

OVEREXPRESSION OF LINC00319 PROMOTE PROLIFERATION, MIGRATION AND INVASION OF HCC.**Peter Kisebo, Christian Cedric Bongolo, Souraka Tapara Dramani Maman and Jian-Cheng Tu***

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ABSTRACT

Objective: Liver cancer is the second largest cancer death cause in the world. In order to improve the survival rate of patients with liver cancer, it is important to study the genes related to tumour metastasis and find new therapeutic targets. More and more studies have shown that long noncoding RNA plays an important role in the regulation and development of tumours. Long intergenic non-protein coding RNA 319 is a new long noncoding RNA, and its role in HCC has never been studied. **Methods:** The expression of linc00319 in hepatocytes was detected for the first time. The expression level of linc00319 was detected by real-time fluorescence quantitative PCR (QRT PCR), and then the appropriate hepatocytes were selected for stable modulation and transfection. Cell proliferation and migration were detected by CCK-8 and Transwell migration test respectively. **Results:** Compared with normal hepatocyte line (L-02), linc00319 was significantly increased in hepatocytes ($P < 0.001$), and its overexpression promoted the proliferation and invasion of hepatocytes. **Conclusion:** linc00319 plays an important role in the growth and development of hepatoma cells.

KEYWORDS: HCC; long non-coding RNA; proliferation; invasion; migration.**INTRODUCTION**

Liver cancer is one of the cancers that causes most deaths in the world. In recent years, the incidence and mortality of liver cancer are on the rise. The high mortality rate of liver cancer is mainly due to the lack of clinical features and early diagnosis.^[2-4] Most patients with liver cancer are in their advanced stage at the time of diagnosis, which limits the choice of treatment methods.^[2,5-7] More and more research shows that the complex causes of liver cancer include not only gene changes, but also epigenetic. Most cancer studies focus on coding genes, proteins, and relatively non-coding RNA genes. Research on their function and molecular mechanism is not in-depth. However, more and more research has shown that non-coding RNA plays an important role in tumour pathophysiology.^[8,9] Long non-coding RNA (lncRNA) is a kind of RNA with many characteristics. One of the features is that it has more than 200 nucleotides.^[10] Long Non-coding RNA plays an important role in the proliferation, differentiation, apoptosis and cell cycle of tumours. Therefore, it can be considered as a new cancer biomarker. Linc00319 (NR 152722) is part of the new lncRNA. It is located on chromosomes 21q22.3. Zhou et al reported its upregulation in lung cancer for the first time in 2017.^[10] In addition, several studies have shown and confirmed that it is involved in the proliferation and migration of lung cancer cells. However, its expression and role in HCC have not been studied. Therefore, the

purpose of this study is to detect the expression of linc00319 in hepatocytes and to explore the relationship between linc00319 and liver cancer cell proliferation, migration and invasion.

METHODOLOGY**cell culture**

HepG2, Huh-7 and hep-3b and health cell L-02 were purchased from cctcc, Wuhan, China. HCCLM3 and MHCC97-L cells have been in our laboratory. All cells were cultured in Dulbecco's modified Eagle medium DMEM (GIBCO, USA), which contained 10% of fetal bovine serum (GIBCO, USA) and 50 u/ml penicillin and streptomycin, and placed in a humidified incubator of 37 °C and 5% CO₂.

RNA extraction and reverse transcription to complementary DNA

According to the extraction method, the total RNA of HepG2, Huh-7, hep-3b, HCCLM3 and MHCC97H hepatocarcinoma cells was extracted by Trizol reagent (Invitrogen, CA, USA), and then the extracted RNA was transcribed and synthesized into complementary DNA (cDNA). Reverse transcription was done using ReverTra Ace qPCR RT Kit (Toyobo, Japan). The reaction conditions were 65 °C 5min, 98 °C 15min, 37 °C 5min.

Real-time quantitative PCR

According to the manufacturer's instructions, quantitative real-time PCR (QRT PCR) was used to detect the expression of linc00319 in hepatocytes. The expression of linc00319 was detected by SYBR[®] Premix Ex Taq kit[™] II real-time PCR kit (Takara) on bio rad CFX PCR machine. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a control to normalize the data to determine the relative expression of target genes. The primers were: LINC00319 5'-GAACCTCAGTTCCTG GCCTC-3'(sens), 5'-GAGGCCAGGAAGTCTGAGGTTTC-3'(anti-sens); GAPDH (sens): 5'- GGTCTCCTCTGACTTCAACA-3'and (anti-sens): 5'-GTGAGGGTCTCTCT- CTTCCT-3'.

