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Article

LncRNA-CR848007.6 regulates the translatome of Cadmium malignant transformed 16HBE cells from a biomechanical perspective

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Abstract: Background: Previous studies found that cadmium (Cd) was an environmental toxicant that not only induces toxicological effects but also disrupts cellular biomechanics, affecting cell stiffness, motility, and mechanotransduction pathways. LncRNA-CR848007.6 played an important regulatory role in cadmium toxicology. However, whether its regulatory mechanism involves the biomechanics and changes of the translatome remains to be elucidated. **Objective:** This study aimed to explore the function and mechanism of LncRNA-CR848007.6 in translation regulation and biomechanical properties during cadmium malignant transformed human bronchial epithelial cells (16HBE cells) through translatome sequencing techniques and bioinformatics methods. **Methods:** RNA interference technology was applied to silence the expression of LncRNA-CR848007.6 in cadmium malignant transformed 16HBE cells. The libraries of mRNA and RNC were constructed, and Ion Proton™ Sequencer was used for transcriptome and translatome sequencing. Mechanical properties of cells, including stiffness and traction forces, were measured using atomic force microscopy and traction force microscopy. Transcriptome and translatome differential expressions were analyzed using R software. The cell cycle and apoptosis were detected by flow cytometry to verify the functions of LncRNA-CR848007.6 regulating the translatome of cadmium malignant transformed 16HBE cells. **Results:** Four libraries were obtained after sequencing, including 24062 genes from the transcriptome of the siR mR group cell and NC mR group cell, the translatome of siR RNC group cell and the NC-RNC group cell. It was found that there was little change in the number of transcriptome genes between the siR RNC group cell and NC-RNC group cell, with 19 differentially expressed genes downregulated and 0 differentially expressed genes upregulated. There were 114 genes in the translatome with a ration < -2 and 65 genes in the translatome with a ratio > 2. There was no intersection between the differential TR expression genes and differential mRNA expression genes. The GO analysis results showed significant changes in the translation ratio of cell cycle and mitotic-related pathways, but no enriched KEGG pathway appeared. The cell cycle progression was regulated and cell apoptosis was signficantly inhibited ($P < 0.05$) after silencing lncRNA-CR848007.6 by siRNA in CdCl2 malignant transformed 16HBE cells. Transcriptome and translatome analyses revealed differential expression of genes involved in cytoskeletal organization and mechanosensitive signaling. **Conclusion:** LncRNA-CR848007.6 plays a critical role in modulating the biomechanical properties of cadmium-malignant transformed 16HBE cells, influencing cell stiffness and motility through translational regulation. This study provides insights into the biomechanical mechanisms underlying lncRNA-mediated cellular responses to cadmium toxicity.

Keywords: cadmium; biomechanics; transcriptome; translatome; LncRNA

1. Introduction

Previous research showed that proteins with biological functions are synthesized through the process of translation, and translation regulation is the most important and significant regulatory link among the many levels of regulation in the central dogma. Its regulatory effect on the proteome may exceed the sum of all other regulatory levels. Therefore, it is necessary to conduct a comprehensive study on translation regulation. In recent years, with the breakthroughs made by scientists in related omics technologies, various processes of translation can be effectively studied globally, forming the emerging discipline of "Transcriptomics" [1]. which refers to all components directly involved in the translation process, including but not limited to ribosomes, translating mRNA (known as RNC mRNA), tRNA, regulatory RNA (such as miRNA, lncRNA, etc.), newborn peptide chains, various translation factors, etc. Translationomics is a new technique for studying all components of the translation process [2,3]. In recent years, technological advancements in detection have brought breakthroughs to the research of translation teams on a global scale, including their composition and dynamics. These technologies have been increasingly applied in research, enabling a more comprehensive study of translation control. The translation process is not limited to converting mRNA coding sequences into polypeptide chains, but also controls the composition of the proteome in a subtle and sensitive way. In recent years, research on transcriptomics has attracted the attention of scholars around the world and has expanded to many fields, including proteomics, cancer research, bacterial stress response, circadian rhythms [4–6].

Cadmium (Cd) is an environmental and industrial toxicant of worldwide public health significance [7,8], and it has been classified as a Class I carcinogen by the International Agency for Cancer. In industry, cadmium is mainly used in mining, smelting, metallurgy, machinery, electroplating, manufacturing, soldering materials, pigments, batteries, and semiconductor components. In daily life, cadmium is mainly absorbed by the human body through air, food, and water. Research found that cadmium can indirectly induce the production of free radicals after entering cells, affecting the transmission of a series of cellular signals, leading to cell apoptosis and damage. Previous studies have shown that the biomechanical aspects of cadmium toxicity are complex, which include that cadmium enters organisms through ion channels or metal transporters and accumulates in tissues for a long time. Cadmium inhibits the activity of key enzymes, thereby disrupting cellular signaling and metabolic regulation. Cadmium induces excessive generation of reactive oxygen species (ROS). At the same time, cadmium can inhibit the activity of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT), leading to oxidative damage and lipid peroxidation, ultimately causing cell apoptosis or necrosis, the genetic mechanisms, such as increased DNA damage and decreased DNA repair function, oxidative damage, and the role of metallothionein. In recent years, researchers found that the toxicity mechanisms of cadmium also involve epigenetic mechanisms such as DNA methylation, histone acetylation modification, chromatin remodeling, and noncoding RNA [9–12].

Our team published the research findings in 2015 [13], which for the first time elucidated the existence of abnormal LncRNA expression profiles in cadmium toxicity.

