FEBS Letters 589 (2015) 1981–1987

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FEBS Letters

journal homepage: www.FEBSLetters.org

Long non-coding RNA HOTAIR modulates c-KIT expression through sponging miR-193a in acute myeloid leukemia

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ARTICLE INFO

Article history:
Received 21 March 2015
Revised 28 April 2015
Accepted 30 April 2015
Available online 12 May 2015

Edited by Tamas Dalmay

Keywords:
HOTAIR
miR-193a
c-KIT
Acute myeloid leukemia

ABSTRACT

HOTAIR is significantly overexpressed in various cancers and facilitates tumor invasion and metastasis. However, whether HOTAIR plays oncogenic roles in acute myeloid leukemia (AML) is still unknown. Here, we report that HOTAIR expression was obviously increased in leukemic cell lines and primary AML blasts. Clinically, AML patients with higher HOTAIR predicted worse clinical outcome compared with those with lower HOTAIR. Importantly, HOTAIR knockdown by small hairpin RNA inhibited cell growth, induced apoptosis, and decreased number of colony formation. Finally, HOTAIR modulated c-KIT expression by competitively binding miR-193a. Collectively, our data suggest that HOTAIR plays an important oncogenic role in AML and might serve as a marker for AML prognosis and a potential target for therapeutic intervention.

1. Introduction

Long non-coding RNAs (LncRNAs) are non-protein coding transcripts longer than 200 nucleotides. Recently, emerging data has reported that LncRNAs are frequently deregulated in multiple human diseases and play important roles in a wide range of biological processes, such as proliferation, differentiation, apoptosis, and cell migration, through diverse molecular mechanisms including chromatin modification, transcriptional regulation, and post-transcriptional regulation [1–4]. Among post-transcriptional regulation, lncRNAs act as competing endogenous RNAs (ceRNAs) to sponge miRNAs, consequently modulating the de-repression of miRNA targets.

Homeobox (HOX) transcript antisense RNA (HOTAIR), a long intergenic non-coding RNA, interacts with the Polycomb Repressive Complex 2 (PRC2) to enhance H3K27 trimethylation, finally leading to decreased expression of multiple genes [5]. HOTAIR was firstly reported to be upregulated in primary breast tumor and facilitated breast cancer invasiveness and metastasis in epithelial cancer cells via epigenetic silencing metastasis suppressor genes [5]. In addition, HOTAIR promoted proliferation and reduced apoptosis in hepatocellular carcinoma, colorectal cancer, and pancreatic cancer tissues, in which HOTAIR expression was significantly higher than that in their adjacent non-cancerous tissues [6–8]. Whereas HOTAIR plays important role in the carcinogenesis of solid cancer, the expression and function role of HOTAIR in AML remains largely unknown.

MicroRNAs are highly conserved non-coding RNAs of approximately 20–24 nucleotides that inhibit gene expression at the post-transcriptional level via binding to imperfect complementary sites within the 3′-untranslated regions (3′UTR) of messenger RNAs (mRNAs) [9,10]. Thus, acting as oncogenes or tumor suppressor genes, miRNAs modulate diverse biological processes including cell cycle progression, proliferation, and apoptosis [11–13]. miR-193a was reported to be downregulated in primary AML blasts due to hypermethylation in its promoter region. Ectopic expression of miR-193a inhibited cell proliferation, facilitated differentiation, and induced apoptosis in AML blasts through directly targeting c-KIT, DNMT3a, CCND1 and MDM2 [14,15]. However, it is unclear whether HOTAIR acts as sponge to modulate miR-193a in AML cells.

Author contributions: XCY, HXQ, XFY, and YZJ performed most of the cellular and biochemical experiments. LHY and ZB partially contributed to the cellular experiments. TLY provided clinical samples. All the authors collected and assembled experimental or clinical data. WJB partially designed the experimental plans. GSM designed experiments, analyzed results, and wrote the manuscript.

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http://dx.doi.org/10.1016/j.febslet.2015.04.061
0014-5793/

2. Materials and methods

2.1. Cell lines

AML cell lines purchased from Shanghai cell bank of Chinese Academy of Sciences were employed for the present study. These cells were cultured in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum (Sigma–Aldrich, St. Louis, MO, USA) in humidified 37 °C incubator with 5% CO_2.

2.2. AML patients cohort

A total of patients (Table S1) with de novo AML (age < 60) were diagnosed and classified according to the French–American–British (FAB) criteria between October 2011 and October 2014 in The First Affiliated Hospital of Wenzhou Medical University. This study was approved by the institutional ethics committee, and a written informed consent in accordance with the recommendations of the Declaration of Helsinki.

2.3. RNA extraction and qRT-PCR

Total RNA was extracted from bone marrow mononuclear cells from AML patients (Table S1) using Trizol (Invitrogen, Carlsbad, CA, USA). The levels of HOTAIR transcript variant 1, 2, and 3 (NR_047517; NR_003716; NR_047518) were determined by quantitative real-time PCR using specific primers (Table S2). The relative HOTAIR expression level was calculated using the $2^{-\Delta\Delta Ct}$ method, with the Ct values normalized using GAPDH as internal control. Mature miR-193a and U6 snRNA were reversely transcribed using Stem-loop RT primer with miscript II RT Kit (Qiagen, Valencia, CA, USA). Real-time PCR was performed using SYBR Green PCR Master Mix (Qiagen) in an Applied Biosystem 7500 instrument.