The relative expression was calculated by $2^{-\Delta CT}$ threshold comparison method.

Plasmid construction and transfection

The PCDH plasmid was transfected into Hep3B cells to produce lentivirus. Lentivirus overexpression of the linc00319 gene was used to construct a stable overexpression cell line of linc00319. The transfection was performed with Lipofectamine 3000 reagent according to the manufacture's instructions.

cell proliferation

After 24 hours of plasmid transfection, the cells were seeded into 96 well microplates, of which about 2000 cells / well were cultured for 0, 24, 48 and 96 hours respectively. 10 μ l CCK-8 solution (cell counting kit-8) was added to each pump, and then incubated at 37 ° C for 2 hours. Finally, the absorbance was determined by 450 nm spectrophotometer (enspire, PerkinElmer, USA).

Transwell experiment

A Transwell migration test was conducted to quantify cell migration. After transfection, the cells were seeded into the upper cell compartment and a chemically attractive solution was placed in the lower cell compartment. The cells were then initially collected and returned to the top chamber (20000 cells/chamber) of the Transwell assay plate (Corning, USA) with 200 μ l DMEM free serum containing 10% fetal bovine serum added to quantify cell migration.

Wound healing assay

The cells were embedded in a 6-well plate, and a monolayer of cells was scratched using the tip of a 200 μ l pipette. Then the cells were washed with phosphate-buffered saline (PBS), and a new full medium was added to make the cells grow. Cell culture continued for 0, 24 and 48 hours after the scratch, the number of cells that migrated into the wound were counted from the two fields and wound healing was observed with a light microscope.

statistical analysis

SPSS 21.0 and graph pad prism 8.0 were used for statistical analysis. P < 0.05 was statistically significant.

Student t-test software was used to evaluate the distribution data.

Ethics approval

This study has been approved by the ethics committee of zhongnan Hospital of Wuhan University. All The experiment was carried out according to systematic ethical standards.

RESULTS

The expression of linc00319 in HCC cell line was significantly increased:

We compared the expression of linc00319 in HCC cell line and normal human hepatoma cell line. The results showed that linc00319 gene was highly expressed in HCC cell lines (MHCC97-H vs. L-02, P < 0.01; hcclm-3 vs. L-02, P < 0.01; hep-3b vs.L-02, P < 0.01; Huh-7 vs. L-02, P < 0.01; HepG2 vs. L-02, P < 0.01);

The expression level of linc00319 increased after transfection

In order to study the role of linc00319 in hepatocellular carcinoma, we transfected hep-3b cells with pcdh-linc00319 plasmid and pcdh negative control plasmid to overexpress linc00319. The transfection efficiency was observed by fluorescence microscope, as shown in Figure 2, and evaluated by quantitative RT- PCR. Compared with the negative control group, the expression level of linc00319 in PCDH linc00319 transfected group was significantly increased.

Overexpression of linc00319 promotes the proliferation, migration and invasion of HCC

As shown in Figure 3, CCK-8 cell proliferation test (cell count kit-8) results showed that overexpression of linc00319 in hep-3B cells significantly promoted its proliferation (Fig. 3a). In addition, the transwell test demonstrated that overexpression of linc00319 initiated the migration of hepatocytes (Fig. 3b).

Overexpression of linc00319 promotes the invasion of HCC

After transfection of the plasmid, we conducted a healing test to evaluate the invasion of cells and compared the healing of cells in a period of time. The results of cell healing test showed that overexpression of linc00319 increased the migration and invasion of HepG2 cells. Image processing allows automatic calculation of healing area (Fig. 4).

Graphics and legends

Fig.1 the level of linc00319 in HCC cell lines: The relative expression of linc00319 was detected by quantitative RT- PCR. The expression of linc00319 in hepatoma cell line was significantly higher than that in normal L-02 cell line. All data were analyzed by Student t-test. *p<0.05, **p<0.01.

Fig. 2: fluorescence of transfected cells: Shortly after transfection, the expression of linc00319 in Hep3B cells transfected with PCDH plasmid increased significantly

compared with the control

Fig. 3: overexpression of linc00319 promotes HCC proliferation and migration.

(a) After transfection, cell proliferation was detected by CCK-8 method; (b) overexpression of linc00319 increased the migration ability of hep-3b cells (blue line),

compared with the control group (red line), $P < 0.01$, $** P < 0.001$.

