Aberrant Long Noncoding RNAs Expression Profiles Affect Cisplatin Resistance in Lung Adenocarcinoma

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1. Introduction

In recent years, a growing proportion of lung adenocarcinoma (LAD) has been diagnosed as non-small cell lung tumor (NSCLC) that is attributable to causes such as environmental pollution. The combination of cisplatin-based chemotherapy plays an important role in comprehensive treatment program [1]. With the widespread use of cisplatin, tumor cells will inevitably lead to its resistance and the chemotherapy effect was significantly reduced [2]. Studies show that 70–80% of patients can temporarily be alleviated in the initial stage of chemotherapy, but long-term use of cisplatin leads to 60% or more of recurrence rate, and drug resistance rate of recurrent lung tumor was significantly increased and chemotherapy response rate was less than 30% [3]. Nowadays, chemotherapy response rate of patients with advanced LAD was only 30–40% and five-year survival rate less than 15% [4]. According to the survey of American Tumor Society, it was shown that more than 90% of tumor deaths in patients were related to varying degrees of drug resistance. The formation of tumor cells once resistant to cisplatin resulted in multidrug resistance to many first-line chemotherapy drugs, for example, Adriamycin, vinblastine, fluorouracil, and mitomycin, so the harm is particularly serious [5]. Cisplatin resistance is the leading cause of LAD chemotherapy failure, affecting the cure rate and long-term survival rate, seriously affecting the prognosis and quality of life, but also aggravating the social and medical burden. Therefore, it is very important to find the biomarkers and molecular targets related to cisplatin resistance in LAD and then to reverse its resistance to improve the prognosis and to avoid and overcome multidrug resistance.

Recent studies have shown that cisplatin is a nonspecific cell cycle cytotoxic drug; it mainly plays a role of inhibition of tumor cell DNA synthesis [6], inducing apoptosis [7]. The mechanism of cisplatin resistance is very complex and it mainly involved some mechanisms [6, 8–10], including changes in intracellular drug transport (such as ATP binding...
cassette protein); reducing drug activity interfering with drug action mechanisms such as glutathione (such as GST-pi) can increase cell detoxification function and affect DNA damage repair (breast tumor-associated gene 1, excision repair cross-complementing gene). Genetic changes of the main signal pathways (PDK/Akt, MAPK/Erk, and Wnt) lead to block apoptosis of drug effects. Unfortunately, despite previous advances in genomics and proteomics, the mechanism of cisplatin resistance has not been elucidated.

Studies have shown that lncRNAs known to be aberrantly expressed in normal cells and tumor cells play a role in the regulation of gene expression; so irregular expression of lncRNAs can result in abnormalities of gene expression and tumorigenesis [11–19]. The abnormal expression of lncRNAs is a symbol of many tumors and has been shown to further the development, invasion, and metastasis of tumors by a variety of mechanisms [20, 21]. LncRNAs can regulate expression from the epigenetic, transcriptional, and posttranscriptional levels [20–22].

It was shown that lncRNAs are related to the mechanisms of resistance to cisplatin in tumors, including lung tumor [23–26], providing an important opportunity to elucidate the mechanisms of cisplatin resistance in tumor cells and to find ways to reverse cisplatin resistance. At present, the research of lncRNA of cisplatin resistance in LAD is still in its infancy. Some lncRNA molecules, including HOTAIR [27], AKI26698 [28], MEG3 [23], H19 [29], and ROR [30], have been screened and identified. It is shown that HOTAIR-mediated LAD cisplatin-resistant mechanism may be through the impact of p21 gene expression to enhance cell apoptosis and G0/G1 phase cell cycle arrest [27], AKI26698 regulate non-small cell lung tumor cisplatin resistance partially by Wnt signaling pathway [28], MEG3 expression by inducing mitochondrial apoptosis pathway p53 protein, and Bcl-xl activation of A549/DDP cells to reduce cisplatin resistance [23]. However, lncRNAs of LAD cisplatin resistance need to be further excavated and their mechanisms need to be clarified.

