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Inactivation of lncRNA HOTAIRM1 caused by histone methyltransferase RIZ1 accelerated the proliferation and invasion of liver cancer

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Abstract. – OBJECTIVE: Liver cancer is the second most common cause of cancer death, causing more than 700,000 deaths every year. It has been demonstrated that Long non-coding RNA (LncRNA) plays an important regulatory role in a series of diseases. However, the regulatory mechanism of LncRNAs in liver cancer has not been fully elucidated. The purpose of this study was to explore the interaction of lncRNA HOTAIRM1 and aberrant histone modification in liver cancer.

MATERIALS AND METHODS: qRT-PCR was used to detect the expression levels of RIZ1 and miR-125b in liver cancer cells. Cell proliferation was measured using the CCK8 assay. ChIP-Real-time PCR confirmed the binding site of the promoter of HOTAIRM1 by H3K9me1. The direct target of HOTAIRM1 and miR-125b in liver cancer cells was measured by a luciferase reporter assay. Cell proliferation was detected by Cell Counting Kit-8 (CCK8). Cell invasion was measured by transwell assays and cell migration was detected by wound healing assay.

RESULTS: The expression level of RIZ1 and miR-125b was upregulated, and HOTAIRM1 was downregulated in liver cancer cells. Transwell and CCK-8 assay showed that RIZ1 expression is associated with the proliferation, invasion and migration of liver cancer cells, silencing of RIZ1 inhibited cell proliferation, migration, and invasion in HEPG2 and HCC-LM3 cells. RIZ1 interference could significantly inhibit H3K9me1 expression. H3K9me1 protein can bind to HOTAIRM1 promoter directly. Furthermore, the bioinformatics prediction and luciferase assay demonstrated that miR-125b can interact with HOTAIRM1 by direct binding. HOTAIRM1 down-expression promoted HEPG2 cell growth and metastasis, which was further strengthened following the co-transfection of miR-125b. Furthermore, overexpressed HOTAIRM1 inhibited HCC-LM3 cell growth and metastasis and a complete reversal of the results seen when transfected with miR-125b.

CONCLUSIONS: For the first time, we found that RIZ1 was upregulated in liver cancer cells and RIZ1-mediated H3K9me1 enrichment on the HOTAIRM1 promoter regulated the growth and metastasis of liver cancer cells by targeting miR-125b, which could further accelerate tumor proliferation, migration and invasion. It may serve as a therapeutic marker for liver cancer treatment.

Key Words:
RIZ1, LncRNA HOTAIRM1, MiR-125b, Liver cancer, H3K9me1.

Introduction

Globally, liver cancer is the second most common cause of cancer death, accounting for more than 700,000 deaths every year[1,2]. It is usually an aggressive malignancy associated with poor prognosis, and the five-year survival rate is estimated to be less than 9%[3,4]. Surgical interventions, including liver resection, liver transplantation and percutaneous ablation, are regarded as the most effective approach with curative potential for liver cancer[5]. Unfortunately, due to numerous lesions, and extrahepatic metastasis, only about 20% of liver cancer patients are suitable for surgery[6]. On the other hand, chemotherapeutic drugs for liver cancer are limited[7,8]. So how to further clarify the pathogenesis of liver cancer and find a new drug target that more effectively block this progressive disease is important.

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As a significant epigenetic regulation mechanism, histone methylation plays an important role in many biological processes[9]. In cells, there are various histone methyltransferases and histone demethylases working cooperatively to regulate the histone methylation state[10,11] Upon histone modification, effector proteins recognize modification sites specifically, and affect gene transcriptional process. Lysine methylation usually occurs at sites 4, 9, 27, 36, and 79 (H3K4, H3K9, H3K27, H3K36, and H3K79) and at sites 20 (H4K20) of histone H4[12-14]. It is generally believed that methylation modification of H3K4, H3K36 and H3K79 mediates transcriptional activation[15], while methylation modification of H3K9, H3K27 and H4K20 mediates transcriptional inhibition[16]. The retinoblastoma-protein-interacting zinc finger protein1 (RIZ1), a methyltransferase, contains the characteristic PR zinc finger domain[17]. RIZ1 can methylate H3K9 of histone, acted as a transcription suppression factor of cancers[18,19]. Increasing numbers of human cancers are reported to hold decreased or absent RIZ1 expression, which is closely related to cancer progression[20].

Long non-coding RNAs (lncRNAs) are a group of non-protein-coding RNAs that are greater than 200 nucleotides in length[21,22]. Increasing evidence indicates that lncRNAs, which may serve as either oncogenes or tumor suppressor genes[23] play a vital role in human diseases' pathophysiology, especially in tumorigenesis and progression[24,25]. HOTAIRM1, as a kind of lncRNA, has been proved to have an anti-cancer function and its expression in liver cancer is significantly reduced[26,27], but the mechanism of its action is still unclear.

This study mainly discussed the interaction between HOTAIRM1 and H3K9 mediating miR-125b in the proliferation, migration and invasion of liver cancer cells, revealing the molecular mechanism of H3K9 in the progression of liver cancer, and provided new ideas for the treatment of liver cancer.

Materials and Methods

The Online Database Gene Expression Profiling (the GEPIA Web Tool)

Based on The Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx) projects, the RNA sequencing expression data related to our project was analyzed using the GEPIA web tool (http://gepia.cancer-pku.cn).

Cell Culture

Human liver cancer cells HEPG2 and HCC-LM3 cells were purchased from The Cell Bank of Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). Normal human liver cell line (LO2) was purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). All cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM; Gibco, Rockville, MD, USA) supplied with 10% fetal bovine serum (FBS; Gibco, Rockville, MD, USA) and 1% penicillin-streptomycin (Gibco, Rockville, MD, USA) and incubated at 37°C in an atmosphere of 5% CO_2.

