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ORIGINAL ARTICLE

Differential Analysis of lncRNA, miRNA and mRNA Expression Profiles and the Prognostic Value of lncRNA in Esophageal Cancer

Hongtao Liu[1] · Qing Zhang[1] · Qianqian Lou[1] · Xin Zhang[1] · Yunxia Cui[1] · Panpan Wang[1] · Fan Yang[1] · Fan Wu[1] · Jing Wang[1] · Tianli Fan[2] · Shenglei Li[3]

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Abstract

Integrative central axis of lncRNA-miRNA-mRNA plays pivotal roles in tumor development and progression. However, the regulatory role of lncRNA-miRNA-mRNA in esophageal cancer remains elusive. TCGA database was utilized to investigate the differential expression of lncRNA, miRNA and mRNA in esophageal cancer (ESCA) and normal esophageal tissues, and GEO database was used to further validate the expression profile of key genes. Differential lncRNAs in TCGA database were submitted to Starbase, and lncRNAs related to overall survival were analyzed using Kaplan-Meier and log-rank test. We found 145 lncRNAs, 112 miRNAs and 2000 protein coding mRNAs were differentially expressed in ESCA samples, which were tightly involved in chromosome segregation, extracellular matrix assembly by GO assay, and KEGG assay revealed the correlation of differentially expressed genes with cell cycle, apoptosis and cGMP-PKG signaling pathway. Furthermore, there were 291 nodes in ceRNA network, which consisted of 40 lncRNAs, 28 miRNAs and 233 mRNAs, and formed 677 relations. Furthermore, 6 of 10 lncRNAs in TCGA database were consistent with GEO database, and expressions of 10 mRNAs in TCGA database all exhibited the same tendency with GEO database. Notably, we found 8 lncRNAs (WDFY3-AS2, CASC8, UGDH-AS1, RAP2C-AS1, AC007128.1, AC016205.1, AC092803.2 and AC079949.2) were correlated with overall survival of the patients with ESCA. The key differentially expressed genes participate in the development and progression of ESCA, and thus the elucidation of functions of lncRNA-miRNA-mRNA will provide new novel therapeutic target for the patients with ESCA.

Keywords Long chain non-coding RNA · MicroRNA · Messagers RNA · Differential expression · ceRNA · Esophageal cancer

Introduction

Esophageal cancer (ESCA), as one of high aggressive neoplasms, is the sixth most deadly tumor in the world [1]. At present, there were two major histological types in ESCA, which comprises of esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (ECA) [2, 3]. ESCC as main histological type of ESCA mainly occurs in Asian, accounting for more than 90% of ESCA, whereas ECA mainly appears in North America and Europe [4]. Due to lack of early diagnostic strategy, the patients with ESCA exhibits at a late stage in the present of diagnosis and their 5-year survival rate is extremely poor [5]. Therefore, it is very necessary to seek for novel biomarker and therapeutic target for the patients with ESCA.

System biology based on gene expression profiles as a whole will provide new strategy for seeking for novel molecular target and prognostic factors. Increasing evidence have

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✉ Hongtao Liu
liuht1230@126.com

✉ Tianli Fan
fantianlipp@163.com

✉ Shenglei Li
lslbljys@126.com

[1] College of Life Sciences, Zhengzhou University, 100 Kexue Road, Zhengzhou 450001, People's Republic of China

[2] Department of Pharmacology, School of Basic Medicine, Zhengzhou University, 100 Kexue Road, Zhengzhou 450001, Henan, China

[3] Department of Pathology, The First Affiliated Hospital of Zhengzhou University, 40 Daxue Road, Zhengzhou 450052, Henan, China

revealed that non-coding RNAs (ncRNAs) and protein coding mRNA play a pivotal in many important biological processes, including cell proliferation, differentiation, gene expression, cell cycle, apoptosis, mesenchymal transition, migration and invasion [6–8], especially in tumorigenesis [9]. MicroRNAs (miRNAs) and long non-coding RNAs are one of the most two typical ncRNA subtypes, the former is a class of ncRNAs with approximately 22 nucleotide, which interact with mRNA to degrade mRNAs or inhibit the translation of mRNA [10], and the latter harboring more than 200 nucleotides functions in a large number of diverse mechanisms [11], such as RNA decay, miRNA sponging, transcription regulation, epigenetic modification, etc. [12, 13]. At present, lncRNA-miRNA-mRNA network has been verified to play an essential role in tumor development and progression, including lung cancer [14], pancreatic cancer [15, 16], gastric carcinoma [17], etc., which may exhibit gigantic clinical prospect for identifying potential biomarkers and therapeutic target for many various type of tumors.