We used a high-throughput microarray to compare the lncRNA and mRNA expression profiles in cisplatin resistance cell A549/DDP and cisplatin-sensitive cell A549. Several candidate cisplatin resistance-associated lncRNAs were verified by real-time quantitative reverse transcription polymerase chain reaction (PCR) analysis. Our results suggest that lncRNA expression patterns may provide new molecular biomarkers for the prediction of cisplatin resistance in LAD.

2. Materials and Methods

2.1. Cell Lines. A549, NCI-H1299 cells and cisplatin-resistant cell line A549/DDP were cultured in RPMI1640 medium containing 10% fetal bovine serum. The cells were transferred into cell culture flasks by pipetting. The cells were incubated at 37°C in a 5% CO2 incubator, while A549/DDP cell was added to 1 μg/ml of cisplatin maintain the drug resistance. The medium was changed every 2–3 days. The cells were observed under the good condition and the cell will be digested as the cell density was 70%–90%.

2.2. RNA Extraction. The test group included three parallel cultured A549/DDP cell and three parallel cultured A549 cell as the control group. Total RNAs of cells were extracted using Trizol reagent (Invitrogen, Carlsbad, CA, USA), based on the manufacturer's protocol. The integrity of the RNA was analyzed by electrophoresis on a denaturing agarose gel. The accurate measurement of RNA concentration (OD260), protein contamination (OD260/OD280 ratio), and organic compound contamination (OD260/OD230 ratio) was analyzed with a NanoDrop ND-1000 spectrophotometer.

2.3. Microarray and Computational Analysis. An Agilent Array analysis platform (Agilent Technologies, Santa Clara, CA, USA) was used for microarray analysis. Slightly, an mRNA-ONLY Eukaryotic mRNA Isolation Kit (Épicentre Biotechnologies, USA) purified mRNA from total RNA after removal of rRNA. Then, each sample was amplified and transcribed into fluorescent cRNA along the whole length of the transcripts without 39 bias with a random priming method. The labeled cRNAs were hybridized onto a Human LncRNA Array v3.0 (8660 K; Arraystar including 30,586 lncRNAs and 26,109 coding transcripts). A specific exon or splice junction probe accurately identified each transcript. For hybridization quality control, the positive probes for housekeeping genes and negative probes were also printed onto the array. The arrays were scanned with an Agilent G2505C scanner after washing the slides, and Agilent Feature Extraction software (version 11.0.1.1) was used to analyze the acquired array images. Quartile normalization and subsequent data analysis were performed with the GeneSpring GX v12.0 software package (Agilent Technologies). Complying with the manufacturer's standard instructions with minor modifications, sample preparation and microarray hybridization were performed [31]. The microarray study was performed by KangChen Bio-tech Corporation, Shanghai, China.

2.4. Functional Group Analysis. GO analysis was derived from Gene Ontology (http://www.geneontology.org), which can provide three structured networks of defined terms describing gene product attributes. The P value hints the significance of GO Term enrichment in the unmorally expressed mRNA list (P ≤ 0.05 was considered obviously significant). We also performed pathway analysis for the unmorally expressed mRNAs based on the latest KEGG (Kyoto Encyclopedia of Genes and Genomes) website. This analysis results allowed us to determine the biological pathways whether a significant enrichment of unmorally expressed mRNAs existed (P ≤ 0.05 was considered obviously significant).

2.5. Construction of the mRNA-lncRNA Gene Coexpression Network. The coexpression network is constructed and used with calculating a pairwise relation matrix between all probe sets across microarray samples. The result of Pearson relation matrix was transformed into an adjacency matrix [31].

