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ORIGINAL PAPER

LncRNA expression profiles of EGFR exon 19 deletions in lung adenocarcinoma ascertained by using microarray analysis

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Abstract Studies showed that long chain non-coding RNAs (lncRNAs) involved in the development and progression of lung cancer. However, the mechanisms of EGFR exon 19 deletion in lung adenocarcinoma were unclear. Lung adenocarcinoma was divided into EGFR exon 19 deletion group and EGFR wild-type group. We studied the differential expression profiles of lncRNAs in EGFR exon 19 deletion in lung adenocarcinoma by high-throughput microarray. Using abundant and varied probes, we were able to assess 30,586 lncRNAs and 26,109 mRNAs in our microarray. Compared with the wild-type EGFR, we found that 1,533 lncRNAs and 1,406 mRNAs were differentially expressed (≥twofold change) in EGFR exon 19 deletion in lung adenocarcinoma, indicating that many lncRNAs were significantly upregulated or downregulated in EGFR exon 19 deletion in lung adenocarcinoma. The 10 lncRNAs were aberrantly expressed in EGFR exon 19 deletion in lung adenocarcinoma compared with wild-type EGFR group validated by real-time RT-PCR. Among these, RP11-325I22.2 and LOC440905 were the most aberrantly expressed in 20 cases of EGFR exon 19 deletion in lung adenocarcinoma samples by real-time RT-PCR. Our study showed lncRNAs expression pattern in EGFR exon 19 deletion in lung adenocarcinoma by microarray. RP11-325I22.2 and LOC440905 might play an important role in the mechanism of EGFR exon 19 deletion in lung adenocarcinoma. The study may provide a new mechanism of EGFR exon 19 deletion in lung adenocarcinoma.

Keywords Non-small cell lung cancer · LncRNAs · Lung adenocarcinoma · Microarray · EGFR gene · Deletion

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Introduction

Lung cancer mortality is the highest in all cancers, and its incidence is gradually growing [1]. Lung adenocarcinoma is an important type of non-small cell lung cancer (NSCLC). The epidermal growth factor receptor (EGFR) is a member of EGF-family of extracellular protein ligands. Gefitinib and erlotinib are reversible EGFR tyrosine kinase inhibitors (EGFR-TKIs) combining with EGFR to inhibit EGFR and its downstream pathways. EGFR overexpression has been observed in 40–80 % of NSCLC patients [2]. It was shown that mutations of EGFR kinase domain were more highly sensitive to TKI than the wild-type EGFR in lung adenocarcinoma patients [3–5]. However, these lung adenocarcinoma patients eventually acquired resistance to EGFR-TKI and progressed after 5–9 months of EGFR-TKI therapy. The mechanisms of EGFR-TKI resistance include primary and acquired drug resistance [6]. Mechanisms of primary resistance to EGFR-TKI therapy include K-ras mutations [7, 8], Serine/threonine-protein kinase B-raf mutations [9], Phosphatase, and tensin homolog inactivation [10, 11]. The reasons of acquired resistance involved

Table 1 The demographics characteristics of 90 cases of lung adenocarcinoma

Parameters	Year/number
Age (years)	63.1 (39–81)
Sex (female/male)	50/40
Histological grade	
Well-differentiated carcinoma	16
Well moderately differentiated Carcinoma	12
Moderately differentiated carcinoma	30
Moderately poorly differentiated carcinoma	12
Poorly differentiated carcinoma	20
TNM clinical stage[a]	
Ia	20
Ib	38
IIa	10
IIb	4
IIIa	18
EGFR gene	
Exon 19 deletion	20
Wild type	70

[a] In the light of TNM clinical stage from American Joint Committee on Cancer (AJCC) and Union for International Cancer Control (UICC) in 2002

EGFR secondary mutations [12–14], MET gene amplification [15, 16], hepatocyte growth factor over-expression [17], insulin-like growth factor 1 receptor precursor over-expression [18], and so on. But the mechanisms of EGFR-TKI resistance are complex and have not been fully elucidated. It was important to understand the mechanisms of EGFR-TKI resistance for the treatment of lung cancer, wherein the TK domains of 19 and 21 of exon usually account for about 90 % of EGFR mutations and to these two sites of mutation observed in the clinical treatment with the EGFR-TKIs most closely related to the efficacy.

Long chain non-coding RNAs (long non-coding RNAs, lncRNAs) are non-coding RNAs longer than 200 nucleotides [19]. Many studies have shown that the lncRNAs were associated with some diseases, including cancer [20–22]. The disorders of lncRNAs are also a feature of many cancers and promote the development, invasion, and metastasis by a variety of mechanisms [20, 23]. Studies have shown lncRNAs involved in the development and progression of lung cancer [24–28]. Nowadays, there is no relevant literature of lncRNAs relative to the mechanisms of EGFR exon 19 deletion in lung adenocarcinoma.

