Identification of key genes and long non-coding RNAs in celecoxib-treated lung squamous cell carcinoma cell line by RNA-sequencing

GANG LI, XUEHAI WANG, QINGSONG LUO and CHONGZHI GAN

Department of Thoracic Surgery, Sichuan Academy of Medical Sciences and Sichuan Provincial People's Hospital, Chengdu, Sichuan 610072, P.R. China

Received November 24, 2016; Accepted August 3, 2017

DOI: 10.3892/mmr.2018.8656

Abstract. Celecoxib is an inhibitor of cyclooxygenase-2, a gene that is often aberrantly expressed in the lung squamous cell carcinoma (LSQCC). The present study aims to provide novel insight into chemoprevention by celecoxib treatment. The human LSQCC cell line SK-MES-1 was treated with or without celecoxib and RNA-sequencing (RNA-seq) was performed on the Illumina HiSeq 2000 platform. Expression levels of genes or long non-coding RNAs (lncRNAs) were calculated by Cufflinks software. Subsequently, differentially expressed genes (DEGs) and differentially expressed lncRNAs (DE-LNRs) between the two groups were selected using the limma package and LNCipedia 3.0, respectively; followed by co-expression analysis based on their expression correlation coefficient (CC). Enrichment analysis for the DEGs and co-expressed DE-LNRs was performed. Protein-protein interaction (PPI) network analysis for DEGs was performed using STRING database. A set of 317 DEGs and 25 DE-LNRs were identified between celecoxib-treated and non-treated cell lines. A total of 12 pathways were enriched by the DEGs, including 'protein processing in endoplasmic reticulum' for activating transcription factor 4 (ATF4), 'mammalian target of rapamycin (mTOR) signaling pathway' for vascular endothelial growth factor A (VEGFA) and 'ECM-receptor interaction' for fibronectin 1 (FN1). Genes such as VEGFA, ATF4 and FN1 were highlighted in the PPI network. VEGFA was linked with Inc-AP000769.1-2:10 (CC=-0.99227), whereas ATF4 and FN1 were closely correlated with Inc-HFE2-2:1 (CC=0.996159 and -0.98714, respectively). lncRNAs were also enriched in pathways such as 'mTOR signaling pathway' for Inc-HFE2-2:1. Several important molecules were identified in celecoxib-treated LSQCC cell lines, such as VEGFA, ATF4, FN1, Inc-AP000769.1-2:10 and Inc-HFE2-2:1, which may enhance the anti-cancer effects of celecoxib on LSQCC.

Introduction

Lung cancer is one of the most frequently diagnosed cancers with high mortality worldwide, with >1,500,000 new cases diagnosed annually (1,2). Based on the cancer statistics data from 2015, lung and bronchial cancers are expected to have the highest mortality among all cancers in the USA, with the estimated mortality rate of 158,040 (3). Lung squamous cell carcinoma (LSQCC) is the second most common type of lung cancer, with an annual mortality rate of ~400,000 worldwide (4). Chemotherapy and radiotherapy are the most common treatments for LSQCC; however, patient responses to these therapies are limited (5). Therefore, there is a requirement for additional agents to be developed that may enhance the response to these treatments.

Cyclooxygenase (COX)-2 serves an important role in the tumorigenesis of various types of cancer, and COX-2 inhibitors may effectively prevent tumor progression (6). Celecoxib is a selective COX-2 inhibitor; at the early stages of non-small-cell lung cancer (NSCLC), celecoxib was reported to increase the anti-cancer properties of preoperative chemotherapies, such as paclitaxel and carboplatin (7). In addition, celecoxib treatment upregulated the expression of death receptor 5 (DR5), decreased cell survival and induced apoptosis in NSCLCs (8).
Increased expression of COX-2 has been reported in LSQCC, and its inhibitor celecoxib is predicted as the beneficial target for chemotherapy (9). Although these previous studies have implied that celecoxib enhances the response sensitivity of chemotherapy in LSQCC, the specific molecular mechanisms of this inhibitor are still unknown. Several previous studies have indicated that celecoxib treatment may result in cell cycle arrest by downregulating the expression of p21 and p27, which may account for the reduction of cyclin-dependent kinase activity (10,11). In addition, celecoxib may contribute to the inhibition of angiogenesis by suppressing the expression of angiogenic factors in tumor cells (12). However, additional studies are required to comprehensively determine the mechanism of celecoxib on chemoprevention.