Construction of Lentivirus and Cell Transfection

Lentiviral Lnc HOTAIRM1 and lnc HOTAIRM1 siRNA were synthesized and constructed by Shanghai GenePharma Co., Ltd (Shanghai, China). For miR analysis, the miR-125b mimic and the negative control were constructed by Shanghai GenePharma Co. Ltd., (Shanghai, China). RIZ1-small interfering RNA (si-RIZ1) and negative control (si-NC) were constructed by Gene Pharma (Shanghai, China). For cell transfection, 1×10^4 cells were seeded in 6-well plates and transfected with corresponding constructs when the confluence was up to 80% following the instructions of Lipofectamine 2000 (Life Technology, Carlsbad, CA, USA). At 6-h post-transfection, cultures were replaced with DMEM containing 10% FBS. The cells without transfection were used as the control (reRIZ1d as to control). Then, at an indicated time point after transfection, cells were harvested for further study.

Chromatin Immunoprecipitation (ChIP)

According to the manufacturer's protocol, the ChIP assay was performed using the ChIP assay kit (Sigma-Aldrich, St. Louis, MO, USA). Cells were crosslinked with 1% formaldehyde for 10 min at room temperature and the reaction was terminated with 125 mM glycine treatment for 10 min. Chromatin extracts were immunoprecipitated with anti-H3K9me1 and anti-IgG antibodies on Protein-A/G-Sepharose beads. After washing, elution, and de-crosslinking, PCR was performed using the primers spanning the putative H3K9me1-binding site on RIZ1 promoter.

RT-qPCR Assays

After treatment, total RNA of cells was extracted by using TRIzol reagent (Life Technologies, Waltham, MA, USA) according to the manufactur-

er's instructions. And samples were stored at room temperature for 30 min. The reverse transcription of cDNA was performed with a PrimeScript™ RT reagent Kit (TaKaRa, Otsu, Shiga, Japan) according to the manufacturer's instructions. And for qRT-PCR, PCR primers were synthesized by GenePharma (ShangHai Gene Pharma, Shang-Hai, China) and sequences were listed in Table I. SYBR Premix Ex Taq II (TaKaRa, Otsu, Shiga, Japan) was used to detect the expression.

CCK-8 Assays

The CCK-8 kit (Dojindo Molecular Technologies, Kumamoto, Japan) was used to measure the proliferation of HEPG2 and HCC-LM3 cells according to the manufacturers' instructions. In brief, 5×10^3 cells were seeded in 96-well plates uniformly. After treated with regulated medium, the medium was removed and cells were washed with PBS solution for 3 times. Then CCK8 dilution was added to the 96-well plates and incubated at 37°C in an atmosphere of 5% CO_2 for 2 hours. After incubation, the plates were taken out, and cell proliferation was measured using multi-detection microplate reader. And the absorbance (OD) value at 450 nm of each well was detected.

Migration and Invasion Assay

To test the migration and invasion ability of tumor cells, transwell plates with a pore size of 8 μm (Millipore Inc, Billerica, MA, USA) were used. Tumor cells were seeded on the upper chamber with a serum-free medium, and the lower chamber was added with DMEM supplemented with 20% FBS. The invading or migrating cells were fixed with 95% ethanol, stained with 0.1% crystal violet. The upper side of the membrane was wiped with a cotton swab to remove the cells that did not migrate, cell numbers in five random fields were counted in each sample.

Luciferase Assays

After transfection for 48 h, the luciferase activities were measured by using the Dual-Luciferase reporter assay system (Promega, Madison, WI, USA) according to the manufacturer's protocol. The wild-type HOTAIRM1 and mutant HOTAIRM1 sequence (mutant in miR-125b binding site) were cloned into pmirGLO plasmid. The pmirGLO-HOTAIRM1 or pmirGLO-HOTAIRM1-mut was co-transfected with miR-125b mimics or miR-NC by Lipofectamine 2000 (Life Technology, Waltham, MA, USA). Subsequently, the recombinant vectors were co-transfected with miR-NC or miR-125b mimics into HEK 293 cells. Luciferase activity was detected by the Dual-luciferase Reporter Assay System (Promega, Madison, WI, USA) according to the manufacturer's protocols after transfection for 48 h.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism 5 V5.01 software (La Jolla, CA, USA). Statistical differences between the two groups were analyzed using the Student's *t*-test. The comparison between multiple groups was made using One-way ANOVA test followed by Post-Hoc Test (Least Significant Difference). Independent experiments were repeated at least three times for each experiment and error bars are mean ± standard deviation ($\bar{x} \pm SD$). $p<0.05$ was considered statistically significant.

Results

RIZ1 Was Upregulated and HOTAIRM1 Was Downregulated in Liver Tumor

We first detected the expression levels of RIZ1 and HOTAIRM1 in liver tumors. From the expression profile of RIZ1 in 33 cancer types, we found that RIZ1 was highly expressed in liver hepatocellular carcinoma (LIHC) compared with non-tumor samples (Figure 1A). Then, in the GEPIA database, the expression level of RIZ1 was significantly higher in LIHC tissues (Figure 1B), while the mRNA levels of HOTAIRM1 were significantly downregulated in LIHC (Figure 1C). Meanwhile, Kaplan-Meier analysis suggested that