To further understand the possible etiology of ESCA and seek for new molecular target, we performed the TCGA database assay for seeking for differentially expressed genes of lncRNAs (161 tumor samples vs 11 normal samples), miRNAs (184 tumor samples vs 13 normal samples) and mRNAs (161 tumor samples vs 11 normal samples) in ESCA and normal samples. Gene ontology (GO) and KEGG assay was used to interrogate the functions of lncRNA, miRNA and mRNA in ESCA. Furthermore, ceRNA network of lncRNA-miRNA-mRNA in ESCA was also investigated to establish the correlations of lncRNA, miRNA and mRNA. Besides, GEO database (GSE89102 datasets) was utilized to validate the data of TCGA. Finally, we found 8 lncRNAs (WDFY3-AS2, CASC8, UGDH-AS1, RAP2C-AS1, AC007128.1, AC016205.1, AC092803.2 and AC079949.2) were correlated with overall survival of the patients with ESCA. Taken together, our data presented herein will provide the novel experimental evidence for further elucidation of lncRNA-miRNA-mRNA functions in ESCA.

Materials and Methods

Data Download

The data of ESCA was downloaded using GDCRNATools [18]. All data comprised of the following three parts: (1) Gene expression profile data: the data (read count) derived from RNA-seq was treated using Htseq [19], which was named as gene ID Ensembl (v 90). Gene types comprised protein_coding, long_non_coding, pseudogene, TEC (To be Experimentally Confirmed), ncRNA, IG (Immunoglobulin gene), etc. A total of 172 samples were obtained, including 161 primary tumors and 11 normal samples. (2) miRNA expression profile data: the data (read count) derived from miRNA-seq was treated using Htseq [19], which was named using miRbase (v21). A total of 197 samples were obtained, including 184 primary tumors and 13 normal samples.

Data Pretreatment and Analysis of Differential Expression

Raw data were standardized using CPM (count per millions) provided by edgeR [20], and more than 50% of expression abundance ratio of gene/miRNA (CPM > 1) was saved, which will be used to investigate the differential expression genes. The differential expression analysis was performed between primary tumor and normal samples using likelihood ratio tests provided by edgeR, which will obtain differential expression gene or miRNA by setting $|\log FC| > 1 \& FDR < 0.05$. All differential expression genes were displayed using heatmap and volcano map.

Prediction of Regulation Correlation of lncRNA, miRNA and mRNA

Three databases (Starbase [21], miRcode [22], mirTarbase [23]) were used to verify the miRNA-protein coding gene regulation correlation. Meanwhile, three databases (Starbase [21], miRcode [22] and spongScan [24]) were utilized to confirm the regulatory correlation of miRNA-lncRNA.

Prediction of ceRNA Network and GO and KEGG Assay

Protein coding genes in ceRNA network was enriched by GO (Gene Ontology, http://www.geneontology.org/) and KEGG database (Kyoto encyclopedia of genes and genomes) using clusterProfiler [25], which was used to indirectly predict the function of ceRNA network. Similarly, according to enrichment analysis of protein coding gene related to lncRNA or miRNA, the functions of lncRNA and miRNA were indirectly predicted. When deciphering ceRNA network, lncRNA or miRNA must correspond to more than 10 protein coding genes. A P value less than 0.05 was considered as significant enrichment.

The Validation of TCGA Database by GEO Datasets

GEO datasets (GSE89102) comprised of 5 ESCA samples and 5 normal samples, and data was treated using limma package [26]. The threshold adj.Pvalue <0.05 and $|\log FC| > 1$ were considered as differential expression genes. The differential results derived from TCGA and GEO were investigated using VENN (http://bioinfogp.cnb.csic.es/tools/venny/index.html) and the expression levels of key differential genes were compared.

Coexpression Assay of lncRNAs and mRNAs in ESCA

LncRNAs and mRNAs selected were used to construct the coexpression relations, and lncRNAs and mRNAs were submitted to Starbase [21] (http://starbase.sysu.edu.cn/panGeneDiffExp.php).

Construction of lncRNAs Related to Overall Survival of ESCA

Differential lncRNAs and mRNAs in TCGA database were submitted to Starbase [21] (http://starbase.sysu.edu.cn/panGeneDiffExp.php). LncRNAs and mRNAs related to overall survival were analyzed using Kaplan-Meier and log-rank test, and statistical significance was set at $P < 0.05$.

Results

Differential Expression of lncRNAs, miRNAs and mRNAs in ESCA

To obtain the differential expression profile of lncRNAs, miRNAs and mRNAs in ESCA, TCGA database was used to investigate the differential expression of lncRNAs, miRNAs and mRNAs in ESCA. A total of 145 lncRNAs were displayed, including 83 upregulations and 62 downregulation (Fig. 1). We found that 38 miRNAs were upregulated and 74 miRNAs were downregulated in ESCA (Fig. 1). Further investigations revealed that protein coding mRNAs were also differentially exhibited, comprised of 1011 upregulations and 989 downregulations (Fig. 1). In addition, the most significant genes of top 30 lncRNA, miRNA and mRNA was screened out using FC > 1 or $P < 0.05$ by heat map and volcano map (Supplementary Figure 1 and Supplementary Tables 1, 2 and 3).

