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Identification of key long non-coding RNAs as competing endogenous RNAs for miRNA-mRNA in lung adenocarcinoma

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Abstract. – OBJECTIVE: RNA-seq data and miRNA-seq data of lung adenocarcinoma (LUAD) were analyzed to identify critical long non-coding RNAs (lncRNAs) and disclose molecular pathogenesis.

MATERIALS AND METHODS: RNA-seq data and miRNA-seq data were downloaded from TCGA. Differentially expressed lncRNAs (DELs) and microRNAs (DEMs) were revealed by two sample *t*-test. |Fold change| > 2 and *p*-value < 0.01 were set as the cutoffs. Univariate Cox regression was performed to disclose prognostic lncRNAs. Information about miRNA-lncRNA interactions and miRNA-mRNA interactions were acquired from miRcode and miRTarBase, respectively. A miRNA-lncRNA-mRNA regulatory network was then constructed, from which regulatory modules were identified. Functional enrichment analysis was performed with DAVID.

RESULTS: A total of 57 DELs and 118 DEMs were identified from 507 LUAD compared with 19 normal samples. Three DELs, including MEG3, MIAT and MIR4697HG, were associated with clinical features, while nine DELs (LINC00115, LINC00265, LINC01001, LINC01002, MIR22HG, NFYC–AS1, SNHG10, THUMPD3–AS1 and TMPO–AS1) were revealed to be prognostic biomarkers. A regulatory network including 61 miRNA-lncRNA interactions and 304 miRNA-mRNA interactions was constructed, from which 19 lncRNA-miRNA-mRNA regulatory modules were identified. Among the modules, MEG3 and MIAT may play important roles in the development of LUAD by interactions with miR-106 which then regulated the MAPK9 to involve in MAPK signaling pathways. LINC00115 might interact with miR-7 to regulate FGF2 to participate in pathways in cancer.

CONCLUSIONS: MEG3, MIAT, LINC00115 may be underlying therapeutic targets for LUAD functioning as ceRNAs for regulation of miRNA-mRNA.

Key Words:

Lung adenocarcinoma, Long non-coding RNAs, microRNAs, Clinical features.

Introduction

Lung adenocarcinoma (LUAD) is the most common histological subtype of non-small cell lung cancer, responsible for more than 600,000 deaths annually worldwide[1]. Since LUAD tends to form metastasis widely at an early stage, the prognosis for patients with LUAD is commonly poor, with the average five-year survival rate less than 20%[2]. Thus, an investigation into the etiology and metastasis mechanism of LUAD remains a hot spot to develop novel effective therapeutic measures.

Recently high-throughput transcriptome analysis has revealed that more than 90% of the transcriptome is transcribed into non-coding RNAs, among which microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) have been identified to be involved in malignant behaviors of LUAD[3]. For example, Qu et al[4] illustrated that the expression of miR-33b is dramatically decreased in LUAD cell lines and tissues. Restoration of miR-33b expression inhibits LUAD cell proliferation, migration, and invasion by specific suppressing the expression of its target gene ZEB1 via binding in the ZEB1 3'-UTR region followed by limiting tumor cell epithelial-mesenchymal transition. Wu et al[5] demonstrated that HNF1A-AS1 is significantly highly expressed in LUAD compared with corresponding non-tumor tissues, and its expression level is significantly associated with TNM stage, tumor size, and lymph node metastasis, leading to worse overall survival. Further *in vitro* and *in vivo* analyses demonstrated HNF1A-AS1 may promote tumor proliferation and metastasis by regulating cyclin D1, E-cadherin, N-cadherin and β-catenin expression.

Furthermore, several authors believed that there is an interplay between lncRNAs and miRNAs during the tumorigenic process, among which the

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competing endogenous RNA (ceRNA) hypothesis attracts more attention[6]. ceRNA hypothesis proposes that lncRNAs may serve as molecular sponges for miRNAs and hence functionally liberate mRNA targeted regulated by aforementioned active miRNAs[7,8]. This hypothesis has been proved in a serial of literatures focusing on lung cancer. For example, Nie et al[9] found that high expression of lncRNA UCA1 in lung cancer up-regulates the expression of miR-193a-3p target gene ERBB4 through competitively 'spongeing' miR-193a-3p, ultimately promoting cell proliferation, while overexpression of miR-193a-3p attenuates the promoting proliferation effect of UCA1. Thus, the disclosure of the lncRNA-miRNA-mRNA interaction network may be conducive to comprehensively understand the etiology and metastasis mechanism of cancer to provide potential therapeutic targets, which have been performed for several cancers[10,11], but not in LUAD.

The goal of this study is to construct the microRNA-lncRNA-mRNA regulatory network and screen modules associated with LUAD by collecting RNA-seq data and miRNA-seq data from a public database. Critical lncRNA were further disclosed by correlation with the clinical characteristics.

Materials and Methods

Raw Data

RNA-seq data and miRNA-seq data of LUAD were downloaded from The Cancer Genome Atlas (TCGA, https://tcga-data. nci. nih. gov/tcga/) data portal on November 18, 2015. The RNA expression data (level 3) were generated from HiSeq 2000 sequencing platform (Illumina Inc, San Diego, CA, USA) by RNASeqV2 post-processing pipelines and demonstrated as RSEM (RNA-Seq by Expectation-Maximization) normalized count data. The miRNA expression data (level 3) were collected by GA as well as HiSeq 2000 sequencing platform (Illumina Inc, San Diego, CA, USA) and demonstrated as reads per million miRNA (RPM) mapped data.

Clinical information of the cases was also collected, including gender, anatomic organ subdivision, location lung parenchyma, tumor status, AJCC tumor pathologic pT, AJCC nodes pathologic pN, AJCC metastasis pathologic pM, AJCC pathologic tumor stage, EGFR mutation status, EML4-ALK translocation status, tobacco smoking history indicator, new tumor event dx indicator and vital status.