Fig.4: linc00319 overexpression promotes HCC invasion and wound healing. pcdh-linc00319 group has the strongest hepatocyte migration. $*p < 0.05$, $**p < 0.01$.

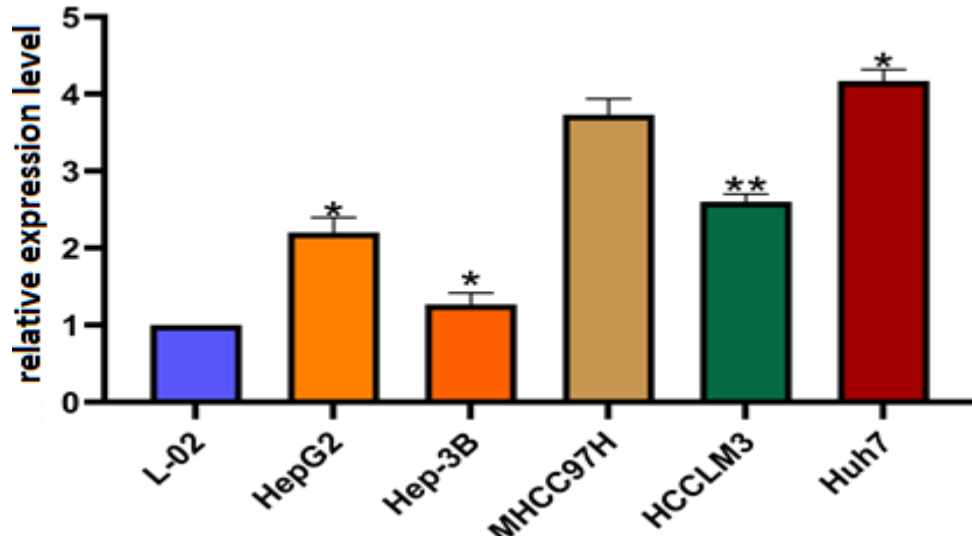


Figure 1.

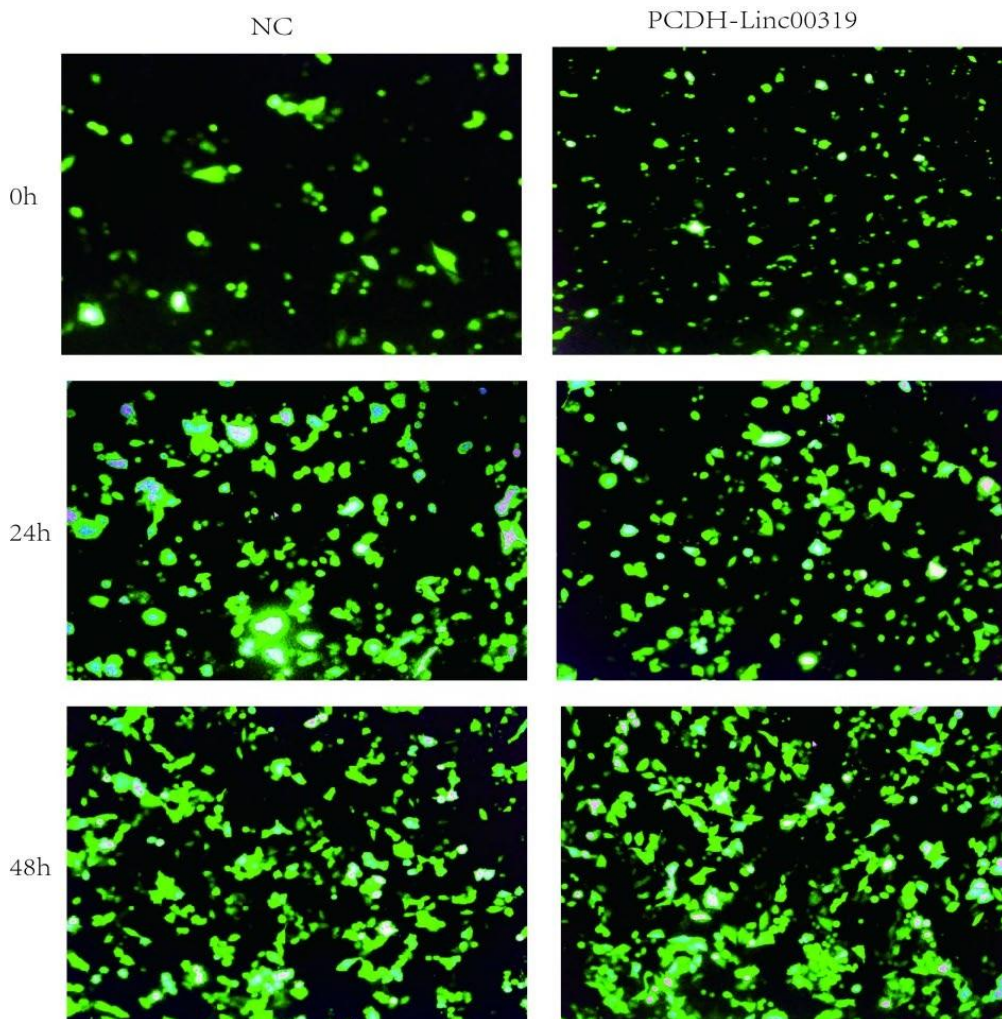
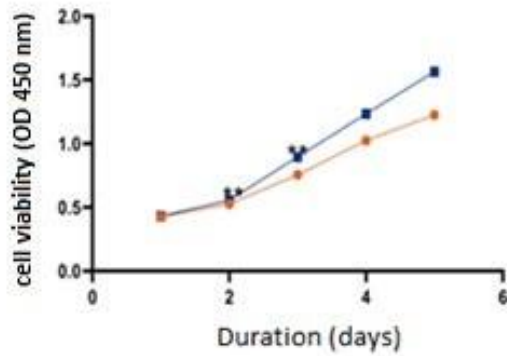


