Original Paper

Knockdown of Long Noncoding RNA HOXA-AS2 Suppresses Chemoresistance of Acute Myeloid Leukemia via the miR-520c-3p/S100A4 Axis

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Key Words

Hoxa-AS2 • AML • Chemoresistance • miR-520c-3p • S100A4 • CeRNA

Abstract

Background/Aims: Among different molecular candidates, there is growing data to support that long noncoding RNAs (lncRNAs) play a significant role in acute myeloid leukemia (AML). HOXA-AS2 is significantly overexpressed in a variety of tumors and associated with anti-cancer drug resistance, however, little is known regarding the expression and function of HOXA-AS2 in the chemoresistance of AML. In this study, we aimed to determine the role and molecular mechanism of HOXA-AS2 in adriamycin-based chemotherapy resistance in AML cells. **Methods:** Quantitative real-time PCR was used to detect HOXA-AS2 expression in the BM samples and ADR cell lines, U/A and T/A cells. Furthermore, the effects of HOXA-AS2 silencing on cell proliferation and apoptosis were assessed *in vitro* by CCK8 and flow cytometry, and on tumor growth *in vivo*. Furthermore, bioinformatics online programs predicted and luciferase reporter assay were used to validate the association of HOXA-AS2 and miR-520c-3p in AML. **Results:** In this study, we showed that HOXA-AS2 is significantly upregulated in BM samples from AML patients after treatment with adriamycin-based chemotherapy and in U/A and T/A cells. Knockdown of HOXA-AS2 inhibited ADR cell proliferation *in vitro* and *in vivo* and promoted apoptosis. Bioinformatics online programs predicted that HOXA-AS2 sponge miR-520c-3p at 3'-UTR with complementary binding sites, which was validated using luciferase reporter assay and anti-Ago2 RIP assay. HOXA-AS2 could negatively regulate the expression of miR-520c-3p in ADR cells. S100A4 was predicted as a downstream target of miR-520c-3p, which was confirmed by luciferase reporter assay. **Conclusion:** Our results suggest that HOXA-AS2 plays

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an important role in the resistance of AML cells to adriamycin. Thus, HOXA-AS2 may represent a therapeutic target for overcoming resistance to adriamycin-based chemotherapy in AML.

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Introduction

Acute myeloid leukemia (AML) is a complex disease with high incidence and recurrence rates [1]. Currently, several chemotherapeutic drugs including adriamycin were predominantly used for the treatment of AML, and the development of chemoresistance is the major problem [2]. Approximately 20% of adult patients with AML fail to achieve remission with initial induction chemotherapy, and approximately 50% ultimately experience relapse after achieving complete remission (CR) [3]. Although nearly 60-80% of the patients achieved CR after cytotoxic therapy, a significant subset presents with primary refractory disease [4-6]. Accordingly, chemoresistance constitutes a critical challenge in the pharmacological treatment of AML. It is therefore critical to understand the molecular mechanisms underlying chemoresistance in AML and develop revolutionary therapeutic strategies for reversing chemoresistance.

Long noncoding RNAs (lncRNAs) are transcripts longer than 200 nucleotides, located within the intergenic stretches or overlapping antisense transcripts of protein coding genes [7]. lncRNAs are involved in multiple stages of gene regulation including chromatin modification, chromatin structure, and mRNA and protein biogenesis during differentiation and development [8]. Similar to protein-coding genes, lncRNAs may have important roles in diverse cellular processes, with their dysregulation already associates with disorders, including leukemia [9]. However, only a few have been mechanistically and functionally characterized in the context of AML-associated aberrant gene networks. Recently, increasing evidence has shown that HOXA cluster antisense RNA 2 (HOXA-AS2), a lincRNA located between and antisense to the human HOXA3 and HOXA4 genes, promote several tumorigenic features including survival, proliferation and invasion [10, 11]. Subsequent studies suggested that HOXA-AS2 is significantly overexpressed in a variety of tumors and associated with poor prognosis of these tumors. Although the role of HOXA-AS2 in anti-cancer drug resistance has been confirmed in many malignancies, however, little is known about the role of HOXA-AS2 in chemoresistance in AML.