Pre-treatment of raw data

Low-abundance RNA and miRNA were removed. RNA with expression value >1 in 70% samples were retained and miRNA with expression value >10 in 80% samples were retained.

Human gene information was downloaded from The HUGO Gene Nomenclature Committee (HGNC, http://www.genenames.org/). Then transcripts were divided into two groups using a perl transcript: protein-coding gene and non-coding RNA. Genes without annotations from HGNC were delivered to Ref Gene to retrieve annotations.

Screening of Differentially Expressed lncRNAs and miRNAs

Differentially expressed lncRNAs (DELs) and miRNAs (DEMs) between LUAD and normal samples, or associated with clinical features were screened by two sample *t*-test. |Fold change| > 2 and p-value < 0.01 were set as the cutoffs.

Survival Analysis

Univariate Cox regression was performed to analyze the correlation between lncRNA and survival time. p-value < 0.05 was set as the cut-off.

Prediction of Target lncRNAs of miRNAs

Information about miRNA-target gene was downloaded from miRcode (http://www.mircode.org/)[12] and miRNA-lncRNA interactions were selected out. The DELs and DEMs were mapped into the interactions. Thus, LUAD-specific miRNA-lncRNA interactions were revealed. In addition, potential regulationship between miRNAs and lncRNAs were also predicted by starBase v2.0 (http://starbase.sysu.edu.cn/index.php)[13].

Prediction of Target mRNAs of mRNAs

Target mRNAs were predicted for DEMs using miRTarBase (http://mirtarbase.mbc.nctu.edu.tw/)[14]. Confident miRNA-mRNA interactions that were validated by at least two experimental methods (assay, western blot, qPCR, microarray, pSILAC and NGS) were selected out and then DEMs were mapped into the interactions.

Construction of the Regulatory Network

The miRNA-lncRNA-mRNA regulatory network was constructed with above interactions. Maximal information coefficient (MIC) method[15] was adopted to filter the network in which MIC > 0.17 as well as MIC-p2 > 0.17 were set as the cut-offs. The network was visualized with Cytoscape software (http://cytoscape.github.io/).

Table I. Correlation between differentially expressed lncRNAs and clinical features.

Comparisons	Down-regulated	Up-regulated
Gender (male vs. female)	NA	MEG3
Anatomic organ subdivision (R vs. L)	NA	NA
Location lung parenchyma (Central vs. Peripheral)	NA	NA
Tumor status (tumor free vs. with tumor)	NA	NA
AJCC tumor pathologic pT (T3+T4 vs. T1 + T2)	NA	MEG3, MIAT
AJCC nodes pathologic pN (N0 vs. N1+N2+N3)	NA	NA
AJCC pathologic tumor stage (S1+S2 vs. S3+S4)	NA	NA
EGFR mutation status (yes vs. no)	NA	NA
EML4 ALK translocation status (yes vs. no)	NA	MEG3
Tobacco smoking history (yes vs. no)	NA	NA
New tumor event dx indicator (yes vs. no)	NA	MIR4697HG

Functional Enrichment Analysis

Functional enrichment analysis, including Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Gene Ontology (GO) Biological Processes term, was performed for the genes from the regulatory network with DAVID (Database for Annotation, Visualization and Integration Discovery, https://david.ncifcrf.gov/)[16]. p-value < 0.05 was set as the threshold.

Regulatory Modules

lncRNAs and directly connected miRNAs, as well as mRNAs directly linked to the miRNAs constituted a regulatory module. The modules were identified by Cytoscape software (http://cytoscape.github.io/).

Results

Pre-treated Data

RNA-seq data contained 575 samples and 20,532 RNAs. GA miRNASeq data included 504 samples while HiSeq miRNASeq contained 63 samples. Both datasets detected 1,046 miRNAs. To ensure the reliability of the research, a total of 532 matched RNA-seq data and miRNA-seq were selected out. Subsequently, two distant metastatic samples and 4 repeated samples were removed, ultimately leading to 507 LUAD and 19 normal samples obtained for the following analysis.

Since they were already normalized by TCGA, no further normalization process was applied to these data. Finally, a total of 13,863 RNA and 286 miRNAs were acquired. Among the 13,863 RNA transcripts, 13,299 and 202 were identified as protein-coding genes and long non-coding genes, respectively. These 13,501 genes were used to construct the miRNA-lncRNA-mRNA network.

Key DELs

A total of 57 DELs and 118 DEMs were identified in LUAD samples compared with normal samples (Table I). Three DELs, including maternally expressed 3 (MEG3), myocardial infarction associated transcript (MIAT) and MIR4697HG, were significantly associated with clinical features. MEG3 was significantly up-regulated in patients with the male sex, advanced T category (T3 + T4), and EML4-ALK translocation. The expression of MIAT was higher in patients with advanced T category (T3 + T4). MIR4697HG was expressed at higher levels in the relapsed patients.

Univariate Cox regression was performed to identify prognostic lncRNAs from the 202 DELs. A total of 34 DELs were shown to be significantly associated with survival time, in which 9 lncRNAs (LINC00115, LINC00265, LINC01001, LINC01002, MIR22HG, NFYC−AS1, SNHG10, THUMPD3−AS1 and TMPO−AS1) were differentially expressed (Table II, Figure 1).

Regulatory Network

A total of 1,631 miRNA-lncRNA interactions were obtained from miRode and 372 interactions were acquired from starBase v2.0. These two groups of interactions were combined and 1,955 miRNA-lncRNA interactions were obtained. DEMs were mapped into the interactions and 778 LUAD specific miRNA-lncRNA interactions were revealed.