2.6. Quantitative PCR. Total RNA was then reverse-transcribed with an RT Reagent Kit (Thermo Scientific, CA, USA), according to the manufacturer's protocols. Total RNA
(2 μg) was reverse-transcribed to cDNA. PCR was performed in a total reaction volume of 20 μl, including 10 μl of SYBR Premix Ex Taq (2x), 2 μl of cDNA template, 1 μl of PCR forward primer (10 mM), 1 μl of PCR reverse primer (10 mM), and 6 μl of double-distilled water. The qPCR reaction included an initial denaturation step of 10 min at 95°C; 40 cycles of 95°C for 15 s, 60°C for 1 min; and a final extension step of 5 min at 95°C, and a final extension step of 5 min at 72°C. The qPCR detected LncRNA with SYBR Premix Ex Taq and an ABI 7200 instrument (ABI Corporation, CA, USA). The candidate LncRNAs were analyzed by qPCR and the sequence information of these gene primers is shown in Table 1. We verified the expression of these LncRNAs by qPCR using GAPDH as a housekeeping gene and by calculating 2^(-ΔΔCT) values [32].

| LncRNA gene | Sense primer (5'-3') | Antisense primer (5'-3') | PCR product length (bp) |
|-------------|---------------------|--------------------------|--------------------------|
| GLYCTK      | CGTCGTGATCTCAGGTGGTA| CTTCAGAAACGTGGCAGGG      | 164                      |
| HSD17B7P2   | GTGACCACTGAGTCATCTTTC | GAGGCTCAGTTCGCCAATC      | 283                      |
| AP001469.9  | TCACACAACATCTCGTGTG | TTTGCTCAAGACTGTTGCAAG    | 309                      |
| NABP1       | GAGAGGTTGGAAGAATTTGAC| CTCGATCTCATCCACACAG      | 260                      |
| RPII-909N173| AGACCCCTGCTATTCAGCATG| AAGGGATGCAGCAGTTCTC      | 102                      |
| UCA1        | AGCTAATGCTGACCTGTTGT| CTGCCGACTGCTCCAGTGT      | 124                      |
| POLD4       | GCACCCTCCTGGCACATC TC | GGTGAGCTCTGACACCTCC      | 180                      |
| XLOC_009833 | AGCCCCCTTATACGTTGCGG | GACATTCAGAGAAGGGGGG      | 147                      |
| CTD-2550O16.2| GAGGAAGGTCCCTTGGTGCG | CAGTGCAGTTTATGCAGT      | 79                       |
| RPII-299H122.5 | AGTCGCCCTTTCCCTTAGGCC | GCAGCCTCATCTGTTGCTT       | 108                     |
| BC033241    | TCTACAACAGCAGCAAGCAT | TTGACAGCTGGTGTGGGAGA     | 107                      |
| AC078883.3  | GTGGCAACATCCCTTCAACCA | ACAGGTTCGTTGTCACCGT      | 245                      |
| GAPDH       | TGACCTCAACAGCAGCAACCA | CACCTGTGCTGATGCACAAAA  | 121                      |

2.7. Lentivirus-Mediated siRNA, Overexpression Vector Construction, and Transfection. We constructed siRNA GV248 lentivirus targeting UCA1 and overexpression GV303 vector targeting UCA1 (Gene Chem, Shanghai, China). siRNA sequences were as follows: siRNA1: CCACCTGTAAGAGAACAGT; siRNA2: GAAGAGTAGAAGACAGGT; siRNA3: GCCGTGACAGAAGACGT. Transfections were performed by seeding 2×10^4 cells in 6-well plate. After 24 h, the medium was replaced, and the cells were incubated with the transfection complex based on the manufacturer’s protocol; the multiplicity of infection (MOI) values was as follows: A549 MOI = 20 and NCI-H1299 MOI = 5. The cells were infected with lentivirus for 72 h, and the siRNA or overexpression efficiency was assessed by qPCR. Puromycin test isolated these cell lines successfully transfected with the lentivirus-mediated vector. The study included NCI-H1299 UCA1 overexpression cell lines (UCA1 OE group), NCI-H1299 was infected with lentivirus negative control LVCON077 vector (NC group), NCI-H1299 (control group), and A549 UCA1 siRNA cell line (UCA1 siRNA group), and A549 was infected with lentivirus negative control LVCON145 vector (NC group) and A549 (control group).

