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A novel long non-coding RNA T-ALL-R-LncR1 knockdown and Par-4 cooperate to induce cellular apoptosis in T-cell acute lymphoblastic leukemia cells

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Abstract

T-cell acute lymphoblastic leukemia (T-ALL) is a hematologic malignancy with a poor prognosis. It has been shown that long non-coding RNA (lncRNA) plays an important role in tumorigenesis. Here, we characterized a novel lncRNA, T-ALL-R-LncR1, with whole-transcriptome deep sequencing from the Jurkat leukemic T-cell line. T-ALL-R-LncR1 was not observed in human normal tissues. However, an obvious expression was observed in some tumor tissues. T-ALL-R-LncR1 was markedly expressed in neoplastic T lymphocytes of 11 cases out of 21 children with T-ALL, indicating that T-ALL-R-LncR1 might be associated with T-ALL. T-ALL-R-LncR1 knockdown predisposed Jurkat cells to undergo pro-apoptotic factor Par-4-induced apoptosis. Further studies revealed that T-ALL-R-LncR1 knockdown facilitated the formation of a Par-4/THAP1 protein complex, resulting in the activation of caspase-3 and an increase of pro-apoptotic Smac protein in T-ALL cells. Our studies indicate a potential role of suppressing the novel long non-coding RNA T-ALL-R-LncR1 in the therapy of human T-ALL.

Keywords: Long non-coding RNA, Par-4, apoptosis, T cell, leukemia

Introduction

T-cell acute lymphoblastic leukemia (T-ALL) is a hematologic malignancy, and the morbidity is approximately 15% of pediatric cases [1]. The mortality from T-ALL is about 20% for children [1,2]. However, the pathogenesis of T-ALL still remains elusive. Although lots of research efforts are devoted to the exploration of its molecular pathology, the detailed oncogenic mechanisms remain unknown [1–3].

Currently, long non-coding RNA (lncRNA) has been the focus of many studies because it plays an important role in regulating gene expression and tumorigenesis [4]. lncRNAs are involved in transcriptional and post-transcriptional gene regulation [5]. The lncRNA UCA1 is up-regulated in bladder carcinoma. The overexpression of UCA1 promoted proliferation, migration and invasion in human bladder transitional cell carcinoma cells [6]. Gutschner et al. demonstrated that the lncRNA MALAT1 was a critical regulator of gene expression governing carcinogenesis in lung cancer and was a biomarker for lung cancer metastasis [7]. The overexpression of lncRNA HOTAIR is related to poor survival for patients with colon and pancreatic cancer. HOTAIR knockdown decreased cell proliferation and induced apoptosis in pancreatic cancer cells [8]. However, little research has been instigated to explore the relationship between lncRNA and T-cell acute lymphoblastic leukemia.

The prostate apoptosis response protein-4 (Par-4) is a pro-apoptotic factor, which was originally found in prostate cancer cells [9]. Human Par-4 is a protein product of the pro-apoptotic gene Par-4 and consists of 342 amino acids. The conserved functional domains of Par-4 include a leucine zipper domain in the C-terminal region and two nuclear localization sequences in the N-terminal region. Increasing Par-4 levels can induce cellular apoptosis through both intrinsic and extrinsic pathways. The decrease of Par-4 is a critical event in tumorigenesis and contributes to the occurrence of human colon cancer, renal cell carcinoma, ovarian cancer and breast cancer. Chaudhry et al. showed that the knockdown of Par-4 reduced caspase-3 activation and apoptosis induction [10]. It was recently demonstrated that dysfunction of Par-4 was involved in tumorigenesis of chronic lymphocytic leukemia [11]. However, the role of Par-4 in the pathogenesis of T-ALL is still unclear.

Par-4 exerts its transcriptional control on target genes through interacting with some Par-4-binding partner proteins, such as Wilms’ tumor (WT1) and protein kinase C (PKC) [9]. It was recently demonstrated that the zinc-finger protein THAP1 is associated with Par-4 [12]. THAP1 belongs to the THAP (thanatos-associated protein) family, which comprises 12 distinct members in humans. As a sequence-specific DNA-binding factor, THAP1 plays
important roles in cell proliferation, apoptosis and transcriptional regulation [12].