A total of 11,576 miRNA-mRNA interactions were collected from miRTarBase and DEMs were then mapped, resulting in 2,833 interactions. Combined with miRNA-lncRNA, miRNA-mR-

Table II. Prognostic lncRNAs differentially expressed in lung adenocarcinoma.

lncRNA	*p*-value (Cox *p*-value)	Up- or down-regulated	Fold change	*p*-value (dif-exp)
LINC00115	0.0257	Up	3.55	2.74E-31
LINC00265	0.0476	Up	2.68	1.67E-11
LINC01001	0.0169	Up	2.33	1.33E-10
LINC01002	0.0393	Up	2.27	4.42E-09
MIR22HG	0.0406	Down	0.35	2.23E-04
NFYC−AS1	0.0086	Up	2.75	9.97E-16
SNHG10	0.0122	Up	2.12	6.18E-11
THUMPD3−AS1	0.0282	Up	2.22	5.24E-18
TMPO−AS1	0.0140	Up	2.67	2.97E-29

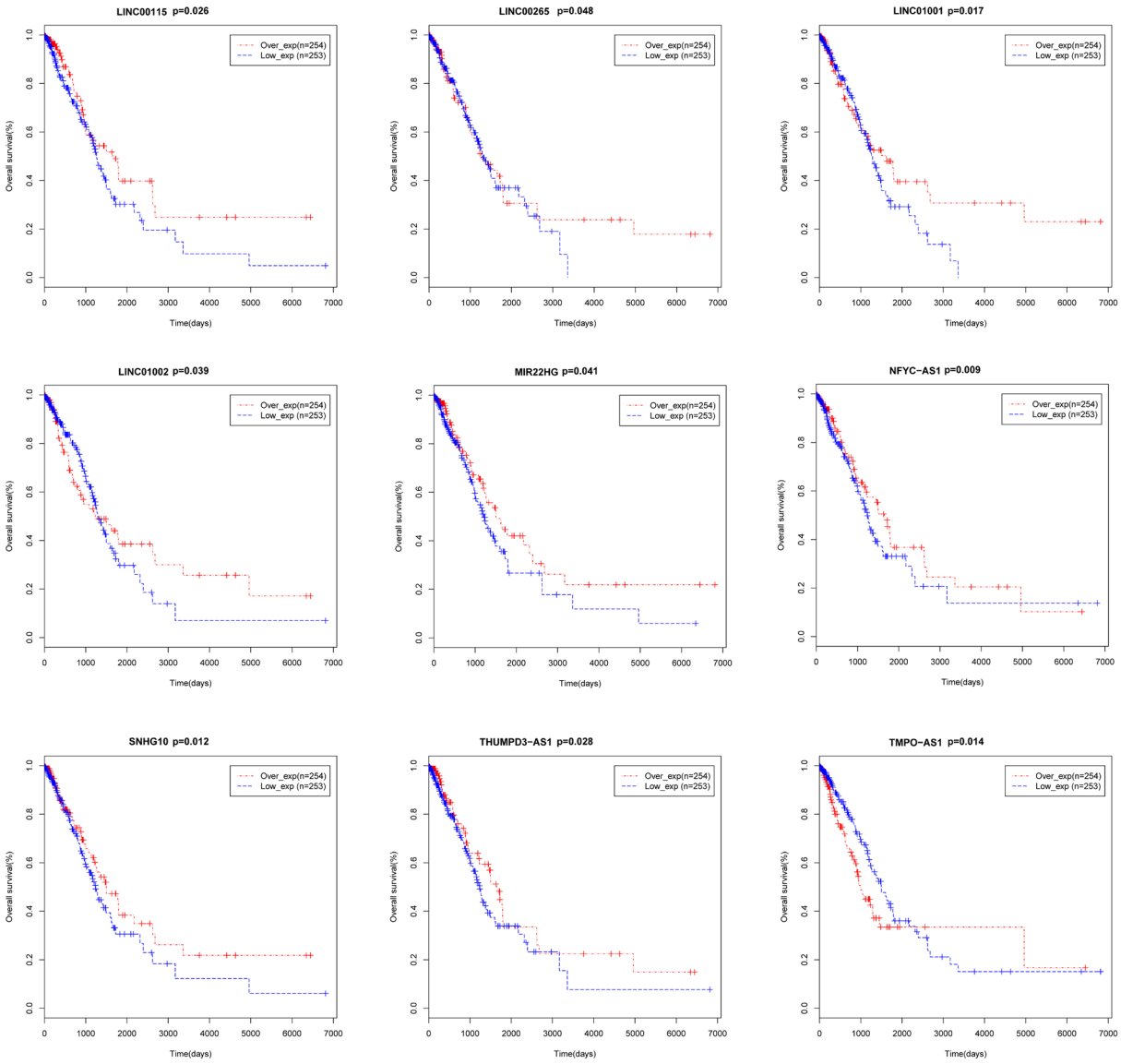

Figure 1. Kaplan-Meier survival curves for nine lncRNAs.