Many studies have shown that lncRNAs can serve as a competing endogenous RNAs (ceRNAs) to play a post-transcriptional regulatory role in miRNA distribution on their targets [12]. MiRNAs are a class of 21–25 nucleotide small non-coding RNA with no protein-coding potential. MiRNAs have crucial roles in the regulation of gene expression through binding to the 3'-UTR of the target genes [13]. MiRNAs are established regulators of hematopoiesis and leukemogenesis, however, whether HOXA-AS2 could serve as a miRNA sponge to regulate the chemoresistance of AML is still unknown.

In this study, we aimed to determine the regulation and function of HOXA-AS2 and its underlying mechanism in chemoresistance of adriamycin-based chemotherapy in AML. Our results suggest that HOXA-AS2 was upregulated following adriamycin-based chemotherapy. Functional and mechanistic analyses demonstrated that HOXA-AS2 functioned as a ceRNA to regulate the expression of S100 calcium-binding protein A4 (S100A4) by competing for miR-520c-3p binding and thereby regulating chemoresistance of AML cells. HOXA-AS2 may represent a therapeutic target for overcoming resistance to adriamycin-based chemotherapy in AML.

Materials and Methods

Clinical samples

We recruited 48 AML patients who received adriamycin-based chemotherapy from Department of Hematology, The Third Affiliated Hospital of Sun Yat-sen University during 2010 to 2016. The initial diagnosis of AML was performed according to the French-American-British classification. Bone marrow (BM) samples were collected from these AML patients. All samples were enriched for mononuclear cells and mononuclear cells were purified from BM samples by standard Ficoll-Hypaque density centrifugation and stored at -80°C until use. The informed consent to perform the biological studies was obtained from all of the examined subjects and the related study was approved by the Ethics Committees of the participating hospitals and the Institutional review Board of The Third Affiliated Hospital of Sun Yat-sen University.

Cell lines and culture

Two AML cell lines, U937 and THP-1 were obtained from the KeyGEN Company (Nanjing, China) and grown in RPMI-1640 medium (Thermo Fisher Scientific, Inc, Waltham, MA) supplemented with 10% fetal bovine serum (FBS; HyClone, Logan, UT), 100 U/mL penicillin and 100 U/mL streptomycin at 37°C in a humidified atmosphere of 95% air/5% CO_2 . The adriamycin-resistant (ADR) sublines were established by adding increasing concentrations of adriamycin (Sigma) to the parental cell. Adriamycin concentrations were from 1 to 5 mg/l for 6 months and the cells were namely U937/ADR (U/A) and THP-1/ADR (T/A), respectively. The phenotype of resistant AML cell lines was maintained by adding 1.0 mg/L adriamycin.

RNA extraction and qRT-PCR assays

Total RNA was isolated from BM samples and AML cell lines by Trizol reagent (Invitrogen, USA). Using a PrimeScript™ RT reagent Kit (TaKaRa), the first strand cDNA was synthesized. The cDNA was amplified by SYBR Premix Ex Taq™ II (TaKaRa). HOXA-AS2 were normalized to GAPDH, miR-520c-3p was normalized to U6. The qRT-PCR assays were conducted on an ABI 7500, and data collected with this instrument. Our qRT-PCR results were analyzed and expressed relative to threshold cycle (CT) values, and then converted to fold changes.

Cell transfection

Cells were seeded in 6-well dishes the night before to give 80-90% confluence for plasmid and 40-50% for miRNA at the day of transfection. The following day cells were transfected with miR-520c-3p mimics (50 nM), miR-520c-3p inhibitor (100 nM), miR-nc (50 nM), inhibitor-nc (100 nM), respectively using Lipofectamine 2000 (Invitrogen), according to the manufacturer's instructions. To perform effective lentivirus-mediated suppression of HOXA-AS2 in cells, HOXA-AS2 shRNA and scrambled control shRNA were inserted into the pLVX-tdTomato-Puro vector (Biowit, Shenzhen, China). After transfection for 6 hours, the medium was replaced with normal culture medium. At 48 h post-transfection, cells were harvested for qRT-PCR analysis.

Cell proliferation assay

Cells were incubated in 10% CCK-8 (DOJINDO, Japan) diluted in culture medium at 37°C until visual color conversion occurred. Proliferation rates were determined at 0, 24, 48, 72, 96 h after transfection. The absorbance of each well was measured with a microplate reader set at 450 nm and 630 nm. All experiments were performed in quadruplicate.