GO and KEGG Assay for the Functions of lncRNA, miRNA and mRNA in ESCA

Gene ontology (GO) and KEGG assay were conducted out to reveal the functions of differentially expressed lncRNAs, miRNAs and mRNA in ESCA. Differentially expressed lncRNAs were involved in cell differentiation, chromosome segregation, protein kinase C and A signaling, extracellular matrix assembly, and chromatin remodeling at centromere, etc. Further investigation by KEGG revealed that lncRNAs participated in the regulations of Gap junction, carbon metabolism, phospholipase D signaling pathway, cell cycle, apoptosis and cGMP-PKG signaling pathway, etc. (Fig. 2). Corresponding miRNA function by GO assay was tightly associated with cell-substrate adhesion, chromosome segregation, epithelial cell maturation, the regulation of mitosis and spindle organization, anoikis, cell cycle G1/S phase transition and extracellular matrix assembly, etc., and KEGG assay demonstrated that miRNAs were related to mismatch repair, apoptosis, DNA replication, gap junction, tight junction, cGMP-PKG signaling pathway, and calcium signaling pathway, etc. (Fig. 2). Furthermore, GO assay revealed the correlation of protein coding mRNAs with sister chromatid segregation, cohesion, mitotic nuclear division, cell-cell junction, extracellular matrix binding, ect, whereas KEGG assay verified the association of protein coding mRNAs with cell cycle, estrogen signaling pathway, cGMP-PKG signaling pathway, tight junction, 2-oxocarboxylic acid metabolism and citrate cycle (TCA cycle), etc. (Fig. 2).

CeRNA Network of lncRNA-miRNA-mRNA Axis in ESCA

According to the prediction correlation of lncRNA, miRNA and mRNA, ceRNA network was obtained using ceRNA mechanism analysis (Fig. 3). There were 291 nodes in ceRNA network, which consisted of 40 lncRNAs, 28 miRNAs and 233 mRNAs, and formed 677 relations (Fig. 3). In addition, a total of top 15 of high nodes regarding lncRNAs, miRNAs and mRNAs were also displayed in Table 1, which will exhibit the important biological significance. ZNF667-AS1 and has-miR-1-3p have similar coding gene, suggesting their similar function, most importantly, ZNF667 may function as sponge of hsa-miR-1-3p, and further regulate the level of protein coding gene (Fig. 3 and Table 1). Among 15 high nodes, we found only hsa-miR-7-5p displayed significant prognosis value in ESCA, and the overall survival ratio of the patients with ESCA harboring low hsa-miR-7-5p level was significantly higher than that exhibiting high hsa-miR-7-5p level ($P = 0.034$) (Supplementary Figure 2).

Validation of lncRNAs and mRNAs Expression Profiles by GEO Database

Ten of expression profiles of Interest of differential genes were summarized in Fig. 4, GEO database was utilized to validate the data of TCGA database. We found that 6 of 10 lncRNAs (HNF1A-AS1, MEG3, RAB11B-AS1, SLC2A1-AS1, TMEM161B-AS1 and ZEB1-AS1) in TCGA database were consistent with GEO database, and expressions of 10 mRNAs including FOXA1, SNAI2, TMSB10, CENPQ, SPC25, CCNA2, RFC4, CKS1B, UBE2C and NEK2 in TCGA database all exhibited the same tendency with GEO database (Fig. 4, and Supplementary Tables 4 and 5).

Associations of lncRNAs with Overall Survival

Kaplan-Meier and log-rank test were employed to determine the relationship between lncRNAs and overall survival in ESCA. A total of 8 differential lncRNAs (WDFY3-AS2,

