patients were diagnosed by histopathological exam. No initiated therapy was observed and recurrent CRC patients were excluded. Patients were grouped into American Joint Committee on Cancer (AJCC) stage I or II ($n = 26$) and III or IV ($n = 36$). Based on AJCC stages, the 62 patients were treated with different therapies. A follow-up (5 years) was performed monthly to record their survival. Informed consent was signed by all patients.

Crc Tissues And Cells

Prior to treatments, CRC and paired non-tumor tissues were obtained from all patients. Following confirmation by histopathological exam, the tissue samples were stored in liquid nitrogen before use.

WiDr (ATCC® CCL-218™) and HT-29 (ATCC® HTB-38™) human CRC cell lines from ATCC (USA) were included for cell experiments and were cultivated following the instructions from ATCC. Cell culture medium was ATCC-formulated McCoy's 5a Medium Modified (Catalog No. 30-2007) with 10% FBS. Cells were cultivated at 37°C.

Vector, Mirna And Cell Transfections

LINC 01436 expression vector (pcDNA3.1, Invitrogen) and miR-466 mimic (Sigma-Aldrich) were transfected into cells through transfections mediated by Lipofectamine 2000 (Invitrogen), which was used to transfect 40 nM miRNA or 1 µg expression vector into 10^8 cells.

Rna Isolations

Total RNA isolation was done by using Ribozol reagent (Invitrogen). DNA removal from RNA was done by incubating with DNase I (Invitrogen) for 80 min at 37°C. Electrophoresis was performed using Urea-PAGE gels (5%) to determine RNA integrity. RNA purity was determined by calculating OD260/280 ratios.

Rt-qpcrs

RNA samples with an OD 260/280 ratio close to 2.0 were used to synthesize cDNA samples, followed by qPCRs with internal control 18S rRNA to study the expression of LINC 01436.

To determine the expression of miR-466 precursor, a sequence-specific reverse primer was used to perform reverse transcriptions (RTs), and sequence-specific forward and reverse primers were used to perform qPCRs. To determine the expression of mature miR-466, miRNAs were first added with poly (A), followed by RTs performed using poly (T) as reverse primer. After that, poly (T) and sequence-specific forward primer were used to perform qPCRs. All steps were completed using All-in-One™ miRNA qRT-PCR reagent kit (GeneCopoeia).

Ct values were subjected to $2^{-\Delta\Delta CT}$ method normalization. Primers were listed in supplemental Table.1.

Cck-8 Assay

At 48h post-transfection, WiDr and HT-29 cells were harvested and were subjected to cell apoptosis analysis through CCK-8 assay using a CCK8 kit (ab228554, Abcam). A 96 well plate was used to cultivate cells (3000 cells per well) at 37°C. At 2h after the addition of CCK-8 (10%) the measurement of OD values (450 nm) was performed.

Statistical analysis

Average values of gene expression in three technical replicates of paired tissues were subjected to paired t test. Mean \pm SD values in three biological replicates were subjected to ANOVA Tukey's test. Correlation analysis was performed by linear regression. With the median level of LINC 01436 in CRC tissues as a cutoff value, patients were classified into high and low level group (n = 31). Log-rank test was used for comparison. P < 0.05 was deemed statistically significant.

Results

LINC 01436 was highly expression in CRC and predicted poor survival

Paired tissues from CRC patients were collected and expression of LINC 01436 was determined by RT-qPCR. LINC 01436 was significantly overexpressed in CRC tissues (Fig. 1A, p < 0.001). Survival analysis illustrated that high LINC 01436 expression was closely correlated poor survival (Fig. 1B). Therefore, LINC 01436 was overexpressed in CRC and the expression levels of LINC 01436 were correlated with patients' survival. In addition, no correlations between cancer stages and LINC 01436 levels were found (data not shown), suggesting that LINC 01436 is an independent prognostic factor for CRC.

