Microarray expression profile of long non-coding RNAs in paclitaxel-resistant human lung adenocarcinoma cells

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Abstract. Paclitaxel (PTX)-based chemotherapy is a standard treatment for human lung adenocarcinoma, but treatment often fails since resistance develops. Recent studies have described the activity of long non-coding RNAs (lncRNAs) in many biological processes and human diseases. Chemotherapy resistance is one of these areas, but the role of lncRNAs in paclitaxel resistance of human lung adenocarcinoma cells has not been reported. A paclitaxel resistance model was established using A549 human lung adenocarcinoma cells. lncRNAs and mRNAs were profiled in parental A549 and paclitaxel-resistant A549/PTX cells by microarray analysis. Real-time quantitative PCR (RT-qPCR) was used to validate the results of the microarray. Chromosomal distribution patterns of differentially expressed lncRNAs and mRNAs were assessed. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses were performed using gene set enrichment. We screened 1,154 lncRNAs and 1,733 mRNAs that had a >3-fold difference in expression in A549/PTX cells compared with A549 cells, most of which were downregulated. Nine lncRNAs and six mRNAs were randomly selected and validated by RT-qPCR. Most aberrantly expressed lncRNAs and mRNAs were located on chromosomes 1, 2, 6, 12 and 17, particularly on chromosome 1. Bioinformatics, GO and KEGG pathway analyses, revealed that some differentially expressed genes regulated classical functions and pathways such as cytosol components, protein binding, gene expression and metabolic pathways. Differential expression of lncRNAs and mRNAs in A549/PTX and A549 cells indicates that various lncRNAs may be useful diagnostic or prognostic markers of resistance to treatment, or future targets for paclitaxel-based chemotherapy, providing a novel rationale for clinical treatment.

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Introduction

Lung cancer is one of the most common human cancers, and with the highest worldwide incidence and mortality (1). Adenocarcinoma is one of the more common pathological types (2). Despite advances in surgery, radiotherapy and targeted treatment, survival is far from satisfactory. Most patients are given paclitaxel (PTX)-based combination chemotherapy as a standard treatment (3). Paclitaxel is a taxane, and its cytotoxic effect depends on microtubule polymerization, and inhibition of microtubule depolymerization leads to a cell cycle block in the G2/M phase resulting in tumor cell apoptosis or necrosis (4). However, eventual development of resistance to paclitaxel is inevitable and leads to treatment failure. The paclitaxel mechanism of resistance is multifactorial and complex, involving changes in drug-efflux, increased drug metabolism, interference with DNA repair and cell cycle regulation, and disorders of cell apoptosis and autophagy (5,6). Despite our knowledge of its anticancer effects, little is known concerning the development of paclitaxel resistance. A broadening of our understanding is essential for improving treatment outcomes.

Much of the human genome is transcribed as long non-coding RNAs (lncRNAs), a type of ncRNA 200 nt in length that is not translated, but does influence the regulation of gene expression and chemotherapy resistance in various ways (7,8). For example, lncRNA n375709 expression was significantly increased in paclitaxel-resistant CNE-2 nasopharyngeal carcinoma compared with parental CNE-2 cells (9). Knockdown of AK126698 activated the canonical Wnt signaling pathway and induced cisplatin resistance in A549 human lung cancer cells (10). lncRNA MEG3 overexpression in drug resistant A549/DDP cells increased their chemosensitivity to cisplatin both *in vitro* and *in vivo* by inhibiting cell proliferation and inducing apoptosis (11). Upregulation of lncRNA GAS5 overcame resistance of human lung adenocarcinoma cells to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) therapy (12). Other lncRNAs, such as HOTTIP, NEAT1, PVT1 and ODRUL have also been reported to modulate chemosensitivity (13-16). It has thus been established that the upregulation and downregulation of lncRNAs is implicated in chemotherapy resistance.

To the best of our knowledge, there have been no studies concerning the roles of lncRNAs in the acquisition of paclitaxel

resistance in lung adenocarcinoma. We investigated differential expression of lncRNAs and mRNAs in paclitaxel-sensitive A549 and paclitaxel-resistant A549/PTX cells using microarray assays. Nine differentially expressed lncRNAs and six mRNAs were randomly selected for validation by real-time quantitative PCR (RT-qPCR). Subsequent bioinformatics evaluation by Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analyses identified classical biological regulatory functions and pathways that were differentially expressed in these cell lines. Our results add to the knowledge concerning the involvement of lncRNAs in the resistance to paclitaxel-based chemotherapy in human lung adenocarcinoma cells, and thus, may provide novel molecular therapeutic targets.