In the present study, we carried out a deep sequencing using high-throughput technology for transcriptome profiling in T-cell acute lymphoblastic leukemia cells. One thousand and two hundred long non-coding RNAs were identified and were designated as T-ALL related long non-coding RNA (T-ALL-R-LncR). T-ALL-R-LncR1 was implicated in apoptosis regulation in T-ALL cells. Knockdown of T-ALL-R-LncR1 by small interfering RNA (siRNA) sensitized cells to Par-4-induced apoptosis and inhibited cell proliferation. The knockdown of T-ALL-R-LncR1 facilitated the formation of a Par-4/THAP1 protein complex, resulting in an increase of the activity of caspase-3 and up-regulated expression of pro-apoptotic Smac protein. Our research might benefit the development of potential therapeutics for T-ALL.

Materials and methods

Reagents and antibodies

Anti-Par-4 and anti-THAP1 antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). The plasmids pcDNA3-myc and pcDNA3-FLAG were obtained from BD Biosciences (Franklin Lakes, NJ). A Nuclear Protein Extraction kit was purchased from Active Motif (Carlsbad, CA). An Endo-free Plasmid Maxi kit was obtained from Qiagen Sciences (Germantown, MD). Restriction endonucleases were purchased from New England Biolabs (Beverly, MA). Lipofectamine 2000 reagent was obtained from Invitrogen (Carlsbad, CA). A caspase-3 assay kit was purchased from Calbiochem (Cambridge, MA).

Whole-transcriptome deep sequencing for T-ALL Jurkat cells

Whole-transcriptome deep sequencing was performed for T-ALL Jurkat cells at the Beijing Genomics Institute, Shenzhen (China) as previously described [13,14]. Briefly, total RNA was obtained from human T-cell acute lymphoblastic leukemia Jurkat cells. Then, whole-transcriptome sequencing libraries were constructed, in which poly(A)+ RNA, poly(A)− RNA and small RNA species were included. The deep RNA-sequencing was performed with Illumina technology. The expression level of a gene by RNA-Seq was normalized by the number of reads per kilobase of exon region per million mapped reads (RPKM). The cut-off value for determining gene transcriptional activity was determined based on a 95% confidence interval for all RPKM values of each gene.

RACE

Total RNA extractions and purifications were performed from Jurkat cells. Rapid amplification of cDNA ends (RACE) was performed using a 5′/3′ RACE Kit (Roche, Indianapolis, IN), according to the manufacturer’s instructions. Three antisense gene-specific primers were needed for first-strand cDNA synthesis and nested polymerase chain reactions (PCRs), including 5′-ACCCAGAGTTCTCACCCACA TTGA-3′, 5′-ATGGTGTCACCCAGATGGAGG-3′ and 5′-GTGTCACCCAGATGGAGG-3′. siRNA experiments

T-ALL-R-LncR1 and scrambled siRNAs were designed using the online Invitrogen Block-iT RNAi Designer. The three siRNAs had the following sequences: T-ALL-R-LncR1-siRNA-1: sense 5′-GGGUCAGAGUUCUGAGAATT-3′, antisense 5′-UCUCAGAGUACACUGACGTT-3′; T-ALL-R-LncR1-siRNA-2: sense 5′-GAACGGAAACGAUGUUAUTT-3′, antisense 5′-UAACAAUCGUUUCCUGUUCTT-3′; T-ALL-R-LncR1-siRNA-3: sense 5′-GCAUAACUUCGACUACAACTT-3′, antisense 5′-UGUGAACUUGCGAUUAUGCTT-3′.

Jurkat cells were transfected with siRNA using Lipofectamine 2000 in Opti-MEM I for 6 h. At 24 h and 48 h after transfection, total RNA was prepared for real-time quantitative PCR analysis. A siRNA-based experiment for THAP1 was similarly performed.

Reverse transcription PCR analysis

Total RNA extractions and purifications were performed using various cells and tissue specimens. Then, 5 μg of RNA was transcribed. Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control. PCR reactions were performed in a 25 μL mixture containing PCR buffer, dNTP and Ex-Taq DNA polymerase. Primers for T-ALL-R-LncR1 were as follows: sense: TGC- CAGCACGCGCTAGGATAAA, antisense: GTGAGGAAACCGTTTTGGA. Amplification protocols for T-ALL-R-LncR1 and GAPDH included pre-denaturing at 98°C for 1 min, 35 repetitive cycles of denaturing at 98°C for 30 s, annealing at 64°C for 30 s, a chain extension at 72°C for 2 min and a final extension at 72°C for 7 min. Amplified cDNA was separated and visualized by 1.2% agarose gel electrophoresis, then visualized under ultraviolet (UV) light.