In this study, we presented the lncRNAs expression pattern in lung adenocarcinoma with EGFR exon 19 deletion samples compared with the wild-type EGFR gene samples, several of which were evaluated by SYBR Green I real-time RT-PCR in a total of 90 lung adenocarcinoma tissues. Our results showed that lncRNAs expression pattern may provide a new mechanism and preliminary data for the study of EGFR exon 19 deletion in lung adenocarcinoma.

Materials and methods

Patient samples

The lung adenocarcinoma with 5 exon 19 deletion patients and 5 wild-type EGFR patients was prospectively collected from the First Affiliated Hospital of Wenzhou Medical University, China, from April 2012 to August 2013. Otherwise, 90 cases of lung adenocarcinoma tissues (including 20 EGFR exon 19 deletion samples and 70 wild-type EGFR gene samples) were collected for SYBR Green I real-time RT-PCR, see Table 1. EGFR mutation was analyzed by ARMS method, conducted by a D×S EGFR mutation Test Kit, according to the manufacturer's recommendations (Amoy Diagnostics Co., LTD, China). The diagnosis of lung adenocarcinoma was confirmed by histopathology result. The Institutional Ethics Review Board of the First Affiliated Hospital of Wenzhou Medical University approved this study, and all patients provided informed consent to this study.

RNA extraction

Lung adenocarcinoma cells were obtained by Laser capture microdissection; we combined the five lung adenocarcinoma with exon 19 deletion samples cells and five samples with the wild-type EGFR cells; and the two groups were subjected to RNA extraction. Total RNA was extracted using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. The integrity of the RNA was assessed by electrophoresis on a denaturing agarose gel. A NanoDrop ND-1000 spectrophotometer was used for the accurate measurement of RNA concentration (OD260), protein contamination (ratio OD260/OD280), and organic compound contamination (ratio OD260/OD230).

Microarray and computational analysis

The Agilent array platform was used for microarray experiments. The sample preparation and microarray hybridization were performed based on the manufacturer's standard protocols. Briefly, mRNA was purified from total RNA after removal of rRNA using an mRNA-ONLY^{TM} Eukaryotic mRNA Isolation Kit (Epicentre Biotechnologies.USA). Then, each sample was amplified and transcribed into fluorescent cRNA along the entire length of the transcripts without 3′ bias using a random priming method.

The labeled cRNAs were hybridized onto a Human lncRNA Array v3.0 (8 × 60 K, Arraystar), designed for 30,586 lncRNAs and 26,109 mRNAs. The lncRNAs were carefully constructed using the most highly respected public transcriptome databases (Refseq, UCSC Known Genes, GENCODE, etc.), as well as landmark publications (Nature, Cell, Science, etc.). Each transcript was accurately identified by a specific exon or splice junction probe. Positive probes for housekeeping genes and negative probes were also printed onto the array for hybridization quality control. After washing the slides, the arrays were scanned using the Agilent Scanner G2505C and the acquired array images were analyzed with Agilent feature extraction software (version 11.0.1.1). Quantile normalization and subsequent data processing were performed using the GeneSpring GX v12.0 software package (Agilent Technologies). The microarray work was performed by KangChen Bio-tech, Shanghai, and People's Republic of China.

Functional group analysis

GO categories were derived from Gene Ontology (www.geneontology.org), which provides three structured networks of defined terms that describe gene product attributes. The *P* value denotes the significance of GO term enrichment in the differentially expressed mRNA list ($P \leq 0.05$ was considered statistically significant). We also performed pathway analysis for the differentially expressed mRNAs based on the latest Kyoto Encyclopedia of Genes and Genomes (KEGG) database. This analysis allowed us to determine the biological pathway for which a significant enrichment of differentially expressed mRNAs existed ($P \leq 0.05$ was considered statistically significant).

SYBR green I real-time RT-PCR

Total RNA was extracted from frozen lung adenocarcinoma tissues with TRIzol reagent (Invitrogen Life Technologies, USA) and then reverse transcribe using Thermo Scientific RT reagent Kit (Thermo Scientific)according to the manufacturer's instructions. lncRNAs expression in lung adenocarcinoma tissues was measured by SYBR Green I real-time RT-PCR using SYBR Premixes Ex Taq on ABI 7000 instrument. Two lncRNAs that significantly expressed (RP11-325I22.2 and LOC440905) were evaluated in all of the patients included in this study. 2 mg of total RNA was transcribed to cDNA. PCR was performed in a total reaction volume of 20 ul, including 10ul SYBR Premix (2×), 2 ul of cDNA template, 1 ul of PCR Forward Primer (10 mM), 1 ul of PCR Reverse Primer (10 mM), and 6 ul of double-distilled water. The SYBR Green I real-time RT-PCR was set at an initial denaturation step of 10 min at 95 °C, and 95 °C (5 s), 60 °C (30 s) in a total of 40 cycles with a final extension step at 72 °C for 5 min. All experiments were done in triplicate, and all samples normalized to GAPDH. The median in each triplicate was used to calculate relative lncRNAs concentrations (ΔCt = Ct median lncRNAs − Ct median GAPDH). Expression fold changes were calculated.