2.4. RNA interference

Gene-specific short hairpin RNA (shRNA) were designed and cloned into pSIREN-RetroQ (Clontech, Palo Alto, CA, USA) retroviral vector. Control shRNA is a non-functional construct provided from Clontech. The sequences of shHOTAIR#1, shHOTAIR#2, and sh-c-KIT were indicated in Table S2.

2.5. Plasmid construction

Expression plasmid for HOTAIR3 were created using PCR amplification with human cDNA as template and then subcloned into lentivirus pLVX-IRES-ZsGreen1 (Clontech) and pcDNA3.1 (Invitrogen). pCDNA-HOTAIR (Mut) and pLVX-HOTAIR (Mut) were generated by the site-directed mutagenesis kit (Stratagene, La Jolla, CA, USA). To produce pMIR-c-KIT3′UTR and pMIR-HOTAIR plasmids, human c-KIT3′UTR and HOTAIR cDNA were amplified by PCR and cloned into pMIR-REPORT vector (Ambion, Dallas, TX, USA). All constructs were confirmed by DNA sequence.

2.6. Western blotting

Western blotting analysis was performed using standard techniques. The following antibody was used: c-KIT (Cell Signaling Technology, Beverly, MA, USA); β-actin antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA).

2.7. RNA binding protein immunoprecipitation (RIP) assay

RNA immunoprecipitation was performed using the EZMagna RIP kit (Millipore, Billerica, MA, USA) following the manufacturer's protocol. THP1 cells were lysed in complete RIP lysis buffer, following incubation with RIP buffer containing magnetic beads conjugated with human anti-Ago2 antibody (RIP Ab+ AGO2 kit, Millipore) or negative control normal mouse IgG (Millipore). Samples were incubated with proteinase k with shaking to digest the protein and then immunoprecipitated RNA was isolated. Purified RNA was subjected to qRT-PCR analysis to demonstrate the presence of the binding targets using respective primers.

2.8. Colony-forming assays

Bone marrow was obtained from 6 patients with AML (2M2, 2M4 and 2M5). Mononuclear cells were separated by Ficoll-Paque liquid and selected by $CD34^+$ (Miltenyi-Biotec, Bergisch Gladbach, German) and suspended in IMDM (Gibco), followed by retrovirus infection. Retrovirus-infected $CD34^+$ cells were seeded into methylcellulose medium (Stem Cell Technologies, Vancouver, Canada) for 14 days. Granulocyte macrophage colony-forming unit (CFU-GM) and erythroid burst-forming unit (BFU-E) were counted according to the manufacturer's protocol.

2.9. Cell growth and apoptosis detection

Cell viability was determined by the trypan-blue exclusion assay, and growth inhibition rate was calculated according to viable cell numbers of treated cells against numbers of untreated cells. cells were plated in triplicate at 2×10^5 cells/mL. After cells were transfected for pSIREN–HOTAIR or pSIREN-NC, apoptosis was detected by annexin V (Invitrogen) in combination with propidium iodide (Invitrogen).

2.10. miR-193a mimic and inhibitor transfection

miR-193a mimic and inhibitor were designed and synthesized by QIAGEN, followed with transfection by HiPerFect transfection reagent (QIAGEN) according to the procedure.

2.11. Luciferase assays

293T cells were seeded in 24-well plates at a density of 2.0×10^5 cells per well, followed growth for 24 h before transfection. Then, each well was transiently cotransfected with pMIR-REPORT reporter plasmid, Scramble or miR-193a mimics and 10 ng internal control vector pRL-SV40 (Promega, Madison, WI, USA) using Hiperfect transfection reagent (Qiagen). Cell lysates were harvested after 24 h transfection, followed by the measurement of firefly and renilla luciferase activities by the Dual-Luciferase Reporter Assay System (Promega). The value of relative luciferase activity indicates the firefly luciferase activity normalized to that of renilla for each assay.

2.12. Statistical analysis

Survival probabilities were estimated by the Kaplan–Meier method and differences in survival distributions were compared using the log-rank test. Cox proportional hazard models were used to estimate hazard ratios (HR) for univariate and analyses for over survival (OS) and disease free survival (DFS). OS was defined as being from the date of diagnosis to death or last follow-up. DFS was defined as the time in CR to relapse or death due to progressive disease. For all the analyses, the P values were two-tailed and a value $P \leqslant 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS 22.0 (SPSS Inc, Chicago, IL, USA).