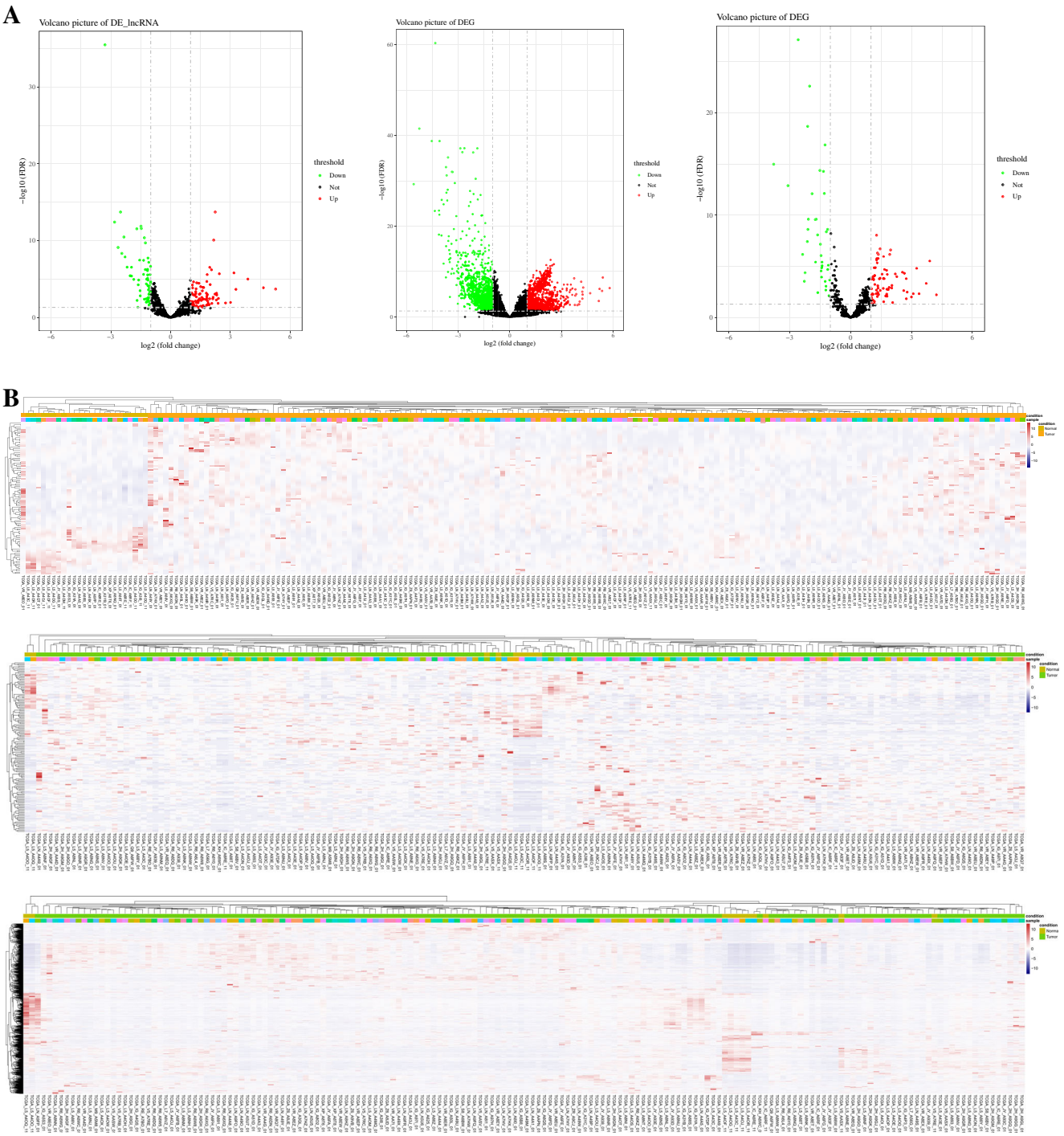

Fig. 1 Differential analysis of lncRNA, miRNA and mRNA in ESCA and normal esophageal tissues. **a** Volcano map analysis for differential expressions of lncRNA, miRNA and mRNA in ESCA, X axis indicates the mean expression differences of lncRNA, miRNA and mRNA, and Y axis represents log transformed false discovery rate (FDR) values. **b** Heatmap analysis for differential expressions of lncRNA, miRNA and mRNA in ESCA and normal samples, X axis represents the samples, and Y axis denotes differential expressions of lncRNAs, miRNAs and mRNAs

CASC8, UGDH-AS1, RAP2C-AS1, AC007128.1, AC016205.1, AC092803.2 and AC079949.2) were found to be correlated with overall survival of the patients with ESCA (Fig. 5). In addition, expression patterns of 8 differential lncRNAs were also investigated using starBase, we found WDFY3-AS2, UGDH-AS1 and RAP2C-AS1 were displayed in low expression level in ESCA samples, whereas CASC8, AC007128.1, AC016205.1, AC092803.2 and AC079949.2 were exhibited in high expression level in ESCA samples. However, prognostic assay revealed that the 10 mRNAs