2.8. CCK-8 Assays. IC50 (half maximal inhibitory concentration) was detected by Cell Counting Kit-8 (CCK-8, Corning Corporation, UAS) abiding by the manufacturer’s protocols. Briefly, 3000 cells were remixed and seeded into a 96-well plate with 10% FBS. The next day, the cells were incubated with CCK-8 for 1h and the absorbance of 450 nm was analyzed.

2.9. Statistical Methods. Statistical analysis was performed for the comparison of two groups in the microarray which was performed with Student's t-test and the fold change. The false discovery rate (FDR) was calculated for correcting the P value. The threshold value used to designate abnormally expressed LncRNAs and mRNAs according to a fold change of ≥2.0 or ≤0.5 (P < 0.05). Differences with P < 0.05 were considered statistically significant.

3. Results

3.1. Overview of LncRNA Profiles. The result showed that there were 1,543 differentially expressed LncRNAs between A549/DDP and A549 cell. Among these, compared to A549 group, the 984 LncRNAs upregulated more than twofold in the A549/DDP group, while 559 lncRNAs downregulated (Supplemental Table 1 in Supplementary Material available online at https://doi.org/10.1155/2017/7498151 and Figure 1). These LncRNAs might play an important role in the cisplatin resistance of LAD.

3.2. LncRNA Classification and Subgroup Analysis. There were 43 differentially intergenic LncRNAs (LincRNAs) (including 31 upregulated and 12 downregulated) expressed (fold change ≥ 2.0, P < 0.05) between A549/DDP cell and A549 cell. We also found some nearby coding genes that may be regulated by these LincRNAs (Supplemental Table 2). LncRNAs with enhancer-like functions (lncRNA-a) were identified with GENCODE annotation. There were 33 LncRNA-a (including 17 upregulated and 16 downregulated) differentially expressed between A549/DDP and A549 cell. We also found some nearby coding genes that may be regulated by these LncRNA-a (Supplemental Table 3). Otherwise, we also found 52 antisense lncRNAs (including
**Figure 1:** Box plots, scatter plots, and heat map showing the variation in lncRNA expression between the A549/DDP and A549 arrays. The values of the X and Y axes in the scatter plot are averaged normalized values in each group (log2-scaled). The lncRNAs above the top green line and below the bottom green line are those with a >3-fold change in expression between tissues. (a) Scatter plots of T2 group versus C2 group. (b) Scatter plots of T3 group versus C3 group. (c) Scatter plots of T5 group versus C5 group. (d) Volcanic map. (e) heat map and hierarchical clustering of lncRNA, and (f) box plots showing the distribution of the lncRNA.
Table 2: Some upregulated or downregulated mRNAs in A549/DDP.

| Probe name         | Fold change | Regulation | Gene symbol |
|--------------------|-------------|------------|-------------|
| ASHG A5P001180     | 39.0517312  | up         | USF2        |
| ASHG A5P016060     | 3.9089125   | up         | EFTUD2      |
| ASHG A5P005374     | 3.2721382   | up         | TTC39C      |
| ASHG A5P034316     | 3.2788507   | up         | ZNF836      |
| ASHG A5P006930     | 2.1690486   | down       | TUBD1       |
| ASHG A5P050222     | 4.1895085   | down       | PAFAH1B3    |
| ASHG A5P006058     | 9.1726844   | down       | TACC3       |
| ASHG A5P010515     | 2.6021545   | down       | QKI         |
| ASHG A5P037999     | 2.193611    | down       | FAM159A     |
| ASHG A5P009559     | 2.2639906   | down       | DIAPH2      |
| ASHG A5P001190     | 3.9636124   | up         | CPA4        |
| ASHG A5P002598     | 2.1978303   | up         | SNX24       |

21 upregulated and 31 downregulated (Supplemental Table 4).