Linc 01436 Was Only Correlated With Mature Mir-466

Mature miR-466 and miR-466 precursor in CRC tissues was detected by RT-qPCR. Correlations analysis illustrated that LINC 01436 and mature miR-466 were inversely correlated (Fig. 2A). However, LINC 01436 and miR-466 precursor were not closely correlated (Fig. 2B). Therefore, LINC 01436 may suppress the maturation of miR-466 in CRC.

Linc 01436 Overexpression Decreased Mature Mir-466 In Crc Cells

To test whether LINC 01436 can affect the maturation of miR-466, WiDr and HT-29 cells were overexpressed with LINC 01436 and miR-466, followed by the measurement of the expression of LINC 01436, mature miR-466 and miR-466 precursor by RT-qPCR. Overexpression of LINC 01436 and mature miR-466 was confirmed by RT-qPCR (Fig. 3A, $p < 0.05$). In addition, overexpression of LINC 01436 mediated the downregulation of mature miR-466 (Fig. 3B, $p < 0.05$), but not miR-466 precursor (Fig. 3C). Moreover, miR-466 did not affect LINC 01436 expression (Fig. 3D). Therefore, LINC 01436 suppressed the maturation of miR-466 in CRC cells.

LINC 01436 overexpression increased the proliferation of WiDr and HT-29 cells through miR-466

CCK-8 assay data illustrated that LINC 01436 overexpression increased cell proliferation, and miR-466 overexpression decreased the proliferation of both WiDr and HT-29 cells. Moreover, LINC 01436 overexpression reduced the inhibitory effects of miR-466 overexpression on cell proliferation (Fig. 4, $p < 0.05$).

Discussion

We studied the crosstalk between LINC 01436 and miR-466 in CRC. We found that LINC 01436 was overexpressed in CRC and expression levels of LINC 01436 predicted the survival of CRC patients. In addition, LINC 01436 may suppress the maturation of miR-466 to promote CRC cell proliferation.

Previous studies have reported that LINC 01436 plays oncogenic roles in both lung cancer and gastric cancer [13, 14]. In lung cancer, LINC 01436 was proven to be a hypoxia-sensitive lncRNA and was regulated by E2F6. In addition, overexpression of LINC 01436 increased cancer cell proliferation, invasion and migration by targeting tumor suppressive miR-30a-3p [13]. LINC 01436 was also overexpressed in gastric cancer and sponged miR-585 and suppressed the translation of FBXO11 by binding to its promoter region to promote tumor growth and metastasis [14]. In this study we first reported the upregulation of LINC 01436 in CRC. Moreover, overexpression of LINC 01436 increased the proliferation of CRC cells. Therefore, LINC 01436 plays oncogenic roles in CRC by increasing cancer cell proliferation.

Treatment of CRC is limited by the low early diagnostic rate, and the early diagnosis of CRC is unlikely to be significantly improved in near future, mainly owing to the lack of sensitive markers [5]. We found that LINC 01436 predicted CRC patients' poor survival. Therefore, LINC 01436 may serve as a marker to guide the determination of therapies to improve the survival of CRC patients. However, clinical trials are needed to test our hypothesis.

MiR-466 is a tumor suppressor in several cancers [15, 16]. In CRC, miR-466 is downregulated and its overexpression suppresses cancer proliferation. This study confirmed the inhibitory effects of miR-466 on CRC cell proliferation. Previous studies mainly investigated the interactions between LINC 01436 and miRNAs [13, 14]. In this study we showed that LINC 01436 could downregulate mature miR-466, but not miR-466 precursor. Therefore, LINC 01436 may suppress the maturation of miR-466 to participate in cancer biology. A recent study showed that lncRNA CCAT2 could suppress the maturation of miR-145 by

suppressing the transportation of miR-145 precursor [17]. LINC 01436 may also suppress the transportation of miR-466 from nucleus to cytoplasm, where mature miR-466 is produced from precursor. However, more studies are needed to validate this hypothesis and explore other possible mechanisms.