Statistical methods

A comparison between the two groups was performed by Mann–Whitney *U* test. $P < 0.05$ was considered to be statistically significant. The fold change and the Student's *t* test were analyzed for statistical significance of the microarray results. The false discovery rate (FDR) was calculated to correct the *P* value. The threshold value we used to designate differentially expressed lncRNAs and mRNAs was a fold change of ≥2.0 or ≤0.5.

Results

Overview of lncRNAs profiles

To study the potential biological functions of lncRNAs in lung adenocarcinoma with EGFR exon 19 deletion samples, we examined the lncRNA and mRNA expression profiles in human lung adenocarcinoma through microarray analysis (Fig. 1a, b). Among these, 539 lncRNAs were found to be upregulated more than twofold in the lung adenocarcinoma with EGFR exon 19 deletion group compared with wild-type EGFR group, while 994 lncRNAs were downregulated more than twofold ($P < 0.05$; Table 2; Fig. 1c, d).

LncRNA classification and subgroup analysis

The expression profiles of 426 intergenic lncRNAs indicated that they were differentially expressed (fold change ≥ 2.0, $P < 0.05$) between EGFR exon 19 deletion group and wild-type EGFR group. Among these, 166 were upregulated and 260 were downregulated. We also identified some nearby coding genes (distance < 300 kb) that may be regulated by these lncRNAs (Table 3). LncRNAs with enhancer-like function (lncRNA-a) were identified using GENCODE annotation. The expression profiles of 46 enhancer-like lncRNAs indicated that they were differentially expressed (fold change ≥ 2.0, $P < 0.05$) between EGFR exon 19 deletion group and wild-type EGFR group. Among these, 15 were upregulated and 31 were downregulated. We also identified some nearby coding genes that may be regulated by these enhancer-like lncRNAs (Table 4). HoxlncRNAs (lncRNA transcribed from Hox loci LncRNAs) profiles: This data table contains 83 HoxlncRNA clusters (Table 5).

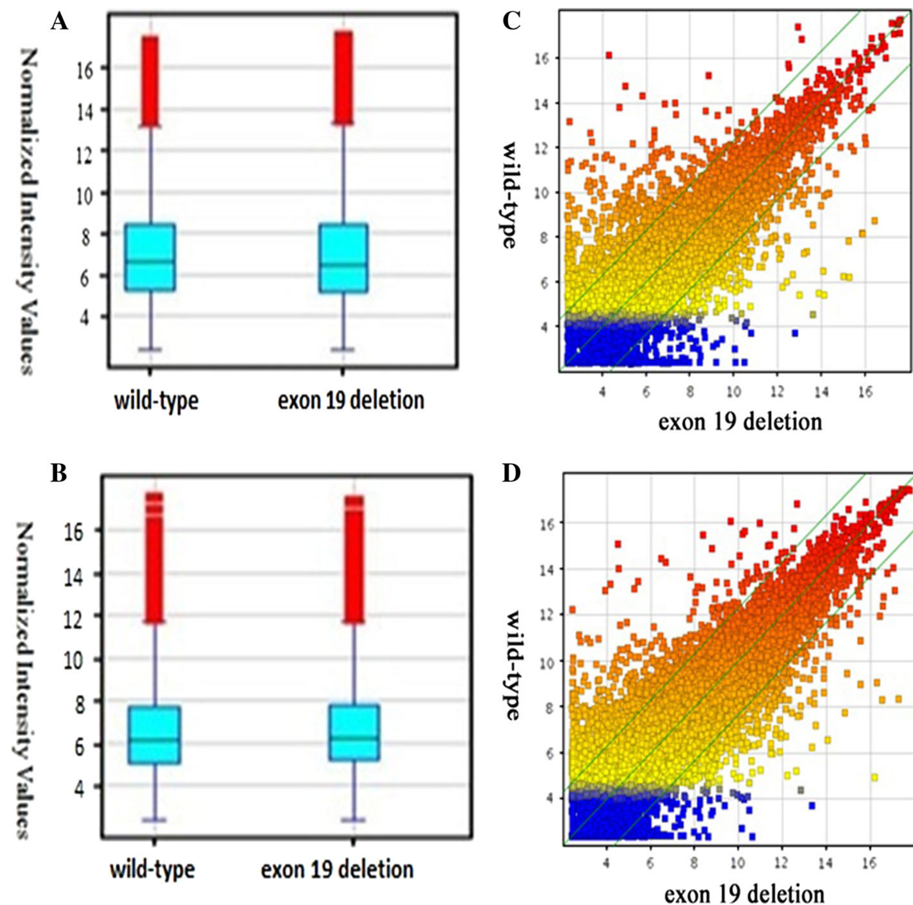

Fig. 1 lncRNA and mRNA differentially expressed profile between EGFR exon 19 deletion and wild-type EGFR gene in the lung adenocarcinoma samples. The box plot is a convenient way to visualize the distribution of a dataset in the lncRNA (**a**) and mRNA (**b**) profiles. After normalization, the distributions of log 2-ratios among the tested samples are nearly the same. The *scatter-plot* is used for assessing the lncRNA (**c**) and mRNA (**d**) expression variation between the lung adenocarcinoma and normal lung compared arrays. The values of *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* indicate more than threefold change of lncRNAs between pairs