Figure 2.

(a)



(b)

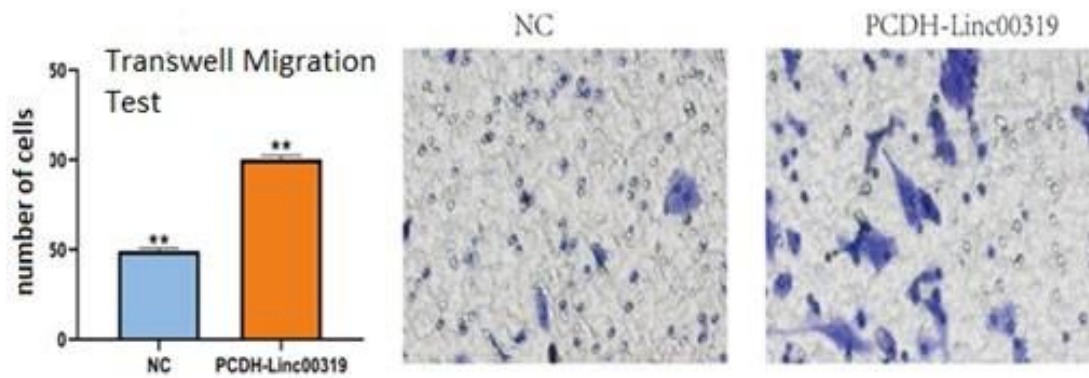


Figure 3.