Table I. Primer sequences for qRT-PCR

Genes	Forward	Reverse	Tm (°C)
RIZ1	5'-CCTAGGATCCGTAGGACGC- 3'	5'-GCAGGATTTAAAGCTGACG-3'	60
miR-125b	5'-GGACTCGTAACCGTCCGAA-3'	5'-AACGGTAATGCTGACGTTGC-3'	61
HOTAIRM1	5'-ACCTGTAACGGCACCGTGC-3'	5'-TTGCGTGCAAGGCTTGCACG-3'	62
GAPDH	5'-GCTAGATTGGACACACGGCAT-3'	5'-GGCATCGATTACTCGTACCG-3'	62

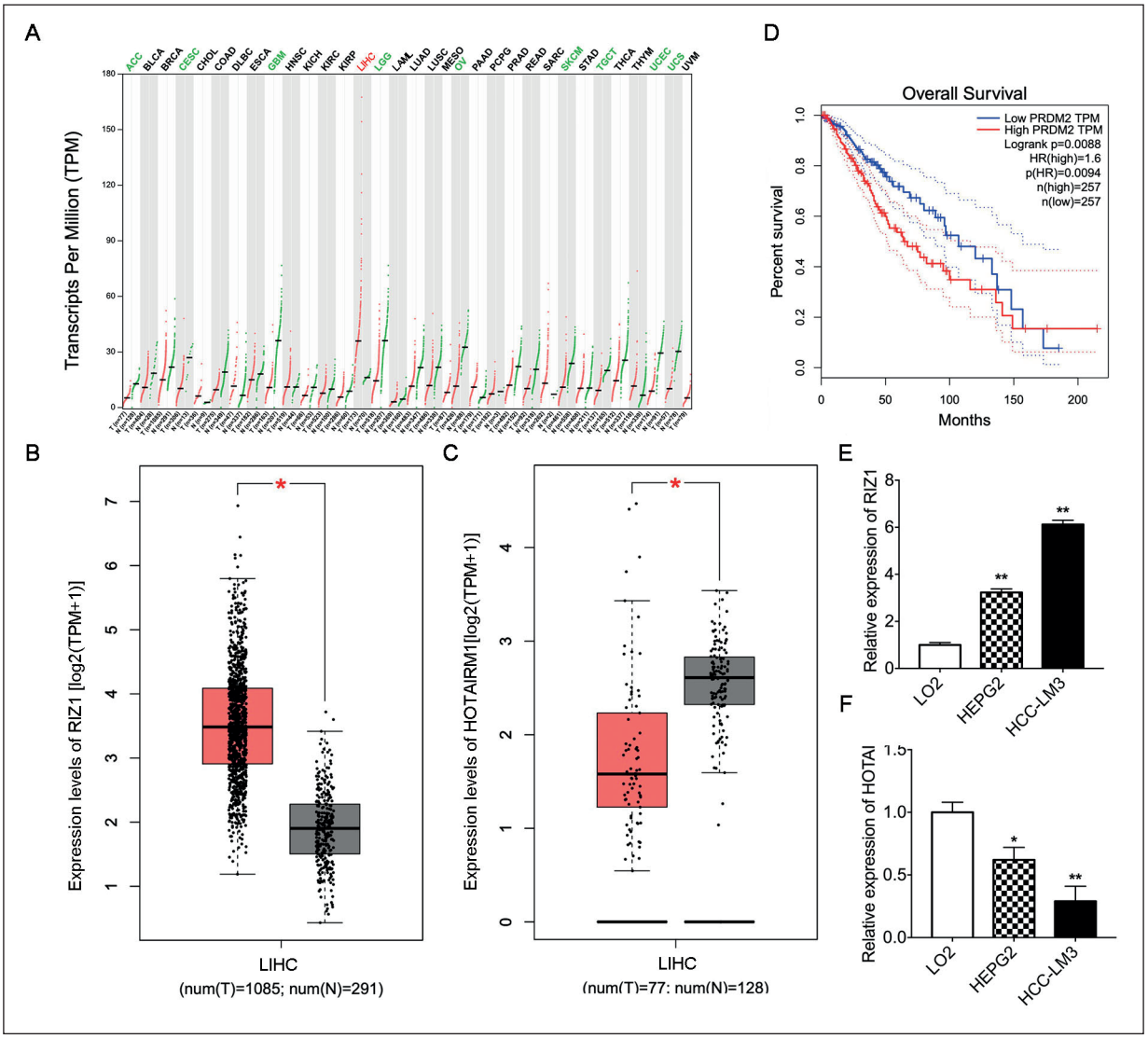

Figure 1. RIZ1 was upregulated and HOTAIRM1 was downregulated in liver tumor. **A,** The expression profile of RIZ1 across 33 cancer types and paired non-tumor samples. Each plot represented a distinct tumor or normal sample. **B,** mRNA levels of RIZ1 in LIHC tissues from GEPIA database. **C,** The mRNA levels of HOTAIRM1 in LIHC tissues from GEPIA database. **D,** Overall survival of liver hepatocellular carcinoma patients in different expression of RIZ1 from GEPIA database. **E,** The mRNA expression levels of RIZ1 were measured by qRT-PCR in liver cancer cell lines (HEPG2 and HCC-LM3) and normal human liver cell line (LO2). **F,** The mRNA expression levels of HOTAIRM1 were measured by qRT-PCR in HEPG2, HCC-LM3, and LO2 cells. The data are expressed as mean ± SD. *p<0.05.

high expression of RIZ1 was associated with reduced overall survival (Figure 1D). Then, we performed qRT-PCR to observe the expressions of RIZ1 and HOTAIRM1 in liver cancer cells. The results revealed that the expression level of RIZ1 was also significantly higher in liver cell lines (HEPG2 and HCC-LM3) than that in a normal human liver cell line (LO2) (Figure 1E). HOTAIRM1 expression was downregulated in HEPG2 and HCC-LM3 compared with LO2 (Figure 1F). Taken together, these data showed that RIZ1 is a potential oncogene in liver cancer and correlated with poor prognosis of liver tumor patients.