3. Results

3.1. Upregulation of HOTAIR in AML cells

To investigate the biological role of HOTAIR in AML, we firstly detected the expression of HOTAIR. Three different isoforms were found including HOTAIR transcript variant 1, HOTAIR transcript variant 2, and HOTAIR transcript variant 3 in NCBI (National Center for Biotechnology Information) database. To distinguish the expression of these different isoforms, three pairs of different primers were designed to measure HOTAIR expression (Fig. 1A). As indicated in Fig. 1B, the levels of all HOTAIR variants were increased in leukemic cell lines compared with those in normal controls. Importantly, HOTAIR variant 3 (HOTAIR3) is mostly increased than HOTAIR variant 1 and 2 in leukemic cell lines. Thus, HOTAIR3 was selected for the following analysis. Further, HOTAIR expression was detected in the bone marrow mononuclear cells from 136 AML patients de novo. HOTAIR expression was significantly higher in AML blasts compared with that in normal controls (Fig. 1C). Finally, we analyzed HOTAIR expression in AML

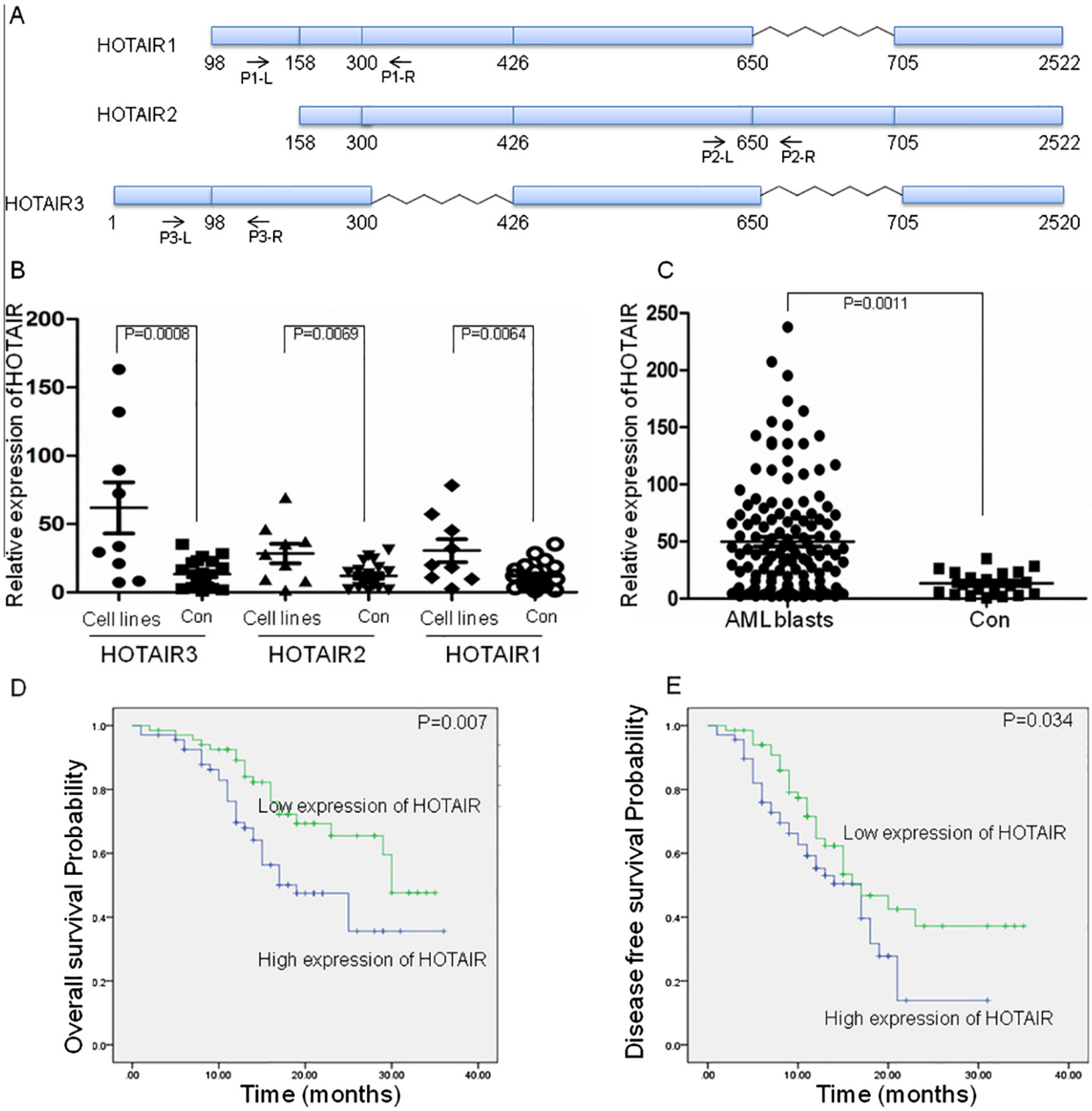

Fig. 1. HOTAIR is highly expressed in AML blasts and higher expression of HOTAIR predicted poor outcome in AML patients. (A) A schematic representation of HOTAIR transcript variant 1, 2, and 3. Primers P1-L and P1-R for HOTAIR1 were set between nucleotide 98–158 and 300–426, respectively, to distinguish the expression of HOTAIR2 and HOTAIR3. Primers P2-L and P2-R for HOTAIR2 were set between nucleotide 426–650 and 650–705 to distinguish the expression of HOTAIR1 and HOTAIR3. Also, primers P3-L and P3-R for HOTAIR3 were set between nucleotide 1–98 and 98–300 to distinguish the expression of HOTAIR1 and HOTAIR2. (B) HOTAIR1, 2, and 3 expressions were detected in normal controls and several leukemia and lymphoma cell lines including K562, HL-60, THP1, NB4, Kasumi, U937, KG-1, HEL, and Jurkat by qRT-PCR. (C) HOTAIR expression was detected by qRT-PCR in bone marrow mononuclear cells from primary AML blasts and normal controls. (D and E) The median expression of HOTAIR was used as the cutoff. Low HOTAIR expression in each of the 68 patients was defined as a value below the 50th percentile. High HOTAIR expression in each of the 68 patients was defined as a value above the 50th percentile. Kaplan–Meier overall survival curve (D) and disease free survival curve (E) were indicated according to HOTAIR expression level.