Conclusion

In conclusion, LINC 01436 is overexpressed in CRC and predicts poor survival of CRC patients. In addition, LINC 01436 may suppress the maturation of miR-466 to promote the proliferation of CRC cells.

Declarations

Ethical Approval and Consent to participate

Informed consent was obtained from all individual participants included in the study. All producers were approved by Ethics Committee of People's Hospital of Baoan District. Procedures operated in this research were completed in keeping with the standards set out in the Announcement of Helsinki and laboratory guidelines of research in China.

Consent to publish

Not applicable.

Availability of supporting data

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to their containing information that could compromise the privacy of research participants.

Competing interests

All other authors have no conflicts of interest.

We declare that we do not have any commercial or associative interest that represents a conflict of interest in connection with the work submitted.

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Not Applicable.

Authors' contributions

HL, DH: study concepts, literature research, clinical studies, data analysis, experimental studies, manuscript writing and review; HL, WD: study design, literature research, experimental studies and manuscript editing; JH: data acquisition, manuscript preparation and data analysis.

All authors have read and approve the submission of the manuscript.

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Not Applicable.

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Figures

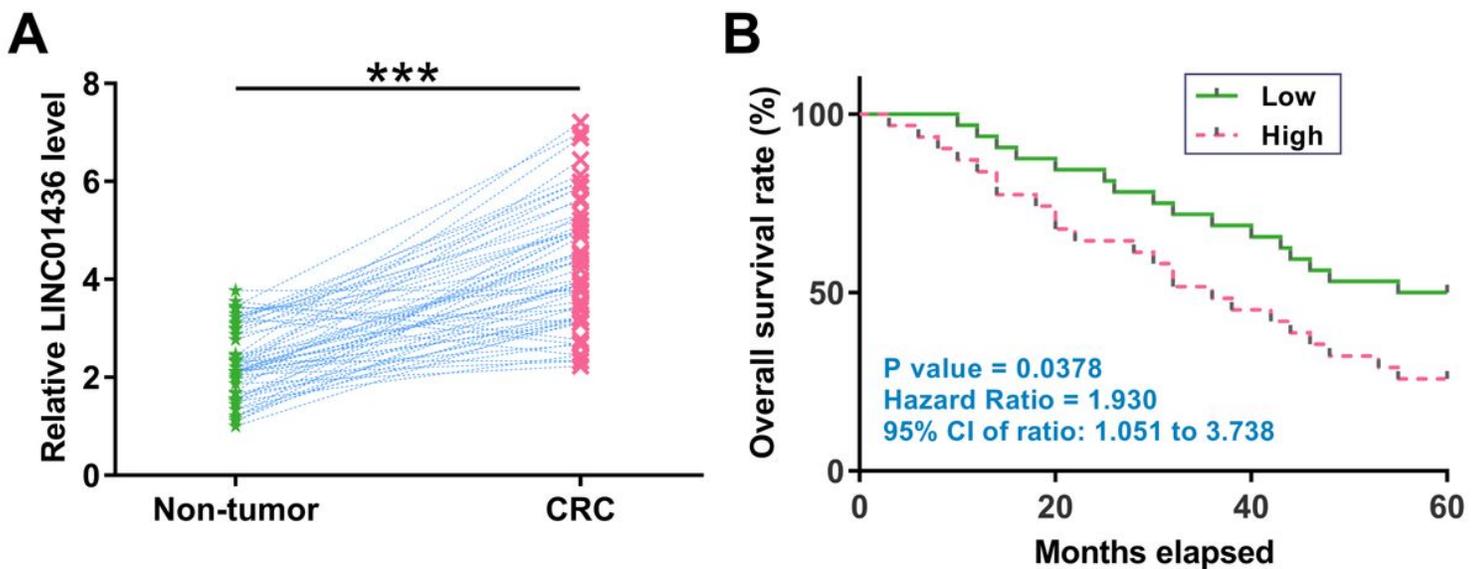


Figure 1

LINC 01436 was overexpressed in CRC and was correlated with patients' survival. Paired CRC and non-tumor tissues from 62 CRC patients were collected and expression of LINC 01436 in these tissue samples was determined by RT-qPCR. QPCRs were performed in three technical replicates and average values were presented. ***, $p < 0.001$ (A). Survival analysis was performed following the methods described in methods section using data of the 5-year follow-up study. The comparison of survival curves was performed using log-rank test (B).