Some LncRNAs were involved in regulating processes such as DNA damage repair, cell proliferation, and apoptosis in cadmium toxicity. This paper was the first international academic paper in the world at that time to elucidate the existence of abnormal LncRNA expression profiles in cadmium toxicity and the involvement of LncRNA in cadmium toxicity regulation. Our research demonstrated that LncRNA-CR848007.6 (its seqname was lncRNA-ENST0000414355) regulated DNA damage and repair, biological cycle, cell cycle progression, metabolism, molecular transducer activity, and other functions during the malignant transformation of cadmium cells. Cell experiments, animal experiments, and population studies have also confirmed that LncRNA-CR848007.6 effectively regulated DNA damage and repair, cell cycle, and apoptosis during the malignant transformation of cadmium cells. This is the only paper today that elucidates the regulatory function of LncRNA-CR848007.6 in the malignant effects of cadmium. We also found that the mechanisms of cadmium toxicity involved changes in translation initiation and other fields [14]. However, it remains to be clarified whether the regulation of LncRNA-CR848007.6 during the malignant transformation of cadmium cells involves translatome changes. Therefore, the current study aims to address this gap by exploring the function and mechanism of LncRNA-CR848007.6 in translation regulation and biomechanical properties during cadmium malignant transformation of human bronchial epithelial cells (16HBE cells) by transcriptome and translatome sequencing technology.

2. Materials and methods

2.1. Cell culture and treatments

Human bronchial epithelial cells (16HBE) were morphologically transformed using $CdCl_2$, as previously described [15]. Untransformed 16HBE cells (controls); Cd-transformed cells at the 5th (5 μmol L^{-1} Cd for 2 weeks), 15th (5 μmol L^{-1} Cd for 6 weeks), and 35th (5 μmol L^{-1} Cd for 14 weeks) passage (Cd malignant transformed cells) were cultured in RPMI-1640 containing L-glutamine, 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Life Technologies) at 37 ℃ in a humidified atmosphere with 5% CO_2. The cells were passaged twice weekly and cells in the logarithmic growth phase (5×10^5 cells/mL) were harvested for the following experiments.

2.2. RNA interference

To inhibit LncRNA-CR848007.6, 50 nM of small interfering RNA (siRNA) (siRNA–CR848007.6-4311, siRNA-CR848007.6-4312, siRNA-CR848007.6-4313, Shanghai Genepharma, China)) were selected based on their predicted specificity and efficiency in silencing the target gene. The siRNAs were transfected into untreated 16HBE cells and Cd malignant transformed cells using Lipofectamine 2000 reagent according to the manufacturer's instructions. Knockdown efficiency was validated by quantitative real-time PCR, with cells transfected with scramble-control siRNA serving as controls. Cells were harvested 72 h after transfection. Compared with controls, siRNA–CR848007.6-4311 and siRNA–CR848007.6-4312 successfully decreased the expression of LncRNA–CR848007.6. The expression levels of

LncRNA-CR848007.6 in cells after siRNA–CR848007.6-4311 and siRNA–CR848007.6-4312 intervention were 0.2 times and 0.13 times of negative control cell. The sequences of LncRNA–CR848007.6 siRNAs and scramble control siRNA were listed in **Table 1**.

Table 1. Primers for RT-PCR and sequences of lncRNA-CR848007.6 siRNA.

LncRNAs	**Primers**
CR848007.6	Forward: 5′−CAGAAAGAAGCCAAACAAGGAG−3’
	Reverse: 5′−AACCACCAAACAGTCAGCAG−3’
β-Actin	Forward: 5′−ACAGAGCCTCGCCTTTGCCGAT-3’
	Reverse: 5′−CTTGCACATGCCGGAGCCGTT -3’
Name	Sequence (5′to 3′)
siRNA-CR848007.6-4311	sense: 5′−AGAAGCCAAACAAGGAGCUTT-3’
	antisense: 5′−AGCUCCUUGUUUGGCUUCUTT-3’
siRNA-CR848007.6-4312	sense: 5′−CCUAGGCACAGAUGCUAAUTT-3’
	antisense: 5′−AUUAGCAUCUGUGCCUAGGTT-3’
siRNA-CR848007.6-4313	sense: 5′−GGAGCUUUCUGCAGAAUGATT-3’
	antisense: 5′−UCAUUCUGCAGAAAGCUCCTT-3’
siRNA-NC	sense: 5′−UUCUCCGAACGUGUCACGUTT-3’
	antisense: 5′−ACGUGACACGUUCGGAGAATT-3’

2.3. Quantitative real-time polymerase chain reaction

Quantitative real-time polymerase chain reaction Total RNA was isolated using the Trizol reagent. Reverse transcription was performed using a TITANIUM real-time polymerase chain reaction (RT-PCR) kit (Clontech, Mountain View, CA) according to the manufacturer’s instructions. The gene expression was quantified using a fluorescence-based quantitative real-time polymerase chain reaction (RT-qPCR) according to the manufacturer’s instructions (Bio-Rad Laboratories). The sequences of primers used for RT-qPCR are shown in **Table 1**.

2.4. Detection of translationomics

2.4.1. Extraction of ribosome neopeptide chain complexes (RNCs)

The cells were treated with 100 μ g/mL of streptomycin for 15 min, then wash the cell surface twice with 4 mL of pre-cooled phosphate buffered saline (PBS), and evenly add 1 mL of pre-cooled cell lysis buffer (containing 1% TritonX-100 ribosomal buffer (20 mM HEPES-KOH (pH 7.4), 15 mM MgCl2, 200 mM KCl, 100 mg/ml cycloheximide, and 2 mM DDT), and lyse on ice for 30 min. Gently scrape off the lysed cells with a sterile cell scraper and transfer them to a pre-cooled 1.5 mL centrifuge tube. Centrifuge at 4 ℃ for 10 min at 16,000 g to remove precipitated cell debris. Transfer the supernatant to a centrifuge tube containing 5 times the volume of 30% sucrose, centrifuge at 110,000 rpm for 45 min at 4 ℃. After centrifugation, carefully discard the supernatant, and the light yellow precipitate at the bottom of the tube was the RNC-RNA complex. After dissolving the precipitate in 100 μl of

deionized water without ribozyme, add 1 ml of TRIzol® LS Reagent, extract RNC-RNA.