Table 2 Some upregulated and downregulated lncRNAs in lung adenocarcinoma with EGFR exon 19 deletion

Probe name	FC (abs) ([D] vs. [C])	Regulation	Gene symbol
ASHGA5P028128	4.506442	Up	RP11-956E11.1
ASHGA5P019657	4.407372	Up	BDNF-AS1
ASHGA5P019657	4.407372	Up	BDNF-AS1
ASHGA5P019657	4.407372	Up	BDNF-AS1
ASHGA5P019657	4.407372	Up	BDNF-AS1
ASHGA5P019657	4.407372	Up	BDNF-AS1
ASHGA5P019657	4.407372	Up	BDNF-AS1
ASHGA5P013553	−2.660376	Down	GBP1P1
ASHGA5P042496	−7.4964657	Down	KCNQ5-IT1
ASHGA5P042496	−7.4964657	Down	KCNQ5-IT1
ASHGA5P042496	−7.4964657	Down	KCNQ5-IT1
ASHGA5P042496	−7.4964657	Down	KCNQ5-IT1
ASHGA5P042496	−7.4964657	Down	KCNQ5-IT1
ASHGA5P020436	−3.6163046	Down	RP11-213H15.3

D EGFR exon 19 deletion group, *C* wild-type EGFR gene group

Overview of mRNAs profiles

In total, 1,406 mRNAs were found to be differentially expressed between EGFR exon 19 deletion group and wild-type EGFR group, including 483 upregulated mRNAs and 923 downregulated mRNAs (Table 6; Figs. 1, 2).

Go analysis

The genes corresponding to the downregulated mRNAs included 160 genes involved in biological processes, 59 genes involved in cellular components, and 51 genes involved in molecular functions. The genes corresponding to the upregulated mRNAs included 296 genes involved in biological processes, 21 genes involved in cellular components, and 69 genes involved in molecular functions. These results imply that biological processes relevant to mRNA might play a more important role in the mechanism of EGFR exon 19 deletion in lung adenocarcinoma than these of cellular components and molecular functions.

Table 3 Some upregulated and downregulated lincRNAs in lung adenocarcinoma with EGFR exon 19 deletion and nearby coding genes regulated by lincRNAs

Transcript ID	Gene symbol	Log fold change -lncRNAs	Regulation-lncRNAs	Nearby gene
ENST00000418403	RP11-462G2.1	−8.530131	Down	NM_001105531
ENST00000421463	AC016995.3	2.482141	Up	NM_022374
ENST00000421619	RP11-114B7.6	−2.7748656	Down	ENST00000373484
ENST00000422178	AC016745.3	2.4745655	Up	NM_012184
ENST00000422825	ZNF815P	−4.2304697	Down	NM_014413
ENST00000423628	ERRFI1-IT1	2.749525	Up	NM_021995
ENST00000423719	LINC00484	−3.384536	Down	NM_003177
ENST00000425151	AC140481.7	−7.16901	Down	NM_032545
ENST00000426393	RP4-792G4.2	4.0767307	Up	ENST00000371116
ENST00000426393	RP4-792G4.2	4.0767307	Up	NM_001172819

Table 4 Some enhancer-like lncRNAs in lung adenocarcinoma with EGFR exon 19 deletion and nearby coding genes regulated by lincRNAs

Transcript ID	Gene symbol	Log fold change -lncRNAs	Regulation-lncRNAs	Nearby gene
ENST00000294715	RP11-14N7.2	4.535548	Up	NM_001102663
ENST00000411595	RP4-737E23.2	3.1275525	Up	ENST00000347397
ENST00000412132	RP3-413H6.2	−2.9948843	Down	NM_002114
ENST00000413975	RP11-475O6.1	2.4344473	Up	NM_024686
ENST00000416894	LINC00568	−2.6944923	Down	NM_000396
ENST00000416894	LINC00568	−2.6944923	Down	NM_018178
ENST00000416894	LINC00568	−2.6944923	Down	ENST00000314136
ENST00000416894	LINC00568	−2.6944923	Down	NM_001136479
ENST00000416894	LINC00568	−2.6944923	Down	NM_004425
ENST00000416894	LINC00568	−2.6944923	Down	NM_004698

lncRNAs with enhancer-like function (LncRNA-a) are identified using GENCODE annotations (Harrow, Denoeud et al. 2006) of human genes