Downregulating the RIZ1 Expression Significantly Decreased the Proliferation, Migration and Invasion of Liver Cancer Cells

To explore the functions of RIZ1 in liver cancer progression, we constructed si-RIZ1 to

decrease the expression of RIZ1 and transfected it into HEPG2 and HCC-LM3 cells. In addition, we also performed qRT-PCR to detect the expression level of RIZ1 in tumor cells. And the results showed that the expression of RIZ1 in the si-RIZ1 group was significantly decreased compared with the control ($p < 0.05$) (Figure 2A). To further investigate the role of RIZ1 in cell proliferation, CCK8 assay was performed on HEPG2 and HCC-LM3 cells after transfected with si-RIZ1. The results showed that inhibition of RIZ1 significantly decreased cell proliferation of HEPG2 and HCC-LM3 compared with the control (Figure 2B, Figure 2C). To investigate whether RIZ1 influenced the migration and invasion of tumor cells, we performed transwell assay to detect the migration ability after the expression of RIZ1 was decreased. Results revealed that after downregulating RIZ1 expression, the number of HEPG2 and HCC-LM3 cells that transHOTAIRM1d through transwell chambers was significantly decreased compared with control group (Figure 2D). Besides, the scratch assay was also performed and found that the migration distance was significantly decreased in si-RIZ1 group (Figure 2E). Taken together, these results demonstrated that RIZ1 can regulate the migration ability of human liver cancer cells, and that downregulated RIZ1 can effectively inhibit the tumor cell proliferation, migration, and invasion.

RIZ1-Mediated H3K9me1 Enrichment on the HOTAIRM1 Promoter

RIZ1 is a specific H3K9me1 histone methyltransferase. To investigate whether RIZ1-mediated H3K9me1 enrichment on the HOTAIRM1 promoter regulated liver tumor proliferation and metastasis, si-HOTAIRM1 or Lnc-HOTAIRM1 was introduced into si-RIZ1 transfected HEPG2 and HCC-LM3 cells. And qRT-PCR was performed to detect the expression level of H3K9me1. The results showed that H3K9me1 expression was inhibited in HEPG2 and HCC-LM3 cells after transfection with si-RIZ1 (Figure 3A). ChIP assay was showed to evaluate the binding of H3K9me1 to the HOTAIRM1 promoter, and we observed an increase in the binding between H3K9me1 and HOTAIRM1 promoter in HEPG2 and HCC-LM3

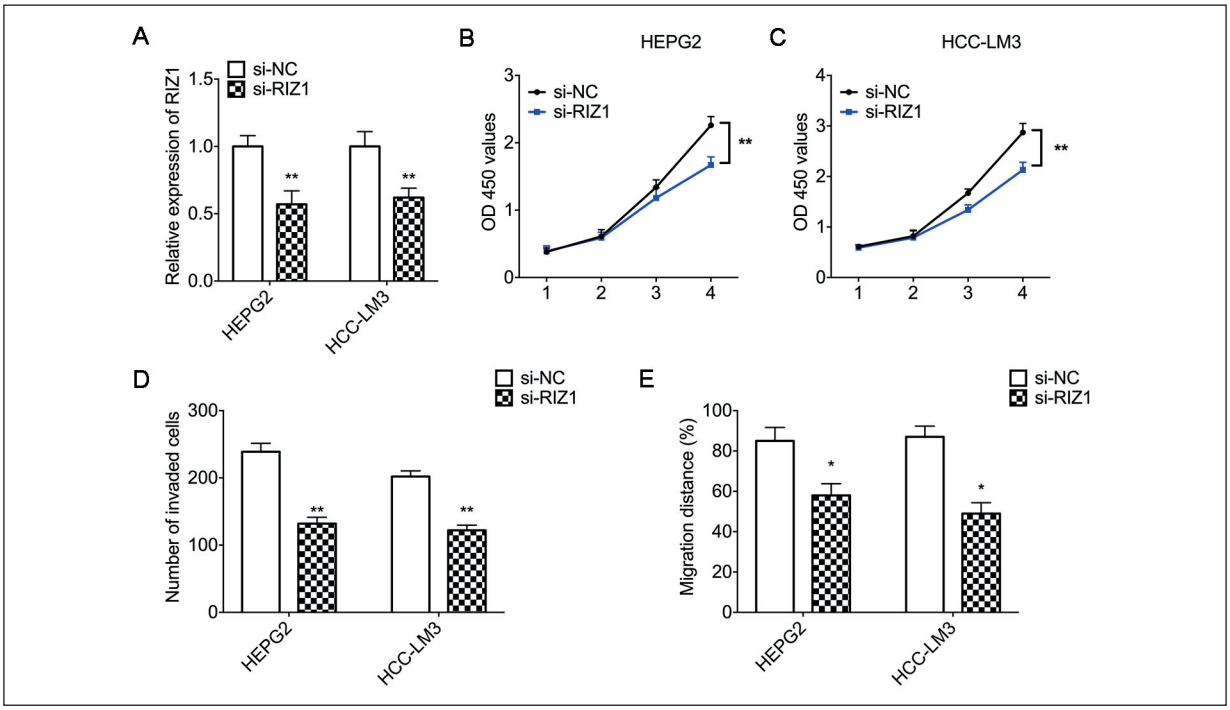

Figure 2. Downregulating the RIZ1 expression significantly decreased the proliferation, migration and invasion of liver cancer cells. **A,** The mRNA expression levels of RIZ1 in HEPG2 and HCC-LM3 cells after transfection with si-RIZ1 were measured by qRT-PCR. **B and C,** The cell proliferation of HEPG2 and HCC-LM3 cells after transfection with si-RIZ1 was measured using CCK-8 assay. **D,** The cell invasion of HEPG2 and HCC-LM3 cells after transfection with si-RIZ1 was measured using transwell assay. **E,** The cell migration of HEPG2 and HCC-LM3 cells after transfection with si-RIZ1 was measured using transwell assay. The data are expressed as mean ± SD. *p<0.05.