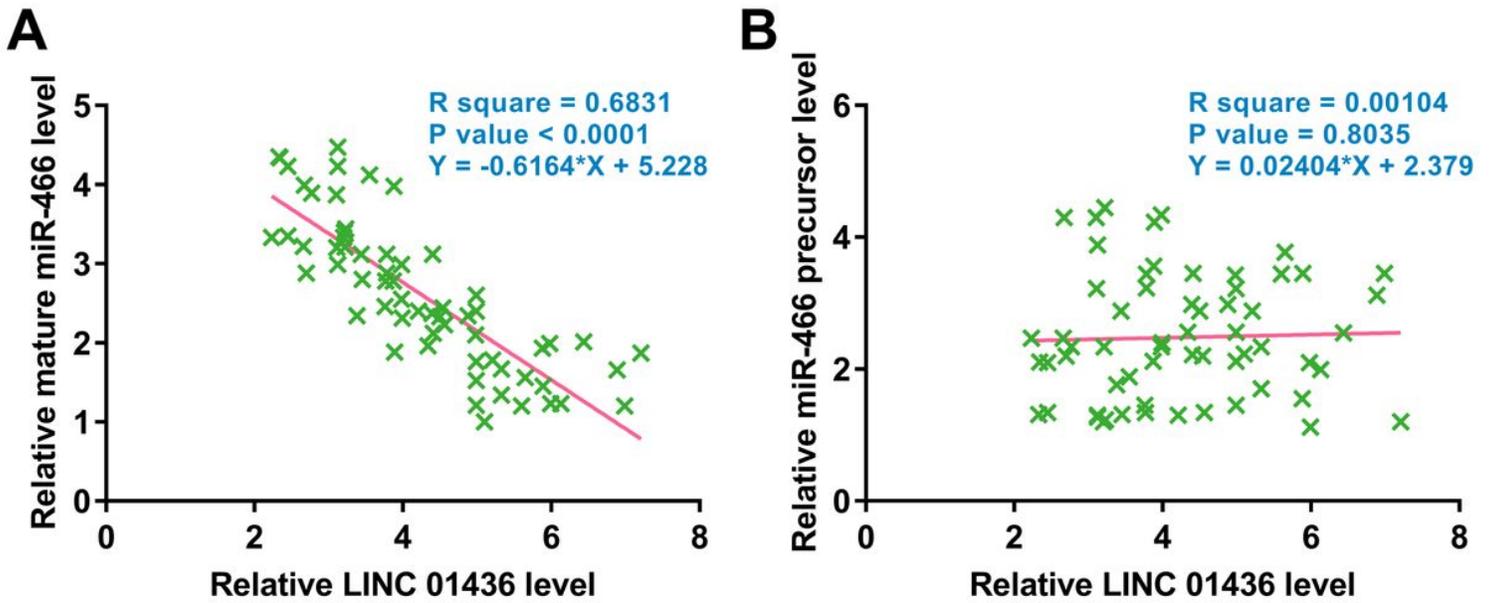


Figure 2

LINC 01436 was inversely correlated with mature miR-466, but not miR-466 precursor across CRC tissues. The expression of mature miR-466 and miR-466 precursor in CRC tissues was determined by RT-qPCR. QPCRs were performed in three technical replicates and average values were presented. Correlations between LINC 01436 and mature miR-466 (A) or miR-466 precursor (B) were analyzed by linear regression.