Materials and methods

Cell lines and cell culture. The A549 human lung adenocarcinoma cell line was obtained from the Cell Bank of the Shanghai Branch of the Chinese Academy of Sciences. A549/PTX cells were established in our laboratory in a stepwise manner by exposing drug-sensitive A549 cells to increasing doses of paclitaxel (PTX; Bristol-Myers Squibb, New York, NY, USA). The cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (both from Gibco, Carlsbad, CA, USA) at 37˚C in humidified incubator with 5% CO_2. The A549/PTX cell culture medium also contained 200 ng/ml PTX to maintain the drug-resistant phenotype. Cells in the logarithmic phase of growth were used in all experimental procedures.

In vitro drug sensitivity assay. To evaluate differences in chemoresistance, cells were seeded into 96-well plates at $5x10^3$ cells/well and incubated with various concentrations of PTX for 48 h. At 48 h, the number of viable cells was assayed with Cell Counting Kit-8 (CCK-8; Dojindo Laboratories, Kumamoto, Japan). CCK-8 reagent (10 μl) was added to each well and incubation followed for 1 h at 37˚C. The number of viable cells was estimated by assessment of the optical density (OD) at 450 nm, and the PTX concentration that produced 50% inhibition of growth (IC_{50}) was estimated from the relative survival curves. Three independent experiments were performed in five duplicate wells.

RNA extraction and RNA quality control. Total RNA was extracted using TRIzol reagent (Takara, Otsu, Japan) following the manufacturer's protocol. RNA quantity and quality were assessed using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Inc., Waltham, MA, USA), and RNA integrity was assessed by electrophoresis on a denaturing agarose gel. Isolated RNAs were stored at -80˚C prior to lncRNA microarray analysis and RT-qPCR.

lncRNA microarray. The GeneChip Human Transcriptome Array 2.0 (Affymetrix, Santa Clara, CA, USA) contains >6.0 million distinct probes of both coding and non-coding transcripts. Approximately 25,000 lncRNAs and 24,500 mRNAs listed in databanks including RefSeq, Ensembl, UCSC (known genes and lincRNA transcripts), NONCODE, the Human Body Map lincRNA and TUCP catalog, and lncRNAdb, were detected.

RNA labeling and microarray hybridization. RNA labeling and microarray hybridization were performed following an Affymetrix GeneChip protocol from GMINIX BioTech (Shanghai, China). Briefly, total RNA was purified after removal of rRNA and tRNA. Each sample was amplified and transcribed into fluorescent cRNA along the entire length of the transcript, without the 3' bias, using a random priming method. After purification, the labeled cRNAs were hybridized at 45˚C for 16 h in an Affymetrix hybridization oven. After being washed, the hybridized arrays were scanned using Affymetrix GeneChip Scanner 3000, and data were extracted using Transcriptome Analysis Console Software.

Validation of aberrantly expressed lncRNAs and mRNAs by RT-qPCR. Total RNA was extracted using TRIzol reagent, and was then reverse-transcribed using a PrimeScript RT reagent kit with gDNA Eraser (Perfect Real Time; Takara) following the manufacturer's instructions. Reactants were incubated for 2 min at 42˚C, 15 min at 37˚C, 5 sec at 85˚C, 7 min at 4˚C, and then stored at -20˚C. The transcripts of nine differentially expressed lncRNAs and six mRNAs were randomly selected for RT-qPCR using a SYBR-Green assay (Takara, Dalian, China); GAPDH was used as an internal control. Specific lncRNA and mRNA primers were designed using Primer 5.0. The RT-qPCR reaction was set at an initial denaturation step of 10 min at 95˚C followed by 40 cycles of 95˚C for 10 sec and 60˚C for 1 min. All experiments were performed three times, and fold changes of expression were calculated using the $2^{-\Delta\Delta Ct}$ method.

Bioinformatics analysis. Affymetrix Transcriptome Analysis Console software was used to analyze the acquired array images. Differentially expressed lncRNAs and mRNAs were identified through fold-change filtering. GO categories (www.geneontology.org) and KEGG pathway analyses (http://www.genome.jp/kegg/) were performed using the standard enrichment computation method.