Long noncoding RNAs (lncRNAs) are a recently described RNA transcript species that is different from mRNAs and microRNAs. They are not transcriptional ‘noise’, but are important genes that function in numerous biological processes (13,14). Currently, several lncRNAs have been identified with significant functions in lung cancer; for example, the upregulation of PVT1 was reported to promote oncogenesis in NSCLCs (15), and prostate cancer-associated transcript 6 was predicted as an oncogenic lncRNA in the growth and invasion of lung cancer cells (16). In addition, a recent study demonstrated that the novel lncRNA onco-lncRNA 230 was able to induce invasion and apoptosis in LSQCC, and it was suggested as a possible new diagnostic marker for the disease (17). These imply that regulation of lncRNAs serves a key role in LSQCC. However, lncRNA expression under celecoxib treatment has not been reported. A previous study demonstrated that celecoxib treatment (50 µM) induced significant overexpression of Bcl-2, Bcl-extra large and survivin following 24 h treatment, whereas no significant alterations in expression were identified in the activation of caspase-3, caspase-8 or caspase-9 (18). Another study revealed that the expression of multidrug resistance-associated-4, a member of the ATP-binding cassette transporters, was significantly upregulated in human LSQCC SK-MES-1 cells following treatment with 5 and 50 µmol/l of celecoxib for 24, 48 and 72 h (19). These data suggested that celecoxib treatment may induce a series of variations in the metabolism of SK-MES-1 cells; however, no lncRNAs have been identified. Therefore, the present study used RNA-sequencing (RNA-seq), which facilitates transcript analysis in various cancers (20), to identify differentially expressed genes (DEGs) and differentially expressed lncRNAs (DE-LNRs) between SK-MES-1 cells cultured with or without celecoxib treatment. In addition, potential correlations were calculated, followed by pathway exploration of the lncRNAs. This study aimed to provide novel insight into celecoxib chemoprevention and to identify potential targeting markers for COX-2 induced LSQCC.

Materials and methods

Cell culture and drug treatment. The human LSQCC cell line SK-MES-1 was purchased from the Cell Bank of Type Culture Collection Chinese Academy of Sciences (Shanghai, China). Cells were cultured in the RPMI-1640 medium (Gibco; Thermo Fisher Scientific, Inc., Waltham, MA, USA) containing 10% fetal bovine serum (Gibco; Thermo Fisher Scientific, Inc.), at 37˚C and 5% CO₂.

Cells at the logarithmic growth phase (at a confluency of 70-75%) were divided into two groups: i) Two identical SK-MES-1 cell samples were treated with 10 µM celecoxib in 1% DMSO medium (celecoxib-treated group); and ii) two identical SK-MES-1 cell samples were treated with equal amounts of DMSO (Control group). Both groups were cultured for 48 h at 37˚C.

RNA extraction and RNA-seq. A total of 5x10⁵ cells were utilized to isolate RNA using the RNeasy kit (catalog no. 74106; Qiagen Sciences, Inc., Gaithersburg, MD, USA) according to the manufacturer's protocol. RNA purity was analyzed with a Nanodrop spectrophotometer (NanoDrop Technologies; Thermo Fisher Scientific, Inc.), and the RNA was reverse transcribed into cDNA for library preparation using NEBNext Ultra RNA Library Prep kit for Illumina (catalog no. E7530L; New England BioLabs, Inc., Ipswich, MA, USA), following the manufacturer's protocol. Briefly, RNA (5 µg) from each sample was sheared into small fragments (200 nucleotides) prior to cDNA synthesis using fragment buffer. Subsequently, the cDNA was blunt-ended and phosphorylated. A single 3’ adenosine moiety and Illumina adapters were added on the repaired ends, followed by 15 cycles of polymerase chain reaction (PCR) according to the protocol of the kit. Pre-amplification were performed using the NEB Phusion DNA polymerase (New England BioLabs, Inc.). RNA-seq was performed on the Illumina HiSeq 2000 Sequencing System (Illumina, Inc., San Diego, CA, USA) using the 2x50 paired-end sequencing method.

Pretreatment of RNA-seq data. Quality control (QC) of raw sequencing reads was performed using the next generation sequencing (NGS) QC Toolkit, as previously described (21). Briefly, the adaptor sequences in the reads were removed, and the low-quality reads with the base quality score <20 were filtered out. High quality sequences were defined having bases with a quality score >20 that accounted for >90% of its length. Subsequently,
the clean reads were aligned against the University of California Santa Cruz Homo sapiens reference genome (hg19 assembly, http://www.genome.ucsc.edu/index.html) using TopHat2 software (v2.0.9; http://ccb.jhu.edu/software/tophat) with the default parameters (22).