cells (Figure 3B). Furthermore, the inhibition of RIZ1 dramatically increased the expression level of HOTAIRM1 in HEPG2 and HCC-LM3 cells (Figure 3C). Besides, RIZ1 down-expression and HOTAIRM1 inhibition in HEPG2 cells reversed the effects of silencing of RIZ1 on liver tumor

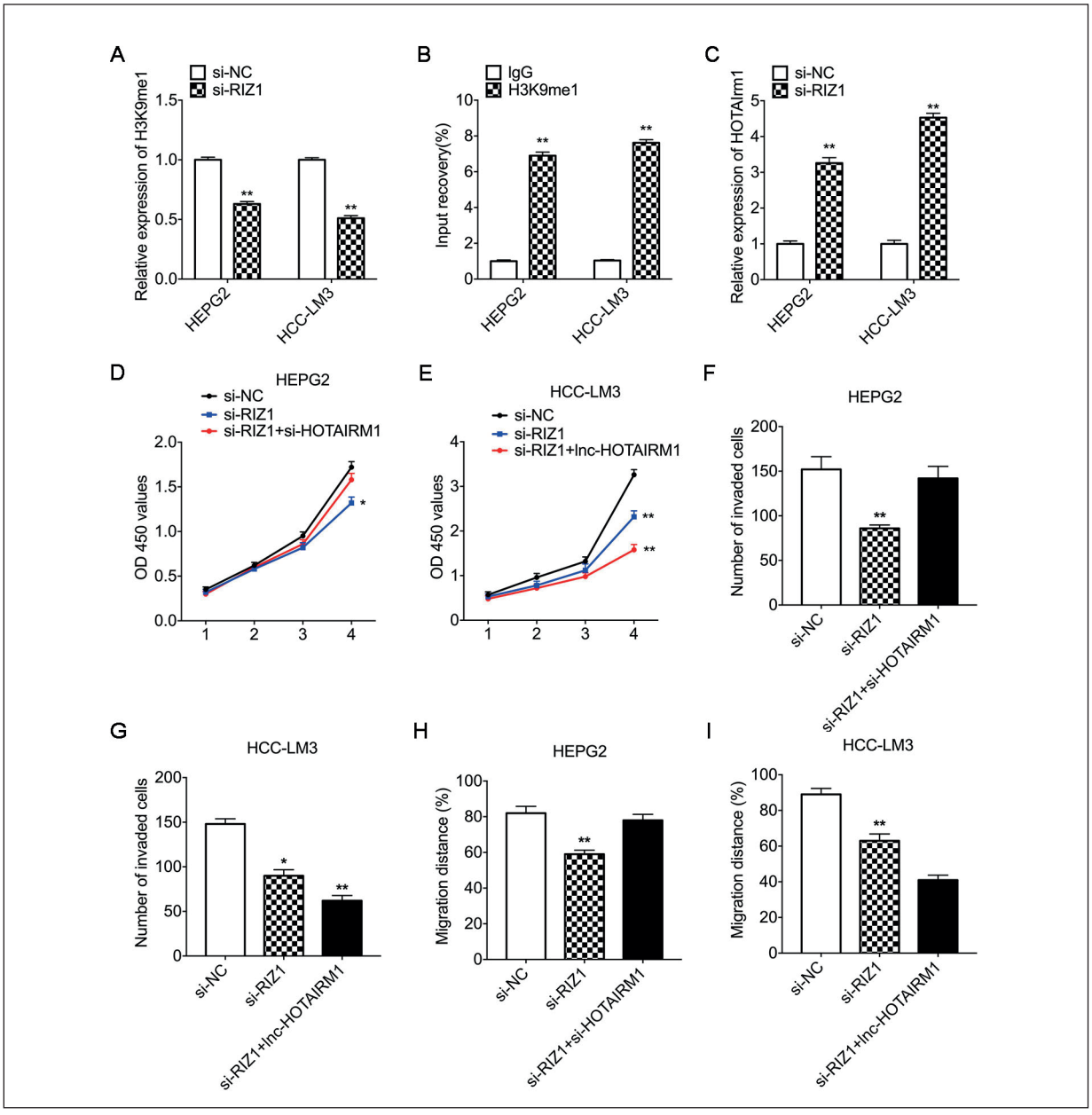

Figure 3. RIZ1-mediated H3K9me1 enrichment on the HOTAIRM1 promoter. **A,** The protein expression levels of H3K9me1 in HEPG2 and HCC-LM3 cells after transfection with si-RIZ1 were measured by qRT-PCR. **B,** The binding of H3K9me1 to the promoter region of HOTAIRM1 was performed by ChIP assay. **C,** The mRNA expression levels of HOTAIRM1 in HEPG2 and HCC-LM3 cells after transfection with si-RIZ1 were measured by qRT-PCR. **D,** The cell proliferation of HEPG2 cells after transfection with si-RIZ1 or si-RIZ1 and si-HOTAIRM1 was measured using CCK-8 assay. **E,** The cell proliferation of HCC-LM3 cells after transfection with si-RIZ1 or si-RIZ1 and LV-HOTAIRM1 was measured using CCK-8 assay. **F,** The cell invasion of HEPG2 cells after transfection with si-RIZ1 or si-RIZ1 and si-HOTAIRM1 was measured using transwell assay. **G,** The cell invasion of HCC-LM3 cells after transfection with si-RIZ1 or LV-RIZ1 and si-HOTAIRM1 was measured using transwell assay. **H,** The cell migration of HEPG2 cells after transfection with si-RIZ1 or si-RIZ1 and si-HOTAIRM1 was measured. **I,** The cell migration of HCC-LM3 cells after transfection with si-RIZ1 or LV-RIZ1 and si-HOTAIRM1 was measured. The data are expressed as mean ± SD. *p<0.05.