Statistical analysis. Statistical analysis was performed using SPSS version 17.0 software (SPSS, Inc., Chicago, IL, USA). Results are presented as the means ± standard deviation (SD) of three separate assays. Differences between groups were assessed by two tailed t-tests. $P<0.05$ was considered as statistically significant.

Results

CCK-8 assay of in vitro drug sensitivity. The paclitaxel-resistance of A549/PTX cells was characterized by determining the IC_{50} value. After treatment with various concentrations of paclitaxel for 48 h in A549 and A549/PTX cells, the cell survival was assayed using CCK-8 (Fig. 1A). The paclitaxel IC_{50} value for the drug-resistant A549/PTX cell line was 1240.12±6.13 and 18.21±0.84 ng/ml for the A549 cell line (Fig. 1B), with a drug-resistance index of A549/PTX relative to A549 cells of 68 ($P<0.01$). A previously reported criterion of high-resistance is an index >20 (17). The A549/PTX cells were revealed to be more resistant to paclitaxel than the A549 cells.

RNA quality control. RNA quantification and quality were spectrophotometrically assayed using the NanoDrop ND-1000.

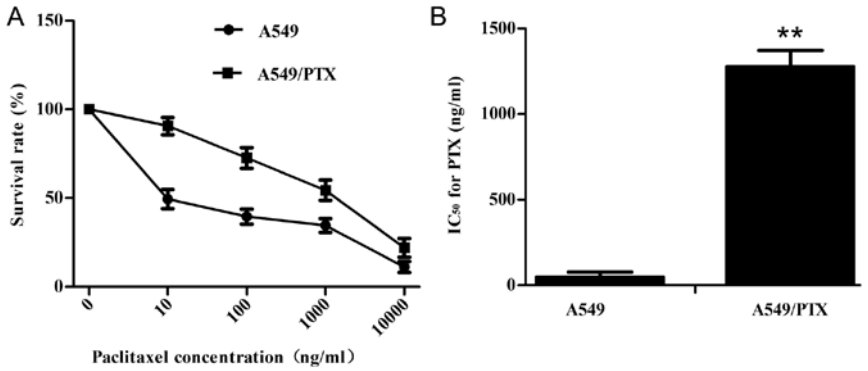

Figure 1. Paclitaxel (PTX)-resistance of A549 and A549/PTX cells. (A) Cells were treated with increasing concentrations of paclitaxel and 48 h later, the cell survival rate was assessed using CCK-8 assay. The A549/PTX cells were more resistant to paclitaxel than the A549 cells. (B) The IC_{50} value of paclitaxel for the A549/PTX cells was 1240.12±6.13 ng/ml, and for the A549 cells the value was 18.21±0.84 ng/ml. The drug-resistance index of A549/PTX cells relative to A549 cells was 68. Data are presented as the mean ± standard deviation; $^{**}P<0.01$.