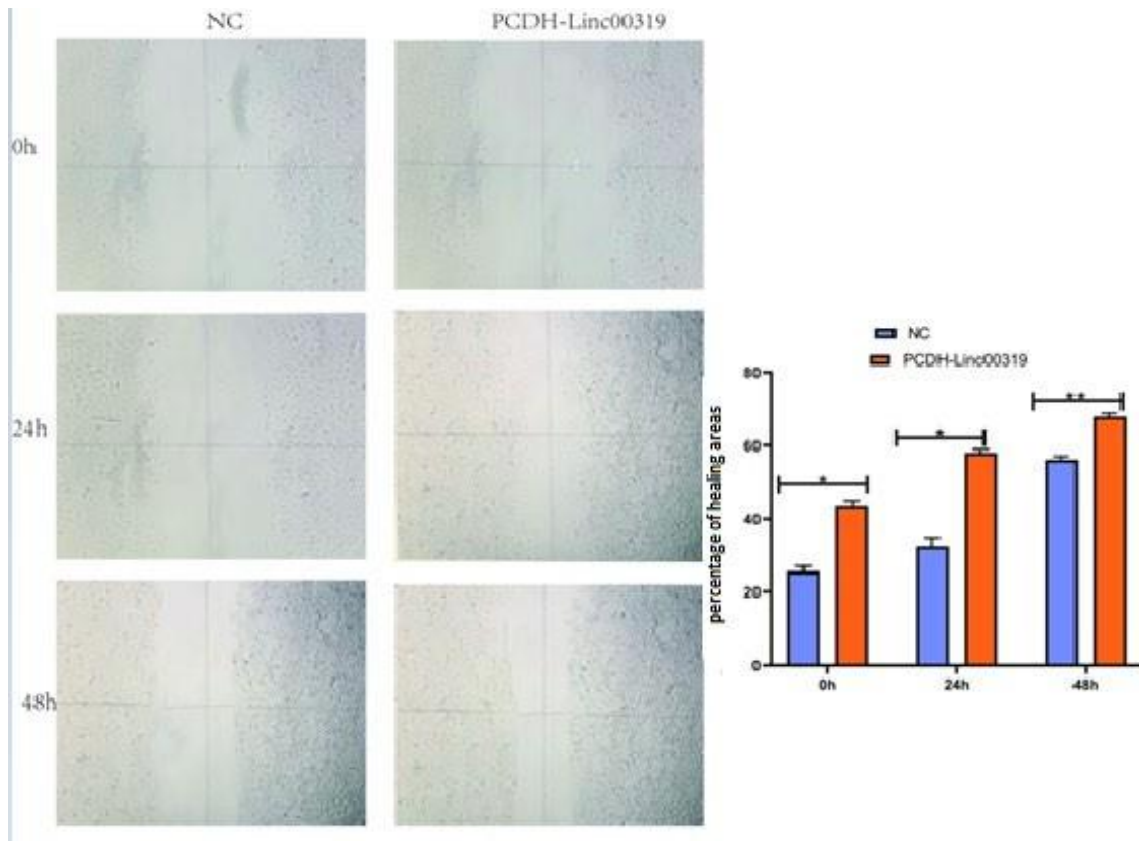


Figure 4.

DISCUSSION

In recent years, numerous studies have found that long non-coding RNA (lncRNA) plays a crucial role in the development of human cancer. Fang et al. Confirmed that lncRNA XIST, a long non-coding RNA, is a carcinogen gene because it induces the occurrence of small cell lung cancer. He also found that lncRNA overexpression in small cell lung cancer is associated with life expectancy and prognosis. Liz et al.^[13] also found out that some long, non-coding lncRNA plays a key role in human cancer. This helps to better understand several important RNA in Cancer. Many studies have confirmed that linc00319, a long non-coding RNA, may play an important role in the development of multiple cancers. Yanni Zhang et al.^[14] found that linc00319 promoted AML leukaemia by enhancing SIRT6 expression, Peng song et al.^[15] found that linc00319 was significantly increased in NPC tissues and cells, and the abnormal expression of linc00319 showed poor prognosis in NPC patients. In addition, the silence of linc00319 inhibited the growth of NPC cells. However, its clinical significance in HCC has not been studied. The purpose of this study was to evaluate the expression of the linc00319 gene in hepatocytes for the first time and to prove that overexpression of long non-coding RNA linc00319 initiated proliferation, the migration and invasion of hepatocytes in HCC. Our results showed that linc00319 in HepG2, hep-3b, HCCLM3, MHCC97H and Huh7 hepatocytes increased compared with normal hepatocytes L-02. In addition, in vitro experiments showed that high expression of linc00319 promoted the proliferation of hep-3b cells, and more importantly, the overexpression of linc00319 promoted the migration, invasion and tumour proliferation of hep-3b cells. It is suggested that linc00319 may be related to the occurrence of liver cancer.

CONCLUSION

In this study, the expression level of linc00319 in HCC cell lines was reviewed for the first time. In addition, according to the in vitro study, the expression level of linc00319 in hepatoma cell lines was much higher than that of normal L-02 cells. Overexpression of linc00319 promotes the proliferation, migration and invasion of HCC. In conclusion, linc00319 may be considered as an oncogene that promotes and initiates the development of HCC. However, the results of this study are based only on a single cell line. In order to verify our conclusions, further experiments are needed on human and animal tissue samples.

Author's contribution

Peter Kisembo and Jian Cheng Tu designed the study. Peter Kisembo and Christian Cedric Christian performed all the experiments. Tapara Dramani souraka analyzed the data. Peter Kisembo and Christian Cedric bongolo wrote the manuscript. Souraka tapara Dramani and peter kisembo edited the manuscript. All authors were responsible for approving the final manuscript submitted.

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