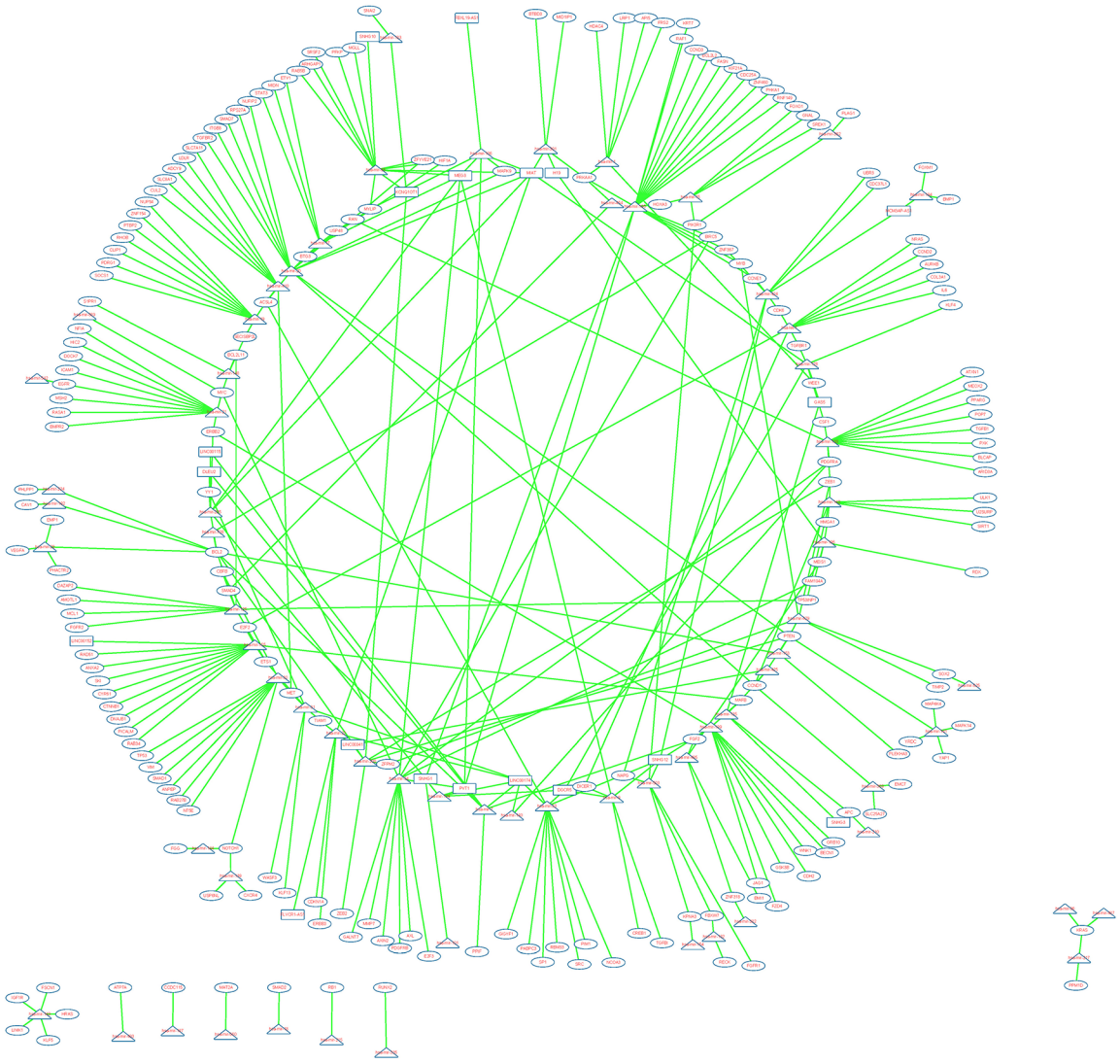

Figure 2. miRNAs-lncRNAs-mRNAs network of lung adenocarcinoma.

NA interactions, 389 miRNA-target genes were screened out, including 61 miRNA-lncRNA interactions and 304 miRNA-mRNA interactions. The regulatory network was then visualized with Cytoscape (Figure 2).

Functional enrichment analysis revealed that the genes in the regulatory network may play important roles in LUAD by involving in 642 significant GO terms (Table III) and 44 KEGG pathways (Table IV), such as pathways in cancer (hsa05200) and MAPK signaling pathway (hsa04010).

Regulatory Modules

Nineteen lncRNA-miRNA-mRNA regulatory modules, including lncRNA MEG3, MIAT, PVT1, DGCR5, DLEU2, FBXL19-AS1, FLVCR1-AS1, GAS5, H19, KCNQ1OT1, LINC00115, LINC00152, LINC00174, LINC00341, MCM3AP-AS1, SNHG1, SNHG3 and SHG10, were identified from the network. Among them, both MEG3 (Figure 3) and MIAT (Figure 4) may exert their roles in LUAD by interactions with miR-106 which then regulated the target mRNA MAPK9 (mitogen-activated protein kinase 9). LINC00115 might function in LUAD through interaction with miR-7 which then regulated the target mRNA FGF2 (fibroblast growth factor 2) (Figure 5).

Discussion

By constructing the miRNA-lncRNA-mRNA network, our findings indicate that MEG3 and

Table III. Top 10 enriched GO biological processes terms of the genes in miRNA-lncRNA-mRNA network of lung adenocarcinoma