Identification of DEGs. Cufflinks (v2.2.1; http://cufflinks.cbcb.umd.edu/index.html) software was used to calculate the fragments per kilobase of exon per million fragments mapped (FPKM), from which the gene expression values were obtained (23). The linear models for microarray analysis (limma; v3.10.3) package in R (http://www.bioconductor.org/packages/release/bioc/html/limma.html) was used to select DEGs between the two groups (24), with the thresholds of q-value<0.05 and |log$_2$(FC)|>0.58; where FC is fold change.

Selection of DE-LNRs. LNCipedia 3.0 (http://www.lncipedia.org), an online storage of lncRNA annotation (25), was used.

---

**Table II. Differentially expressed lncRNAs in the celecoxib-treated group and Control group.**

### A. Upregulated

| lncRNA                | Value_1 | Value_2 | Log$_2$(FC) | q-value    |
|-----------------------|---------|---------|-------------|------------|
| lnc-C14orf166B-3:4    | 183.118 | 400.277 | 112.823     | 5.35x10$^{-4}$ |
| lnc-CNN3-3:1         | 112.248 | 179.237 | 0.675       | 5.06x10$^{-8}$ |
| lnc-CTSL1-2:2        | 327.718 | 549.936 | 0.747       | 2.63x10$^{-2}$ |
| lnc-CXCL3-1:1        | 128.057 | 34.035  | 141.023     | 8.89x10$^{-13}$ |
| lnc-ENTPD6-2:1       | 101.243 | 188.522 | 0.897       | 1.7x10$^{-7}$  |
| lnc-ERN1-1:1         | 750.668 | 164.504 | 113.187     | 8.84x10$^{-9}$ |
| lnc-HES1-10:1        | 258.042 | 694.213 | 142.777     | 5.96x10$^{-4}$ |
| lnc-HFE2-2:1         | 343.839 | 851.858 | 130.888     | 6.09x10$^{-4}$ |
| lnc-KIAA1257-3:1     | 132.568 | 21.211  | 0.678       | 1.57x10$^{-2}$ |
| lnc-KS1-1:1          | 108.716 | 165.887 | 0.610       | 2.81x10$^{-2}$ |
| lnc-MOGAT2-5:1       | 930.449 | 140.722 | 0.597       | 2.03x10$^{-8}$ |
| lnc-MT2A-1:2         | 1990.13 | 3034.1  | 0.608       | 1.22x10$^{-8}$ |
| lnc-RAB44-3:1        | 517.827 | 879.917 | 0.765       | 1.57x10$^{-2}$ |
| lnc-RBM3-1:1         | 726.858 | 139.598 | 0.942       | 1.89x10$^{-2}$ |
| lnc-RP11-231C14.2.1-3:1 | 164.477 | 551.775 | 0.676       | 3.0x10$^{-3}$  |
| lnc-RSPH9-4:1        | 658.082 | 105.15  | 0.676       | 7.30x10$^{-3}$ |
| lnc-TRIB3-1:2        | 366.972 | 569.299 | 0.634       | 1.4x10$^{-2}$  |

### B. Downregulated

| lnc-AP000769.1-2:10  | 895.237 | 589.174 | -0.604      | 6.98x10$^{-4}$ |
| lnc-BOLA3-2:2        | 136.839 | 0       | -1.80x10$^{-8}$ | 4.31x10$^{-2}$ |
| lnc-E2F2-1:1         | 200.807 | 118.647 | -0.759      | 1.50x10$^{-6}$ |
| lnc-FOXG1-7:1        | 39.749  | 0       | -1.80x10$^{-8}$ | 4.16x10$^{-13}$ |
| lnc-GNLY-4:2         | 90.639  | 306.009 | -156.656    | 3.67x10$^{-3}$ |
| lnc-KIAA0226-2:1     | 219.713 | 0.367   | -258.257    | 1.62x10$^{-4}$ |
| lnc-LTP3-2:5         | 11.504  | 730.114 | -0.656      | 5.95x10$^{-3}$ |
| lnc-TOR1A-2:1        | 23.017  | 131.966 | -0.803      | 1.40x10$^{-2}$ |

FC, fold change; lncRNA, long noncoding RNA.