patients according to gender, FAB subtype, cytogenetics, and molecular genetic abnormality. However, no significant differences were found between these factors (Table S1).

3.2. Higher expression of HOTAIR predicts poor outcome in AML patients

To determine whether HOTAIR expression is associated with clinical outcome in AML patients, AML patients were divided into two different groups according to the median HOTAIR level. HOTAIR expression above or below the median was considered as high or low expression. As indicated in Fig. 1D and E, AML patients with higher expression of HOTAIR predicted worse OS (HR = 2.033; 95% CI = 1.164–3.549; $P = 0.007$) and DFS (HR = 0.609; 95% CI = 0.372–0.997; $P = 0.034$) compared with those with lower HOTAIR expression, respectively.

3.3. HOTAIR knockdown inhibits proliferation and induces apoptosis in AML cells

The significant increase of HOTAIR in AML blasts prompted us to explore the possible biological roles of HOTAIR in AML. To analyze it, endogenous HOTAIR was knocked down by two pairs of shRNA in THP1 and HL-60 cell lines, in which HOTAIR expression is mostly highest (Fig. S1). qRT-PCR analysis showed that HOTAIR expression was effectively reduced by shRNA (Fig. 2A). HOTAIR silencing significantly decreased proliferation (Fig. 2B and C) and induced apoptosis (Fig. 2D and E) in leukemic cells. Further, we asked whether HOTAIR knockdown reduced colony formation in hematological progenitors. $CD34^+$ stem cells were isolated from 6 AML patients and then transfected with shHOTAIR#1, shHOTAIR#2, or sh-NC. As indicated in Fig. 2F and G, HOTAIR knockdown significantly decreased the number of CFU-GM and BFU-E in 4 of 6 AML patients, respectively.

3.4. HOTAIR binds with miR-193a

Recently, several long non-coding RNAs have been reported to function as competing endogenous RNAs (ceRNAs) via competitively binding microRNAs [16,17]. miR-193a has been reported to inhibit cell proliferation and induce apoptosis by targeting c-KIT in AML cells [15]. Similarly, our data also showed ectopic overexpression of miR-193a significantly decreased c-KIT protein level in leukemic cells (Fig. S2A). Also, overexpression of miR-193a