Term	Count	Genes	*p* value
GO:0042127~Regulation of cell proliferation	60	BMI1, HRAS, E2F3, PPARG, MMP7, FOXO1, JAG1, ZEB1, PTEN, TGFB1, CTNNB1, CUL2, S1PR1, FGF2, MYC, EGFR, TP53, CDK6, RB1, PPM1D….	2.46E-28
GO:0010604~Positive regulation of macromolecule metabolic process	55	HRAS, E2F3, PPARG, FOXO1, ZEB1, CBFB, TGFB1, CTNNB1, CCNE1, S1PR1, YAP1, MYC, FGF2, RPS27A, RAN, TP53, RB1, HMGA1, RAD51, CCND1….	3.41E-22
GO:0051173~Positive regulation of nitrogen compound metabolic process	46	HRAS, E2F3, SOX2, PPARG, FOXO1, ZEB1, CBFB, TGFB1, CTNNB1, CCNE1, IGF1R, S1PR1, YAP1, RUNX2, MYC, FGF2, RPS27A, KLF5, EGFR, ICAM1….	3.75E-20
GO:0045935~Positive regulation of nucleobase, nucleoside, nucleotide and nucleic acid metabolic process	44	HRAS, E2F3, SOX2, PPARG, FOXO1, ZEB1, CBFB, TGFB1, CTNNB1, CCNE1, IGF1R, S1PR1, YAP1, RUNX2, MYC, FGF2, RPS27A, KLF5, IL6, MAFB….	5.05E-19
GO:0009891~Positive regulation of biosynthetic process	46	HRAS, E2F3, SOX2, PPARG, FOXO1, ZEB1, CBFB, TGFB1, CTNNB1, CCNE1, IGF1R, S1PR1, PRKAA1, YAP1, RUNX2, MYC, FGF2, RPS27A, KLF5, EGFR….	7.41E-19
GO:0010628~Positive regulation of gene expression	42	E2F3, CSF1, SOX2, PPARG, FOXO1, ZEB1, CBFB, TGFB1, CTNNB1, CCNE1, S1PR1, YAP1, RUNX2, MYC, FGF2, RPS27A, KLF5, IL6, MAFB, RAN….	1.65E-18
GO:0031328~Positive regulation of cellular biosynthetic process	45	HRAS, E2F3, SOX2, PPARG, FOXO1, ZEB1, CBFB, TGFB1, CTNNB1, CCNE1, IGF1R, S1PR1, YAP1, RUNX2, MYC, FGF2, RPS27A, KLF5, EGFR, ICAM1…..	2.65E-18
GO:0001568~Blood vessel development	29	CAV1, DICER1, COL3A1, FOXO1, ANPEP, JAG1, CDH2, PTEN, CTNNB1, S1PR1, HOXA3, CXCR4, RHOB, FGF2, RASA1, CYR61, KLF5, RECK, SMAD7, TGFBR1….	3.56E-18
GO:0007167~Enzyme linked receptor protein signaling pathway	33	FGFR2, FGFR1, ERBB3, ERBB2, COL3A1, BMPR2, FOXO1, PTEN, SRC, TGFB1, IGF1R, TIAM1, FRS2, FGF2, GIGYF1, PIK3R1, EGFR, SMAD7, CREB1….	4.64E-18
GO:0001944~Vasculature development	29	CAV1, DICER1, COL3A1, FOXO1, ANPEP, JAG1, CDH2, PTEN, CTNNB1, S1PR1, HOXA3, CXCR4, RHOB, FGF2, RASA1, CYR61, KLF5, RECK, SMAD7….	6.79E-18
GO:0010557~Positive regulation of macromolecule biosynthetic process	43	E2F3, HRAS, SOX2, PPARG, FOXO1, ZEB1, CBFB, TGFB1, CTNNB1, CCNE1, IGF1R, S1PR1, YAP1, RUNX2, MYC, FGF2, RPS27A, KLF5, IL6, MAFB…..	1.81E-17
GO:0045941~Positive regulation of transcription	40	E2F3, SOX2, PPARG, FOXO1, ZEB1, CBFB, TGFB1, CTNNB1, CCNE1, S1PR1, YAP1, RUNX2, MYC, FGF2, RPS27A, KLF5, IL6, MAFB, RAN, KLF13….	2.58E-17
GO:0045893~Positive regulation of transcription, DNA-dependent	37	E2F3, SOX2, PPARG, FOXO1, ZEB1, CBFB, TGFB1, CTNNB1, CCNE1, S1PR1, YAP1, MYC, FGF2, RUNX2, IL6, RAN, KLF13, MAFB, CREB1, SMAD4….	3.27E-17
GO:0051254~Positive regulation of RNA metabolic process	37	E2F3, SOX2, PPARG, FOXO1, ZEB1, CBFB, TGFB1, CTNNB1, CCNE1, S1PR1, YAP1, MYC, FGF2, RUNX2, IL6, RAN, KLF13, MAFB, CREB1, SMAD4….	4.26E-17
GO:0048514~Blood vessel morphogenesis	26	CAV1, DICER1, ANPEP, JAG1, CDH2, PTEN, CTNNB1, S1PR1, HOXA3, CXCR4, RHOB, FGF2, RASA1, CYR61, KLF5, SMAD7, TGFBR1, TGFBR2, ANXA2, ATP7A, NOTCH1….	1.07E-16
GO:0045944~Positive regulation of transcription from RNA polymerase II promoter	32	SOX2, PPARG, FOXO1, ZEB1, TGFB1, CBFB, CTNNB1, S1PR1, YAP1, MYC, FGF2, RUNX2, IL6, KLF13, MAFB, CREB1, SMAD4, TP53, SMAD2, RB1…..	5.09E-16
GO:0042325~Regulation of phosphorylation	35	CAV1, ERBB2, CSF1, BMPR2, ZEB2, PTEN, TGFB1, KRAS, CXCR4, BCL2, PRKAA1, FRS2, FGF2, APC, EGFR, IL6, SMAD7, TGFBR1, MET, SOCS1…..	8.73E-16
GO:0008284~Positive regulation of cell proliferation	33	BMI1, FGFR2, FGFR1, E2F3, HRAS, ERBB2, CSF1, SOX2, TGFB1, IGF1R, KRAS, S1PR1, HOXA3, BCL2, MYC, FGF2, RUNX2, KLF5, EGFR, IL6, TGFBR1…..	1.40E-15
GO:0051174~Regulation of phosphorus metabolic process	35	CAV1, ERBB2, CSF1, BMPR2, ZEB2, PTEN, TGFB1, KRAS, CXCR4, BCL2, PRKAA1, FRS2, FGF2, APC, EGFR, IL6, SMAD7, TGFBR1, MET, SOCS1, TGFBR2….	2.94E-15

Table IV. Top 20 enriched KEGG pathways in miRNA-lncRNA-mRNA network of lung adenocarcinoma.