---

Figure 1. Scatter plot of FPKM in celecoxib-treated SK-MES-1 cells and untreated Control cells. Red dots represent upregulated genes in the celecoxib-treated group; green dots represent downregulated genes; and blue dots represent genes without differential expressions. The x-axis denotes cells in the Control group, and the y-axis denotes celecoxib-treated cells. FPKM, fragments per kilobase of exon per million fragments mapped.
to acquire information on IncRNAs. Subsequently, Cufflinks was used to screen the DE-LNRs. Similar to the selection of DEGs, the cut-off values were \( q<0.05 \) and \(|\log_2(FC)|>0.58\).

**Enrichment analysis of the DEGs.** To explore potential functions and pathways that the DEGs may participate in, function enrichment and pathway enrichment were implemented based on the Gene Ontology (GO; http://www.geneontology.org) (26) database and the Kyoto Encyclopedia of Genes and Genomes (KEGG; http://www.genome.jp/kegg/pathway.html) database, respectively, and the Database for Annotation, Visualization and Integration Discovery (DAVID; v6.8; http://david.abcc.ncifcrf.gov) (27). Selection criteria for a significant GO or KEGG pathway category were \( P<0.05 \) with \( \geq 2 \) genes enriched in a category.

**Protein-protein interaction (PPI) network construction.** The Search Tool for the Retrieval of Interacting Genes (STRING; v10.0, http://string-db.org) database was searched to discover potential interactions of proteins encoded by the identified DEGs (28). With the selection criterion of a combined score \( >0.7 \), a PPI network was established, which was drawn using Cytoscape (v3.2.0; http://cytoscape.org) software (29).

**Co-expression analysis of IncRNAs and mRNAs.** For the identified DE-LNRs and DEGs, their correlation coefficient (CC) was calculated by Pearson correlation. The co-expressed DE-LNRs and DEGs were selected under the condition of \(|CC|>0.98\). Subsequently, enrichment analysis of the co-expressed DEGs was performed to predict biological functions of the DE-LNRs.

**Validation of identified DEGs and IncRNA.** To further confirm the identification of DEGs and DE-LNRs, expression levels of fibronectin 1 (FN1), vascular endothelial growth factor A (VEGFA) and IncAP000769.1-2:10 were determined using reverse transcription-quantitative PCR (RT-qPCR) in SK-MES-1 cells treated with 10 µM celecoxib or DMSO for 48 h. Total RNA was isolated from cells at a confluency of 70-75% using TRizol agent (Takara Biotechnology Co., Ltd., Dalian, China), and reverse transcribed to cDNA using the PrimeScript RT Reagent kit (Takara Biotechnology Co., Ltd.), according to the manufacturer’s protocol. qPCR was

### Table III. Top 10 identified KEGG enrichment pathways of identified DEGs.

| KEGG ID | Pathway name                             | Count \(^a\) | Genes                                                                 | P-value          |
|---------|------------------------------------------|--------------|----------------------------------------------------------------------|-----------------|
| 970     | Aminoacyl-tRNA biosynthesis               | 10           | AARS, CARS, EPRS, GARS, IARS                                        | \(4.49\times10^{-7}\) |
| 4141    | Protein processing in endoplasmic reticulum | 15           | ATF4, DDTI3, DNAJB2, ERN1, HERPUD1                                   | \(1.05\times10^{-6}\) |
| 260     | Glycine, serine and threonine metabolism  | 6            | CBS, CTH, PHGDH, PSAT1, P5PH, SHMT2                                  | \(3.78\times10^{-3}\) |
| 3060    | Protein export                            | 4            | HSPA5, SEC11C, SEC63, SRPRB                                         | \(1.09\times10^{-3}\) |
| 520     | Amino sugar and nucleotide sugar metabolism | 5            | GFT51, GFT2, GMPPB, HKDC1, NAGK                                     | \(2.79\times10^{-3}\) |
| 250     | Alanine, aspartate and glutamate metabolism | 4            | ASNS, GFT51, GFT2, GPT2                                            | \(3.84\times10^{-3}\) |
| 4150    | mTOR signaling pathway                    | 5            | DDIT4, EIF4E, EBP1, RPS6KA2, ULK1, VEGFA                              | \(3.97\times10^{-3}\) |
| 860     | Porphyrin and chlorophyll metabolism      | 4            | EPRS, FTH1, HMOX1, UROS                                            | \(1.11\times10^{-2}\) |
| 5020    | Prion diseases                            | 3            | EGR1, HSPA5, IL1A                                                   | \(3.39\times10^{-2}\) |
| 450     | Selenocompound metabolism                 | 2            | CTH, MARS                                                           | \(4.62\times10^{-2}\) |

\(a\) The number of genes that were enriched in a specific pathway category. KEGG, Kyoto Encyclopedia of Genes and Genomes; DEG, differentially expressed gene.