Real-time quantitative PCR

Total RNA extraction was performed using various cells and tissue specimens using TRIzol reagent followed by ethanol precipitation. Real-time quantitative PCR was performed using the StepOnePlus™ Real-Time PCR System and a SYBR® Premix Ex Taq™ Kit (Takara Biotechnology, Otsu, Shiga, Japan). Each reaction consisted of SYBR® Premix Ex Taq™, forward primer, reverse primer, ROX Reference Dye and cDNA solution in a 20 μL reaction. Amplification conditions were as follows: 95°C for 30 s, 40 cycles at 95°C for 5 s, 60°C for 30 s, 95°C for 15 s, 60°C for 1 min and 95°C for 15 s. Average threshold cycle (Ct) values from the triplicate PCR reactions for T-ALL-R-LncR1 were normalized against the average Ct values for GAPDH from the same cDNA sample. The fold change of T-ALL-R-LncR1 transcript levels between T-ALL-R-LncR1-siRNA-1, 2, 3 and the control was \( 2^{-\Delta\Delta Ct} \), where \( \Delta Ct = Ct_{\text{T-ALL-R-LncR1}} - Ct_{\text{GAPDH}} \) and \( \Delta\Delta Ct = \Delta Ct_{\text{T-ALL-R-LncR1-siRNA}} - \Delta Ct_{\text{control}} \). Real-time quantitative PCR for THAP1 was similarly performed.

Amplification conditions were as follows: 95°C for 30 s, 40 cycles at 95°C for 5 s, 60°C for 30 s, 95°C for 15 s, 60°C for 1 min and 95°C for 15 s. Average threshold cycle (Ct) values from the triplicate PCR reactions for T-ALL-R-LncR1 were normalized against the average Ct values for GAPDH from the same cDNA sample. The fold change of T-ALL-R-LncR1 transcript levels between T-ALL-R-LncR1-siRNA-1, 2, 3 and the control was \( 2^{-\Delta\Delta Ct} \), where \( \Delta Ct = Ct_{\text{T-ALL-R-LncR1}} - Ct_{\text{GAPDH}} \) and \( \Delta\Delta Ct = \Delta Ct_{\text{T-ALL-R-LncR1-siRNA}} - \Delta Ct_{\text{control}} \). Real-time quantitative PCR for THAP1 was similarly performed.
Analysis of protein-coding potential of T-ALL-R-LncR1

Analysis of the protein-coding potential of T-ALL-R-LncR1 was done using an in vitro translation system kit (TNT® Quick Coupled Transcription/Translation Systems; Promega, Madison, WI) as described previously [15]. Briefly, the full-length cDNA of T-ALL-R-LncR1 was cloned into an expression vector pFS-EF1-LCS-T2A and transfected into human 293T cells with Lipofectamine 2000. Western blots were performed with anti-V5 antibody. β-Galactosidase (beta-gal) and luciferase (Luc) were used as controls.

For analysis in vivo, the full-length cDNA of T-ALL-R-LncR1 was cloned into an expression vector pFS-EF1-LCS-T2A and transfected into human 293T cells with Lipofectamine 2000. Western blots were performed with anti-V5 antibody. Beta-gal was used as a positive control. β-Actin was used as a loading control.

Analysis of apoptosis by flow cytometry

Jurkat cells were co-transfected with pcDNA3-Par-4 plasmids containing a full-length coding sequence (CDS) of Par-4 together with or without siRNA for T-ALL-R-LncR1. Cells were collected and washed in phosphate buffered saline (PBS). After incubation with Annexin V–allophycocyanin (APC) and 7-aminoactinomycin D (7AAD), the cells were analyzed by flow cytometry. The percentages of apoptosis in the cells were calculated.