Term	Count	Genes	*p* value
hsa05200:Pathways in cancer	47	FGFR2, E2F2, FGFR1, HRAS, E2F3, ERBB2, PPARG, FOXO1, PTEN, TGFB1, CTNNB1, CCNE1, IGF1R, CUL2, KRAS, BCL2, AXIN2, MYC, FGF2, PIK3R1….	7.46E-29
hsa05210:Colorectal cancer	25	EGFR, MSH2, TGFBR1, MET, TGFBR2, TP53, SMAD4, RAF1, BIRC5, SMAD2, FZD4, TGFB1, CTNNB1, IGF1R, CCND1, KRAS, GSK3B, BCL2, PDGFRA, PDGFRB….	2.52E-22
hsa05215:Prostate cancer	25	FGFR2, E2F2, FGFR1, HRAS, E2F3, ERBB2, FOXO1, PTEN, CTNNB1, IGF1R, CCNE1, KRAS, BCL2, PIK3R1, EGFR, CREB1, TP53, RAF1, RB1, NRAS….	1.17E-21
hsa05218:Melanoma	20	EGFR, E2F2, FGFR1, HRAS, E2F3, MET, TP53, RAF1, CDK6, RB1, PTEN, NRAS, IGF1R, CCND1, CDKN1A, KRAS, PDGFRA, PDGFRB, FGF2, PIK3R1	3.51E-17
hsa05212:Pancreatic cancer	20	EGFR, E2F2, E2F3, ERBB2, TGFBR1, TGFBR2, TP53, SMAD4, RAF1, CDK6, SMAD2, RB1, STAT3, TGFB1, RAD51, CCND1, KRAS, VEGFA, MAPK9, PIK3R1	4.68E-17
hsa05214:Glioma	17	EGFR, E2F2, HRAS, E2F3, TP53, RAF1, CDK6, RB1, PTEN, NRAS, IGF1R, CCND1, CDKN1A, KRAS, PDGFRA, PDGFRB, PIK3R1	3.49E-14
hsa05219:Bladder cancer	14	EGFR, E2F2, E2F3, HRAS, ERBB2, TP53, RAF1, RB1, NRAS, CCND1, CDKN1A, KRAS, VEGFA, MYC	6.16E-13
hsa05213:Endometrial cancer	15	EGFR, HRAS, ERBB2, TP53, RAF1, PTEN, CTNNB1, NRAS, CCND1, KRAS, GSK3B, AXIN2, MYC, PIK3R1, APC	6.49E-13
hsa05220:Chronic myeloid leukemia	17	E2F2, HRAS, E2F3, TGFBR1, TGFBR2, TP53, SMAD4, RAF1, CDK6, RB1, TGFB1, NRAS, CCND1, CDKN1A, KRAS, MYC, PIK3R1	6.70E-13
hsa04510:Focal adhesion	22	EGFR, HRAS, CAV1, ERBB2, COL3A1, MET, RAF1, PTEN, SRC, CTNNB1, IGF1R, CCND1, CCND3, CCND2, ITGB8, GSK3B, BCL2, VEGFA, PDGFRA, MAPK9….	2.37E-10
hsa05223:Non-small cell lung cancer	13	EGFR, E2F2, NRAS, HRAS, E2F3, CCND1, KRAS, ERBB2, TP53, RAF1, CDK6, RB1, PIK3R1	3.76E-10
hsa04110:Cell cycle	17	E2F2, E2F3, TP53, SMAD4, CDK6, SMAD2, RB1, TGFB1, WEE1, CDC25A, CCNE1, CCND1, CDKN1A, CCND3, CCND2, GSK3B, MYC	2.17E-09
hsa04520:Adherens junction	13	EGFR, IGF1R, FGFR1, WASF3, TGFBR1, ERBB2, TGFBR2, MET, SMAD4, SMAD2, SNAI2, SRC, CTNNB1	2.75E-08
hsa04012:ErbB signaling pathway	13	EGFR, HRAS, ERBB3, ERBB2, RAF1, SRC, NRAS, CDKN1A, KRAS, GSK3B, MAPK9, MYC, PIK3R1	1.12E-07
hsa05211:Renal cell carcinoma	11	NRAS, CUL2, HRAS, HIF1A, KRAS, ETS1, MET, VEGFA, RAF1, PIK3R1, TGFB1	9.87E-07
hsa05216:Thyroid cancer	8	NRAS, HRAS, CCND1, KRAS, PPARG, TP53, MYC, CTNNB1	1.15E-06
hsa04010:MAPK signaling pathway	19	EGFR, FGFR2, FGFR1, HRAS, TGFBR1, TGFBR2, TP53, RAF1, TGFB1, MAP4K4, NRAS, KRAS, MAPK14, PDGFRA, MAPK9, PDGFRB, FGF2, MYC, RASA1	4.34E-06
hsa05222:Small cell lung cancer	11	E2F2, CCNE1, E2F3, CCND1, BCL2, TP53, CDK6, RB1, MYC, PTEN, PIK3R1	5.44E-06
hsa04310:Wnt signaling pathway	14	TP53, SMAD4, MMP7, SMAD2, FZD4, CTNNB1, CCND1, CCND3, CCND2, GSK3B, MAPK9, AXIN2, MYC, APC	7.46E-29