Detecting apoptosis by flow cytometry

An annexin V-allophycocyanin (APC)/ 4',6-diamidino-2-phenylindole (DAPI) double staining kit (Thermo Fisher Scientific) was used to analyze cellular apoptosis. Transfected cells were seeded in 6-well plates (5×10^5 cells/well) and treated with 1.0 mg/L adriamycin. Cells were then digested with trypsin (Gibco® trypsin-EDTA, Thermo Fisher Scientific), washed with PBS three times, suspended in 500 μl of binding buffer, and then incubated with 5 μl of FITC-conjugated annexin V and 3 μl of PI for 15 min at room temperature in the dark. The stained cells were detected using the BD FACS Aria II flow cytometer (BD Biosciences, Hercules, CA, USA).

Antitumor activity in xenograft model

The male nude mice (5-week old) were obtained from the Shanghai Experimental Animal Center of the Chinese Academy of Sciences (Shanghai, China). U/A and T/A cells transfected with shHOXA-AS2 or negative control shRNA were subcutaneously injected into the right flanks of nude mice. The tumor volume was monitored and calculated according to the following formula: Volume = Length × Width² / 2. All animal experiments were approved by the Institutional Animal Care and Use Committee of The Third Affiliated Hospital of Sun Yat-sen University.

RNA immunoprecipitation (RIP) assay

The RIP assay was performed to explore the interaction between HOXA-AS2 and miR-520c-3p by using EZ-Magna RIP RNA-binding protein immunoprecipitation kit (Millipore). Cells were lysed, and the cell lysis was then incubated with anti-human Ago2 antibodies (Millipore) coated on magnetic beads in RIP buffer. Input and normal IgG were used as controls. The precipitated RNAs were isolated and reverse transcribed in cDNA to analyze HOXA-AS2 and miR-520c-3p level using qRT-PCR.

Luciferase reporter assay

The predicted wild-type (wt) miR-520c-3p binding sequences in HOXA-AS2 or 3'-UTR S100A4 were synthesized and cloned into downstream of the luciferase gene in the pmirGLO luciferase vector (Promega) to generate HOXA-AS2-wt and S100A4-wt. Their corresponding mutated sequences (mut) were created using GeneTailor Site-Directed Mutagenesis System (Invitrogen) and cloned into the same vector, namely HOXA-AS2-mut, and S100A4-mut. Prior to transfection, cells were seeded in 24-well plates (1×10^3 cells/well) and cultured for 24 h. Cells were then transiently cotransfected with 100 ng wt or mut of HOXA-AS2 or S100A4 3'-UTR and 10 nM miR-520c-3p or miR-NC together with 20 ng Renilla luciferase vector (Promega) using Lipofectamine 2000 (Invitrogen). Cells were collected at 48 h post-transfection and the luciferase activity was measured by luciferase reporter assay using the Dual-Luciferase reporter assay system (Promega). Firefly luciferase activity was normalized to Renilla luciferase activity.

Statistics

Student's t-test (two-tailed) was performed to analyze the data. A two-sided P-value of less than 0.05 was considered statistically significant. All statistical computations were performed using SPSS (SPSS Inc., USA).

Results

Expressional of HOXA-AS2 in AML patients and cell lines

To address the role of HOXA-AS2 in regulating the chemoresistance of AML, we initially analyzed the expression of HOXA-AS2 in the BM samples from 48 AML patients before or after treatment with adriamycin-based chemotherapy by qRT-PCR. As compared with AML patients before chemotherapy, significant upregulation of HOXA-AS2 in AML patients treated with adriamycin based chemotherapy was observed ($P < 0.01$, Fig. 1A). Additionally, we established two ADR cell lines, U937/ADR (U/A) and THP-1/ADR (T/A), by continuously exposing U937 and THP-1 cells to increasing concentrations of adriamycin. The expression levels of HOXA-AS2 in these two ADR cell lines compared to parent cell lines were analyzed by qRT-PCR. We found that HOXA-AS2 was significantly upregulated in U/A and T/A cell lines compared with those of drug-susceptible parental cells lines U937 and THP-1 ($P < 0.01$, Fig. 1B-C), indicating that HOXA-AS2 may play an important role in resistance of adriamycin-based chemotherapy.