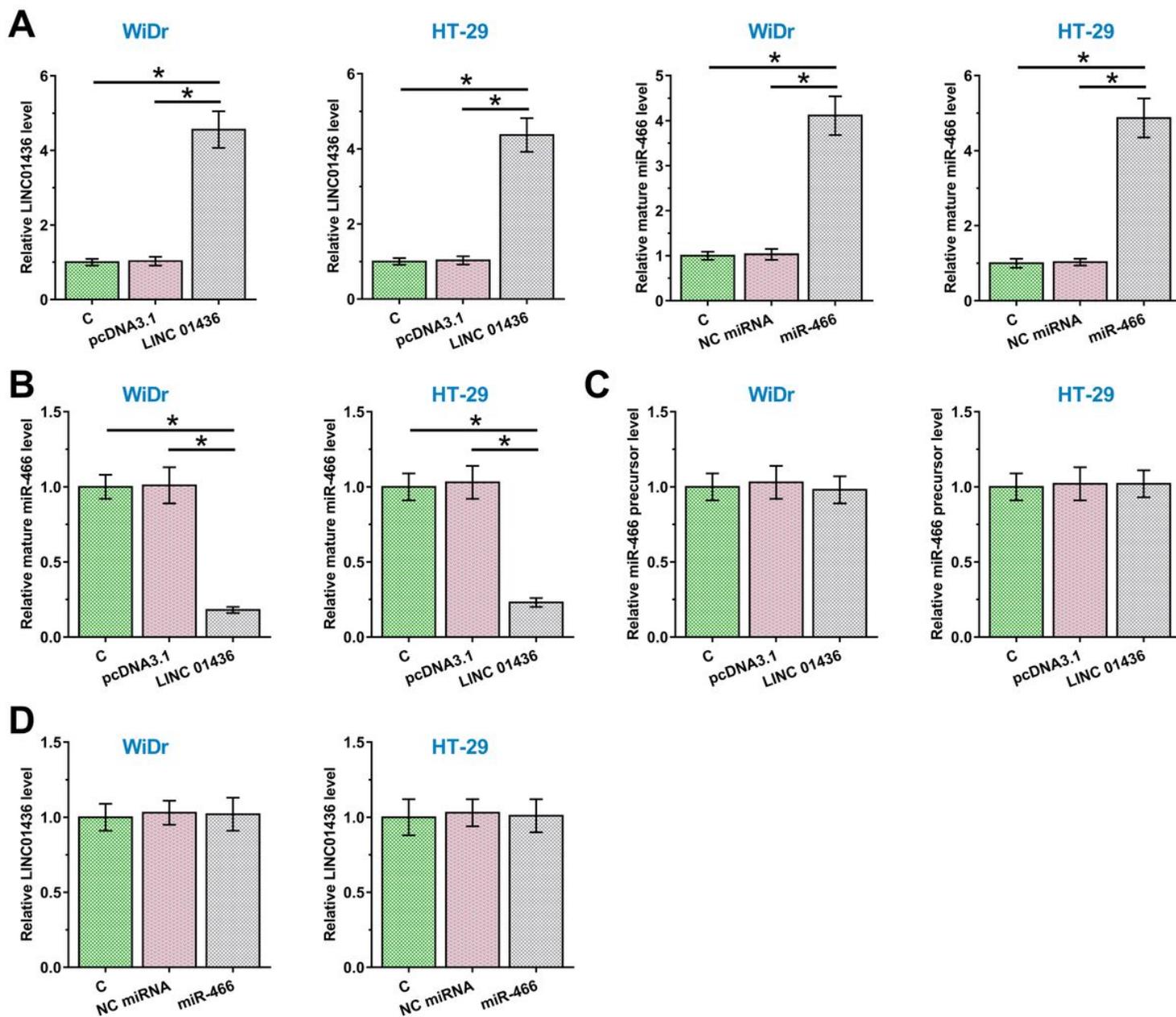