---

LI et al. GENES AND IncRNAs AFFECT LSQCC SENSITIVITY TO CELECOXIB 6459
Figure 2. Protein-protein interaction network of the differentially expressed genes. Red circles represent upregulated genes, and green circles represent downregulated genes.

Figure 3. Enriched pathways of the co-expressed lncRNAs. Red indicates that the lncRNA was enriched in a specific pathway. Rows indicate pathways and columns indicate lncRNAs. lncRNA, long noncoding RNA.
performed using a SYBR Green kit (Applied Biosystems; Thermo Fisher Scientific, Inc.) on a Viia7 PCR instrument (Applied Biosystems; Thermo Fisher Scientific, Inc.) with the following thermocycling conditions: 1 cycle of 50˚C for 3 min and 95˚C for 3 min; followed by 40 cycles of 95˚C for 10 sec and 60˚C for 30 sec. GAPDH was used as the internal control during expression analysis, and gene expression was calculated using the 2^{-∆∆Cq} method (30). Primer sequences are provided in Table I.

Statistical analysis. DEGs were screened using the limma package in R with the thresholds of q<0.05 and |log_{2}(FC)|>0.58; DE-LNRs were screened using Cufflinks software with the same thresholds. Continuous variables are presented as the mean ± standard deviation, and difference between groups was calculated using Student's t-test. P<0.05 was considered to indicate a statistically significant difference.

Results

DEGs and DE-LNRs identification. According to the aforementioned criteria, a set of 261 upregulated and 56 downregulated DEGs were identified in celecoxib-treated group, compared with the untreated Control cells. Gene expressions in each group are presented in a scatter plot of FPKM (Fig. 1). Based on the predefined selection criterion, 17 lncRNAs were upregulated and 8 lncRNAs were downregulated in celecoxib-treated group compared with the untreated Control cells (Table II).

Pathway enrichment analysis of DEGs. KEGG pathway analysis indicated that the upregulated DEGs were significantly enriched in 12 pathway categories, including Aminoacyl-tRNA biosynthesis, protein processing in endoplasmic reticulum (ER), protein export, amino sugar and nucleotide sugar metabolism and mammalian target of rapamycin (mTOR) signaling pathway. The downregulated DEGs were enriched in 17 pathways, such as ‘transforming growth factor (TGF)-β signaling pathway’, ‘extracellular matrix (ECM)-receptor interaction’, ‘cytokine-cytokine receptor interaction’ and ‘p53 signaling pathway’ (Table III).

PPI network of DEGs. In the established PPI network (Fig. 2), several genes were highlighted that had high degrees; that is, a high number of connections between one gene and the others, such as VEGFA (degree=23), activating transcription factor (ATF)-4 (degree=19), FN1 (degree=16), urokinase-type plasminogen activator PLAU (degree=13), ATF3 (degree=11) and serpin E1 (SERPINE1; degree=10).

Co-expressed DE-LNRs and DEGs and their enriched functions. Using the criterion of |CC|>0.98, the co-expressed DE-LNRs and DEGs were screened out. As presented in Table IV, SERPINE1 was co-expressed with Inc-CTSL1-2:2 (CC=0.988577) and Inc-CXCL3-1:1 (CC=0.986928); VEGFA was linked with Inc-HES1-10:1 (CC=0.98906) and Inc-AP000769.1-2:10 (CC=0.998602); Inc-HF2-2:1 was co-expressed with ATF4 (CC=0.996159) and FN1 (CC=-0.98714); ATF3 was co-expressed with Inc-KIAA1257-3:1 (CC=0.990012), Inc-KSR1-1:1 (CC=0.990212) and Table IV. The most highly correlated co-expressed DE-LNRs and DEGs.