HOXA-AS2 modulates the chemosensitivity of AML cells in vitro and in vivo

To analyze the role of HOXA-AS2 in AML, U/A and T/A cells were stably transfected with HOXA-AS2 shRNAs (respectively shHOXA-AS2 #1, shHOXA-AS2 #2, or shHOXA-AS2 #3) or or negative control shRNA (sh-NC) to silence HOXA-AS2 expression. We detected HOXA-AS2 expression at 48h post-transfection by qRT-PCR analysis to analyze efficiency and revealed

Fig. 1. A: qRT-PCR showing expression level of HOXA-AS2 in the BM samples from 48 AML patients before or after treatment with ADR-based chemotherapy; B: qRT-PCR showing expression level of HOXA-AS2 in U937/ADR (U/A) cell lines; C: qRT-PCR showing expression level of HOXA-AS2 in THP-1/ADR (T/A) cell lines; D: HOXA-AS2 shRNAs was used to enhance efficiency of HOXA-AS2 knockdown in U/A and T/A cells; E: CCK8 assay showing knockdown of HOXA-AS2 inhibited cell proliferation of U/A cells; F: CCK8 assay showing knockdown of HOXA-AS2 inhibited cell proliferation of T/A cells.

that HOXA-AS2 expression was significantly decreased in U/A and T/A cells. Moreover, shHOXA-AS2 #3 had higher efficiency of interference than shHOXA-AS2 #1 and #2 group ($P < 0.01$, Fig. 1D), so we chose shHOXA-AS2 #3 subsequently for the following experiments.

CCK-8 assays revealed that knockdown of HOXA-AS2 expression significantly suppressed cell proliferation of U/A and T/A cells after treatment with adriamycin, compared with the respective controls ($P < 0.01$, Fig. 1E-F). Moreover, shRNA targeting HOXA-AS2, significantly enhanced the ability of chemotherapy-induced apoptosis in U/A and T/A cell lines ($P < 0.01$, Fig. 2A-B). To further assess the chemosensitivity to adriamycin *in vivo*, mouse xenograft studies were performed. In the HOXA-AS2 shRNA model, down-expression of HOXA-AS2 significantly inhibited tumor growth. The volumes of tumors derived from HOXA-AS2-silenced cells were significantly reduced compared to those of tumors from control cells ($P < 0.01$, Fig. 2C-F). A similar trend in tumor weight was observed after about one month ($P < 0.01$, Fig. 2G-H).

HOXA-AS2 functioned as a ceRNA by directly binding to miR-520c-3p in AML cells

To investigate the effect of HOXA-AS2 on the expression of miRNAs, U/A cells were transfected with either empty vector or shHOXA-AS2 for 48 hrs. Hierarchical clustering of differentially expressed miRNAs is shown in Fig. 3A. Then, we applied the online software starBase v2.0. to predict the miRNAs that interacted with HOXA-AS2. The bioinformatics analysis revealed potential combination of HOXA-AS2 and miR-520c-3p, the putative binding sites as shown in Fig. 3B. The qRT-PCR analysis implicated that miR-520c-3p expression was

Fig. 2. A: HOXA-AS2 knockdown significantly enhanced the ability of chemotherapy-induced apoptosis in U/A cell lines; B: HOXA-AS2 knockdown significantly enhanced the ability of chemotherapy-induced apoptosis in T/A cell lines; C-F: HOXA-AS2 knockdown inhibits AML tumour growth in vivo. The tumour volume curve of nude mice was analysed. G-H, The tumour weights of nude mice were measured.

demonstrated to be abnormally downregulated in two ADR cell lines compared to parent cell lines, suggesting the potential association between miR-520c-3p and adriamycin resistance ($P < 0.01$, Fig. 3C-D).

To investigate the role of miR-520c-3p in adriamycin resistance, miR-520c-3p mimics or inhibitor was transfected into U/A and T/A cells and the proliferation curves were performed using CCK8 assays. Our results showed that miR-520c-3p overexpression markedly inhibits the cell growth in both U/A and T/A cells when compared with cells transfected with miR-nc, whereas U/A and T/A cells transfected with miR-520c-3p inhibitor grew at a dramatically higher rate as compared with controls (Fig. 3E-H), indicating the opposite result to HOXA-AS2 expression.