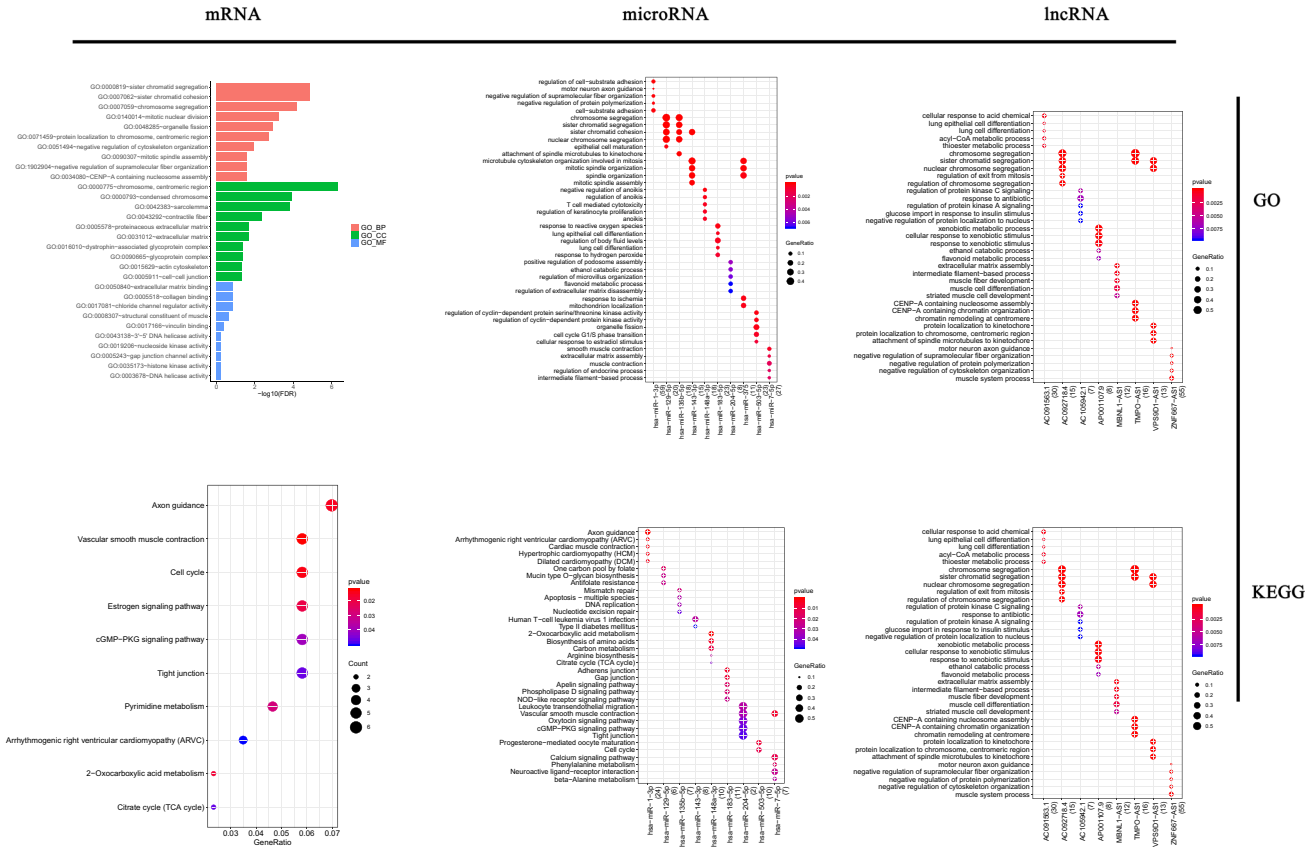

Fig. 2 GO terms and KEGG interpretation for functions of lncRNA, miRNA and mRNA in ESCA. BP: biological pathway; CC: cellular component; MF: molecular function

including CCNA2, CENPQ, CKS1B, FOXA1, NEK2, RFC4, SNAI2, SPC25, TMSB10 and UBE2C were not correlated with prognosis of the patients with ESCA (Supplementary Figure 3).

Coexpression Assay of lncRNAs and mRNAs in ESCA

LncRNAs and mRNAs selected for consistency assay in TCGA and GEO database in Fig. 4 were used to construct the coexpression relations. We found HNF1A-AS1 were negatively correlated with CCNA2, CKS1B, RFC4 and SNAI2, but positively associated with FOXA1 (Fig. 6). MEG3 exhibited negative correlation with CKS1B and RFC4, but was positively related to SNAI2 (Fig. 6). In addition, SLC2A1-AS1 was positively associated with CKS1B, RFC4 and SNAI2, but was negatively related to FOXA1 (Fig. 6). TMEM161B-AS1 was negatively associated with TMSB10 and SNAI2, but was positively correlated with FOXA1 (Fig. 6). Further investigation showed that ZEB1-AS1 was positively correlated with CENPQ, RFC4 and SPC25, but was negatively correlated with TMSB10 (Fig. 6). These findings suggest that these lncRNAs may establish a series of pivotal regulatory network through several important mRNAs in ESCA.

Discussion

With the rapid development of sequencing techniques, it is very imperative to interpret the function of genes, especially non-coding RNA in a wide several of tumors. It is well documented that lncRNA-miRNA-mRNA widely participate in a plethora of biological processes in a number of tumors [14, 16, 27–30], and thus it is very necessary to elucidate the differential expressed profiles of lncRNA, miRNA or mRNA and identify the important regulatory axis of lncRNA-miRNA-mRNA in the development and progression of ESCA, which will contribute to the acceleration of the discovery of new molecular target and prognostic factors. Although several lncRNAs implicated in the development, progression, metastasis of ESCA have been defined, such as HOTTIP [31], HOTAIR [32], AK001796 [33], MALAT1 [34], etc., more novel lncRNAs or their involvement in the regulation of miRNA, or mRNA remains to be uncovered. At present, TCGA database is ideal tumor related database that is used to obtain the expression profiles of non-coding RNA or protein coding gene and related prognostic information, AA et al. found the 1250 differential lincRNA by TCGA database in BB tumors, verified the important biological significance, which will provide novel basis for further elucidation of these