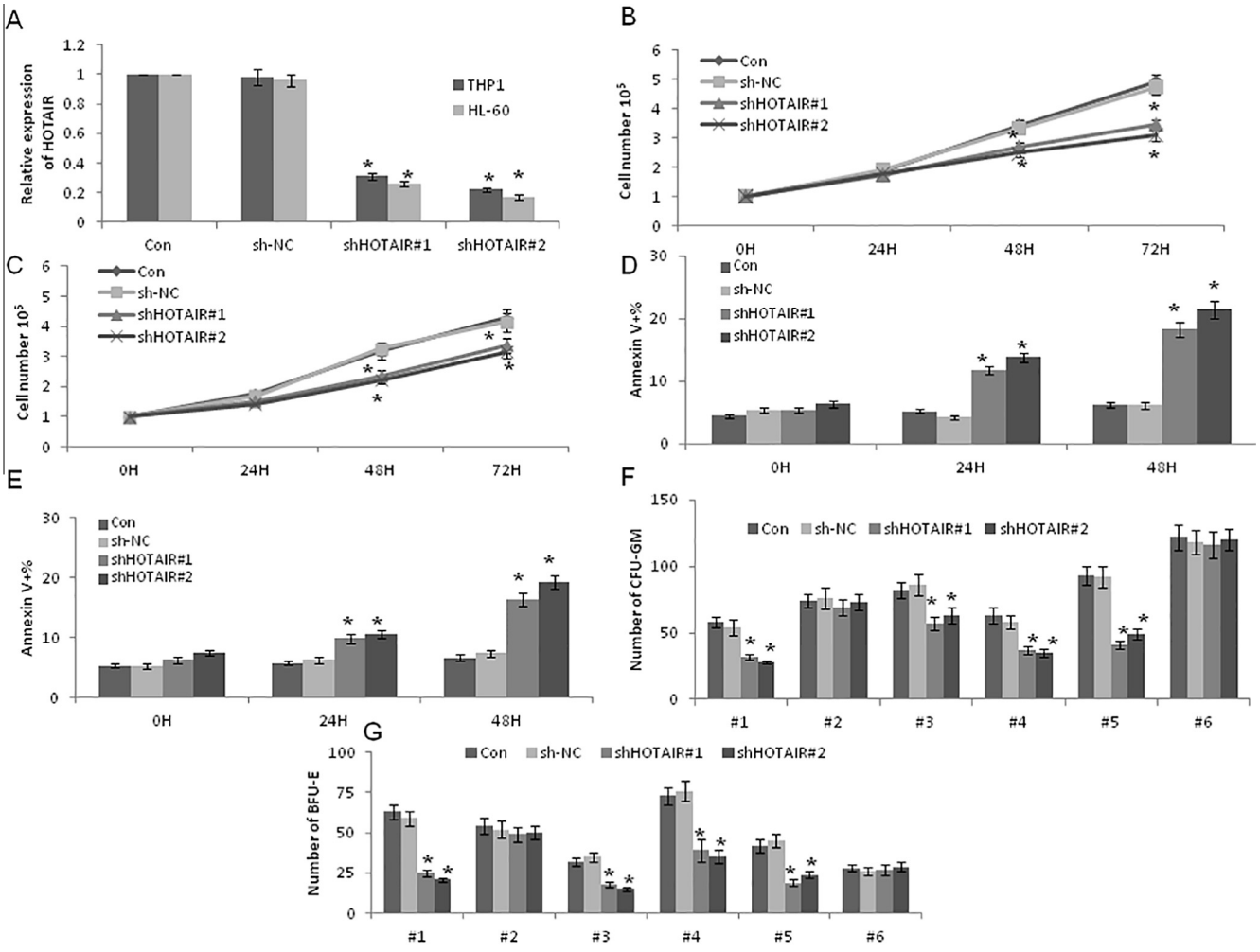

Fig. 2. The effects of HOTAIR on cell growth and apoptosis in leukemic cells. (A) qRT-PCR analysis of HOTAIR expression following transfection of sh-NC, shHOTAIR#1, or shHOTAIR#2 in THP1 and HL-60 cells. (B and C) THP1 (B) and HL-60 cells (C) were transfected with sh-NC, shHOTAIR#1, or shHOTAIR#2 for the indicated times, followed by the direct cell count using the trypan-blue exclusion assay. $^*P < 0.01$ versus NC. (D and E) Apoptosis was detected in THP1 (D) and HL-60 cells (E) transfected with sh-NC, shHOTAIR#1, or shHOTAIR#2 by Annexin V/PI assay. $^*P < 0.01$ versus NC. (F and G) $CD34^+$ stem cells from 6 patients with AML including 2 M2, 2 M4, and 2 M5 were isolated and infected with sh-NC, shHOTAIR#1, or shHOTAIR#2 for colony assay as described in Section 2. The numbers of colonies containing >40 cells in each dish were counted. All experiments were done twice using triplicate plates per experimental point. $^*P < 0.01$ versus NC.