2.4.2. Extraction of total RNA and RNC-RNA

Through TRIzol® LS Reagent lysate (Ambion) and chloroform extraction, ethanol freeze precipitation was used to extract the RNC-RNA mixture and total RNA, and a micro UV spectrophotometer and 1% agarose gel were used to detect the concentration and quality of RNA. According to Dynabeads® MRNA Purification Kit (Ambion) operating instructions, captured RNC and mRNA using polyA tail, quantified and controled concentration using Qubit® 2.0 fluorescence analyzer (Invitrogen).

2.4.3. mRNA enrichment

According to Dynabeads® Instructions for mRNA Purification Kit (Ambion): 2 μg RNA was used for mRNA enrichment. Through Qubit® Due to the fact that mRNA accounts for 2%–5% of total RNA, the amount of mRNA that can be enriched from 2 μg RNA did not exceed 100 ng for concentration quantification using a 2.0 fluorescence analyzer (Invitrogen).

2.4.4. Library construction

According to the operating instructions of Ion Total RNA Library v2 (life technologies) kit, constructed a RNA sequencing library was constructed with an average length of 200 bp using a starting amount of 100 ng RNA. Connect different Ion Xpress to each of the four samples™ RNA BarCode adapters. After preliminary quantification using the Qubit 2.0 Fluorometer, the constructed library was uniformly diluted to 1 ng/μl and then analyzed using Agilent® High Sensitivity DNA Kit operation instructions, performing library quality analysis on the Agilent 2100 bioanalyzer. The concentrations of the four groups of cells were 7674 pM/μ l for the siRNA RNC (RNC in LncRNA-CR848007.6 expression silencing cells) library, the NC-RNC (RNC blank control group) library was 7424 pM/μ l, the siR mRNA library was 7414 pM/μ l, and the NC mRNA library was 7390 pM/μ l.

2.4.5. Transcriptome and translation sequencing

According to the operating instructions of the Ion Total RNA Library v2 (life technologies) kit, constructed libraries from the same batch. Used RNase III to fragment RNA, ensuring coverage of the 100–300 bp insertion fragment range. Hybridization and ligation of tag adapters, reverse transcription into cDNA. Add RNA 5′ and 3′ Barcode primers, amplify and enrich DNA fragments with adapters using ultra-high fidelity enzymes, and purify and select fragments using Nucleic Acid Binding Beams. The constructed library was quantified using a Qubit 2.0 Fluorometer (Invitrogen) and an Agilent 2100 Bioanalyzer (Agilent), and finally diluted to a dilution factor of 100 pM. Next, used Ion OneTouch™ 2 Systems (life technologies) for template preparation and positive template enrichment. Chose 500 flow and P1 chips; Ion Proton™ Sequencer (life technologies) was used for sequencing. The sequencing data was ultimately filtered and processed by the computing server to generate high-quality sequence fastq data files and QC quality control files.

2.5. Bioinformatics analysis

2.5.1. Sequence alignment

A gene cloud analysis platform based on FANSE2 algorithm (http://www.chi-biotech.com) was used，mapping the sequence onto the mouse reference genome (mm10). Four libraries were obtained after sequencing, including genes counts from the transcriptome of siR mR group cells and NC mR group cells, the translatome of siR RNC group cells and NC-RNC group cells. Normalized the expression levels of all genes and calculated the RPKM value. Finding differentially expressed genes in the readings, importing the readings into R, and plotting a density plot of RPKM values.

2.5.2. Transcriptome differential expression analysis

R software was used to analyze the transcriptional expression differences between the LncRNA-CR848007.6 expression silencing cells (siR mR) and the control group (NC mR), draw a density plot, and the edge R software package was used to search for expression differences genes. Select a biological variation of 0.3 and draw a density plot. Filtered out genes with gene readings less than 10 cpm (count per million) and locate expression differences in genes with FDR less than 0.05. PlotSmear and volcano plot were used for plotting. The result file was in result.xls. The upregulated genes sorted by FDR from small to large were listed in the up worksheet, the downregulated genes were listed in the down worksheet, and all expression differences genes were listed in the all worksheet.

2.5.3. Translation ratio (TR) differential expression analysis

The TR value ($TR=\frac{\text{RNC-mRNA(RPKM)}}{\text{mRNA(RPKM)}}$) of each gene was calculated, comparing the ratio of two groups of samples, and the transcription rate of the sample/control was obtained. Calculated the logarithm of the sample/control again and named it the ratio. The higher the TR value, the greater the protein expression level. If the ratio value was positive, it indicated that the RPKM value of RNC mRNA was greater than that of mRNA. The number of genes with a ratio > 2 (TR value upregulated by more than 4 times after drug addition) and the number of genes with a ratio of < −2 (TR value downregulated by 4 times after medication) were caculated, and the intersection of TR expression differential genes and mRNA expression differential genes was analyzed.

2.5.4. Gene Ontology (GO) and pathway analysis

GO analysis was performed on genes with differential TR by the David tool, obtaining the results of biological process enrichment shown in the BP worksheet, the results of cellular components GO shown in the CC worksheet, and the results of molecular functions GO shown in the MF worksheet. KEGG pathway analysis was performed on genes with TR differences by the David tool.

2.6. Flow cytometric analysis of cell cycle

16HBE cell, Cd malignant transformed 16HBE cells after transfected with siRNA LncRNA-CR848007.6 for 72 h were harvested through trypsin digestion and fixed in 70% ethanol at −20 ℃. Before analysis, cells were incubated with RNase A (20 micrograms [μg] per mL) and stained with propidium iodide (PI) (50 μg/mL) for 5 min. Samples were immediately analyzed using flow cytometry with a FACSCalibur

flow cytometer (BD Biosciences, San Jose, CA, USA). A total of 10,000 events were recorded per sample at FL2 peak emission values (FL2-H) (wavelength 575 ± 26 nanometers [nm]), and the cell fractions in the sub-G_1, G_1, S, and G_2/M cell cycle phases were quantified in histograms with WinMDI software (version 2.9, Joseph Trotter).