cell proliferation (Figure 3D), invasion (Figure 3F), and migration (Figure 3H). However, RIZ1 inhibition and HOTAIRM1 over-expression in HCC-LM3 cells further inhibited cell proliferation (Figure 3E), invasion (Figure 3G), and migration (Figure 3I). Taken together, these results indicated that RIZ1-mediated H3K9me1 enrichment on the HOTAIRM1 promoter regulating the growth and metastasis of liver tumor cells.

HOTAIRM1 Can Interact With MiR-125b in Liver Cancer Cells

To investigate the detailed mechanism of HOTAIRM1 in liver cancer progression, we used StarBase 2.0 database to predict the target miRNA of HOTAIRM1 and found that miR-125b was a target miRNA of HOTAIRM1 (Figure 4A). Then we constructed HOTAIRM1-wt luciferase reporter vector and HOTAIRM1-mut 3'UTR lu-

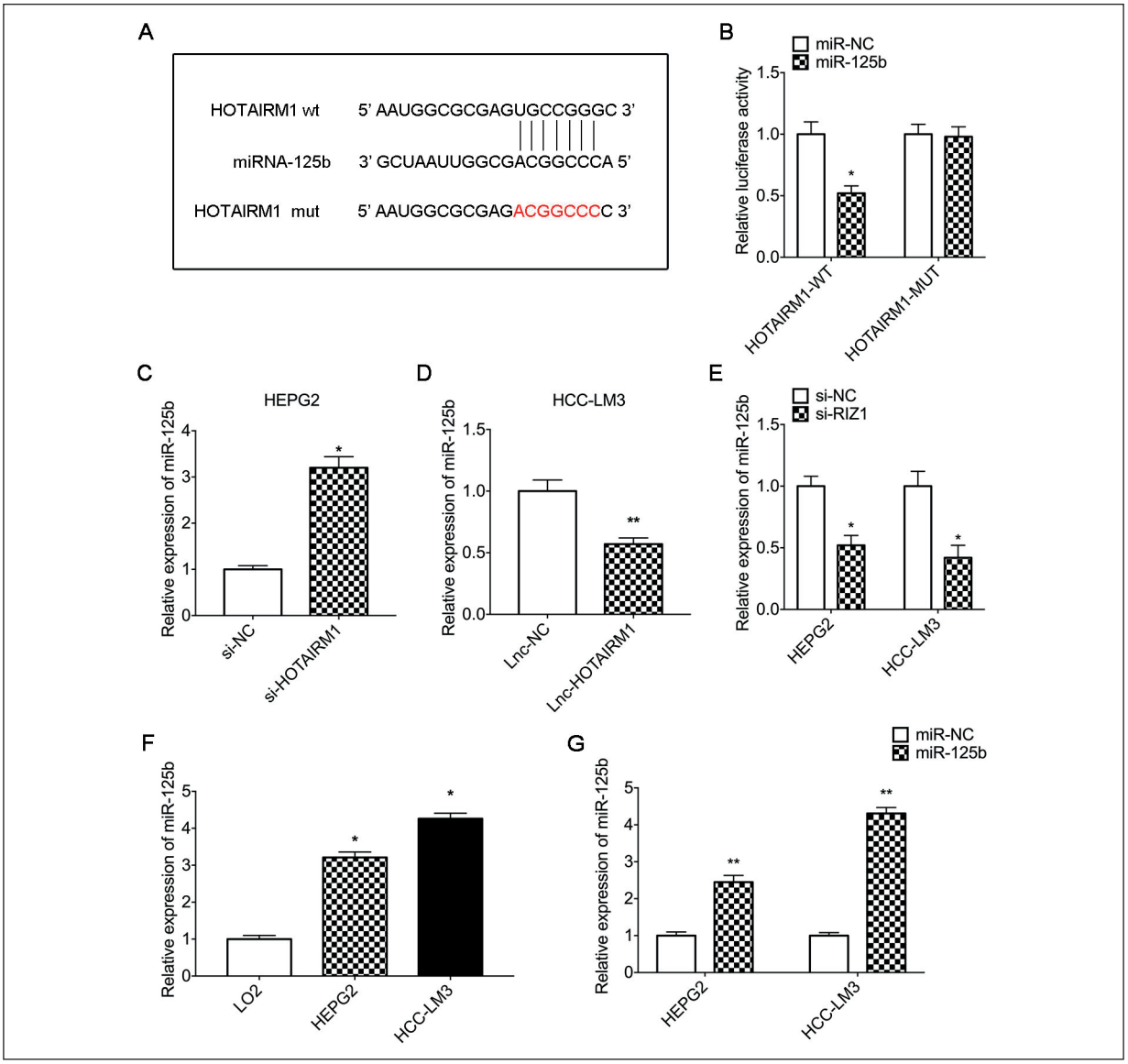

Figure 4. HOTAIRM1 can interact with miR-125b in liver cancer cells. **A,** The predicted miR-125b binging sites in HOTAIRM1 3'-UTR. **B,** Effects of miR-125b on the luciferase activity of HOTAIRM1-WT and HOTAIRM1-MUT reporter were detected by luciferase assays. **C,** The expression levels of miR-125b were determined by qRT-PCR in HEPG2 cells after transfection with si- HOTAIRM1. **D,** The expression levels of miR-125b were determined by qRT-PCR in HCC-LM3 cells after transfection with LV-HOTAIRM1. **E,** The expression levels of miR-125b were determined by qRT-PCR in HEPG2 and HCC-LM3 cells after transfection with si-RIZ1. **F,** The mRNA expression levels of miR-125b were measured by qRT-PCR in HEPG2, HCC-LM3, and LO2 cells. **G,** The mRNA expression levels of miR-125b were measured by qRT-PCR in HEPG2 and HCC-LM3 cells after transfection with miR-125b mimics. The data are expressed as mean ± SD. *p<0.05.

ciferase reporter vector and performed luciferase reporter assay. The results showed that miR-125b over-expression led to a significant decrease in luciferase activity of HOTAIRM1-WT, but not in HOTAIRM1-MUT (Figure 4B). These results suggested that HOTAIRM1 could directly bind to miR-125b. In addition, overexpression of HOTAIRM1 significantly inhibited miR-125b expression and HOTAIRM1 downregulation reversely supported miR-125b expression in liver cancer cells (Figure 4C, 4D). Furthermore, the silencing of RIZ1 dramatically suppressed miR-125b expression level in HEPG2 and HCC-LM3 cells (Figure 4E). To further verify the expression levels of miR-125b in liver cells, qPCR results showed miR-125b expression was increased in HEPG2 and HCC-LM3 compared with LO2 cells (Figure 4F). To explore the function of miR-125b in liver cells, miR-125b mimics was used to elevate the expression of miR-125b in HEPG2 and HCC-LM3 cells and the transfection efficiency was tested using qRT-PCR. The results showed that the expression level of miR-125b was significantly increased in HEPG2 and HCC-LM3 cells transfected with miR-125b mimics. These results showed that HOTAIRM1 suppressed miR-125b expression by direct interaction.

MiR-125b Acts as Functional Target of HOTAIRM1 that Affects the Growth and Metastasis of Liver Cancer Cells