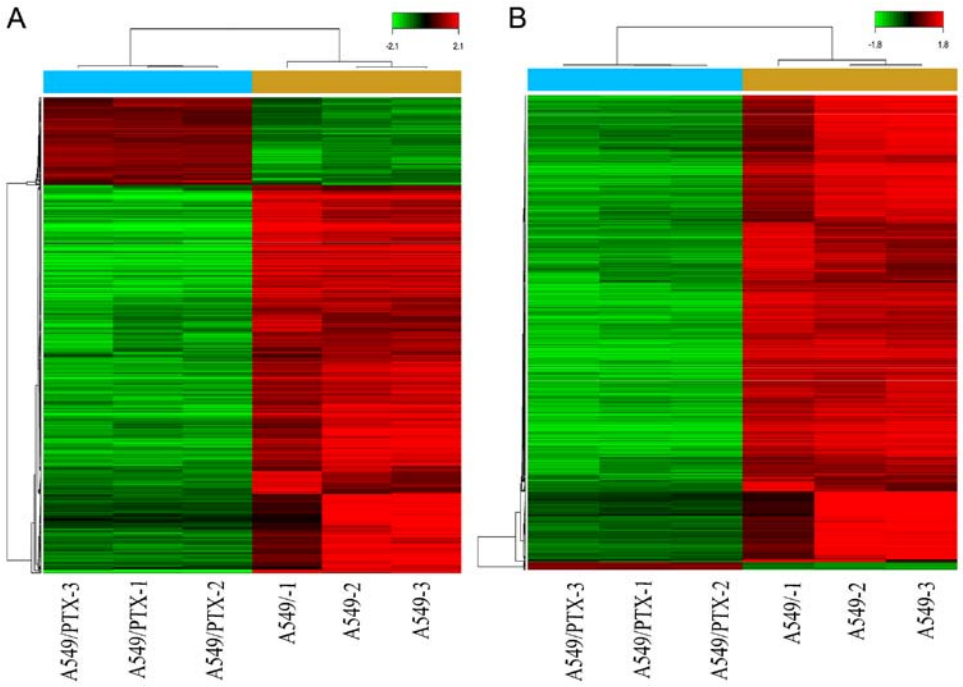

Figure 2. Microarray expression profiling of differentially expressed long non-coding RNAs (lncRNAs) and mRNAs in A549 and paclitaxel-resistant A549 (A549/PTX) cells. (A) Hierarchical clustering of all target lncRNAs. (B) Hierarchical clustering of all target mRNAs. Each row represents one lncRNA or mRNA, and each column represents one cell line sample. 'Red' indicates high relative expression, and 'green' indicates low relative expression. There were 1,154 lncRNAs and 1,733 mRNAs that exhibited a >3-fold differential expression in A549/PTX cells compared with A549 cells. Among them, more were downregulated.

The OD A260/A280 ratio of the total RNA was between 1.8 and 2.1. RNA integrity was assessed by electrophoresis on a denaturing agarose gel. The 28s and 18s ribosomal RNA bands were fairly sharp and intense, and the 28s rRNA band was about twice as intense as the 18s rRNA band. The lncRNA and mRNA samples from the A549 and A549/PTX cells were found to be suitable for microarray expression profiling.

Differentially expressed lncRNAs and mRNAs. The Affymetrix GeneChip Human Transcriptome Array 2.0 was used for profiling both parental A549 and A549/PTX cells. For microarray analysis, ~25,000 lncRNAs and 24,500 mRNAs were detected. After quantile normalization and data filtering, we found a >3-fold difference in the expression of 1,154 lncRNAs and 1,733 mRNAs in the A549/PTX cells compared with the A549 cells. Of the 1,154 differentially expressed lncRNAs, 119 were upregulated and 1,035 were downregulated (P<0.01); 28 of the mRNAs were upregulated and 1,705 were downregulated (P<0.01). The hierarchical cluster analysis of the expression of the lncRNAs and mRNAs that were identified is shown in Fig. 2. The differentially expressed lncRNAs and mRNAs are listed in Table I, and the 10 lncRNAs and mRNAs with the greatest relative upregulation or downregulation are shown in Tables II and III. lncRNA n334075 (fold change=8.77) was the most significantly upregulated and lncRNA n335556 (fold change=-308.61) was the most significantly downregulated. Norrin cystine knot growth factor (NDP) mRNA (fold change=6.75) was the most

Table I. Number of differentially expressed lncRNAs and mRNAs.

lncRNAs and mRNAs	Fold change >3	Total
lncRNAs		1,154
Upregulation	119	
Downregulation	1,035	
mRNAs		1,733
Upregulation	28	
Downregulation	1,705	

lncRNAs, long non-coding RNAs.

Table II. Ten most significantly upregulated and downregulated lncRNAs.

Upregulated in A549/PTX		Downregulated in A549/PTX	
lncRNAs	Fold change	lncRNAs	Fold change
n334075	8.77	n335556	-308.61
n333225	8.76	n333732	-191.67
n335243	8.04	n385730	-154.98
ENST00000455973	7.86	n342204	-120.84
ENST00000416226	7.77	ENST00000544920	-110.94
NR_045483	7.66	NR_002206	-95.60
ENST00000486726	7.62	ENST00000363046	-86.91
ENST00000503218	6.98	NR_002569	-82.88
n335614	5.86	NR_002555	-79.82
ENST00000439079	5.21	NR_029396	-76.72

A549/PTX vs. parental A549 cells. lncRNAs, long non-coding RNAs; A549/PTX, paclitaxel-resistant A549 cells.

significantly upregulated and histone cluster 2 H2A family member a4 (HIST2H2AA4) (fold change=-99.71) was the most significantly downregulated. Downregulated lncRNAs and mRNAs were more common than upregulated lncRNAs and mRNAs in our microarray data.