2.7. Flow cytometric analysis of apoptosis cells

To explore the effect of LncRNA-CR848007.6 on Cd malignant transformed cells, detection of apoptosis in 16HBE cells and Cd malignant transformed cells was carried out after transfection with siRNA-CR848007.6 for 72 h. Apoptotic cells were analyzed using a flow cytometer (CYTOMICS FC 500, Beckman Coulter) after incubating with a reagent containing Annexin V-FITC and Propidium Iodide (BD Bioscience, San Jose, CA) for 15 min in darkness at room temperature. Each study was repeated three times.

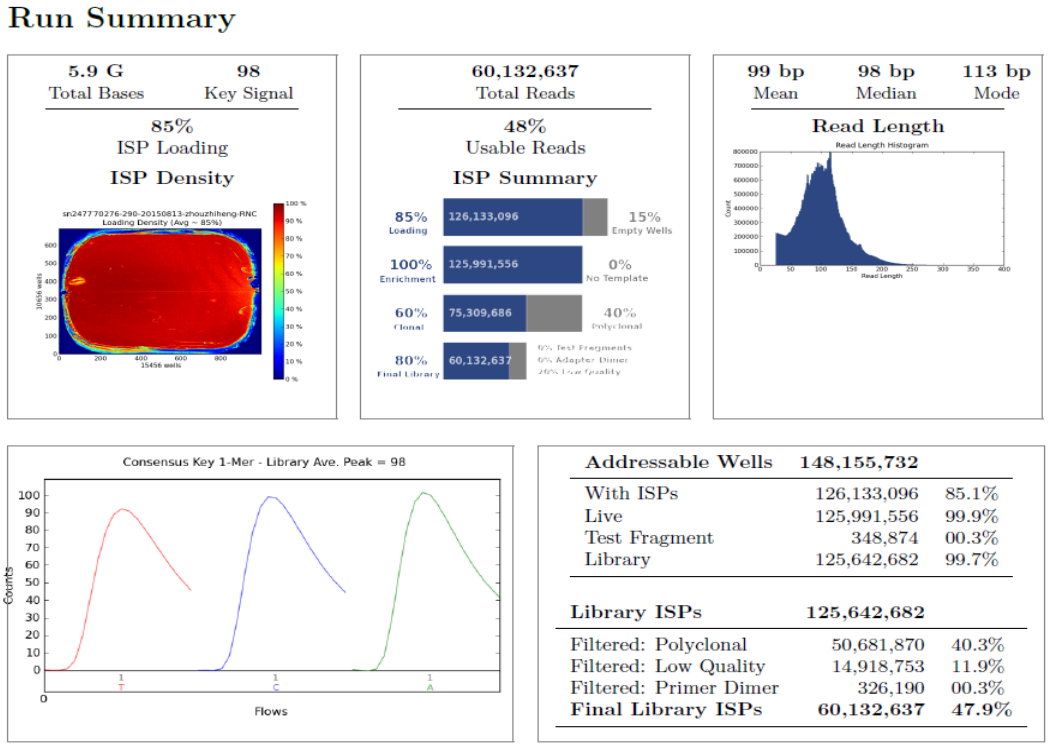
3. Results

3.1. Abnormally high LncRNA-CR848007.6 expression in $CdCl_2$ transformed 16HBE cells

Real-time PCR was performed to detect the LncRNA-CR848007.6 expressions in $CdCl_2$ transformed 16HBE cells at different stages. Results showed the LncRNA-CR848007.6 expression increased over time in $CdCl_2$ transformed 16HBE cells. The LncRNA-CR848007.6 expressions in 16HBE cells of the 5th passage,15th passage, and 16HBE cells of the 35th passage ($CdCl_2$ malignant transformed 16HBE cells) were 2.8, 7.2, and 10.1 times that in the control group ($P < 0.05$). These suggested that there was abnormally high expression of LncRNA-CR848007.6 in $CdCl_2$ transformed 16HBE cells.

3.2. Results of mRNA and RNC sequencing

Ion Proton™ Sequencer was used to perform the sequencing, generating the high-quality sequence fastq data files and QC quality control files. The gene cloud analysis platform based on the FANSE2 algorithm (http://www.chi-biotech.com) was used to map the sequence onto the reference genome (mm10), the read counts of 24,062 genes from four libraries (transcriptome of the siR mR group and NC mR group, translatome of the siR RNC group and NC-RNC group) were obtained. The expression levels of all genes were standardized, and the RPKM values were calculated. The main sequencing Data shown in Supplementary 1 and the run summary results were shown in **Figure 1**.



Barcode Name	Sample	Bases	≥ *Q*20	Reads	Mean Read Length
No barcode	none	221,208,293	145,644,592	2,118,065	104 bp
IonXpressRNA_009	siR-RNC	1,358,086,677	995,840,799	14,437,306	94 bp
IonXpressRNA_010	NC-RNC	1,610,309,374	1,156,746,108	16,426,262	98 bp
IonXpressRNA_011	siR-MR	1,647,178,585	1,186,522,383	16,526,488	100 bp
IonXpressRNA_012	NC-MR	1,138,024,264	808,682,628	10,621,245	107 bp

Test Fragment	Reads	Percent 50AQ17	Read Length Histogram
TF_C	**70,870**	**89%**	
TF_1	**244,937**	**82%**	

Figure 1. The run summary results of mRNA and RNC sequencing.

3.3. Transcriptome differential expression analysis

The transcriptome gene readings count of the siR mR group and the NC mR group did not change significantly, while the transcriptome gene readings count of the siR mR group was downregulated (**Figure 2A**), and searched for differentially expressed genes. The genes for which biological variation was 0.3 and its readings counted less than 10 cpm (counts per million) were chosen; 7465 genes remained. There were 19 differentially expressed genes located with FDR less than 0.05. All of the 19 differentially expressed genes were downregulated (mRNA up worksheet in Supplementary materials **Table S1**), while there were no differentially expressed genes that were upregulated (mRNA up worksheet in Supplementary materials **Table S1**). PlotSmear and volcano plot were used for plotting (**Figure 2B**,**C**). The results indicated that the effect of LncRNA-CR848007.6 on the transcriptome during

cadmium transformation of cells was very limited and may regulate the function and phenotype of the cells by translation regulation.