To further confirm whether HOXA-AS2 function as a ceRNA of miR-520c-3p, anti-Ago2 RIP assay, and luciferase reporter assay were conducted. RIP assay showed that HOXA-AS2 and miR-520c-3p were preferentially enriched in Ago2-containing beads in U/A and T/A cells relative to control IgG immunoprecipitates (Fig. 4A). Results from luciferase reporter assay demonstrated that miR-520c-3p overexpression significantly reduced the luciferase activities of HOXA-AS2-wt reporter vector in both U/A and T/A cells, but had no obvious inhibitory effect on HOXA-AS2-mut reporter vector (Fig. 4B). To explore the regulatory relationship between HOXA-AS2 and miR-520c-3p, U/A and T/A cells were transfected with either empty vector or shHOXA-AS2 #3, and qRT-PCR was used to detect the expression level of miR-520c-3p. The results showed that silencing of HOXA-AS2 increased the expression level of miR-520c-3p in U/A and T/A cells ($P < 0.01$, Fig. 4C).

Fig. 3. A: U/A cells were transfected with shHOXA-AS2 #3 for 48 hrs. Hierarchical clustering revealed systematic variations in the expression of miRNAs. Numerous differentially expressed miRNAs between control and shHOXA-AS2 #3 transfected cells are shown on a scale from green (low) to red (high); B: StarBase v2.0 results showing the sequence of HOXA-AS2 with highly conserved putative miR-520c-3p binding sites; C: qRT-PCR showing expression level of miR-520c-3p expression in U/A cells; D: qRT-PCR showing expression level of miR-520c-3p expression in T/A cells; E: CCK8 assay showing miR-520c-3p overexpression markedly inhibits the cell growth in U/A cells; F: CCK8 assay showing miR-520c-3p overexpression markedly inhibits the cell growth in T/A cells; G: CCK8 assay showing miR-520c-3p miR-520c-3p inhibitor markedly promotes the cell growth in U/A cells; G: CCK8 assay showing miR-520c-3p miR-520c-3p inhibitor markedly promotes the cell growth in T/A cells.

HOXA-AS2 affects expression of endogenous miR-520c-3p target, S100A4

Bioinformatics analysis using targeting algorithms (TargetScan and microRNA.org) showed that miR-520c-3p had candidate binding sites in the 3'-UTR of S100A4 mRNA. To confirm the predictions, a wild type or mutant S100A4 3' UTR luciferase reporter vector was conducted. S100A4-wt or S100A4-mut was co-transfected with miR-520c-3p mimics or negative control into HEK293T cells. The relative luciferase activity was remarkably reduced in cells co-transfected with the S100A4-wt luciferase reporter and miR-520c-3p mimic than in the negative control cells. However, inhibitory effects were abolished when 3'-UTRs that contained both mutant-binding sites were co-transfected with miR-520c-3p, confirming that S100A4 is a target of miR-520c-3p ($P < 0.01$; Fig. 4D). Furthermore, the results of qRT-PCR revealed that HOXA-AS2 silencing and miR-520c-3p overexpression reduced mRNA expression of S100A4 in U/A and T/A cells ($P < 0.01$; Fig. 4E-F).

Fig. 4. A: HOXA-AS2 and miR-520c-3p simultaneously existed in the production precipitated by anti-AGO2; B: The wild-type or mutant miR-520c-3p-binding sites in HOXA-AS2 were inserted into pMIR-report luciferase vector. Luciferase activity was detected in cells co-transfected with miR-520c-3p or negative control (miR-control) and reporter plasmids containing WT-HOXA-AS2 (wild type) or MUT-HOXA-AS2 (mutant type). The normalized luciferase activity in the miR-control group was used as the relative luciferase activity; C: silencing of HOXA-AS2 increased the expression level of miR-520c-3p in U/A and T/A cells; D: Luciferase reporter assay demonstrated miR-520c-3p mimics significantly decreased the luciferase activity of S100A4-wt in HEK293T cells; E: silencing of HOXA-AS2 decreased the expression level of S100A4 in U/A and T/A cells; F: overexpression of miR-520c-3p decreased the expression level of S100A4 in U/A and T/A cells.

Discussion

In this study, we showed that HOXA-AS2 is upregulated in AML patients after treatment with adriamycin-based chemotherapy and in two ADR cell lines, U/A and T/A. Additionally, to determine whether HOXA-AS2 has the ability to confer resistance to AML cells exposed to adriamycin, we knocked down HOXA-AS2 expression in ADR cells. The results showed that when HOXA-AS2 was inhibited, proliferation was suppressed and apoptosis was promoted in ADR cells. Functional and mechanistic analyses demonstrated that HOXA-AS2 functioned as a ceRNA to regulate the expression of S100A4 by competing for miR-520c-3p binding and thereby regulating chemoresistance of AML cells.