3.3. Overview of mRNA Profiles. In total, 1,712 mRNAs were found to be differentially expressed between the A549/DDP and A549 cell, including 795 mRNAs upregulated and 917 mRNAs downregulated (Table 2 and Figure 2). These mRNAs might play a role in the cisplatin resistance of LAD.

3.4. GO Analysis. The genes corresponding to downregulated mRNAs included 979 genes involved in biological processes, 120 genes involved in cellular components, and 137 genes involved in molecular functions (Figures 3(a)–3(c)). The genes corresponding of upregulated mRNAs included 558 genes involved in biological processes, 93 genes involved in cellular components, and 77 genes involved in molecular functions (Figures 3(d)–3(f)).

3.5. Pathway Analysis. The 30 upregulated pathways were found, including chemical carcinogenesis, drug metabolism, and p53 signaling pathway (Figure 3(g) and Table 3). 37 downregulated pathways were identified, like DNA replication, cell cycle, Fanconi anemia pathway, and so on. (Figure 3(b) and Table 3). These pathways might play a role in the cisplatin resistance of LAD.

3.6. LncRNA-mRNA Coexpression Network. We build the lncRNA-mRNA coexpression network. See Figure 4(a). The results imply that UCA1 (uc002nbr.3), ENST00000443252, ENST00000510562, ENST00000565689, ENST0000058690, ENST00000397340, ENST00000440955, ENST00000507916 are closely related to many mRNAs and they together prompted resistance of cisplatin in LAD.

3.7. Real-Time Quantitative PCR Validation. Based on the features (such as fold difference, gene locus, and nearby encoding gene, and so on.) of the differentially expressed lncRNAs, we initially identified a number of interesting candidate lncRNAs for further analysis (including HSD17B7P2, GLYCTK, NABPI, AP001469.9, RPII-909NI73, UCA1, POLD4, XLOC_009833, CTD-2555016.2, RPII-299H22.5, BC033241, and AC078883.3). We found that the microarray results for several of the lncRNAs were consistent with the results of RT-PCR (Figure 4(b)). Of these, UCA1 exhibited significantly changed expression in 20 samples from A549/DDP and A549 cell. The expression of UCA1 in cisplatin-resistant A549/DDP cells was significantly higher than that in cisplatin-sensitive A549 cells (t = 71.14, P = 0.0002, Figure 4(c)). These results suggest that UCA1 and candidate lncRNAs may play an important role in cisplatin resistance in LAD.

3.8. UCA1 Significantly Reduces the IC50 of Cisplatin in A549/DDP Cell after Knockdown. We used CCK-8 method to detect the sensitivity of A549/DDP cells to cisplatin. The results showed that the IC50 of A549 was 2.09 μg/ml ± 0.08 μg/ml, IC50 of A549/DDP was 10.7 μg/ml ± 0.28 μg/ml, and the resistance index was 5.2 (Figure 4(d)). The IC50 of cisplatin in UCA1 siRNA group was significantly lower than that in NC group (t = 17.51, P < 0.0001), control group (t = 37.65, P < 0.0001). The IC50 of A549/DDP cells decreased from 10.7 μg/ml ± 0.28 μg/ml to 3.6 μg/ml ± 0.12 μg/ml after UCA1 knockdown, as shown in Figure 4(f). The results showed that UCA1 siRNA, A549/DDP cisplatin resistance can be significantly reversed.

3.9. UCA1 Overexpression Significantly Increased the IC50 of Cisplatin in NCI-H1299 Cell. The results showed that the IC50 of cisplatin in UCA1 OE group was significantly higher than that in NC group (t = 23.21, P < 0.0001), control group (t = 29.34, P < 0.0001). The IC50 of NCI-H1299 cells increased from 1.20 μg/ml ± 0.04 μg/ml to 4.5 μg/ml ± 0.13 μg/ml after UCA1 was overexpressed, as shown in Figure 4(f). The results showed that UCA1 was overexpressed; NCI-H1299 cisplatin resistance can be significantly increased.