Table 5 Some Hox clusters in lung adenocarcinoma with EGFR exon 19 deletion

Probe name	[D](normalized)	[C](normalized)	Transcript ID	ccdsID	Gene symbol
ASHGA5P021981	12.762258	12.976499	NM_014212	CCDS8867.1	HOXC11
ASHGA5P033182	6.4961843	5.8385425	ENST00000460041		HOXB-AS3
ASHGA5P053003	7.6894193	7.881022	NM_006735	CCDS5403.1	HOXA2
ASHGA5P019568	4.9816904	5.367425	ENST00000492897		HOXB-AS3
ASHGA5P049730	4.522396	4.606554	ENST00000474040		HOXB-AS3
ASHGA5P005899	7.3475404	7.703401	NM_024017	CCDS11534.1	HOXB9
ASHGA5P032505	10.274893	8.870909	NM_024015	CCDS11529.1	HOXB4
ASHGA5P053007	6.5090766	5.9945984	NM_152739	CCDS5409.1	HOXA9
ASHGA5P053006	8.323707	7.6041813	NM_006896	CCDS5408.1	HOXA7
ASHGA5P036265	6.098909	5.9653845	NM_014213	CCDS2267.2	HOXC11

D EGFR exon 19 deletion group, *C* wild-type EGFR gene group

Pathway analysis

The 27 upregulated pathways were identified, including metabolism of xenobiotics by cytochrome p450, drug metabolism-cytochrome p450, chemical carcinogenesis, and so on. 27 downregulated pathways were identified, including alcoholism, systemic lupus erythematosus, viral carcinogenesis, transcriptional misregulation in cancer, and so on (Fig. 3; Tables 7, 8).

Table 6 Some upregulated and downregulated mRNAs in lung adenocarcinoma with EGFR exon 19 deletion

Probe Name	FC (abs) ([C] vs. [D])	Regulation	Gene symbol
ASHGA5P001120	3.0600486	Up	ECH1
ASHGA5P038260	6.2264023	Up	FOXD3
ASHGA5P041370	2.3954492	Up	SOWAHA
ASHGA5P002066	2.7287827	Up	GADD45G
ASHGA5P006552	2.47164	Up	PRTFDC1
ASHGA5P014873	2.389154	Up	CCND3
ASHGA5P048249	2.6033182	Up	GPR152
ASHGA5P011329	−3.6167855	Down	FAM122B
ASHGA5P052221	−3.602097	Down	OSMR
ASHGA5P012440	−3.866324	Down	TAF2
ASHGA5P017814	−4.538289	Down	ZDHHC13
ASHGA5P020058	−2.6459877	Down	SMG7
ASHGA5P004831	−2.3655446	Down	NLGN4Y
ASHGA5P054443	−2.340054	Down	PTPN20A

D EGFR exon 19 deletion group, *C* wild-type EGFR gene group

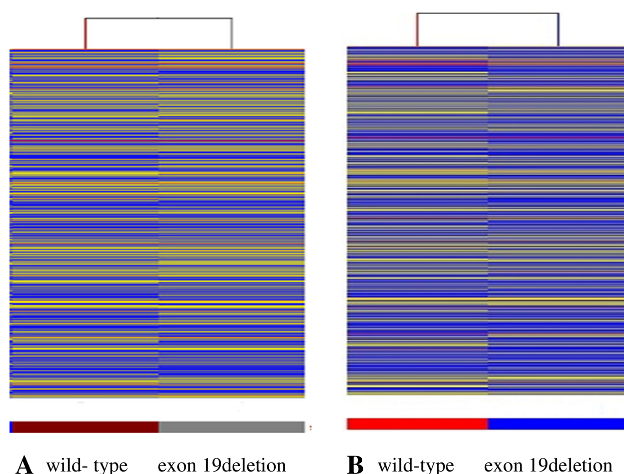

Fig. 2 Heat map and hierarchical clustering of lncRNAs and mRNA differential expression profile between EGFR exon 19 deletion and wild-type EGFR gene in the lung adenocarcinoma samples. **a** lncRNAs, **b** mRNA. Heat map and Hierarchical Clustering is one of the most widely used clustering methods for analyzing lncRNAs or mRNA expression data. Cluster analysis arranges samples into groups based on their expression levels, which allows us to hypothesize the relationships among samples. The dendrogram shows the relationships among LncRNAs or mRNA expression patterns of samples. "*Red*" indicates high relative expression, and "*blue*" indicates low relative expression

SYBR green I real-time RT-PCR validation

According to fold difference, gene locus, and so on, we initially identified a number of candidated lncRNAs (including LOC440905, AC018865.8, RP11-325I22.2, AFAP1-AS1, CYP4F43P, RP11-133F8.2, AC073135.3, XLOC_003318, BLNK, XLOC_005621) and verified the expression of lncRNAs by SYBR Green I real-time RT-PCR with GAPDH as reference gene, by calculating the $2^{-\Delta\Delta CT}$. We found that multiple lncRNAs of gene microarray are consistent with results of SYBR Green I real-time RT-PCR, see Fig. 4. The RP11-133F8.2 and LOC440905 were the most significantly changed lncRNAs of these candidated lncRNAs from 20 cases EGFR exon 19 deletions in lung adenocarcinoma patients and 70 cases wild-type EGFR gene patients. According to Fig. 5, RP11-133F8.2 expression of EGFR exon 19 deletions in lung adenocarcinoma was significantly higher than wild-type EGFR tissues (Mann–Whitney $U = 107.00$, $P = 0.01$), while LOC440905 expression of EGFR exon 19 deletions in lung adenocarcinoma was significantly lower than wild-type EGFR tissues (Mann–Whitney $U = 189.21$, $P = 0.001$).