Co-immunoprecipitation experiments

Jurkat cells were plated at density of $\times 10^5$ cells/well in six-well plates and co-transfected with 5 μg of pcDNA3-Par-4-Myc plasmids and 5 μg of pcDNA3-THAP1-FLAG plasmids together with or without siRNA for T-ALL-R-LncR1 using Lipofectamine 2000. After 24 h, the Jurkat cells were washed and lysed for 1 h on ice with immunoprecipitation buffer (20 mM Tris–HCl, pH 7.5, 150 mM NaCl, 0.25% deoxycholic acid, 10% glycerol, 1% Nonidet P-40, 1 mM ethylenediaminetetraacetic acid [EDTA]). The lysed cells were centrifuged for 10 min at 15 000 × g. The supernatant was saved for immunoprecipitation using anti-Myc antibody or anti-FLAG antibody conjugated to Sepharose beads overnight. The beads were centrifuged and washed with cold lysis buffer. Then, the beads were resuspended in electrophoresis sample buffer and boiled for 10 min to release bound proteins. Proteins were analyzed by SDS-PAGE and immunoblotting. Similarly, immunoprecipitation/immunoblot analyses were performed with anti-THAP1 antibody and anti-Par-4 antibody for co-immunoprecipitation between endogenous Par-4 and THAP1.

Western blotting analysis

Jurkat cells were transfected by T-ALL-R-LncR1 siRNA together with or without pcDNA3-Par-4. At 24 h, 48 h and 72 h following transfection, cells were lysed and total protein was prepared. The protein concentrations were measured with a bicinchoninic acid (BCA) assay kit. SDS-PAGE was performed. The transferred membranes were blocked and incubated with primary antibodies. Membranes were washed three times and incubated with secondary antibody. Blots were developed using an enhanced chemiluminescence (ECL) kit.

Determination of caspase-3 activation

Jurkat cells were transfected with T-ALL-R-LncR1 siRNA and pcDNA3-Par-4. After 24 h and 48 h, cell lysates were obtained and caspase-3 activities were assayed with a Caspase Cellular Activity Assay kit according to the manufacturer’s instructions (Merck-Calbiochem, Darmstadt, Germany). The formation of Ac-DEVD-pNA was monitored at 405 nm. One unit defines the amount of enzyme that will cleave 1.0 nmol of the colorimetric substrate Ac-DEVD-pNA per hour at 37°C under saturated substrate concentrations.

Results

Putative intergenic lncRNAs were obtained from T-ALL Jurkat cells by whole-transcriptome deep sequencing

For whole-transcriptome deep sequencing, we first obtained total RNAs from human T-cell acute lymphoblastic leukemia Jurkat cells. Then whole-transcriptome sequencing libraries were constructed, in which poly(A) + RNA, poly(A) − RNA and small RNA species were included. The deep RNA-seq was performed with Illumina technology. Our deep sequencing of the T-ALL cell transcriptome covered 99.91% of the available human full-length cDNA data [Figures 1(A) and 1(B)]. Extensive read mapping and clustering analysis revealed 15 500 alternative pre-mRNA splicing events of 29 549 genes, including exon skipping, intron retention, alternative 5′ splicing and alternative 3′ splicing [Figure 1(C)]. A total of 1207 novel transcript units had not been previously identified. By alignment, 277 putative intergenic lncRNAs were obtained with respect to the transcriptomes of human mRNAs, RefSeq Genes, lncRNAdb database, LncRNADisease database and Ensembl Gene tracks in the UCSC (University of California, Santa Cruz) Genome Browser. Among these, seven out of 20 randomly selected lncRNAs were validated using reverse transcription PCR and Sanger sequencing.

T-ALL-R-LncR1 was a novel lncRNA

T-ALL-R-LncR1 was one of these putative lncRNAs. The 3′-cDNA end and 5′-cDNA end were confirmed to be 243 bp and 159 bp in length using RACE experiments, respectively [Figure 2(A)]. The full-length cDNA was mapped to 6q24.3 with two exons [Figure 2(B)]. The cloned full-length cDNA of T-ALL-R-LncR1 gene was 1468 bp [Figure 2(C)]. Because T-ALL-R-LncR1 showed a stable secondary structure by computational prediction with RNAfold structure software and the free energy of the thermodynamic ensemble was $-506.04$ kcal/mol [Figure 2(D)], we performed a series of experiments for further identification and focused on functional research.