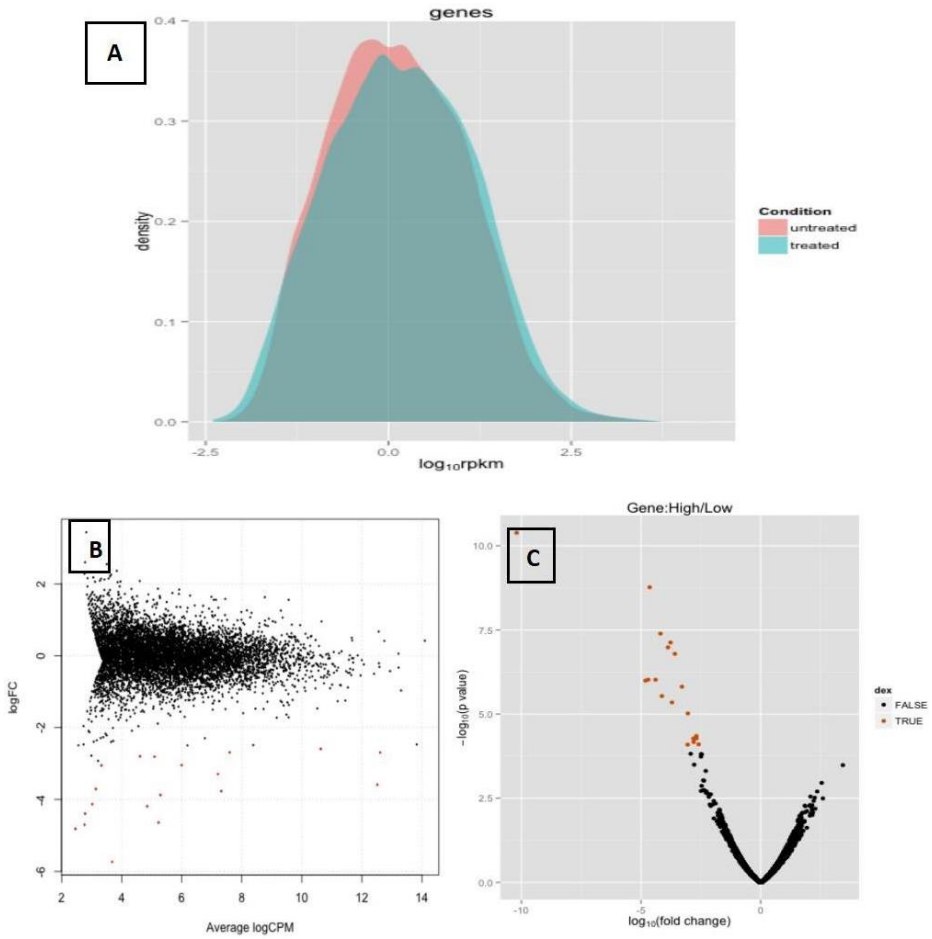


Figure 2. Differential expressed genes by transcriptome sequencing. **(A)** Density plot, blue represented the number of transcriptome genes in the SiR mR group, and brown red represented the number of transcriptome genes in the NC mR group; **(B)** PlotSmear plot; **(C)** volcano plot function plot.

The red dots in the plot represented genes with differential expression between the SiR mR group and the NC mR group, while the black dots represented the genes with no differential expression between the two groups.

3.4. Translation ratio (TR) differential expression analysis

The TR value and ratio value of each gene were calculated in the siRNA RNC and NC-RNC groups. Took the number of genes more than 2 or less than −2, and analyzed the intersection of TR expression differential genes and mRNA expression differential genes. There were 114 genes for a ratio < −2 (shown at TR down worksheet in Supplementary materials **Table S1**) and 65 genes for a ratio > 2 (shown at TR up worksheet in Supplementary materials **Table S1**). There was no intersection between genes with differential TR expression and genes with differential mRNA expression. The results indicated that LncRNA-CR848007.6 directly regulated the translation, and there were no genes found to regulate both transcriptome and translation simultaneously (see **Figure 3**).

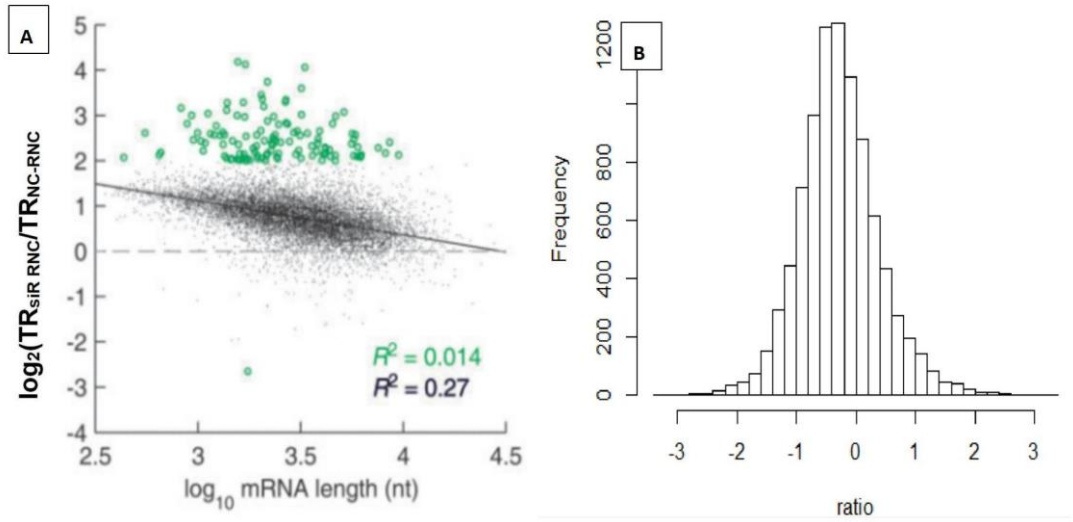


Figure 3. The distribution of TR value and ratio value in translasome sequencing. **(A)** scatter plot of $\log_{10}$ mRNA length(nt) and $\log_2(TR_{siRNA\ RNC}/TR_{NC\text{-}RNC})$; **(B)** histogram of ratio distribution.