Discussion

The most frequent mutations of EGFR gene, as delE746-A750, delL747-p753inss, delL747-T75linss, delL747-S752ins, T790 M mu, L858R mu, occurred in NSCLC patients. These mutations are detected mainly in adenocarcinoma tissue [29–32], wherein the TK domains of 19 and 21 of exon usually accounts for about 90 % of EGFR mutations and were most closely related to the efficacy.

Nowadays, there was no literature about the relation of lncRNAs to EGFR gene mutation in lung adenocarcinoma. In this study, we obtained 1,533 differentially expressed lncRNAs in EGFR exon 19 deletions in lung adenocarcinoma, and our data showed that these lncRNAs may participate in EGFR exon 19 deletions in lung adenocarcinoma.

We fatherly studied the classification and subgroup of lncRNAs and acquired the expression profiles of linRNAs, enhancer-like lncRNAs, and Hox lncRNA cluster. Systematic comparison of the expression pattern of every lncRNA with its immediate 5′ and 3′ Hox gene neighbor showed that the vast majority of lncRNAs (90 %) are coordinately induced with their 3′ Hox genes, while only 10 % of instances are lncRNA expression anticorrelated with 3′ Hox gene expression [33]. LncRNAs in the Hox loci became systematically dysregulated during some cancer progression [20]. LncRNAs are known to function via a variety of mechanisms; however, a common and important function of lncRNAs is to alter the expression of nearby coding genes by affecting the process of transcription [34–36] or directly playing an enhancer-like role [37, 38]. We also identified some nearby coding genes(<300 kb) that may be regulated by lincRNAs and

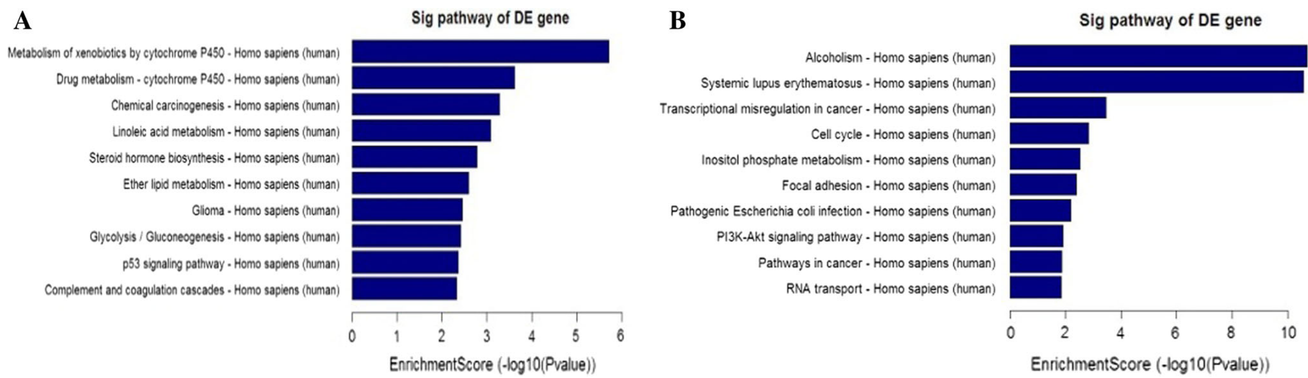

Fig. 3 Pathway analysis of mRNA differential expression profile between EGFR exon 19 deletion and wild-type EGFR gene in the lung adenocarcinoma samples. **a** unregulated pathway, **b** downregulated pathway

enhancer-like lncRNAs. We studied also the relationship of lincRNA and nearby coding genes.

We found that 10 candidated lncRNAs of microarray are consistent with results of SYBR Green I real-time RT-PCR; it indicated that lncRNAs expressed unusually during EGFR exon 19 deletions in lung adenocarcinoma. The RP11-325I22.2 was most significantly upregulated, and LOC440905 were most downregulated of these candidated lncRNAs, and these of EGFR exon 19 deletions in lung adenocarcinoma was significantly higher or lower than

Table 7 The pathway analysis of upregulated mRNA in lung adenocarcinoma with EGFR exon 19 deletion