RT-qPCR validation of microarray data. To validate the microarray data, nine differentially expressed lncRNAs and six mRNAs were randomly selected for RT-qPCR assay of RNA isolated from both resistant A549/PTX and sensitive A549 cells. The primers used for PCR validation are listed in Table IV. As shown in Fig. 3, the expression of lncRNA ENST00000455973, ENST00000416226, ENST00000486726 and ENST00000503218 was upregulated and the expression of ENST00000544920, NR-002206, ENST00000363046, NR-002555 and ENST00000500843 was downregulated. The expression of DDR2 and TNF mRNA was upregulated and the expression of ABCC2, MRPS30, NEDD4, and CASP2 was downregulated. The RT-qPCR results were thus, consistent with the microarray data.

Table III. Ten most significantly upregulated and downregulated mRNAs.

Upregulated in A549/PTX		Downregulated in A549/PTX	
mRNAs	Fold change	mRNAs	Fold change
NDP	6.75	HIST2H2AA4	-99.71
TNF	6.12	ALDH1A1	-92.66
DDR2	5.76	FTL	-77.03
WNT6	5.69	PTPLAD1	-76.91
NCAM1	4.58	CYP24A1	-73.79
LOXL1	4.47	AKR1C2	-58.71
KRTAP5-6	3.42	NQO1	-55.83
IGLV7-43	3.39	NETO2	-46.82
IGHV3-48	3.38	RSL1D1	-46.32
SPPL2C	3.34	HIST1H1E	-41.36

A549/PTX vs. parental A549 cells. A549/PTX, paclitaxel-resistant A549 cells.

Chromosomal distribution of differentially expressed lncRNAs and mRNAs. Since chromosomal imbalances have been associated with drug resistance, a distribution plot of chromosomal location was developed to show the chromosomal locations of the differentially expressed lncRNAs and mRNAs. As shown in Fig. 4, the 1,154 lncRNAs and 1,733 mRNAs were distributed throughout the genome, and were associated with every chromosome. In the present study, aberrantly expressed lncRNAs and mRNAs were most frequently found on chromosomes 1, 2, 6, 12 and 17, however, particularly on chromosome 1.

GO and KEGG pathway analyses. GO analysis was applied to identify the functions of the differentially expressed mRNAs. The GO database includes the primary functional classifications of the National Center for Biotechnology Information (NCBI), and includes three categories, i.e., cellular components, molecular functions and biological processes. The microarray data obtained in the present study revealed that the differentially expressed genes were enriched for GO terms related to the cytosol, cytoplasm, nucleus and nucleolus (cellular components); protein, ATP and RNA bindings, and structural constituents of ribosomes (molecular functions); and gene expression, mitotic cell cycle, small-molecule metabolic process, and translation (biological processes). The top 10 GO terms within each of the three categories, cellular components, molecular functions and biological processes, are shown in Fig. 5A-C.

We conducted pathway analysis of the differentially expressed mRNAs using the latest KEGG database, which identified the biological pathways involved in paclitaxel resistance. We found 96 pathways corresponding to aberrant mRNAs, and the predominant pathways are shown in Fig. 5D. The top 10 pathways were metabolic, ribosomal, RNA transport, cell cycle, endocytosis, pathways in cancer, oxidative phosphorylation, purine metabolism, mismatch repair and DNA replication.

Table IV. Primers used for RT-qPCR validation.

lncRNAs and mRNAs	Forward primer sequence	Reverse primer sequence
ENST00000363046	GGACTCTGTTCCTCCCCTTTC	GAGCCCCGTGTGGTTGG
ENST00000500843	CCTGGCTGAGGTGAATAA	TTGGACCCGAACATCTG
ENST00000544920	AAAGATGAGGCAGAGGTCCAAG	CGATCAGAGGGCGATGAAG
NR-002555 (LOC613037)	GGGCAGAGGACTACCACAAATG	TGTTGTTGAGTTGGAGGAGGTG
NR-002206 (GTF2IP1)	GCTGTGTGGTGGTTGATGG	CTCTTTTATTTCTTCTGTGGCTGGA
ENST00000455973	GATGTGGGAAACAGTGGC	GTAAGGCAGCAGGGAGG
ENST00000416226	AGGAGAAACTCATCAGGC	ATCTCTTCTACGGTGGCT
ENST00000486726	CCTGTCTGGTGTCCTTGC	CAGCAGGAGAGGCATCAG
ENST00000503218	GCAAGTGAAGCCTGATACC	AAAGCGTCTGTGAGCCTAA
TNF	GTGACAAGCCTGTAGCCCATGTT	TTATCTCTCAGCTCCACGCCATT
DDR2	CCCAGCTGTCAGATGAACAGGTTA	TCAGGACAAATGGCTGGTTGAG
CASP2	TGGCATGCATCCTCATCATC	TCTGGCTGAAACTGCCCACT
ABCC2	AGTGATCACCATCGCCCACA	GTTCACATTCTCAATGCCAGCTTC
MRPS30	CGAACCCGAACCTGAACCT	GATATGACCTCGCTCTCCTCGT
NEDD4	TGAAGCCCAATGGGTCAGAAATA	GGACCCTGTTCACAAATCTCCAC
GAP	TGCACCACCAACTGCTTAGC	GGCATGGACTGTGGTCATGAG