First, we found that there were multiple short predicted open reading frames (ORFs) in all three frames of the novel transcript T-ALL-R-LncR1 [Figure 2(E)]. However, the 5′ ends of all ORFs lacked a Kozak sequence for translation initiation. Moreover, all candidate amino acid sequences of these ORFs did not contain any known protein motif and did not share any sequence similarities with other proteins in any species.
T-ALL-R-LncR1 might be associated with T-ALL
Further, we examined T-ALL-R-LncR1 expression patterns using a normal human cDNA library from various tissues (Clontech Inc., Mountain View, CA) and tumor tissues by PCR analysis. Our results demonstrated that T-ALL-R-LncR1 expression levels differed in different histiocytes. As shown in Figure 4(A), T-ALL-R-LncR1 was not observed in liver, spleen, skeletal muscle, heart, brain, placenta, lung, kidney, colon, pancreas, ovary, leukocyte, thymus, testis, prostate or small intestine. Compared with corresponding non-cancerous tissues, an obvious expression was observed in some tumor tissues, such as bladder carcinoma, prostate carcinoma and rhabdomyosarcoma [Figure 4(B)]. Interestingly, we found that T-ALL-R-LncR1 was markedly expressed in neoplastic T lymphocytes of 11 cases out of 21 children with T-cell acute lymphoblastic leukemia [Figure 4(C)], suggesting that T-ALL-R-LncR1 might be associated with T-ALL.

Together, these results indicated that T-ALL-R-LncR1 was a novel T-ALL-related lncRNA.

T-ALL-R-LncR1 silencing predisposed T-ALL Jurkat cells to undergo Par-4-induced apoptosis
Since the expression of T-ALL-R-LncR1 was up-regulated in T-cell acute lymphoblastic leukemia cells as compared with normal T lymphocytes, we next addressed whether knockdown of T-ALL-R-LncR1 affected the survival and proliferation of T-ALL Jurkat cells. Jurkat cells were transfected with either T-ALL-R-LncR1 specific siRNA, scrambled siRNA or no siRNA as control. Real time quantitative PCR analysis was performed for measuring the mRNA expression levels of T-ALL-R-LncR1 in transfected cells. As shown in Figure 5(A) and Supplementary Figure S1 to be found online at http://informahealthcare.com/doi/abs/10.3109/10428194.2013.829574, T-ALL-R-LncR1-siRNA-2 caused an effective reduction of T-ALL-R-LncR1 mRNA and 92.1% of T-ALL-R-LncR1 was suppressed. However, flow cytometry analysis showed that knockdown of T-ALL-R-LncR1 did not induce significant cellular apoptosis and the apoptotic rate was only 4.69% (early apoptotic cells 4.2%, late apoptotic cells 0.49%). We therefore investigated whether T-ALL-R-LncR1 knockdown sensitized Jurkat cells to Par-4-induced apoptosis. A vector with the full-length CDS sequence of Par-4 was generated and transfected into Jurkat cells. Flow cytometry demonstrated that Par-4 overexpression led to a low degree of apoptosis and the apoptotic rate was 15.79% (early apoptotic cells 8.70%, late apoptotic cells 7.09%). Interestingly, knockdown of T-ALL-R-LncR1 by siRNA drastically raised the apoptotic rate to 35.87% (early apoptotic cells 12.60%, late apoptotic cells 23.27%) in Par-4-overexpressed Jurkat cells [Figure 5(B) and Supplementary Figure S2 to be found online at http://informahealthcare.com/doi/abs/10.3109/10428194.2013.829574]. These results indicated that T-ALL-R-LncR1 silencing predisposed T-ALL Jurkat cells to undergo Par-4-induced apoptosis.

Knockdown of T-ALL-R-LncR1 facilitated formation of Par-4/THAP1 protein complex
Next, we further explored the molecular mechanisms by which T-ALL-R-LncR1 knockdown enhanced Par-4-induced apoptosis.
apoptosis in T-cell acute lymphoblastic leukemia cells. Because Par-4 could associate with pro-apoptotic protein THAP1 within promyelocytic leukemia nuclear bodies and promoted cellular apoptosis [12], co-immunoprecipitation experiments were conducted to examine the interaction between Par-4 and THAP1 in Jurkat cells after exposure to T-ALL-R-LncR1 knockdown.