Pathway ID	Definition	Fisher-*P* value	FDR	Enrichment_score
hsa05034	Alcoholism—homo sapiens (human)	1.92885E−11	3.41818E−09	10.7147
hsa05322	Systemic lupus erythematosus—Homo sapiens (human)	2.59937E−11	3.41818E−09	10.58513
hsa05202	Transcriptional misregulation in cancer—Homo sapiens (human)	0.000352254	0.03088096	3.453144
hsa04110	Cell cycle—Homo sapiens (human)	0.001505832	0.09900846	2.822223
hsa00562	Inositol phosphate metabolism—Homo sapiens (human)	0.003062809	0.1611038	2.51388
hsa04510	Focal adhesion—Homo sapiens (human)	0.003978883	0.1744077	2.400239
hsa05130	Pathogenic *Escherichia coli* infection—Homo sapiens (human)	0.006507748	0.2445054	2.186569
hsa04151	PI3 K-Akt signaling pathway—Homo sapiens (human)	0.01244746	0.3804784	1.904919
hsa05200	Pathways in cancer—Homo sapiens (human)	0.01344603	0.3804784	1.871406
hsa03013	RNA transport—Homo sapiens (human)	0.01446686	0.3804784	1.839626
hsa04115	p53 signaling pathway—Homo sapiens (human)	0.0199179	0.3805891	1.700756
hsa05203	Viral carcinogenesis—Homo sapiens (human)	0.02118207	0.3805891	1.674032
hsa05410	Hypertrophic cardiomyopathy (HCM)—Homo sapiens (human)	0.02173687	0.3805891	1.662803
hsa05100	Bacterial invasion of epithelial cells—Homo sapiens (human)	0.02300768	0.3805891	1.638127
hsa04512	ECM-receptor interaction—Homo sapiens (human)	0.02315371	0.3805891	1.635379
hsa05222	Small cell lung cancer—Homo sapiens (human)	0.02315371	0.3805891	1.635379
hsa04012	ErbB signaling pathway—Homo sapiens (human)	0.02618024	0.3822144	1.582026
hsa05220	Chronic myeloid leukemia—Homo sapiens (human)	0.02823947	0.3822144	1.549143
hsa05216	Thyroid cancer—Homo sapiens (human)	0.02858884	0.3822144	1.543804
hsa04650	Natural killer cell-mediated cytotoxicity—Homo sapiens (human)	0.03090261	0.3822144	1.510005
hsa03015	mRNA surveillance pathway—Homo sapiens (human)	0.03122099	0.3822144	1.505553
hsa05332	Graft versus host disease—Homo sapiens (human)	0.03197231	0.3822144	1.495226
hsa04514	Cell adhesion molecules (CAMs)—Homo sapiens (human)	0.03373929	0.3858014	1.471864
hsa04360	Axon guidance—Homo sapiens (human)	0.04034095	0.4420696	1.394254
hsa00670	One carbon pool by folate—Homo sapiens (human)	0.04553065	0.464967	1.341696
hsa04350	TGF-beta signaling pathway—Homo sapiens (human)	0.04597057	0.464967	1.33752
hsa00310	Lysine degradation—Homo sapiens (human)	0.04773426	0.464967	1.32117

Table 8 The pathway analysis of downregulated mRNA with EGFR exon 19 deletion in lung adenocarcinoma

Pathway ID	Definition	Fisher-*P* value	FDR	Enrichment_score
hsa00980	Metabolism of xenobiotics by cytochrome P450—Homo sapiens (human)	1.92009E−06	0.000504984	5.716679
hsa00982	Drug metabolism—cytochrome P450—Homo sapiens (human)	0.000239087	0.03143996	3.621444
hsa05204	Chemical carcinogenesis—Homo sapiens (human)	0.000532954	0.04672229	3.27331
hsa00591	Linoleic acid metabolism—Homo sapiens (human)	0.000833026	0.05477145	3.079341
hsa00140	Steroid hormone biosynthesis—Homo sapiens (human)	0.001648052	0.08668753	2.783029
hsa00565	Ether lipid metabolism—Homo sapiens (human)	0.002530971	0.1109409	2.596713
hsa05214	Glioma—Homo sapiens (human)	0.003542907	0.1255046	2.45064
hsa00010	Glycolysis/Gluconeogenesis—Homo sapiens (human)	0.003825101	0.1255046	2.417357
hsa04115	p53 signaling pathway—Homo sapiens (human)	0.004439106	0.1255046	2.352704
hsa04610	Complement and coagulation cascades—Homo sapiens (human)	0.004772039	0.1255046	2.321296
hsa00480	Glutathione metabolism—Homo sapiens (human)	0.005446149	0.126449	2.26391
hsa00640	Propanoate metabolism—Homo sapiens (human)	0.005769537	0.126449	2.238859
hsa00620	Pyruvate metabolism—Homo sapiens (human)	0.01506088	0.2847503	1.82215
hsa04974	Protein digestion and absorption—Homo sapiens (human)	0.01515781	0.2847503	1.819364
hsa00564	Glycerophospholipid metabolism—Homo sapiens (human)	0.01763788	0.2896251	1.753554
hsa00590	Arachidonic acid metabolism—Homo sapiens (human)	0.01823105	0.2896251	1.739188
hsa00592	alpha-Linolenic acid metabolism—Homo sapiens (human)	0.01872101	0.2896251	1.727671
hsa05031	Amphetamine addiction—Homo sapiens (human)	0.02164262	0.314884	1.66469
hsa04110	Cell cycle—Homo sapiens (human)	0.02328676	0.314884	1.632891
hsa00030	Pentose phosphate pathway—Homo sapiens (human)	0.02537306	0.314884	1.595627
hsa04151	PI3 K-Akt signaling pathway—Homo sapiens (human)	0.02549613	0.314884	1.593526
hsa05030	Cocaine addiction—Homo sapiens (human)	0.02694598	0.314884	1.569506
hsa00983	Drug metabolism—other enzymes—Homo sapiens (human)	0.02873466	0.314884	1.541594
hsa04978	Mineral absorption—Homo sapiens (human)	0.02873466	0.314884	1.541594
hsa04360	Axon guidance—Homo sapiens (human)	0.03028938	0.3186443	1.51871
hsa04512	ECM-receptor interaction—Homo sapiens (human)	0.04672929	0.4726847	1.330411
hsa04911	Insulin secretion—Homo sapiens (human)	0.04870131	0.4743868	1.312459