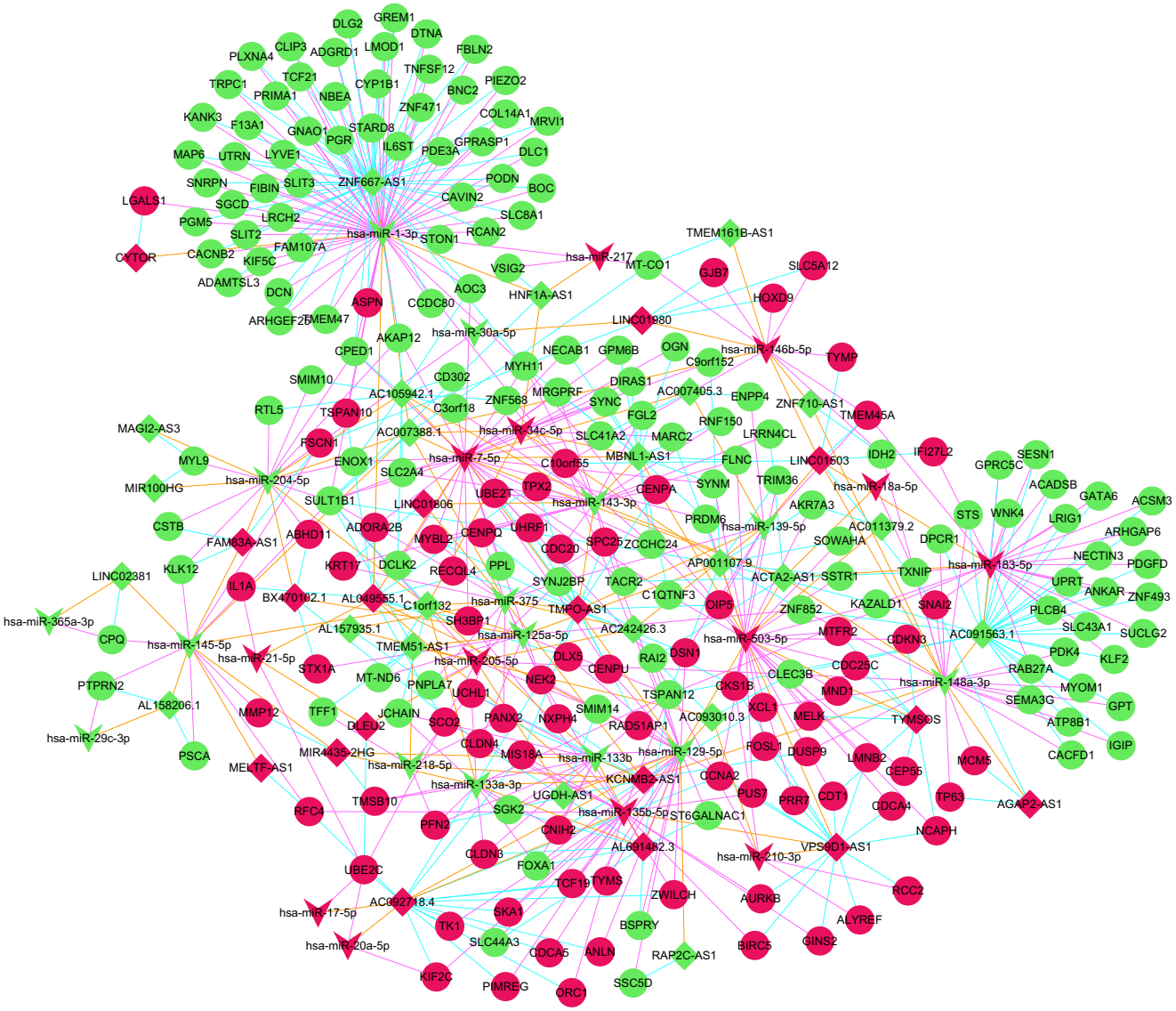

Fig. 3 The regulatory network of ceRNA. Red: upregulation; green: downregulation; the node of V shape: miRNA; the node of prismatic shape: lncRNA; the node of cycle: protein coding gene; connecting line of pink: miRNA-protein coding gene; connecting line of blue: lncRNA-protein coding gene; connecting line of orange: miRNA-lncRNA

lncRNA in BB tumors. Moreover, CC et al. also confirmed that lincRNA or miRNA differential expression in DD tumor. Although several research groups have revealed abnormal lncRNA expression profile in ESCA, and hundreds of lncRNAs have been identified, the data all studies above were from a single transcript, either lncRNA, or mRNA, or two combinations of lncRNA and mRNA [35–37]. It is well known that lncRNA as a competing endogenous RNA (ceRNA) can interact with miRNA to control the expression level of protein coding genes, which further affects tumor development and progression [38–40], however, lncRNA-miRNA-mRNA connections remain elusive in ESCA. Therefore, to preliminarily dissect the differential expression profiles, 161 ESCA samples and 11 normal samples from TCGA database were used to seek for differential expression genes of lncRNA or protein coding genes, and 181 tumor samples and 13 normal samples from TCGA data base were utilized to investigate the differential miRNA expression profile. Our results revealed 83 upregulated lncRNAs and 62 downregulated lncRNAs in ESCA samples, coupled with 1011 upregulations and 989 downregulations in protein coding mRNA. In addition, 38 upregulated miRNAs and 74 downregulated miRNAs were also found in ESCA samples. These differential expression profiles of lncRNA, miRNA and protein coding gene will provide the novel insights into the understanding of the molecular mechanisms of the development and progression of ESCA.

GO and KEGG will provide the understanding the gene function and related pathway, which will provide the novel basis of further interpretation of gene function in ESCA.