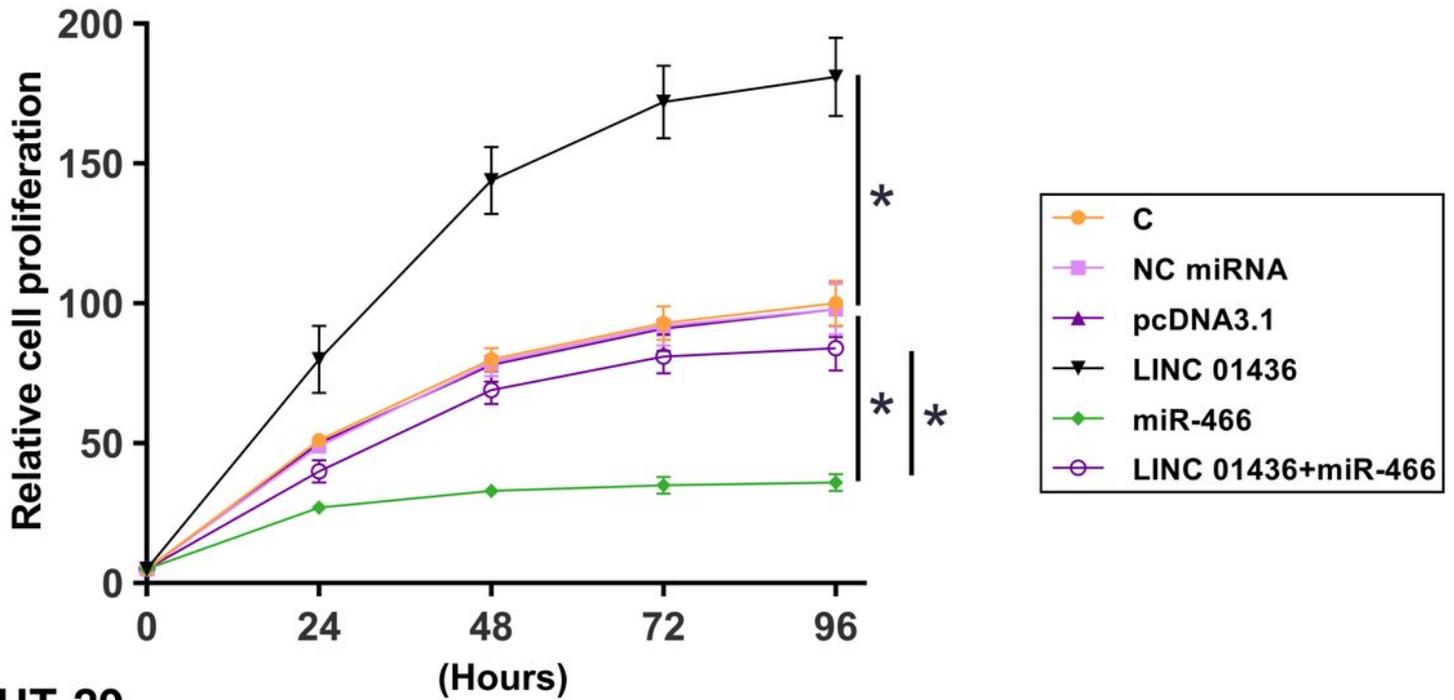