| DE-LNR                  | DEG         | CC       |
|------------------------|-------------|----------|
| Inc-C14orf166B-3:4     | VEGFA       | 0.999351 |
| Inc-CNN3-3:1           | ACOT8       | 0.994477 |
| Inc-CTSL1-2:2          | SERPINE1    | 0.988577 |
| Inc-CXCL3-1:1          | SERPINE1    | 0.986928 |
| Inc-ENTPD6-2:1         | ACVR1       | 0.995922 |
| Inc-ERN1-1:1           | ASPN        | -0.98602 |
| Inc-HES1-10:1          | VEGFA       | 0.890606 |
| Inc-HF2-2:1            | ATF4        | 0.996159 |
| Inc-KIAA1257-3:1       | ATF3        | 0.990212 |
| Inc-KSR1-1:1           | ATF3        | 0.996555 |
| Inc-MOGAT2-5:1         | ACVR1       | 0.986953 |
| Inc-MT2A-1:2           | AASR        | 0.991019 |
| Inc-RAB44-3:1          | ACOT8       | 0.997679 |
| Inc-RBM3-1:1           | ACOT8       | 0.990015 |
| Inc-RP11-231C14.2.1-3:1| ATP3       | 0.993415 |
| Inc-RSPH9-4:1          | ATOT8       | 0.990293 |
| Inc-TRIB3-1:2          | ACOT8       | 0.988663 |
| Inc-AP000769.1-2:10    | ASPN        | 0.987202 |
| Inc-BOLA3-2:2          | CCNF        | 0.892722 |
| Inc-E2F2-1:1           | AMER1       | 0.897329 |
| Inc-FOXG1-7:1          | AMER1       | 0.888729 |
| Inc-GNLY-4:2           | COL1A1      | 0.893597 |
| Inc-GNLY-4:2           | CDH13       | -0.98936 |
| Inc-KIAA0226-2:1       | FN1         | 0.891655 |
| Inc-LTPB3-2:5          | ASPN        | 0.93248 |
| Inc-TOR1A-2:1          | ATOH8       | 0.895423 |
|                        | CDH13       | -0.99533 |

CC, correlation coefficient; DEG, differentially expressed gene; DE-LNR, differentially expressed long noncoding RNA.
Inc-RP11-23IC14.2:1:3:1 (CC = 0.993415); Inc-BOLA3-2:2 was linked with PLAU (CC = -0.99288); and Inc-KIAA0226-2:1 was co-expressed with FN1 (CC = 0.981655).

According to the enrichment analysis results of the co-expressed DEGs, the co-expressed DE-LNRs were mainly enriched in the ‘p53 signaling pathway’ (for example, Inc-HES1-10:1, Inc-HFE2-2:1, Inc-KIAA1257-3:1 and Inc-KSR1-1:1; Fig. 3) and the ‘mTOR signaling pathway’ (for example, Inc-CTSL1-2:2, Inc-CXCL3-1:1, Inc-ERN1-1:1, Inc-HES1-10:1 and Inc-HFE2-2:1; Fig. 3).

Validations of DEGs and IncRNA. To verify the identification of possible DEGs and DE-LNRs, the expression levels of VEGFA, FN1 and Inc-AP000769-1:2:10 were analyzed by RT-qPCR. The results revealed that the expression level of VEGFA was significantly increased in celecoxib-treated SK-MES-1 cells compared with untreated Control cells (Fig. 4A). However, the expression levels of FN1 and Inc-AP000769-1:2:10 were significantly decreased in the celecoxib-treated group compared with the Control group (Fig. 4B and C, respectively). These results were consistent with the aforementioned bioinformatics analytical results.

Discussion

The present study identified a number of genes and IncRNAs that exhibited differential expression levels in celecoxib-treated human LSQCC SK-MES-1 cells, compared with untreated cells, including the genes VEGFA, ATF4 and FN1, and the IncRNAs Inc-AP000769-1:2:10 and Inc-HFE2-2:1. Notably, many of the identified DEGs and DE-LNRs were significantly enriched in pathways like ‘protein processing in endoplasmic reticulum’, ‘mTOR signaling pathway’ and ‘ECM-receptor interaction’.