To further verify whether the effects of HOTAIRM1 in liver cancer progression were mediated by miR-125b, si-HOTAIRM1 mimics and miR-125b were co-transfected into HEPG2 cells and LV-HOTAIRM1 mimics and miR-125b were co-transfected into HCC-LM3 cells. CCK8 assay revealed that HOTAIRM1 down-expression in HEP2 cells significantly promoted cell proliferation (Figure 5A), invasion (Figure 5C), and migration (Figure 5E), which was further strengthened following the co-transfection of si-HOTAIRM1 and miR-125b. Meanwhile, overexpression of HOTAIRM1 inhibited HCC-LM3 cell proliferation (Figure 5B), invasion (Figure 5D), and migration (Figure 5F) and a complete reversal of the results seen in co-transfection of LV-HOTAIRM1 and miR-125b. All these data suggested that HOTAIRM1 blocked liver cancer cell growth and metastasis via down-regulating miR-125b. Taken together, RIZ1-mediated H3K9me1 enrichment on the lncRNA HOTAIRM1 promoter, regulating the proliferation, invasion, migration of cancer cells through targeting miR-125b.

Discussion

Liver cancer is a serious threat to human health and life. The clinical manifestations of primary hepatocellular carcinoma are extremely atypical, and the symptoms are usually not obvious, especially in the early stages of the disease[28]. Although the survival rate of liver cancer has improved in recent years, it is still not optimistic[29]. Therefore, our research aims to find a feasible alternative to the treatment of liver cancer. Epigenetic changes mean that DNA sequences do not change while gene expression is genetically altered. The main mechanism is induced by other inheritable materials except for genetic information in cells and can be transmitted stably during cell development and proliferation. There are many epigenetic phenomena, but such epigenetic phenomena as DNA methylation and histone modification are closely related to the development of malignant tumors[30]. RIZ1, a specific H3K9me1 histone methyltransferase was significantly overexpressed in liver cancer cell lines that we found in our study[31,32]. Meanwhile, the silencing of RIZ1 dramatically suppressed liver cell proliferation, invasion, and migration[33]. Above all, these findings suggest that RIZ1 may be involved in liver cancer progression and is a gene responsible for liver cancer.

A microRNA (abbreviated miRNA) is a small non-coding RNA molecule (containing about 22 nucleotides) found in plants, animals and some viruses that function in RNA silencing and post-transcriptional regulation of gene expression[34-37].

MiRNAs are defined as small non-coding RNAs that regulate gene expression through post-transcriptional regulation and promote cell differentiation, growth, and invasion[19]. MiRNAs can act as oncogenes or tumor suppressors in human cancers by regulating processes associated with tumorigenesis[18,38]. miR-125b is one of the oncogenes miRNAs, which is associated with multiple tumors and HOTAIRM1 is the molecular sponge of miR-125b which regulates the growth and metastasis of liver cancer cells. To further reveal whether the effect of HOTAIRM1 on liver cancer progression was mediated by miR-125b, si-HOTAIRM1 mimics and miR-125b were co-transfected into HEPG2 cells, and Lnc-HOTAIRM1 mimics and miR-125b were co-transfected into HCC-LM3 cells. The results showed that HOTAIRM1 expression was down-regulated in HEPG2 cells, which promoted cell proliferation, invasion and migration. The expression of si-HOTAIRM1 was further enhanced after