3.5. GO and pathway analysis

Based on the biological process GO results, there were significant changes in the translation ratio of cell cycle and mitotic-related pathways, including GO: 0051301~cell division. GO:0007049~cell cycle, GO:0000279~M phase, GO:0022402~cell cycle process. There was the upregulation of translation ratios of common cell cycle molecular indicators such as MIS12, which was consistent with the upregulation of death and apoptosis molecular indicators such as Adora1 (bp worksheet in Supplementary materials **Table S1**). David was used to perform KEGG pathway analysis on genes with TR differences, and no enriched KEGG pathways were found. The results suggested that LncRNA-CR848007.6 regulated the translation of cell cycle and apoptosis-related genes during the malignant transformation of cadmium cells.

3.6. Effect of LncRNA-CR848007.6 on cell cycle in $CdCl_2$ transformed 16HBE cells

To further validate the implications of translationomics results, and determine the effect of LncRNA-CR848007.6 in the cell cycle of $CdCl_2$ transformed 16HBE cells, the cell cycle distribution was measured with flow cytometric analysis. **Figure 4** showed the flow analysis data (10,000 events). The percentage of cells in the G1 phase significantly increased with LncRNA-CR848007.6 knockdown in 16HBE cells, and $CdCl_2$ transformed 16HBE cells ($P < 0.05$). The results suggested that LncRNA-CR848007.6 controls cell cycle progression in $CdCl_2$ transformed 16HBE cells.

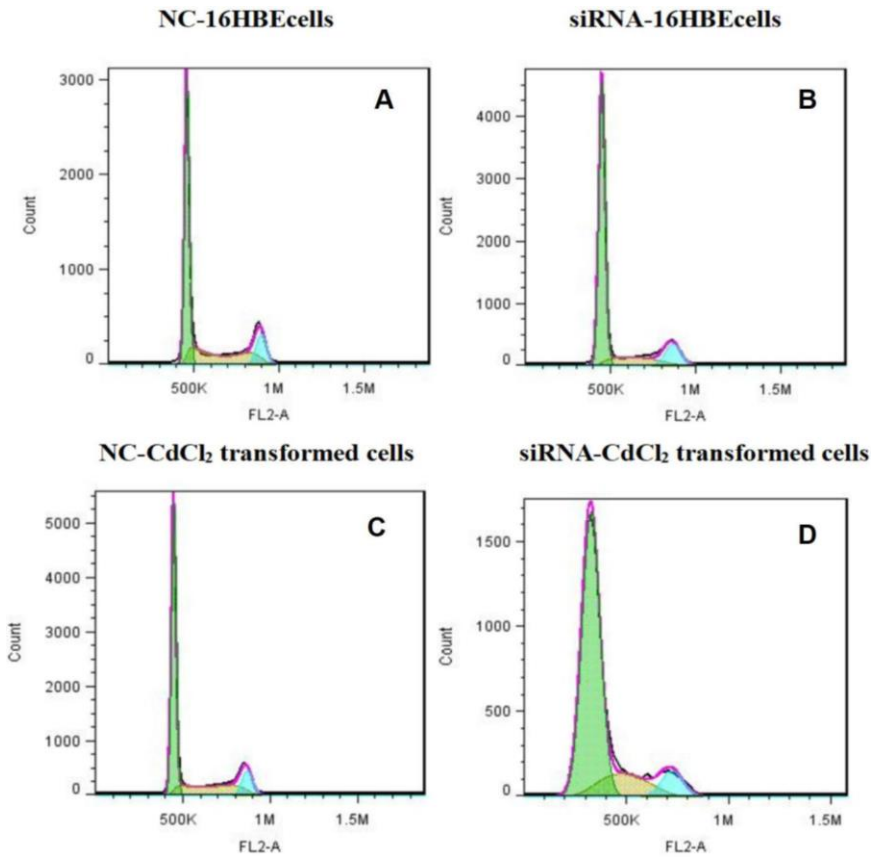


Figure 4. LncRNA-CR848007.6 regulates the cell cycle in 16HBE cells and $CdCl_2$ transformed 16HBE cells. The cell cycle of **(A)** negative control 16HBE cells; **(B)** LncRNA-CR848007.6 knockdown i16HBE cells; **(C)** negative control $CdCl_2$ transformed 16HBE cells; **(D)** LncRNA-CR848007.6 knockdown $CdCl_2$ transformed 16HBE cells detected by flow cytometry.

3.7. LncRNA-CR848007.6 regulates cell apoptosis in $CdCl_2$ transformed 16HBE cells

Flow cytometry was performed to detect the apoptosis of 16HBE cells before and after LncRNA-CR848007.6 silencing. When compared with the control group, the number of apoptotic cells and apoptosis rate reduced in CdCl2 transformed 16HBE cells after LncRNA-CR848007.6 silencing ($P < 0.05$) (**Figure 5**). These results suggested that LncRNA-CR848007.6 regulated the apoptosis of $CdCl_2$ transformed 16HBE cells.