First, we carried out immunoprecipitation experiments to confirm the interaction between exogenous Par-4 and THAP1. We constructed expression plasmids pcDNA3-Par-4-Myc and pcDNA3-THAP1-FLAG. The Myc- and FLAG-tagged expressed proteins could be recognized with mono-specific Myc and FLAG antibodies, respectively. Then, Jurkat cells were co-transfected with pcDNA3-Par-4-Myc, pcDNA3-THAP1-FLAG, and siRNA for T-ALL-R-LncR1 followed by immunoprecipitation with anti-Myc antibody or anti-FLAG antibody. Western blot analysis with the anti-FLAG antibody or anti-Myc antibody demonstrated that T-ALL-R-LncR1 knockdown increased the interaction between Par-4 and THAP1 [Figure 6(A)].

To confirm the interaction between exogenous Myc-Par-4 and FLAG-THAP1 observed in the above experiments, Jurkat cells were subjected to siRNA transfection for T-ALL-R-LncR1 and nuclear extracts were precipitated with an anti-Par-4 antibody and the precipitants were blotted by an anti-THAP1 antibody. Figure 6(B) shows that endogenous Par-4 co-immunoprecipitated with endogenous THAP1 protein. Similar results were obtained when sequential immunoprecipitation/immunoblot analyses were performed with anti-THAP1 antibody and anti-Par-4 antibody in a reverse order, validating the reinforcing effects of T-ALL-R-LncR1 knockdown on the interaction between Par-4 and THAP1.

These results indicated that the knockdown of T-ALL-R-LncR1 facilitated the formation of the Par-4/THAP1 protein complex in human T-cell acute lymphoblastic leukemia cells.
Knockdown of T-ALL-R-LncR1 cooperated with Par-4 to increase the activity of caspase-3 and expression of pro-apoptotic Smac protein

Because caspase-3 is an apoptosis-related cysteine protease and is a common downstream effector of various apoptotic pathways [16], we next transfected T-ALL-R-LncR1 siRNA to Jurkat cells together with or without pcDNA3-Par-4. Then, the activation of caspase-3 was determined. As shown in Figure 7(A), T-ALL-R-LncR1 knockdown significantly enhanced the activation of caspase-3 induced by Par-4 overexpression in human T-cell acute lymphoblastic leukemia cells. However, co-transfection of THAP1 siRNA dramatically alleviated the
implicated in the regulation of gene expression at the transcriptional, post-transcriptional and epigenetic levels [4–6]. However, the functional roles of long non-coding RNAs have not yet been well elucidated. In this study, we obtained 1207 novel transcript units by high throughput sequencing and identified T-ALL-R-LncR1 as a novel long non-coding RNA. First, a Basic Local Alignment Search Tool (BLAST) search and alignment with the National Center for Biotechnology Information (NCBI) Reference Sequence (RefSeq) database showed that T-ALL-R-LncR1 is a novel transcript. Second, T-ALL-R-LncR1 showed a stable secondary structure (RNA minimum free energy) by computational prediction with RNAfold software. Third, the 5' ends of all ORFs of T-ALL-R-LncR1 lacked a Kozak sequence for translation initiation. Fourth, all T-ALL-R-LncR1 ORFs were not translated into any proteins using a mammalian expression system. Therefore, T-ALL-R-LncR1 is a novel long non-coding RNA.

Recently, some studies have been devoted to analysis of the expression patterns of long non-coding RNAs, and tissue specific expression was determined [7,8]. In agreement with these studies, our data demonstrated that high-level enhancement of caspase-3 activity, indicating that reinforcement of T-ALL-R-LncR1 knockdown on Par-4-induced apoptosis was at least partially dependent on THAP1.

It has been well documented that Par-4 can exert its pro-apoptotic function by up-regulating the expression of pro-apoptotic Smac protein [17]. Hence we observed the role of T-ALL-R-LncR1 knockdown in Par-4-induced Smac expression. Western blotting assays showed that co-transfection with T-ALL-R-LncR1 siRNA and pcDNA3-Par-4 plasmids containing full-length CDS sequence of Par-4 together with or without siRNA for T-ALL-R-LncR1. Analysis of apoptosis was performed with flow cytometry.