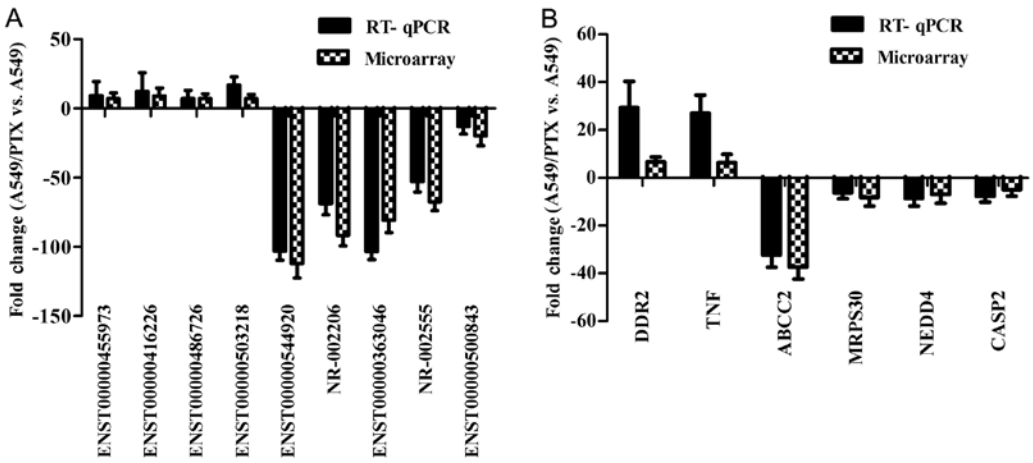

Figure 3. Validation of microarray data by RT-qPCR. (A) Nine long non-coding RNAs (lncRNAs) and (B) six mRNAs differentially expressed in A549 cells compared with paclitaxel-resistant A549 (A549/PTX) cells by microarray were randomly selected and validated by RT-qPCR. The heights of the columns in the chart represent the mean fold change of the expression. The fold change was positive when the expression was upregulated (A549/PTX vs. A549 cells) and negative when the expression was downregulated. Data are presented as the mean ± standard deviation.

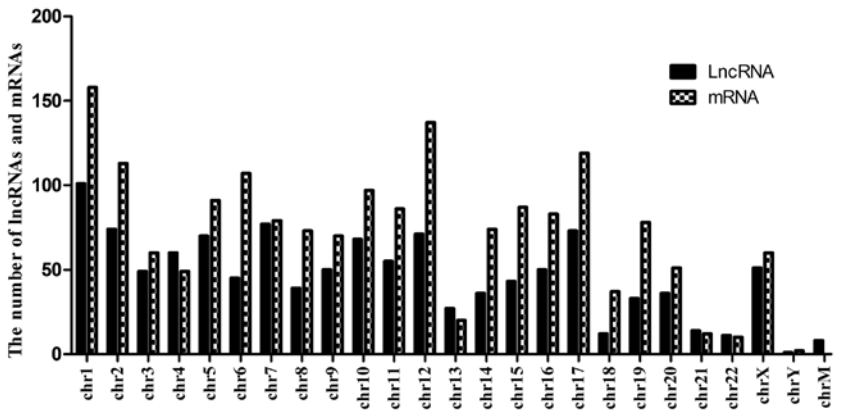

Figure 4. Chromosomal distribution of the differentially expressed long non-coding RNAs (lncRNAs) and mRNAs. The y-axis indicates the number of upregulated and downregulated lncRNAs and mRNAs. Aberrantly expressed lncRNAs and mRNAs were found to be mainly located on chromosomes 1, 2, 6, 12 and 17. 'chr', chromosome.