VEGFA is an essential growth factor for stimulating angiogenesis, which often accompanies tumoral growth (31). A previous study reported that by upregulating the expression of FLJ10540, VEGFA activates the phosphatidylinositol 3-kinase/AKT signaling pathway, subsequently promoting cell invasion and migration in lung cancer (32). mTOR is located downstream of AKT signaling and functions in the control of angiogenesis and cell proliferation during tumor progression (33). VEGFA was also reported to activate the downstream mTOR signaling pathway to promote cancer growth (34). Notably, several therapeutic drugs for lung cancer have been reported to function through this pathway. 2-((18)F)-fluoro-2-deoxy-d-glucose ((18)F-FDG) is a radiolabelled sugar molecule that is commonly used to monitor the therapeutic effects of chemotherapy for many malignant tumors, and the accumulation of (18)F-FDG is regulated by the activation of mTOR signaling in NSCLC (35). Cis-diaminedichloroplatinum (II) (CDDP; also known as cisplatin) is an effective drug for the treatment of many cancers (36). However, resistance to this drug limited its potential use in lung cancer treatment. It was previously reported that overexpression of AKT activates the mTOR signaling pathway, which induces CDDP resistance in lung cancer cells (37). Therefore, inhibitors of the AKT/mTOR signaling pathway may provide a promising therapeutic target. In lung cancer, targeting of mTOR signaling may be suggested as an effective method in developing therapeutic drugs (38). Curcumin, a natural extract of turmeric, is considered as an antitumoral agent, which was reported to enhance the anti-cancer ability of chemotherapy in
LSQCC by regulating multiple pathways such as VEGF signaling (39). Notably, VEGFA was also indicated to be enriched in the mTOR signaling pathway (36). In the present study, VEGFA was identified as an upregulated DEG in celecoxib-treated LSQCC cells, and was demonstrated to be significantly enriched in the mTOR signaling pathway. These data suggested that VEGFA may be a sensitive gene in response to celecoxib, and the increased expression may inhibit the activation of mTOR signaling, which may improve the anti-tumor effects of celecoxib on LSQCC cells.

In addition, IncRNAs may also serve crucial roles in the amplification of anti-tumor effects. Nuclear paraspeckle assembly transcript 1 (Neat1; ENST00000501122.2) is a factor required for the assembly of paraspeckle compartments in the cell (40). Neat1-containing paraspeckles were reported to be responsible for the regulation of chemosensitivity and may be induced by p53 (41). The biofunction of Neat1 is similar to Inc-AP000769.1-2-3. However, no information about Inc-AP000769.1-2:10 has yet been reported. In the present study, Inc-AP000769.1-2:10 was closely correlated with VEGFA, which was enriched in the mTOR signaling pathway following celecoxib treatment. In addition, the expression of Inc-AP000769.1-2:10 was significantly decreased in celecoxib treated SK-MES-1 cells. Therefore, the present study hypothesized that this IncRNA may regulate VEGFA gene expression in the mTOR signaling pathway, which may facilitate to the enhancement of anti-tumor effect of celecoxib for LSQCC treatment.

ATF4 was reported to be associated with cisplatin sensitivity in lung cancer cell lines (42). Celecoxib has been demonstrated to induce the expression of DR5 (43). In addition, C/EBP homologous protein (CHOP) was revealed to serve a crucial role in celecoxib-induced DR5 expression and may also be upregulated by celecoxib (44). Inhibition of ATF4 expression by small interfering RNAs was able to abolish CHOP induction, which indicated the involvement of ATF4 in celecoxib-induced apoptosis (45). The ER is an essential site for protein processing. In many types cancer, the ER serves an important role in the structural maintenance of proteins in pivotal signaling pathways (46). Control of these proteins may offer promising target therapies. In the present study, ATF4 was indicated as enriched in the protein processing in ER pathway, which suggested that it may influence the sensitivity of celecoxib in LSQCC through the regulation of protein processing.

The FN1 protein may be involved in several cellular activities, such as cell adhesion and migration (47,48). In lung cancer, knockdown of FN1 was previously reported to increase the chemosensitivity of cisplatin and promote apoptosis in tumor cells (49). Notably, in the present study, FN1 expression was downregulated in the celecoxib-treated LSQCC cells, which suggested that the reduced expression of this gene may also enhance the sensitivity of tumor cells in response to celecoxib. In addition, FN1 has been implicated in pathways such as the ECM-receptor interaction pathway in many types cancer (50,51). Consistent with these results, FN1 was significantly enriched in the ECM-receptor interaction pathway in the present study, which indicated that FN1 may exert its function through its involvement in this pathway. Results from the present study also predicted that both FN1 and ATF4 were targets of Inc-HF2-2:1, which suggested that the two genes may be regulated by this IncRNA in LSQCC cells following celecoxib treatment. However, there is still little information available about this IncRNA. Based on the present results, Inc-HF2-2:1 may be a novel target to predict sensitivity of celecoxib for the treatment of LSQCC, through the regulation of FN1 and ATF4 expressions.