Discussion

Long non-coding RNAs are non-protein-coding transcripts of more than 200 nucleotides. Accumulating evidence supports the notion that long non-coding RNAs are implicated in the regulation of gene expression at the transcriptional, post-transcriptional and epigenetic levels [4–6]. However, the functional roles of long non-coding RNAs have not yet been well elucidated. In this study, we obtained 1207 novel transcript units by high throughput sequencing and identified T-ALL-R-LncR1 as a novel long non-coding RNA. First, a Basic Local Alignment Search Tool (BLAST) search and alignment with the National Center for Biotechnology Information (NCBI) Reference Sequence (RefSeq) database showed that T-ALL-R-LncR1 is a novel transcript. Second, T-ALL-R-LncR1 showed a stable secondary structure (RNA minimum free energy) by computational prediction with RNAfold software. Third, the 5' ends of all ORFs of T-ALL-R-LncR1 lacked a Kozak sequence for translation initiation. Fourth, all T-ALL-R-LncR1 ORFs were not translated into any proteins using a mammalian expression system. Therefore, T-ALL-R-LncR1 is a novel long non-coding RNA.
expression of T-ALL-R-lncR1 was observed in tumor tissues. Especially, T-ALL-R-lncR1 was markedly up-regulated in T-cell acute lymphoblastic leukemia cells. Thus, we could conclude that T-ALL-R-lncR1 might be a novel T-ALL related lncRNA.

With advancements in high throughput sequencing and transcriptome profiling, increasing evidence shows that the aberrant expression of long non-coding RNAs is associated with cancer. Long non-coding RNAs play potential roles in both tumor-suppressive and oncogenic pathways [4,7,8]. Here, it was found that T-ALL-R-lncR1 was markedly up-regulated in T-cell acute lymphoblastic leukemia cells, indicating that altered expression of this long non-coding RNA might potentially enhance oncogenesis. Although knockdown of T-ALL-R-lncR1 by siRNA did not induce significant cellular apoptosis and the apoptotic rate was only 4.69%, T-ALL-R-lncR1 knockdown drastically raised the apoptotic rate in Par-4-overexpressed Jurkat cells. Par-4 is a pro-apoptotic factor and functions as a tumor suppressor [9–11]. Par-4 offers an attractive target for the development of tumor therapy. Our finding demonstrated that T-ALL-R-lncR1 knockdown predisposed T-ALL Jurkat cells to undergo Par-4-induced apoptosis.

In the present study, we investigated the molecular mechanisms responsible for the synergistic effect of...
T-ALL-R-LncR1 knockdown and Par-4 overexpression. Our results showed that knockdown of T-ALL-R-LncR1 facilitated formation of the Par-4/THAP1 protein complex in human T-cell acute lymphoblastic leukemia cells. THAP1 is a novel nuclear pro-apoptotic factor belonging to the zinc-finger superfamily. Recent research showed that THAP1 potentiated tumor necrosis factor-α (TNF-α)-induced apoptosis by interaction with Par-4. Endogenous Par-4 co-localized with ectopic THAP1 in primary endothelial cells and fibroblasts [12]. A zinc-dependent sequence specific DNA-binding domain contributed to the pro-apoptotic activity of THAP1 [12]. Our finding indicated that acceleration of Par-4-induced apoptosis by T-ALL-R-LncR1 knockdown might be attributed to THAP1. This conclusion was confirmed by THAP1 siRNA experiments. Although the knockdown of T-ALL-R-LncR1 cooperated with Par-4 to promote the increase of caspase-3 activity and expression of pro-apoptotic Smac protein, co-transfection of THAP1 siRNA dramatically alleviated this promotion.

In summary, we found a novel T-ALL-related long non-coding RNA, T-ALL-R-LncR1, which was highly expressed in human T-cell acute lymphoblastic leukemia cells and some tumor tissues. The knockdown of T-ALL-R-LncR1 cooperated with Par-4 to increase apoptosis in human T-cell acute lymphoblastic leukemia cells. The knockdown of T-ALL-R-LncR1 facilitated the formation of the Par-4/THAP1 protein complex, resulting in an increase of caspase-3 activity and the expression of pro-apoptotic Smac protein. Our studies indicate a potential role of suppressing the novel long non-coding RNA T-ALL-R-LncR1 in the therapy of human T-cell acute lymphoblastic leukemia.

**Potential conflict of interest:** Disclosure forms provided by the authors are available with the full text of this article at www.informahealthcare.com/lal.
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References

Supplementary material available online
Supplementary Figures showing further results

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