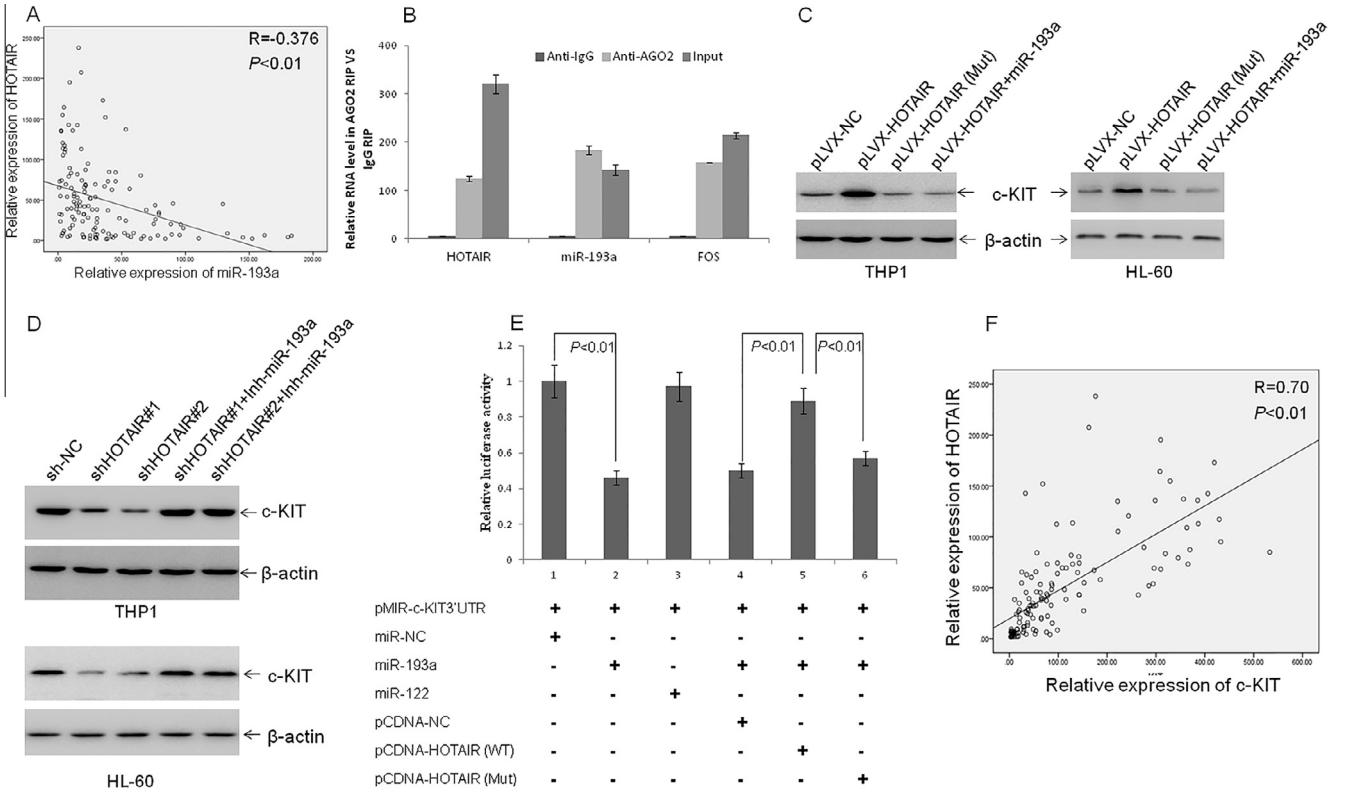

Fig. 3. HOTAIR modulates c-KIT expression via competitively binding miR-193a. (A) The relative expression of HOTAIR and miR-193a were detected in AML blasts by qRT-PCR, respectively. Plotting of HOTAIR and miR-193a expressions showed an inverse correlation between them by Pearson's method. (B) RIP with monoclonal anti-Ago2, preimmune IgG, or 10% input from THP1 lysate. RNA levels of HOTAIR, miR-193a, and FOS were shown as fold enrichment in Ago2 relative to IgG immunoprecipitates by qRT-PCR. (C) THP1 and HL-60 cells were transfected with pLVX-HOTAIR (WT) or pLVX-HOTAIR (Mut), and then positive clones were selected by flowcytometry. Cells with pLVX-HOTAIR (WT) were transfected with miR-193a mimics for 48 h. All cell lysates were extracted for the detection of c-KIT protein. (D) THP1 and HL-60 cells were transfected with sh-NC, shHOTAIR#1, or shHOTAIR#2. The stable knockdown of HOTAIR clone was selected by puromycin and transfected with inhibition of miR-193a. After 48 h transfection, Western blotting was taken to detect c-KIT expression. (E) 3′UTR of c-KIT was cloned into pMIR vector, followed with the transfection with Scramble or miR-193a mimics together with pcDNA-HOTAIR (WT), pcDNA-HOTAIR (Mut), or pcDNA-NC into 293T cells. Luciferase activity was determined using the dual luciferase assay and shown as the relative luciferase activity normalized to renilla activity. miR-122 was purchased as negative control. Histogram presents the relative luciferase activity after trasnfection for 48 h. (F) The relative expression of HOTAIR and c-KIT were detected in AML blasts, respectively. Plotting of HOTAIR and c-KIT expression indicated a positive correlation between them by Pearson's method.

inhibited proliferation (Fig. S2B and C) and induced apoptosis in THP1 and HL-60 cells (Fig. S2D and E). Furthermore, knockdown of c-KIT by specific shRNA (Fig. S2F) decreased cell growth (Fig. S2G and H) and induced apoptosis (Fig. S2I and J). To explore whether HOTAIR binds miR-193a, subsequent bioinformatic analysis by miRmap (http://mirmap.ezlab.org) indicated four putative complementary sequences for miR-193a in HOTAIR (Fig. S3A). Among these four predicted miR-193a sites in HOTAIR, the sites with starting nucleotide at position 1363 and 1809 are an offset 6-mer seed site with strong compensatory base-pairing for the 5′end of miR-193a (Fig. S3A). The other two sites are non-canonical binding (Fig. S3A).

To determine whether HOTAIR actually binds with miR-193a, HOTAIR and miR-193a levels were measured by qRT-PCR in primary AML blasts, respectively. A significant inverse correlation between HOTAIR and miR-193a was found in these cells (Fig. 3A). miRNA is known to be present in the cytoplasm in the form of miRNA ribonucleoprotein complex (miRNP) containing argonaute-2 (Ago2), the core component of the RNA-induced silencing complex (RISC) [18]. To determine if HOTAIR is binding with miRNP, RIP experiment was taken using anti-AGO2 antibody. HOTAIR and miR-193a were significantly enriched by about 25-fold and 36-fold in AGO2-containing immunoprecipitates compared with control immunoglobulin G (IgG) immunoprecipitates (Fig. 3B), respectively. Successful immunoprecipitation of Ago2-associated RNA was confirmed by using human FOS primers in RIP Ab+ AGO2 kit (Fig. 3B).