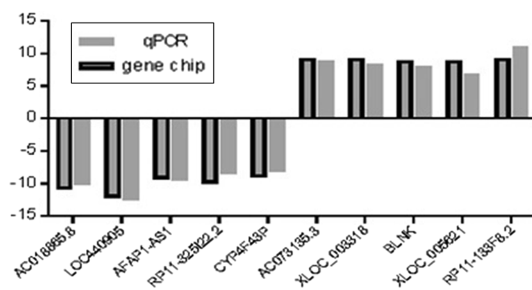

Fig. 4 Comparison between gene chip data and SYBR Green I real-time RT-PCR result. AC018865.8, LOC440905, AFAP1-AS1, RP11-325I22.2, CYP4F43P, AC073135.3, XLOC_003318, BLNK, XLOC_005621, RP11-133F8.2 determined to be differentially expressed EGFR exon 19 deletions and wild-type EGFR gene in the lung adenocarcinoma samples in five patients with lung adenocarcinoma by microarray were validated by SYBR Green I real-time RT-PCR. The validation results of the 10 lncRNAs indicated that the microarray data were correlated with the SYBR Green I real-time RT-PCR results

wild-type EGFR by RT-PCR. This result suggested that RP11-325I22.2 and LOC440905 might play in EGFR exon 19 deletions in lung adenocarcinoma; we would further study the biological function of RP11-325I22.2 and LOC440905.

In order to obtain new sight into the function of targets of lncRNAs, GO analysis and KEGG pathway annotation were applied to this target gene pool. GO analysis revealed that the number of genes corresponding to downregulated mRNA was larger than that corresponding to upregulated mRNA. KEGG annotation showed that there are 27 upregulated pathways (metabolism of xenobiotics by cytochrome p450, drug metabolism-cytochrome p450, chemical carcinogenesis, and so on.) and 27 downregulated pathways (including alcoholism, systemic lupus erythematosus, viral carcinogenesis, transcriptional misregulation in cancer, and so on). Our data denoted that these pathways might play important roles in EGFR exon 19 deletions in lung adenocarcinoma.

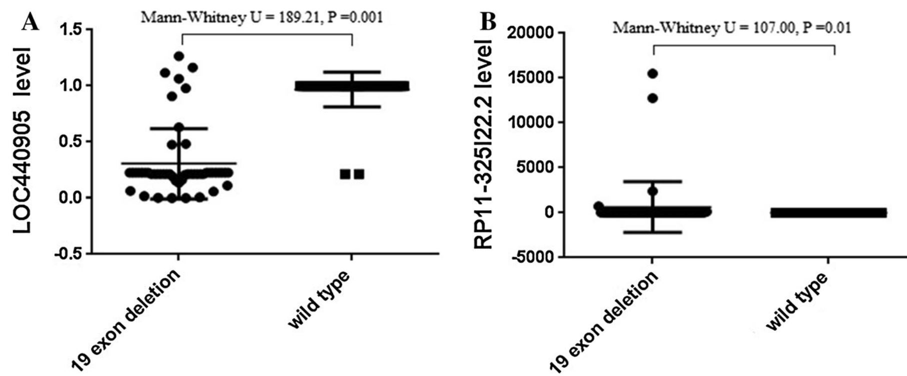

Fig. 5 The expression level of LOC440905 and RP11-325I22.2 between 20 cases from EGFR exon 19 deletion and 70 cases wild-type EGFR gene in the lung adenocarcinoma samples. **a** RP11-133F8.2 expression of EGFR exon 19 deletion group was significantly higher than the wild-type EGFR gene group (Mann–Whitney $U = 107.00$, $P = 0.01$). **b** LOC440905 expression of EGFR exon 19 deletion group was significantly lower than the wild-type EGFR gene group (Mann–Whitney $U = 189.21$, $P = 0.001$)

Conclusions

Our study revealed that a set of lncRNAs are differentially expressed EGFR exon 19 deletions in lung adenocarcinoma. The differentially expressed lncRNAs may be closely related to EGFR exon 19 deletions in lung adenocarcinoma, and we would further study these lncRNAs. This result suggested that RP11-325I22.2 and LOC440905 might play an important role in the mechanism of EGFR exon 19 deletions in lung adenocarcinoma.

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Conflict of interest The authors declare that they have no competing interests.

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