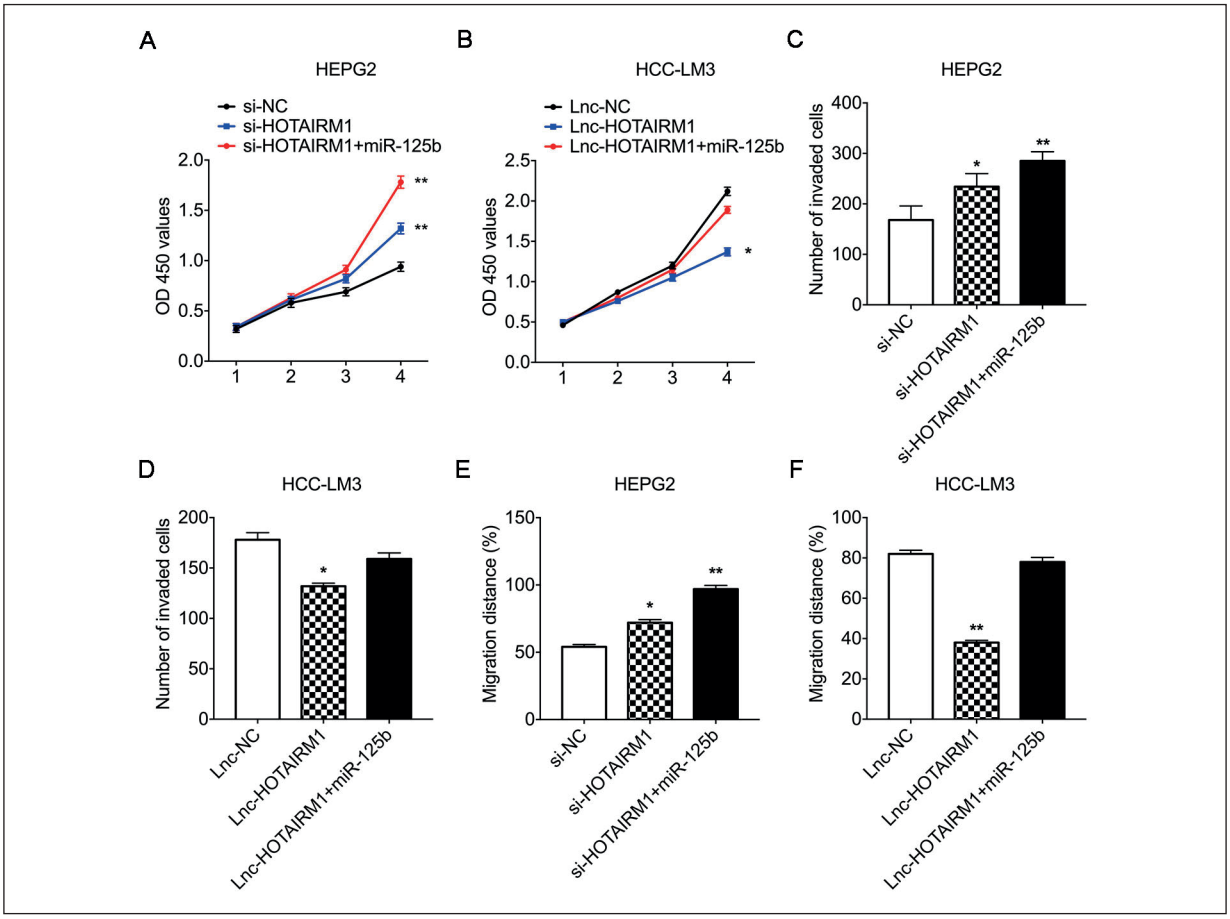

Figure 5. miR-125b acts as a functional target of HOTAIRM1 that affects the growth and metastasis of liver cancer cells. **A,** The cell proliferation of HEPG2 cells after transfection with si-HOTAIRM1 or si-HOTAIRM1 and miR-125b mimics was measured using CCK-8 assay. **B,** The cell proliferation of HCC-LM3 cells after transfection with LV- HOTAIRM1 or LV-HOTAIRM1 and miR-125b mimics was measured using CCK-8 assay. **C,** The cell invasion of HEPG2 cells after transfection with si-HOTAIRM1 or si-HOTAIRM1 and miR-125b mimics was measured using transwell assay. **D,** The cell invasion of HCC-LM3 cells after transfection with LV- HOTAIRM1 or LV- HOTAIRM1 and miR-125b mimics was measured using transwell assay. **E,** The cell migration of HEPG2 cells after transfection with si-HOTAIRM1 or si- HOTAIRM1 and miR-125b mimics was measured. **F,** The cell migration of HCC-LM3 cells after transfection with LV- HOTAIRM1 or LV- HOTAIRM1 and miR-125b mimics was measured using. The data are expressed as mean ± SD. *p<0.05.

co-transfection with miR-125b. Overexpressed HOTAIRM1 inhibited the growth and metastasis of HCC-LM3 cells, completely reversing the co-transfection of Lnc-HOTAIRM1 with miR-125b. All these data suggest that HOTAIRM1 inhibits the growth and metastasis of HCC cells by downregulating miR-125b.

Conclusions

In our study, we found that the expression of RIZ1 was upregulated in liver cancer cells and the expression of HOTAIRM1 was downregulated in liver cancer cells. RIZ1 mediated H3K9me1 enrichment on the HOTAIRM1 that regulated liver cancer cells' growth and metastasis by targeting miR-125b. Thus, our investigation revealed that RIZ1 could epigenetically regulate the expression of lncRNA HOTAIRM1 to promote the proliferation and invasion of liver cancer.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Acknowledgements

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