3.5. HOTAIR affects expression of endogenous miR-193a target

Because HOTAIR shares regulatory miR-193a with c-KIT, we then asked whether HOTAIR could modulate c-KIT expression. Ectopic expression of HOTAIR (WT) but not HOTAIR (Mut) increased the expression of c-KIT (Fig. 3C). However, miR-193a prevented the upregulation of c-KIT by HOTAIR (Fig. 3C). By contrast, HOTAIR knockdown significantly decreased c-KIT (Fig. 3D) expression. However, HOTAIR knockdown plus inhibition of miR-193a restored the decreased c-KIT expression (Fig. 3D). Furthermore, we determined whether HOTAIR acts as sponge to prevent miR-193a-induced degradation of c-KIT mRNA. As indicated in Fig. 3E, ectopic expression of HOTAIR restored the luciferase activity, which was reduced by miR-193a, suggesting that ectopically expressed HOTAIR specifically sequestered endogenous miR-193a, thereby preventing it from inhibiting luciferase activity. When the sequences of HOTAIR at site 1363–1368 and 1809–1814 were both mutated (Fig. S3B), HOTAIR (Mut) lost the ability of binding miR-193a (Fig. 3E chart 6 verse chart 5).

Since HOTAIR increased the expression of c-KIT, we next determine whether HOTAIR is co-expressed with c-KIT in leukemic cells. The expression levels of HOTAIR and c-KIT were detected in

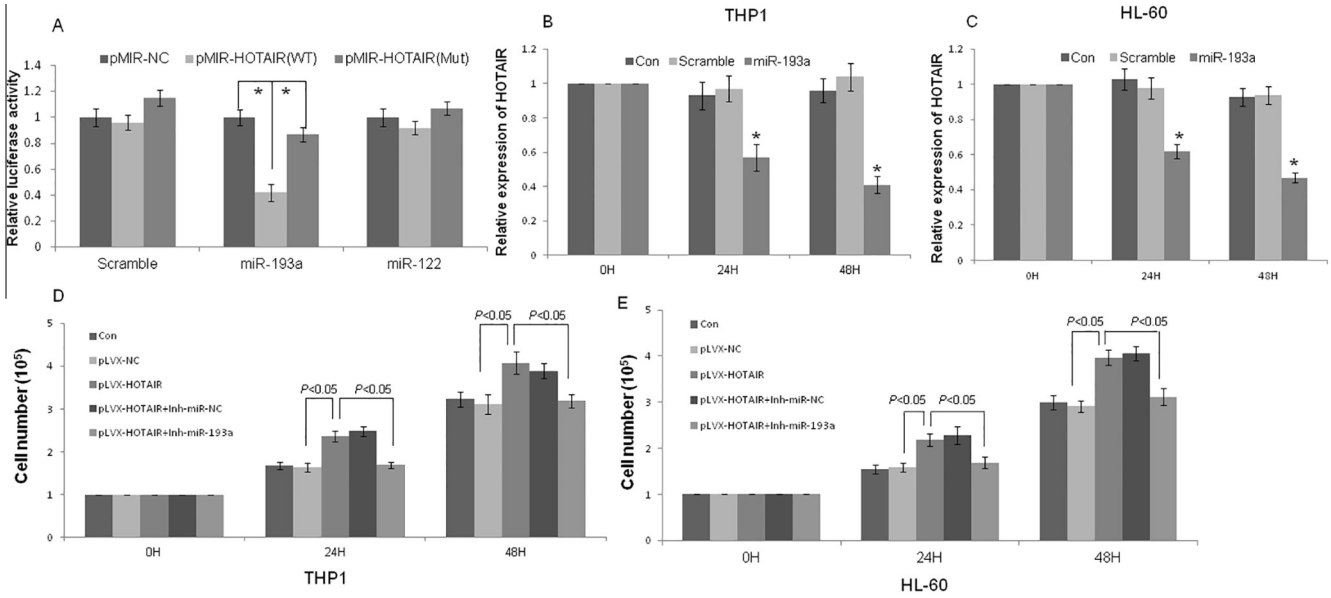

Fig. 4. miR-193a reduces the luciferase activity of HOTAIR and HOTAIR facilitates malignant phenotypes of AML cells (A) HOTAIR cDNA (WT) and HOTAIR cDNA (Mut) were cloned into pMIR vector named by pMIR-HOTAIR (WT) and pMIR-HOTAIR (Mut), which were transfected with Scramble, miR-193a mimics, or miR-122 mimics into 293T cells. Luciferase activity was determined using the dual luciferase assay and shown as the relative luciferase activity normalized to renilla activity. miR-122 was purchased as negative control. $^{*}P < 0.01$ (B and C) HOTAIR levels were measured in THP1 and HL-60 cells transfected with Scramble or miR-193a mimics for 24 and 48 h. $^{*}P < 0.01$ verse Scramble. (D and E) THP1 and HL-60 cells were transfected with pLVX-HOTAIR or pLVX-NC, and then positive clones were selected by flowcytometry. Viable cell numbers were counted in leukemic cells with pLVX-HOTAIR transfected with inhibition of miR-193a or inhibition of miR-NC for 24 and 48 h.

primary AML blasts, and bivariate correlation analysis showed that expression of c-KIT was significantly correlated with HOTAIR transcript level (Fig. 3F).