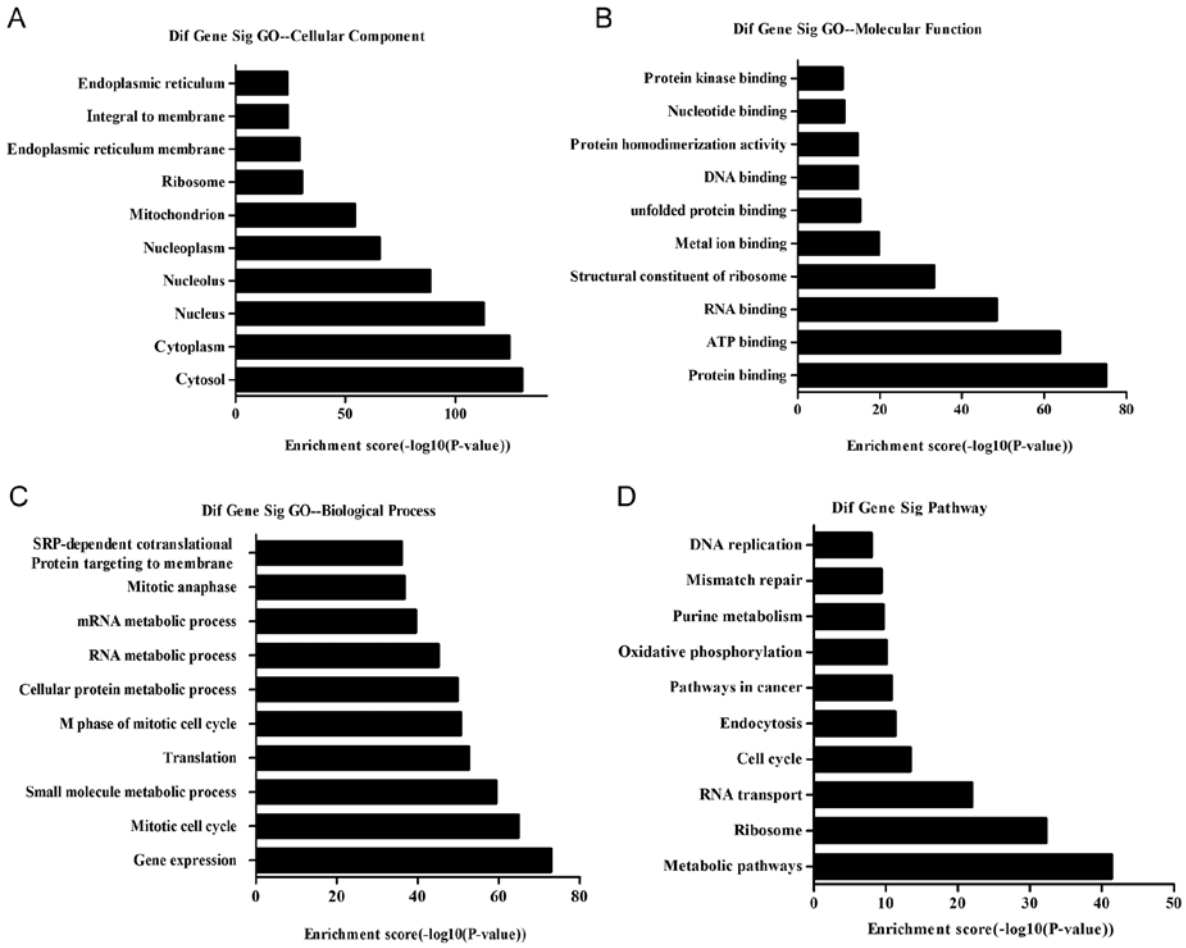

Figure 5. Analysis of significant GO and Kyoto Encyclopedia of Gene and Genome (KEGG) pathways. (A-C) GO and (D) KEGG pathways analyses. The histograms show the top 10 significant GO and KEGG pathways of the differentially expressed genes. The P-value (Fisher P-value) denotes the significance of the GO and the KEGG pathway enrichment. The lower the P-value, the more significant was the difference in the GO and KEGG pathways (the recommend P-value cut-off is 0.05).

Discussion

Paclitaxel is the standard and most effective chemotherapy drug used for lung adenocarcinoma, but drug resistance is a major clinical obstacle that limits its clinical benefits (18). Although some molecular mechanisms of paclitaxel resistance in various types of cancers have been reported in the past few decades (19), the precise cause of paclitaxel resistance in lung adenocarcinoma remains unclear. Research is urgently warranted to fully understand and overcome it. Evidence that lncRNAs are involved in chemotherapy resistance is increasing (20-22), but to the best of our knowledge there is little data on the correlations between lncRNAs and paclitaxel resistance in human lung adenocarcinoma.