4. Discussion

LncRNAs are involved in many biological processes, as X-chromosome inactivation, gene imprinting [33, 34]. Otherwise, lncRNAs are important factors in the control of gene
Figure 2: Box plots, scatter plots, and heat map showing the variation in mRNA expression between the A549/DDP and A549 arrays. The values of the X and Y axes in the scatter plot are averaged normalized values in each group (log2-scaled). The mRNAs above the top green line and below the bottom green line are those with a >3-fold change in expression between tissues. (a) Scatter plots of T2 group versus C2 group, (b) Scatter plots of T3 group versus C3 group, (c) Scatter plots of T5 group versus C5 group, (d) Volcanic map, (e) Heat map and hierarchical clustering of IncRNA, (f) Box plots showing the distribution of the IncRNA.
Figure 3: Continued.
RNA expression in tumor [35] and play an important role in the development, progression, and drug resistance of tumors [36]. Recently, disease-lncRNA association prediction is a recent trend for the identifying potential disease-related lncRNAs [37, 38]. Developing powerful computational models for potential disease-related lncRNAs identification would benefit biomarker identification and drug discovery for human disease diagnosis, treatment, prognosis, and prevention [37, 38].

In this study, we analyzed lncRNA abnormal expression profiles and ascertained the potential role of cisplatin resistance in LAD. High-throughput microarray techniques revealed a variety of differentially expressed lncRNAs, including 984 lncRNAs upregulated and 559 lncRNAs downregulated in A549/DDP cell compared to A549 cell. LncRNAs are usually divided into five categories: sense, antisense, bidirectional, intronic, and intergenic. LncRNAs are known to function by a variety of mechanisms. However, a common and important function of lncRNAs is to change the expression of nearby mRNAs by influencing process of transcription [39] or directly playing an enhancer-like role [40, 41]. In the study, we increased the accuracy of target prediction by comparing...
Table 3: Pathways analysis of mRNA of A549/DDP cell.