It is becoming evident that lncRNAs have been demonstrated to play an important role in the malignant phenotype of cancer cells and drug-resistance potential. HOXA-AS2 was first identified in all-trans retinoic acid-treated NB4 promyelocytic leukemia cells, and the functional role of HOXA-AS2 was demonstrated in various types of cancers [14, 15]. Lian et al. demonstrated that lncRNA-HOXA-AS2/EZH2/LSD1 complex may function as an oncogene in pancreatic cancer cell proliferation, and also provides a potential therapy target for pancreatic cancer [11]. Gao et al. found that HOXA-AS2 knockdown inhibited malignant glioma behaviors and VM formation via the miR-373/EGFR axis [16]. However, data on the expression and biological function of HOXA-AS2 in AML adriamycin resistance are scarce. In this study, we showed that HOXA-AS2 is upregulated in the BM samples from 48 AML patients following adriamycin-based chemotherapy. Elevated levels of HOXA-AS2 have been observed in two ADR cell lines, U937/ADR (U/A) and THP-1/ADR (T/A), compared with U937 and THP-1. Additionally, to determine whether HOXA-AS2 has the ability to confer resistance to AML cells exposed to adriamycin, we knocked down HOXA-AS2 expression in ADR cells. The results showed that when HOXA-AS2 was inhibited, proliferation was suppressed and apoptosis was promoted, suggesting that HOXA-AS2 knockdown inhibited adriamycin resistance in AML cells. Our results are consistent with those of previous studies demonstrating that knockdown of HOXA-AS2 reduces proliferation in various cell types. In this study, HOXA-AS2 knockdown inhibits the tumor growth *in vivo*, and adriamycin treatment decreased the tumor volume of HOXA-AS2 shRNA model.

The ceRNA hypothesis proposed by Pandolfi et al. suggests that endogenous RNA molecules have miRNA binding sites, which could interact with miRNAs seed sequences through miRNA-binding sites to reduce their regulatory effect on target mRNA [8, 12]. The ceRNA hypothesis confers new and more extensive biological functions of mRNAs and non-coding RNAs. To further determine the molecular mechanism by which HOXA-AS2 contributes to adriamycin resistance of AML cells, we performed a microarray to screen the HOXA-AS2-regulated miRNAs in U/A cells. Then, bioinformatics analysis (Starbase 2.0, RNA22) of miRNA recognition sequences on HOXA-AS2 revealed the presence of more than 30 miRNAs binding sites. Based on these results, miR-520c-3p stood out through detailed survey. The anti-Ago2 RIP assay and luciferase reporter assay confirmed that miR-520c-3p is HOXA-AS2-targeting miRNA. More importantly, the gain and loss function assay indicated that knockdown HOXA-AS2 increased the expression level of miR-520c-3p in U/A and T/A cells. We also found that miR-520c-3p was a suppressor gene of adriamycin resistance in AML by suppressing cell proliferation of U/A and T/A cells, which was consistent with results of knockdown of HOXA-AS2 expression.

In general, miRNAs regulate cell proliferation, apoptosis and differentiation [17]. MiRNAs also played an important role in the ceRNA network through binding the miRNAs regulatory elements located in 3'-UTR of target mRNAs. Bioinformatics software analysis revealed that S100A4 might be a target gene of miR-520c-3p. Moreover, we further used luciferase reporting assay to illustrate S100A4 as a potential target gene of miR-520c-3p. S100A4 was previously reported to be an important protein with the capacity to promote invasion and metastasis of many human neoplasms, including AML. Furthermore, the results of qRT-PCR revealed that HOXA-AS2 silencing and miR-520c-3p overexpression reduced

mRNA expression of S100A4 in U/A and T/A cells. Collectively, these results demonstrated that knockdown of HOXA-AS2 suppresses chemoresistance of AML by regulating miR-520c-3p /S100A4 pathway.

Conclusion

In summary, our results showed that HOXA-AS2 expression increases the resistance of AML cells to adriamycin, perhaps through the miR-520c-3p /S100A4 pathway. This indicates that the targeting of HOXA-AS2 could be used to sensitize AML cells to adriamycin-based chemotherapy. HOXA-AS2-targeted therapy may therefore be an attractive strategy for use in combination with adriamycin-based chemotherapy.

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Disclosure Statement

The authors declare no conflicts of interest.

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