Figure 3

LINC 01436 overexpression decreased the expression of mature miR-466 in CRC cells. To test whether LINC 01436 can affect the maturation of miR-466, WiDr and HT-29 cells were transfected with LINC 01436 expression vector or miR-466 mimic, followed by the measurement of the expression of LINC 01436, mature miR-466 and miR-466 precursor by RT-qPCR. Overexpression of LINC 01436 and mature miR-466 was confirmed by RT-qPCR (A). The effects of LINC 01436 overexpression on the expression of mature miR-466 (B) and miR-466 precursor (C) were analyzed by RT-qPCR. Moreover, the effects of miR-466 overexpression on the expression of LINC 01436 (D) were also analyzed by RT-qPCR. All experiments were performed in three biological replicates. *, $p < 0.05$.

WiDr



HT-29

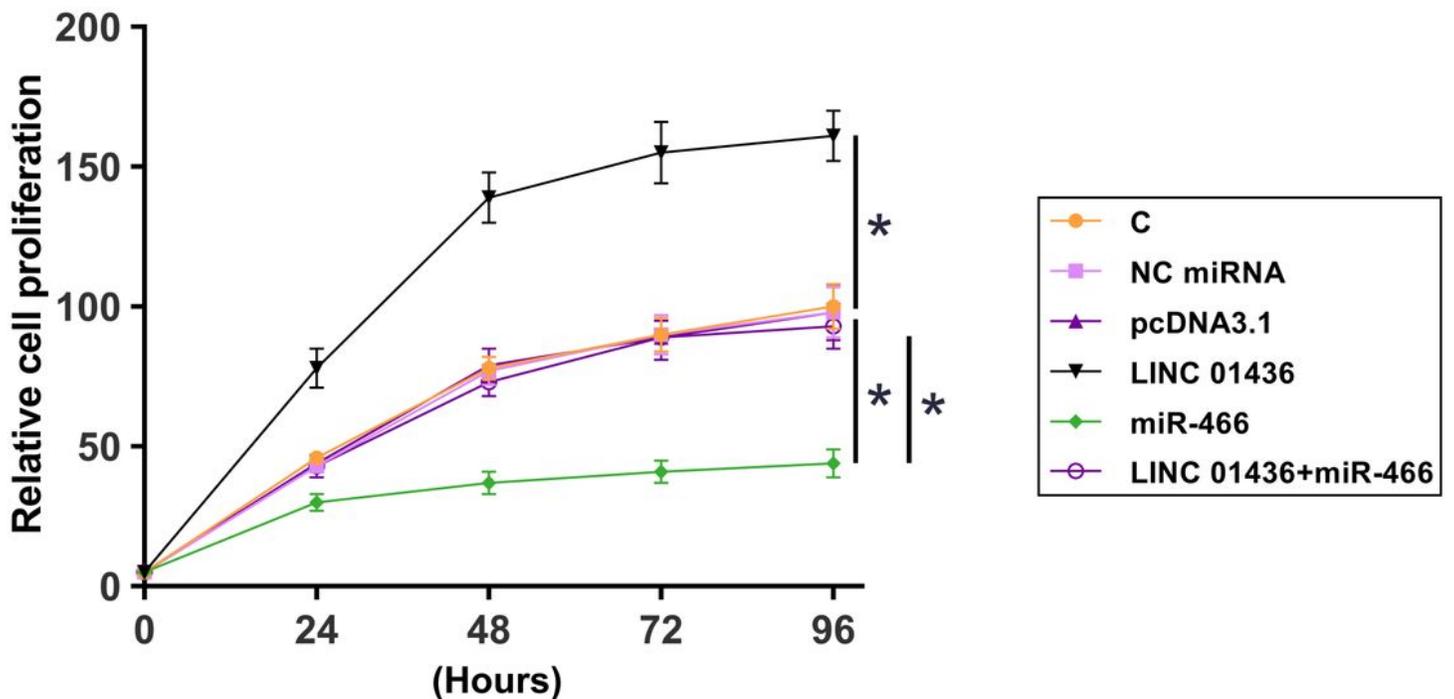


Figure 4

LINC 01436 overexpression increased the proliferation of WiDr and HT-29 cells through miR-466. The role of LINC 01436 and miR-466 in regulating the proliferation of WiDr and HT-29 cells was analyzed by CCK-8 assay. Cell proliferation was reflected by OD values at 450 nm, which were measured every 24h until 96h. Mean±SD values were used to expression data of three biological replicates. *, $p < 0.05$. Supplemental Table.1 Sequences of primers used in RT-qPCR.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementalTable.1.docx](#)