| Pathway ID | Definition | Fisher $P$ value | Count | FDR   | Enrichment Score |
|------------|------------|------------------|-------|-------|------------------|
| hsa03030   | DNA replication, *Homo sapiens* (human) | 2.51659E−12 | 36    | 7.39876E−10 | 11.599188       |
| hsa04110   | Cell cycle, *Homo sapiens* (human) | 6.86608E−11 | 124   | 1.00931E−08 | 10.163291       |
| hsa04115   | p53 signaling pathway, *Homo sapiens* (human) | 1.51925E−10 | 68    | 1.48887E−08 | 9.81837         |
| hsa03460   | Fanconi anemia pathway, *Homo sapiens* (human) | 9.53284E−09 | 53    | 7.00664E−07 | 8.020778        |
| hsa05322   | Systemic lupus erythematosus, *Homo sapiens* (human) | 1.60137E−08 | 136   | 9.41604E−07 | 7.795509        |
| hsa05034   | Alcoholism, *Homo sapiens* (human) | 5.43613E−07 | 180   | 2.66371E−05 | 6.26471         |
| hsa05204   | Chemical carcinogenesis, *Homo sapiens* (human) | 1.11406E−06 | 82    | 4.67904E−05 | 5.953093        |
| hsa00983   | Drug metabolism, other enzymes, *Homo sapiens* (human) | 8.07034E−06 | 46    | 0.000296585 | 5.093108        |
| hsa00980   | Metabolism of xenobiotics by cytochrome P450, *Homo sapiens* (human) | 0.000084192 | 74    | 0.000463605 | 4.847956        |
| hsa00140   | Steroid hormone biosynthesis, *Homo sapiens* (human) | 3.3875E−05 | 58    | 0.000995926 | 4.47012         |
| hsa00982   | Drug metabolism, cytochrome P450, *Homo sapiens* (human) | 6.55397E−05 | 68    | 0.001751697 | 4.183496        |
| hsa04114   | Oocyte meiosis, *Homo sapiens* (human) | 0.000202216 | 113   | 0.004954299 | 3.69484         |
| hsa03430   | Mismatch repair, *Homo sapiens* (human) | 0.000264734 | 23    | 0.005987061 | 3.57719         |
| hsa00830   | Retinol metabolism, *Homo sapiens* (human) | 0.00049597 | 65    | 0.01041537 | 3.304545        |
| Pathway ID | Definition                                                                 | Fisher P value | Count | FDR          | Enrichment Score |
|-----------|-----------------------------------------------------------------------------|----------------|-------|--------------|------------------|
| hsa04978  | Mineral absorption, *Homo sapiens* (human)                                 | 0.000531877    | 51    | 0.01042478   | 3.274189         |
| hsa03410  | Base excision repair, *Homo sapiens* (human)                               | 0.000844241    | 33    | 0.01551292   | 3.073534         |
| hsa00053  | Ascorbate and aldarate metabolism, *Homo sapiens* (human)                   | 0.00090023     | 27    | 0.01556869   | 3.045646         |
| hsa03440  | Homologous recombination, *Homo sapiens* (human)                           | 0.00173489     | 28    | 0.01916699   | 2.930521         |
| hsa00500  | Starch and sucrose metabolism, *Homo sapiens* (human)                      | 0.00294299     | 56    | 0.02002757   | 2.887965         |
| hsa00240  | Pyrimidine metabolism, *Homo sapiens* (human)                              | 0.00511088     | 105   | 0.022213     | 2.82071          |
| hsa03420  | Nucleotide excision repair, *Homo sapiens* (human)                         | 0.003406297    | 47    | 0.04768816   | 2.467717         |
| hsa00860  | Porphyrin and chlorophyll metabolism, *Homo sapiens* (human)               | 0.00512004     | 42    | 0.06831497   | 2.291409         |
| hsa05200  | Pathways in cancer, *Homo sapiens* (human)                                 | 0.006405562    | 398   | 0.07929988   | 2.193443         |
| hsa00040  | Pentose and glucuronate interconversions, *Homo sapiens* (human)           | 0.00647346     | 36    | 0.07929988   | 2.188864         |
| hsa05203  | Viral carcinogenesis, *Homo sapiens* (human)                               | 0.006997658    | 206   | 0.08229246   | 2.155047         |
| hsa05222  | Small cell lung cancer, *Homo sapiens* (human)                             | 0.00779351     | 86    | 0.08812662   | 2.108267         |
| hsa05219  | Bladder cancer, *Homo sapiens* (human)                                     | 0.009084523    | 38    | 0.09892036   | 2.041698         |
| Pathway ID | Definition                                                                 | Fisher P value | Count | FDR   | Enrichment Score |
|-----------|------------------------------------------------------------------------------|----------------|-------|-------|------------------|
| hsa05134  | Legionellosis, *Homo sapiens* (human)                                        | 0.01081157     | 55    | 0.1135215 | 1.966111         |
| hsa04621  | NOD-like receptor signaling pathway, *Homo sapiens* (human)                 | 0.0138298      | 57    | 0.1402056 | 1.859184         |
| hsa05202  | Transcriptional misregulation in cancer, *Homo sapiens* (human)             | 0.01531221     | 179   | 0.1500597 | 1.814962         |
| hsa05143  | African trypanosomiasis, *Homo sapiens* (human)                             | 0.01618672     | 34    | 0.1535128 | 1.790841         |
| hsa05162  | Measles, *Homo sapiens* (human)                                             | 0.01985443     | 134   | 0.1824125 | 1.702143         |
| hsa04914  | Progesterone-mediated oocyte maturation, *Homo sapiens* (human)             | 0.02189761     | 88    | 0.1950878 | 1.659603         |
| hsa00410  | beta-Alanine metabolism, *Homo sapiens* (human)                            | 0.03354188     | 31    | 0.2900386 | 1.474413         |
| hsa00480  | Glutathione metabolism, *Homo sapiens* (human)                             | 0.04770932     | 51    | 0.4007582 | 1.321397         |
Figure 4: Some lncRNA expression in LAD and A549/DDP, A549 cell. (a) lncRNA-mRNA coexpression network, the results imply that UCA1, ENST00000443252, ENST00000510562, ENST00000565689, ENST00000558690, ENST000004397340, ENST00000440955, and ENST00000507916 are also closely related to many mRNA molecules. (b) Comparison between gene chip data and qPCR result. The validation results of the 12 lncRNAs indicated that the microarray data correlated well with the qPCR results. UCA1 expression level in LAD and A549/DDP, A549 cell. (c) The expression of UCA1 in cisplatin-resistant A549/DDP cells was significantly higher than that in cisplatin-sensitive A549 cells. (d) We used CCK-8 method to detect the sensitivity of A549/DDP cells to cisplatin. (e) The IC50 of cisplatin in UCA1 siRNA group was significantly lower than that in NC group, control group. (f) The IC50 of cisplatin in UCA1 overexpression group was significantly higher than that in NC group, control group.
abnormally expressed mRNAs with differentially expressed lncRNAs. The expression profiles of 43 intergenic lncRNAs (lncRNAs) hinted that they were differentially expressed between A549/DDP cell and A549 cell. Among these, 31 were upregulated and 12 were downregulated. The expression profiles of 33 lncRNA-a indicated that they were differentially expressed between A549/DDP and A549 cell. Among these, 17 were upregulated and 16 were downregulated. Otherwise, we found 52 antisense lncRNAs, among these, 21 were upregulated and 31 were downregulated. So as to get insights into lncRNA target gene function, GO analysis and KEGG pathway annotation were applied to the lncRNA target gene pool. GO analysis uncovered that the number of genes corresponding to downregulated mRNAs was larger than that relating to upregulated mRNAs. KEGG annotation unveiled that there were 30 upregulated pathways (including chemical carcinogenesis, drug metabolism, and p53 signaling pathway) and 37 downregulated pathways (including DNA replication, cell cycle, and Fanconi anemia pathway). These pathways might be involved in the occurrence and development of cisplatin resistance in LAD.