Table 1 Top 15 of high nodes in ceRNA network

node_name_1	node_name_2	type	regulate	degree
hsa-miR-1-3p	hsa-miR-1-3p	mir	down	67
hsa-miR-7-5p	hsa-miR-7-5p	mir	up	36
hsa-miR-503-5p	hsa-miR-503-5p	mir	up	31
hsa-miR-129-5p	hsa-miR-129-5p	mir	down	30
hsa-miR-183-5p	hsa-miR-183-5p	mir	up	27
ENSG00000166770	ZNF667-AS1	lnc	down	58
ENSG00000254343	AC091563.1	lnc	down	33
ENSG00000257167	TMPO-AS1	lnc	up	21
ENSG00000261061	AC092718.4	lnc	up	19
ENSG00000229619	MBNL1-AS1	lnc	down	18
ENSG00000173597	SULT1B1	pc	down	12
ENSG00000106025	TSPAN12	pc	down	8
ENSG00000117650	NEK2	pc	up	6
ENSG00000175063	UBE2C	pc	up	6
ENSG00000133392	MYH11	pc	down	6

Here, GO assay found dysregulated lncRNAs were involved in cell differentiation, chromosome segregation, protein kinase C and A signaling, extracellular matrix assembly, and chromatin remodeling at centromere, etc. Further investigation by KEGG revealed that lncRNAs participated in the regulations of Gap junction, carbon metabolism, phospholipase D signaling pathway, cell cycle, apoptosis and cGMP-PKG signaling pathway, etc. GO assay demonstrated that miRNA function was tightly associated with cell-substrate adhesion, chromosome segregation, epithelial cell maturation, the regulation of mitosis and spindle organization, anoikis, cell cycle G1/S phase transition and extracellular matrix assembly, etc., and KEGG assay demonstrated that miRNAs were related to mismatch repair, apoptosis, DNA replication, gap junction, tight junction, cGMP-PKG signaling pathway, and calcium signaling pathway, etc. Furthermore, GO assay revealed the correlation of protein coding mRNAs with sister chromatid segregation, cohesion, mitotic nuclear division, cell-cell junction, extracellular matrix binding, ect, whereas KEGG assay verified the association of protein coding mRNAs with cell cycle, estrogen signaling pathway, cGMP-PKG signaling pathway, tight junction, 2-oxocarboxylic acid metabolism and citrate cycle (TCA cycle), etc. These data above suggest that lncRNA-miRNA-mRNA may participate in the regulation of cGMP-PKG signaling pathway, which may be a pivotal target triggered by lncRNA-miRNA-mRNA axis in ESCA.

The discovery of novel LncRNA-miRNA-mRNA network contributed to the understanding the nosogenesis of tumors and provided new molecular target for tumor therapy, which

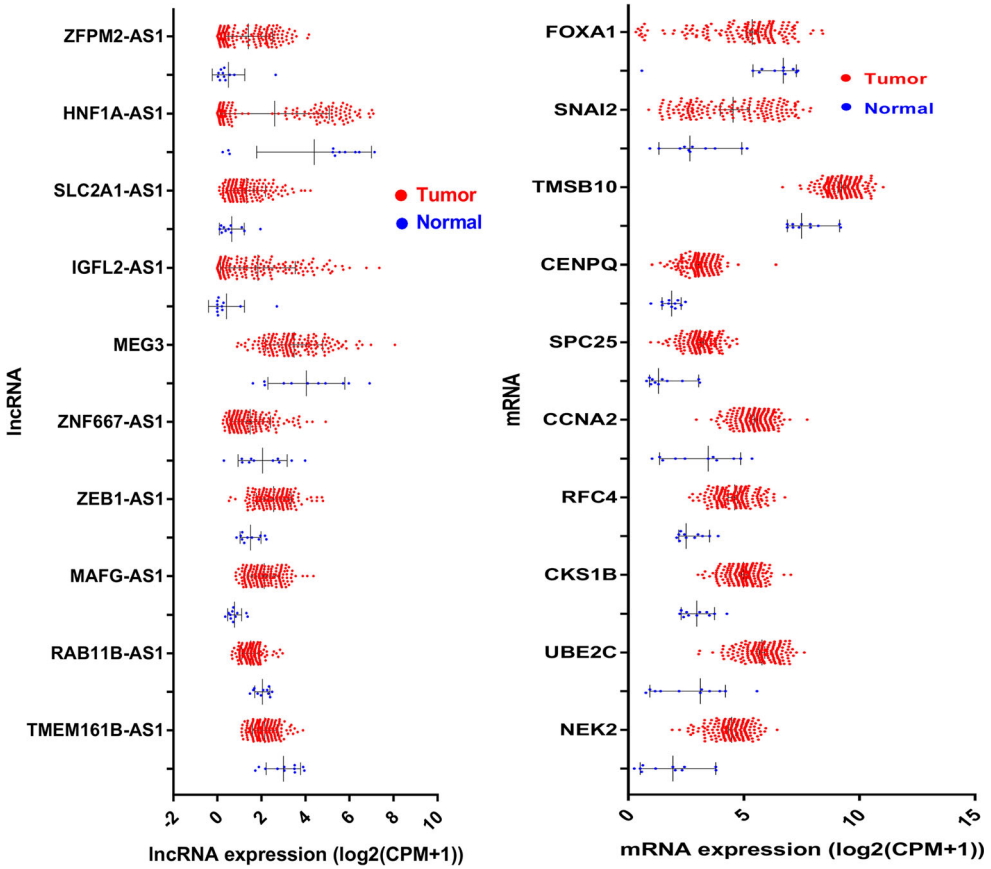

Fig. 4 Expression patterns of candidate lncRNAs and mRNAs in TCGA database. 10 lncRNAs including HNF1A-AS1, MEG3, RAB11B-AS1, SLC2A1-AS1, TMEM161B-AS1 and ZEB1-AS1 were selected for expression analysis, and 10 mRNAs including FOXA1, SNAI2, TMSB10, CENPQ, SPC25, CCNA2, RFC4, CKS1B, UBE2C and NEK2 were included in this study