3.6. miR-193a reduces the luciferase activity of HOTAIR

To further confirm the direct interaction between HOTAIR and miR-193a, the cDNA of HOTAIR (WT) and HOTAIR (Mut) were cloned downstream of the luciferase gene (pMIR-HOTAIR) and transfected with miR-193a mimics or Scramble into 293T cells. As indicated in Fig. 4A, miR-193a reduced the luciferase activity of pMIR-HOTAIR (WT) by 58% compared with the empty vector control. miR-122 served as negative control. However, when the putative two miR-193a-binding sites were mutated (Fig. S3B), miR-193a failed to reduce the luciferase activity of pMIR-HOTAIR (Mut). Meanwhile, we found ectopic overexpression of miR-193a decreased the expression of HOTAIR mRNA levels in leukemic cells (Fig. 4B and C).

3.7. HOTAIR maintenance of the malignant phenotypes of AML cells is dependent on the interaction between HOTAIR, miR-193a, and c-KIT

Finally, we explore whether HOTAIR maintenance of the malignant phenotypes of AML cells is dependent on the interaction between HOTAIR, miR-193a, and c-KIT. We found ectopic overexpression of HOTAIR promoted the proliferation of leukemic cells (Fig. 4D and E). However, HOTAIR-promoted proliferation was prevented by the inhibition of miR-193a (Fig. 4D and E), suggesting that HOTAIR maintenance of the malignant phenotype of AML cells might depend on the interaction of HOTAIR, miR-193a, and c-KIT.

4. Discussion

As a new discovered non-coding RNAs, long non-coding RNAs have been reported to be deregulated in various human diseases, especially cancer. In present study, we find that a well HOTAIR act as natural sponge to bind miR-193a and inhibit its function. The physiological significance of interaction is underscored that HOTAIR knockdown suppresses cell proliferation and induces apoptosis, suggesting that HOTAIR may act as an oncogenic long non-coding RNA.

The importance of lncRNAs in human disease might be correlated with their ability to impact cellular functions through different mechanisms [5,19]. Although HOTAIR was firstly discovered in the nucleus to bind PRC2 complex and alter H3K27 methylation [5], the subcellular location analysis by RNA fluorescence in situ hybridization assay indicated that HOTAIR was distributed in both the nucleus and cytoplasm [20]. Further, HOTAIR was reported to act as scaffold in the cytoplasm to induce ubiquitin-mediated proteolysis by facilitating the ubiquitination of Ataxin-1 and Snurportin-1 [21]. Recently, HOTAIR has been reported to bind different miRNAs in several cancer cells. For example, HOTAIR competitively bound miR-331-3p to regulate HER2 expression in gastric cancer [22]. In support with the interaction of HOTAIR and miRNAs, HOTAIR-mediated oncogenic activity was at least partly through suppression of miR-193a in AML cells. Thus, the mechanistic heterogeneity of HOTAIR in the cytoplasmic form is far from being fully elucidated.

Previous data showed miR-193a is an important tumor-suppressive miRNAs, which was significantly decreased in primary AML blasts due to hypermethylation in miR-193a promoter region [15]. In addition, HOTAIR bound miR-193a to further inhibit its function. Thus, miR-193a expression and biological activity were both inhibited by DNA hypermethylation and high expression of HOTAIR. Therefore, restoration of miR-193a through both hypomethylation and HOTAIR knockdown might completely restore the anti-tumor activity of miR-193a.

Whereas lncRNAs modulate epigenetic gene regulation, metastasis, and prognosis in solid tumors, little data showed their roles in AML. Garzon et al. investigated whether lncRNA expression profile was associated with clinical features, molecular abnormalities, and outcome in older patients with cytogenetically normal AML

and found that patients with unfavorable lncRNA score had shorter OS and DFS compared with those with favorable lncRNA score [23]. These results are consistent with our report that AML patients with higher expression of HOTAIR predicted worse outcome compared with those with lower expression of HOTAIR. Garzon et al. also reported distinctive lncRNA signature in recurrent mutations such as nucleophosmin (NMP) and internal tandem duplications (FLT3-ITDs) [23]. However, we failed to find that HOTAIR expression was associated with such mutations. Obviously, it is urgent to explore if HOTAIR can contribute to understanding the genetic diversity of AML and stratify AML samples for a better clinical prognostication.

In summary, our data indicate that HOTAIR may function as the endogenous sponge to modulate c-KIT expression through competitively binding miR-193a in AML cells. Understanding the precise molecular mechanism is vital for exploring new potential strategies for early diagnosis and therapy. Our experimental data also suggest that targeting the HOTAIR-miR-193a-c-KIT axis may represent a novel therapeutic application in AML.

Conflict of interest disclosure

The authors of this manuscript have no conflicts of interest to disclose.

Acknowledgements

This work was supported by National Natural Science Foundation of China (81200350); The Fund of Wenzhou Science and Technology Bureau (Y20130090, Y20130044).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2015.04.061.

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