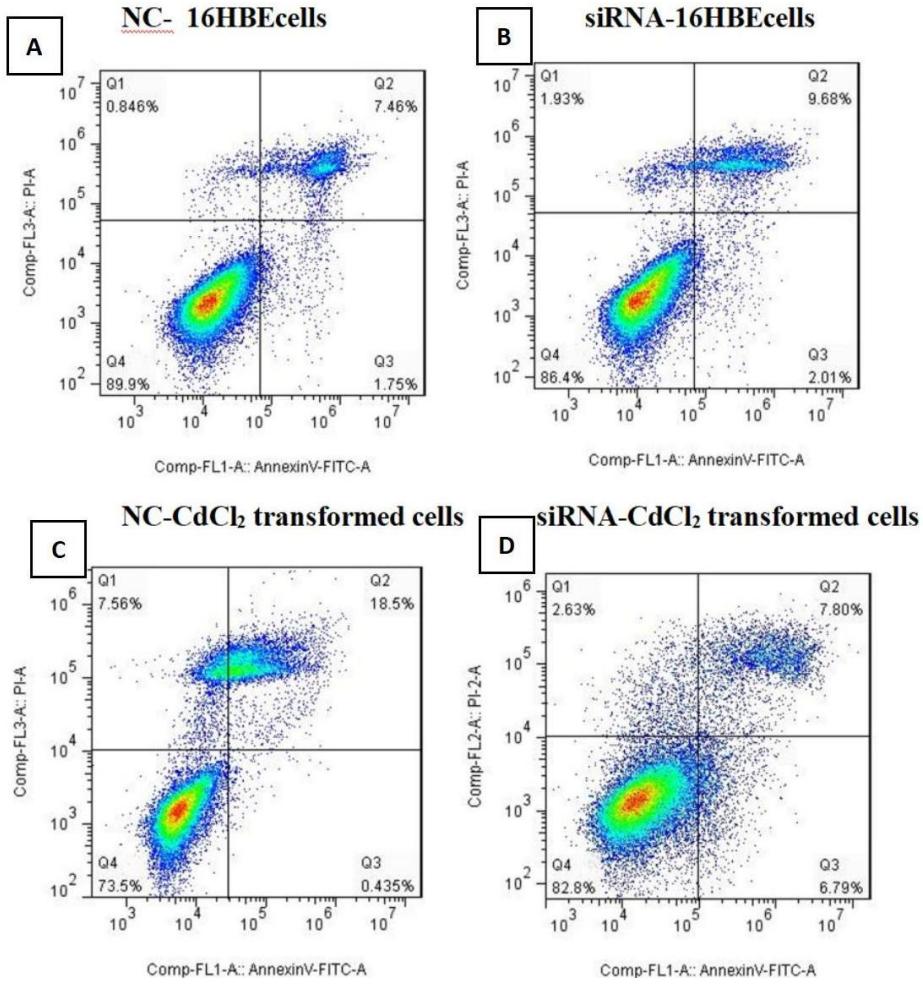


Figure 5. LncRNA-CR848007.6 regulates cell apoptosis in $CdCl_2$ transformed 16HBE cells. The cell apoptosis of **(A)** negative control 16HBE cells; **(B)** LncRNA-CR848007.6 knockdown i16HBE cells; **(C)** negative control $CdCl_2$ transformed 16HBE cells; **(D)** LncRNA-CR848007.6 knockdown $CdCl_2$ transformed 16HBE cells detected by flow cytometry.

4. Discussion

Cadmium is one of the most toxic pollutants found in occupational factors and ecosystems. Scientists have extensively explored the genetic mechanisms of DNA damage, DNA repair, oxidative damage, as well as epigenetic mechanisms such as DNA methylation and histone acetylation modification in cadmium toxicity. In recent years, there have been many reports on the regulatory functions and mechanisms of non-coding RNAs, including miRNA and LncRNA in cadmium toxicity [16].

Long non-coding RNA (lncRNA) is an RNA with a length exceeding 200 nt, which plays an important role in the occurrence and development of diseases by regulating gene expression during transcription or post-transcriptional/translational and post-translational levels. There were many studies reporting the mechanism of action of lncRNA in acute heavy metal exposure. The study showed that lncRNA-UCA1 regulated autophagy through a ceRNA competition mechanism to protect liver cells from damage caused by heavy metal arsenic exposure [17,18]. In addition, studies found that lncRNA-MTIDP alleviated cadmium-induced oxidative stress and

nephrotoxicity by stabilizing MIR-365 and reducing the expression of nuclear factor-related factor 2 (NRF2) in red blood cells [19,20].

Our previous studies [21] found that there was an abnormal LncRNA expression profile in cadmium toxicity, and long-term cadmium exposure significantly increased the expression of lncRNA-ENST0000414355 (its gene symbol was CR848007.6) in rat serum, which is located at chromosome 9 and its size is 149,891 bp. It is an intergenic lncRNA, and is 44,021,071 bp at the 3′ end of the gene encoding apoptosis inhibitor 5, and 44,021,414 bp at the 5′ end of the gene encoding the leucine-rich repeat-containing protein 4C precursor. We found that LncRNA-CR848007.6 regulated DNA damage and repair, cell cycle, and apoptosis in cell models, animal models, and cadmium-exposed occupational populations. However, the regulatory mechanism of LncRNA-CR848007.6 in cadmium toxicity has not been elucidated, and whether it involves translation regulation is still unknown.

Although gene expression in humans and animals exposed to Cd has been studied at the transcriptional and non-coding RNA levels, little is known about Cd's impacts at the translational level. Previous studies have explored the effects of cadmium toxicity on translation regulation. Huang Kaiyao et al. used Chlamydomonas reinhardtii as the research object and revealed the molecular mechanism of algae tolerance and adaptation to cadmium toxicity through ribosome profiling technology and physiological experiments. Translatome data showed differential expression of various transporters under cadmium stress, including potassium, phosphorus, calcium transporters and multiple ABC transporters. Yu Kyoung Park et al. [22] found that cadmium specifically induced the expression of COX-2 by both transcriptional and co-transcriptional (N-glycation) regulation in C6 cells, which is closely related to oxidative stress-dependent activation of the family of MAPKs and the cadmium-induced expression of both N-glycated and unglycosylated COX-2 proteins and translation-dependent. Zhang [23] found that cadmium caused endogenous sulfur starvation, leading to an increase in mRNA mistranslation, which contributed to the resistance of yeast cells to cadmium. In 2016, we found that translation (eukaryotic) initiation factor 3 (eIF3 or TIF3) regulated migration, invasion and apoptosis in cadmium-transformed 16HBE cells and was a novel biomarker of cadmium exposure in a rat model and in workers [14]. These studies indicated that cadmium toxicity affected the translation process of genes, but these studies only focused on some translation-related factors or certain pathways in the translation process, and did not fully elucidate the impact of cadmium on translation regulation from a translationalomics perspective.