There were some limitations to the present study. First, although some genes and IncRNA had been validated in this study, the regulatory relationships between IncRNAs and DEGs have yet to be confirmed with in vitro experiments. Second, as LSQCC may develop from a number of lung cell dysfunctions, SK-MES-1 cells may not reflect the wider results of celecoxib. Therefore, different types of LSQCC cell lines should be used in future studies, and the intersection of DEGs and IncRNAs may be focused. Finally, an appropriate animal model should be used to confirm the identified DEGs and DE-LNRs, including investigations on the predicted signaling pathways.

In conclusion, genes (such as VEGFA, ATF4 and FN1), and IncRNAs (such as Inc-AP000769.1-2:10 and Inc-HF2-2:1) may be crucial molecules to enhance the anti-cancer effects of celecoxib treatment on LSQCC, and may be used as predictors for chemosensitivity of celecoxib. However, additional validation experiments are required in further studies.

References
1. Henley SJ, Richards TB, Underwood JM, Eheman CR, Plessia M and McAfee TA; Centers for Disease Control and Prevention (CDC): Lung cancer incidence trends among men and women-United States, 2005-2009. MMWR Morb Mortal Wkly Rep 63: 1-5, 2014.
2. de Groot P and Munden RF: Lung cancer epidemiology, risk factors, and prevention. Radiol Clin North Am 50: 863-876, 2012.
3. Siegel RL, Miller KD and Jemal A: Cancer statistics, 2015. CA Cancer J Clin 65: 5-298, 2015.
4. Cancer Genome Atlas Research Network: Comprehensive genomic characterization of squamous cell lung cancers. Nature 489: 519-525, 2012.
5. Bendale Y, Bendale V, Birari-Gawande P, Kadam A and Gund P: Tumor regression with ayurvedic rasayana therapy in squamous cell carcinoma of lungs. Rasamruta 7: 1-5, 2015.
6. Mascaux C, Martin B, Verdebout JM, Ninane V and Sculier JP: COX-2 expression during early lung squamous cell carcinoma oncocogenesis. Eur Respir J 26: 198-203, 2005.
7. Altorki NK, Keresztes RS, Port JL, Libby DM, Korst RJ, Flieder DB, Ferrara CA, Yankelevitz DF, Subbaramiah K, Pasmanter MW and Dannenberg AJ: Celecoxib, a selective cyclooxygenase-2 inhibitor, enhances the response to preoperative paclitaxel and carboplatin in early-stage non-small-cell lung cancer. J Clin Oncol 21: 2645-2650, 2003.
8. Liu X, Yue P, Zhou Z, Khuri FR and Sun SY: Death receptor regulation and celecoxib-induced apoptosis in human lung cancer cells. J Natl Cancer Inst 96: 1769-1780, 2004.
9. Gold KA, Kim ES, Lee JJ, Wistuba II, Farhangfar CJ and Hong WK: The BATTLE to personalize lung cancer prevention through reverse migration. Cancer Prev Res (Phila) 4: 962-972, 2011.
10. Fu SL, Wu YL, Zhang YP, Qiao MM and Chen Y: Anti-cancer effects of COX-2 inhibitors and their correlation with angiogenesis and invasion in gastric cancer. World J Gastroenterol 10: 1971-1974, 2004.
11. Mineo TC, Ambrogi V, Cufari ME and Pompeo E: May cyclooxygenase-2 (COX-2), p21 and p27 expression affect proliferation and therapeutic strategy of patients with malignant pleural mesothelioma? Eur J Cardiothorac Surg 38: 245-252, 2010.
Pimentel H, Salzberg SL, Rinn JL and Pachter L: Biology of next generation sequencing data. PLoS One 7: e30619, 2012.

Frati L, and aspirin in lung cancer cell lines. Failure of apoptosis and activation on NFkappaB by celecoxib induces apoptosis and invasion in lung squamous cell carcinoma. Abstract 971: A novel long noncoding RNA, onco‑lncRNA 230, induction and invasion in lung cancer cells. Oncol Res 24: 161-170, 2016.

Wan L, Zhang L, Fan K, Cheng ZX, Sun QC and Wang JJ: Increased expression of the lncRNA PVT1 promotes tumori