To investigate the regulatory effects of lncRNAs in paclitaxel resistance of lung adenocarcinoma, we established a paclitaxel-resistant A549/PTX cell line and assayed its PTX resistance. Microarray expression profiling of lncRNAs and mRNAs in parental A549 and paclitaxel resistant A549/PTX cells identified a total of 1,154 differentially expressed lncRNAs. We also found aberrant expression of 1,733 mRNAs in A549/PTX cells compared with the parental A549 cells. Various of the differentially expressed lncRNAs and mRNAs including, MALAT1, TUG1, HOTAIR, ABCB1 (MDR1) and Wnt6 have been previously reported in other chemoresistant cancers (23-27). Whether the molecular mechanisms of chemoresistance are the same as in the A549/PTX cells warrants further investigation. However, in our screening results, the 10 predominantly upregulated and downregulated lncRNAs have not been previously related to treatment resistance. Consequently, their mechanism of resistance regulation and their function in resistant cells are not clear. lncRNAs regulate neighboring protein-coding genes (28), and we found that some of the aberrantly expressed lncRNAs may play important roles in paclitaxel resistance by regulating nearby coding genes. For example, upregulated lncRNA ENST00000447028 was found to be located near VEGF-B mRNA. VEGF-B mRNA was reported by Yang *et al* to take part in the resistance of lung cancer cells to the chemotherapeutic drug EGFR-TKI (29). We found that VEGF-B mRNA was strongly upregulated, therefore, ENST00000447028 may influence paclitaxel resistance by regulating VEGF-B mRNA. Another upregulated lncRNA, ENST00000416226, was found to be located near Wnt6 mRNA, which was upregulated in our results, and is involved in both oncogenesis and chemoresistance in human cancers (30). We thus, speculate that lncRNAs may influence paclitaxel resistance in human lung adenocarcinoma by regulating the expression of their nearby

coding genes. Further studies of the effect of overexpression or knockdown of lncRNAs and western blot analyses should be performed to investigate the precise relationships.

We found that the aberrantly expressed lncRNAs and mRNAs were distributed throughout the genome and could be found in every chromosome, particularly on chromosome 1. Perhaps, all the chromosomes participate in paclitaxel resistance. The importance of chromosome 1 in the occurrence of paclitaxel resistance in lung adenocarcinoma warrants investigation.

The biological functions and signaling pathways associated with the lncRNAs and mRNAs identified in the present study, were evaluated by GO and KEGG pathway analyses. The most enriched GOs were cytosol (cellular components), protein binding (molecular function) and gene expression (biological processes). In addition, many of the identified GO terms in our results have been reported in other cancer chemoresistance studies. For example, protein and nucleotide binding, and metabolic processes have been reported to be involved in the chemoresistance of lung and colorectal cancer (31). This suggests that lncRNAs among those we identified may have regulated chemoresistance of the A549/PTX cells by influencing the expression of these GO database genes. Pathway analysis revealed a total of 96 pathways corresponding to all the differentially expressed mRNAs. Some of the top 10 pathways that we identified have previously been associated with chemoresistance. For example, Wu *et al* using genome-wide microarrays found that the metabolic pathway participated in EGFR-TKI resistance of lung adenocarcinoma (32). Zhu *et al* reported that the cell cycle pathway was associated with the development of doxorubicin resistance in the MG63/DXR human osteosarcoma cell line (33). Zhou *et al* found that the DNA replication pathway contributed to the occurrence of gemcitabine resistance in SW1990 pancreatic cancer cells (34). These data revealed that the differentially expressed lncRNAs may regulate paclitaxel resistance in lung adenocarcinoma through these classical pathways.

In conclusion, the present study reveals, for the first time, numerous differentially expressed lncRNAs and mRNAs in A549 and A549/PTX cells which may play an important role in regulating paclitaxel resistance through various biological functions and signaling pathways. We described a novel approach to clarify the molecular mechanisms of paclitaxel resistance in human lung adenocarcinoma. The results provide a novel rationale for studies on reversal of paclitaxel resistance and for identifying patients who can benefit the most from chemotherapy. Evaluation of clinical samples for *in vivo* validation is warranted.

Acknowledgements

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