The generalized concept of translatome referred to all components directly involved in the translation process, including but not limited to ribosomes, translating mRNA (also known as RNC mRNA), tRNA, regulatory RNA (such as miRNA, lncRNA, etc.), newborn peptide chains, various translation factors, etc. The narrow concept of translatome referred to the mRNA being translated. Therefore, detecting and analyzing the mRNA being translated is crucial for studying translationomics. The technologies that have emerged in the past include polysome profiling, translating ribosome affinity purification (TRAP), ribosome profiling, and translatome profiling. However, most of these methods cannot obtain ribosome position, density, reading frame position, translation pause region, uORFs, start codon detection, or accurately

determine the number of ribosomes on the same mRNA or the mRNA being translated [24,25]. Therefore, they were not widely applied. In recent years, with the development of high-throughput sequencing technology and the application of RNC seq in the detection and analysis of RNC mRNA, all mRNA information being translated under specific translation states can be obtained. Translationalomics sequencing has achieved the goal of studying translation regulation as a whole. This technology could calculate the translation ratio (TR) value of each gene by balancing the mRNA and RNC mRNA of the same group of samples. In eukaryotic cells, translation initiation is the main rate-limiting step in protein synthesis. Therefore, the TR value approximately represented the efficiency of translation initiation. At present, translationomics has been established for the study of tumor occurrence and development, microbial stress resistance mechanisms, guidance for proteomic identification and discovery of new proteins, and optimization of protein folding efficiency using translation pause theory. Therefore, translational genomics sequencing technology effectively connects the key links from genome to proteome [26–28].

This study constructed libraries of mRNA and RNC, and translationalomics sequencing was carried out by the Ion Proton™ sequencer. R software was used to analyze the transcriptome differential expression between siRNA-treated group cells and control group cells, TR value and ratio value were calculated, and the regulatory function of differentially expressed genes was analyzed by GO and pathway analysis. Flow cytometry was used to detect cell cycle and apoptosis, and verify the regulatory functions of the translation group. The results showed that after transcriptome sequencing, there was little change in the number of transcriptomes between the cadmium-transformed cells in the LncRNA-CR848007.6 silencing expression group and the control group cells, with only 19 transcription genes downregulated and no gene differential expression upregulated. The results of translation sequencing and informatics analysis showed that 65 genes had a translation ratio (ration) > 2, and 114 genes had a translation ratio (ration) < −2. It was also found that genes with differential translation expression did not intersect with genes with differential mRNA expression. The results suggested that lncRNA-CR848007.6 directly regulated the transcriptome during cadmium transformation of 16HBE cells. These findings have significant implications for understanding how LncRNA-CR848007.6 modulates the proteomic landscape during cadmium toxicity. Further bioinformatics analysis revealed that genes with differential translation expression mainly involve cell cycle and mitotic-related pathways. We confirmed through flow cytometry that lncRNA-CR848007.6 regulated the cell cycle and apoptosis during cadmium transformation of 16HBE cells. However, how LncRNA-CR848007.6 specifically modulates the translation of target genes and how this regulation affects cell biomechanics is still unclear. Our team recently found in tumor research that LncRNA-CR848007.6 could regulate the cell cycle and its protein expression of target genes through the LYN-mediated PI3K/AKT signaling pathway (the results of this study have not been published). Whether this mechanism also appears in cadmium toxicity remains to be confirmed by further research. By regulating translation, LncRNA-CR848007.6 may influence cell stiffness and motility, which are crucial for understanding the biomechanical aspects of cadmium toxicity. Furthermore, our results suggest potential therapeutic targets for

intervening in cadmium-related diseases by modulating LncRNA-CR848007.6 expression.

To our knowledge, this is the first paper exploring the regulatory function of lncRNA in cadmium toxicity using high-throughput sequencing technology in the transcriptome, which will provide a reference for other scholars to conduct related research in the future.

Through the application and development of ribosome analysis and sequencing technologies, advanced studies [29] have discovered the translation of lncRNAs. LncRNAs were originally defined as noncoding RNAs, but many lncRNAs actually contain small open reading frames and lncRNA-encoded peptides/proteins that were translated into peptides [30,31]. These opened a broad area for the functional investigation of lncRNAs. Importantly, the role of lncRNA-encoded peptides/proteins holds promise in cancer and toxicology research, but some potential challenges remain unresolved, which will have great significance for further expanding the biological functions of lncRNAs.

5. Limitations

This study conducted a beneficial exploration of LncRNA-CR848007.6 and the translatome in cadmium-malignant transformed 16HBE cells, and found some interesting results, but some limitations remain. First of all, this study focused on 16HBE cells, and it is unclear whether the findings can be generalized to other cell types or in vivo models. Second, the potential off-target effects of siRNA-mediated silencing of LncRNA-CR848007.6 should be addressed. Third, the regulatory mechanism of LncRNA-CR848007.6 on the translatome in cadmium toxicity still needs further study.

6. Conclusion

In conclusion, our study reveals that LncRNA-CR848007.6 plays a pivotal role in modulating the translatome and biomechanical properties of cadmium-malignant transformed 16HBE cells. By directly regulating translation, LncRNA-CR848007.6 influences cell cycle progression and apoptosis, which have significant implications for understanding cadmium toxicity at the molecular level. Future research should focus on elucidating the underlying molecular mechanisms of LncRNA-CR848007.6 regulation of the translatome in 16HBE cells and other models of cadmium toxicity and exploring its potential as a therapeutic target in cadmium-related diseases.

Supplementary materials: Transcriptome analysis, Translation ratio (TR) analysis, GO analysis dada.

Author contributions: Conceptualization, ZZ and HG; methodology, HG; software, HG; validation, ZZ and SZ; formal analysis, SZ; investigation, SZ; resources, ZZ; data curation, SZ; writing—original draft preparation, ZZ; writing—review and editing, SZ; visualization, HG; supervision, ZZ; project administration, SZ; funding acquisition, ZZ. All authors have read and agreed to the published version of the manuscript.

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