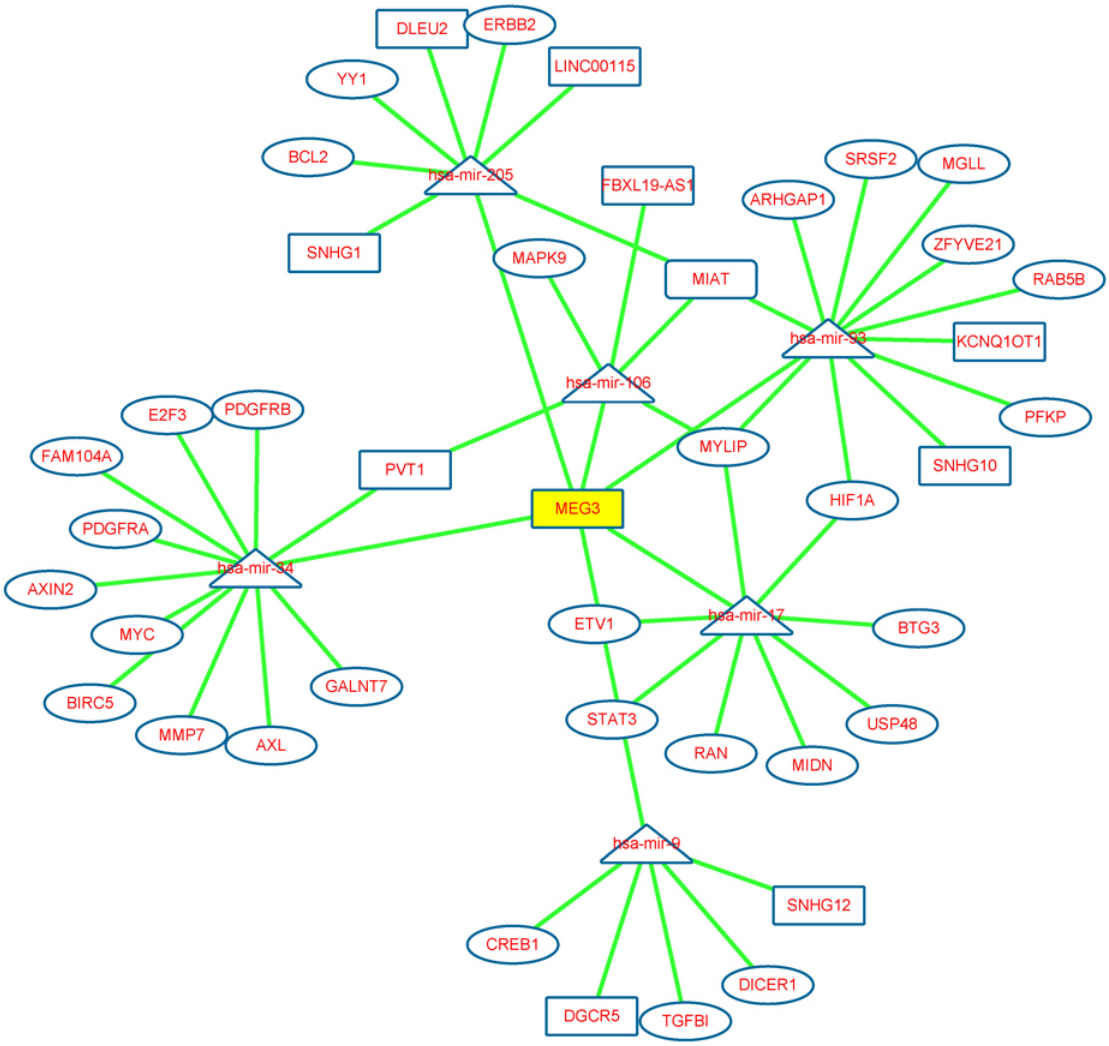

Figure 3. The regulatory module of MEG3.

MIAT, tumor proliferation related lncRNAs, may play important roles in the development of LUAD by interactions with hsa-mir-106, which then regulated the MAPK9 to involve in MAPK signaling pathways. LINC00115 might be a prognosis biomarker in LUAD through interactions with hsa-mir-7 which then regulated FGF2 to participate in pathways in cancer.

Increasing evidence has demonstrated that MEG3 acts as a tumor suppressor gene to inhibit tumor cell proliferation and induce apoptosis. Down-regulation of MEG3 contributes to chemotherapy resistance and is associated with poor prognosis[17-20]. Further function and molecular mechanism study indicate that MEG3 may function as a ceRNA to bind with miR-181s and prevent the inhibition effect of miR-181s for Bcl-2, ultimately leading to the up-regulation of Bcl-2 and subsequent suppressing gastric carcinogenesis[21]. Similarly, Zhang et al[22] also find that over-expression of MEG3 reduces the level of miR-21-5p expression, causing decreased proliferation and increased apoptosis in cervical cancer cells. However, in this study, we observed a significantly up-regulated expression of MEG3 in LUAD, indicating its carcinogenic role. Although this conclusion still needs further experimental confirmation, we believe it is possible because we predict that MEG3, as a ceRNA, may down-regulate miR-106 and then up-regulate MAPK9 to promote the development of lung cancer, while the same expression trends of hsa-mir-106 and MAPK9 has been proved in several cancers. For example, Ni et al[23] report that miR-106b can be markedly down-regulated during breast cancer bone metastasis. The lower expression of miR-106b leads to the abundant accu-

mulation of MMP2, which then activates the ERK signaling cascade and adjusts the bone microenvironment to favor osteoclastogenesis and bone metastasis. Zheng et al[24] also show down-regulation of hsa-mir-106 induces epithelial-mesenchymal transition which confers cells migratory and invasive properties. As a kinase, MAPK9 fulfills its purpose by phosphorylating diverse substrates, such as c-Jun which has been considered as a proto-oncogene to promote cancer cells proliferation and migration[25]. Targeted inhibition of JNK2/ c-Jun signaling pathway increases the sensitivity to chemotherapeutic drugs and heightens cell apoptosis, achieving the therapeutic aim for colorectal cancer cells[26].

MIAT is a lncRNA that was initially identified to be associated with myocardial infarction[27], but recent studies have implicated that MIAT is also involved in paranoid schizophrenia[28], diabetes-related diseases[29,30] and cancer[31,32]. lncRNA MIAT is shown to be highly expressed in diabetic retinas and endothelial cells cultured in high glucose medium. Silencing of MIAT significantly inhibits endothelial cell proliferation, migration and ameliorates diabetes-induced retinal microvascular dysfunction[29], indicating a cancer-promoting gene. This hypothesis has been demonstrated by several studies[32,33] to screen lncRNA associated with cancer pathogenesis, in which MIAT is highly upregulated. However, the expression and mechanism of MIAT involved in lung cancer remain unclear. As reported by Yan et al[29] we predicted MIAT may function as a competing endogenous RNA to form a feedback loop with MAPK9 and miR-106 to regulate proliferation, invasion and migration of lung cancer cells, which, to our knowledge, has not been reported and needs further confirmation.