We found that 12 of the lncRNAs identified in the microarray analysis were confirmed by qPCR to be aberrantly expressed in A549/DDP cell. Among these lncRNAs, UCA1 was the significantly upregulated. Furthermore, we built the lncRNA-mRNA coexpression network and it is shown that UCA1 and some lncRNAs were individually related to some mRNAs; it hinted that lncRNA-mRNA coexpression network might contribute to the development of cisplatin resistance in LAD. UCA1 has also been reported to be related to cisplatin resistance in bladder carcinoma [42, 43]. The expression of UCA1 in A549/DDP cells was significantly higher than that in A549 cells, suggesting that UCA1 may play an important role in cisplatin resistance in LAD. Subsequently, we found the IC50 of A549/DDP cells decreased from 10.7 μg/ml ± 0.28 μg/ml to 3.6 μg/ml ± 0.12 μg/ml after UCA1 knockdown, while the IC50 of NC1-H2199 cells increased from 1.20 μg/ml ± 0.04 μg/ml to 4.5 μg/ml ± 0.13 μg/ml after UCA1 was overexpressed; it hinted that UCA1 might contribute to the development of cisplatin resistance in LAD and further study of the biological function of UCA1 will be required to confirm this notion.

Our study revealed a set of lncRNAs with differential expression from cisplatin resistance in LAD. Furthermore, potential roles for these lncRNAs in the regulation of chemical carcinogenesis and DNA replication signaling pathways were identified. Moreover, we found that UCA1 might contribute to the development of cisplatin resistance in LAD.

Conflicts of Interest
The authors declare that no conflicts of interest exist.

Authors’ Contributions
Lijuan Hu and Jian Chen contributed equally to this work.

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