LINC00115 is a lncRNA fewly reported, except one study of Zhang et al[34] which revealed down-regulated LINC00115 may be specific for lung squamous carcinoma (SCC). However, in this study, we found LINC00115 was up-regulated in LUAD (fold change, 3.55; *p*-value, 2.74E-31). This finding illustrates LINC00115 seem to be a potential biomarker to distinguish LUAD from SCC. However, the expression and mechanism

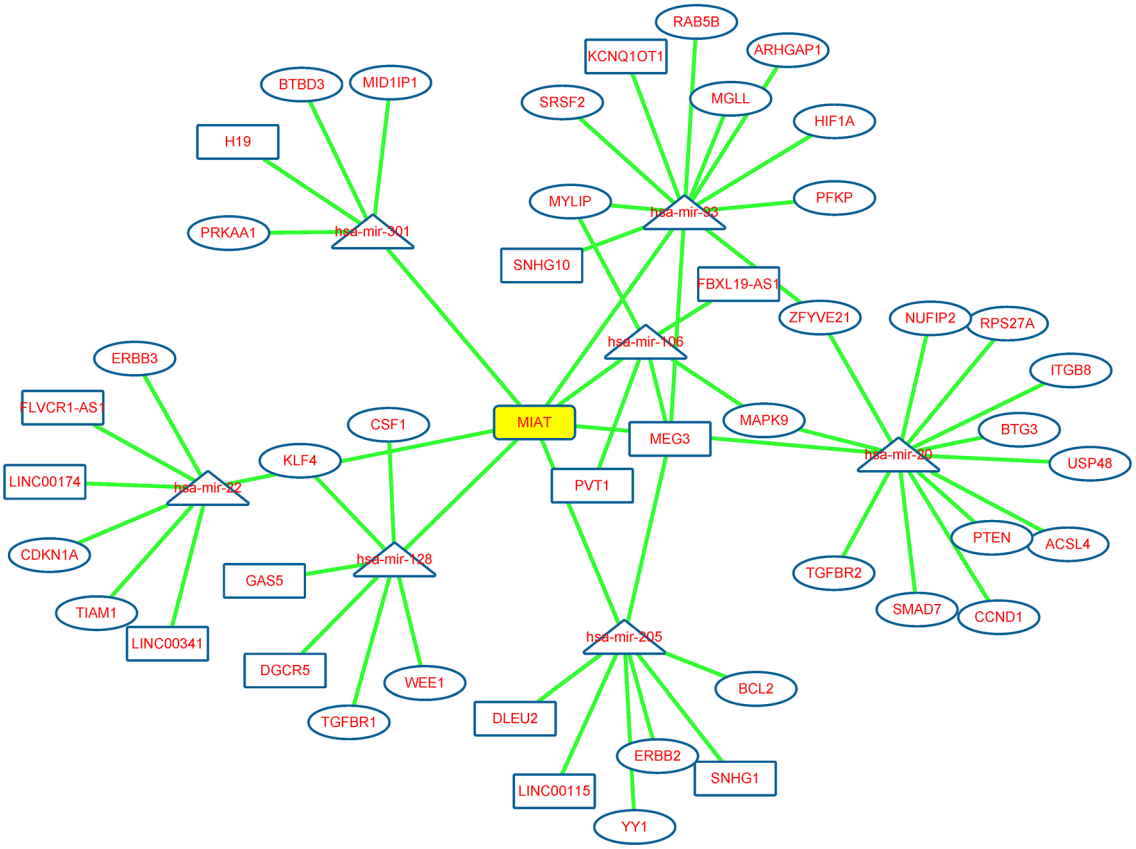

Figure 4. The regulatory module of MIAT.

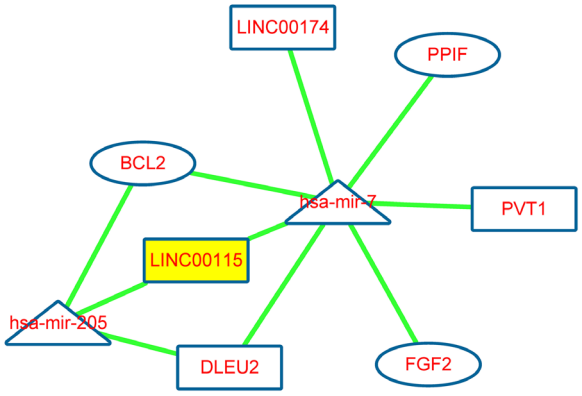

Figure 5. The regulatory module of LINC00115.

of MIAT involved in lung cancer remain unclear. In the present study, we supposed LINC00115 might participate in LUAD through competitive interactions with miR-7, leading to the low expression of hsa-mir-7 followed by up-regulating FGF2. A large amount of research has documented that miR-7 is down-regulated in cancer specimens and cell lines. miR-7 overexpression inhibited cell proliferation, invasion and metastasis, but induced cell apoptosis, in which epidermal growth factor receptor (EGFR) expression suppression mechanism was involved[35,36]. Similar to the EGFR growth factor, FGFR also promotes cell malignant transformation after combination with its ligand FGF2[37]. Thus, FGF2 concentration is commonly elevated in cancer compared with control, including lung cancer. Patients with higher FGF-2 level exhibited significantly shorter survival than patients with low FGF-2 (7.5 months vs. 16 months, $p=0.034$)[38]. However, the investigation of the regulatory relationship among LINC00115, FGF2 and miR-7 remains necessary.

Conclusions

We have identified several key lncRNAs (MEG3, MIAT, LINC00115) associated with LUAD and disclosed they exert important roles in carcinogenesis as ceRNAs for regulation of miRNA-mRNA network. Our findings may propose several new prognostic markers as well as therapeutic targets for LUAD.

Conflicts of interest

The authors declare no conflicts of interest.

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