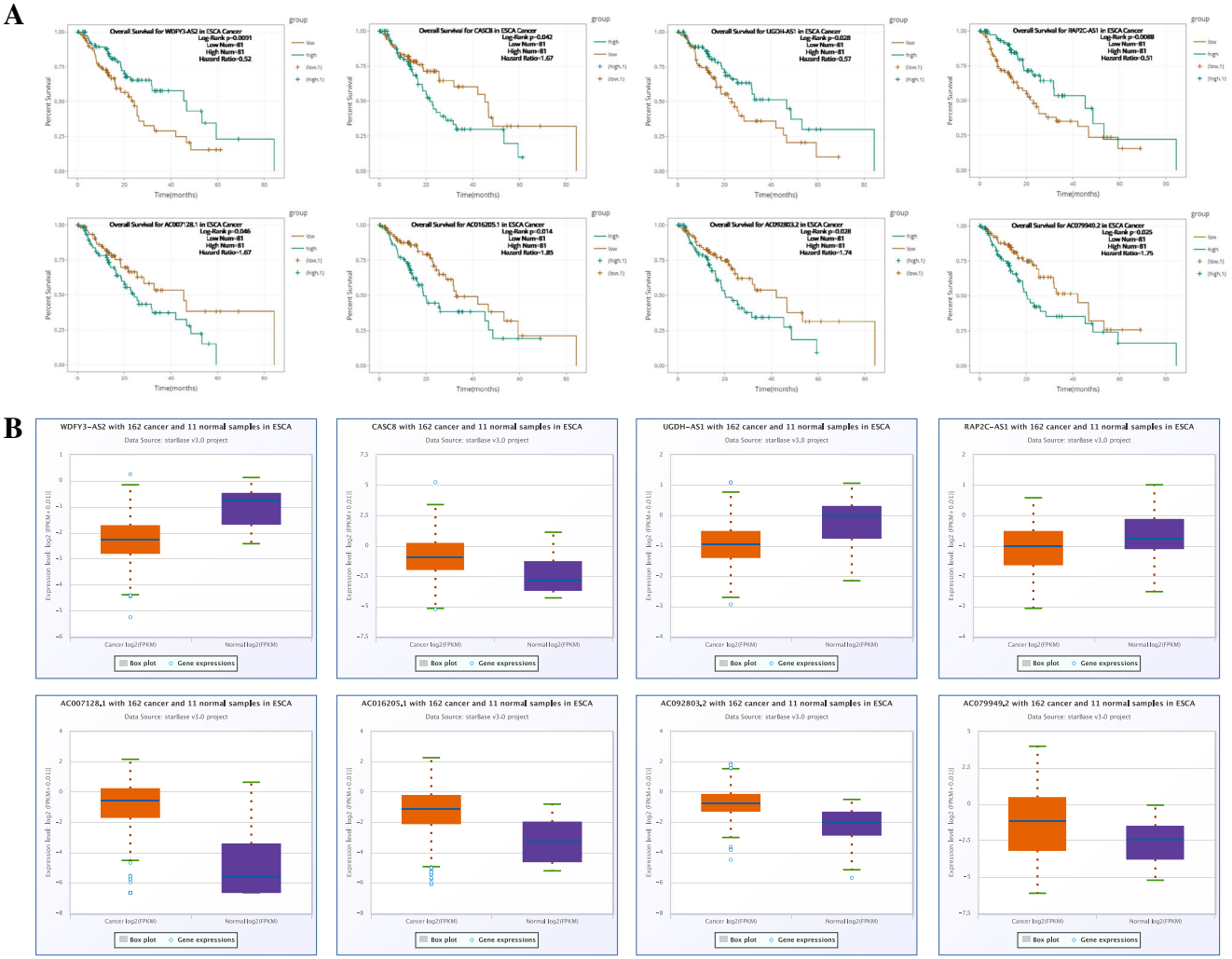

Fig. 5 Expression patterns of prognosis-related lncRNAs in ESCA samples. A: Kaplan-Meier survival analysis for the correlation of differential expression lncRNAs with overall survival of the patients with ESCA. Log-rank test was utilized to determine the survival differences. B: StarBase was employed to investigate the expression patterns of differential lncRNAs in ESCA samples

have been verified in a plethora of documents. Wang P, et al. revealed a novel lncRNA-miRNA-mRNA triple network in gastric carcinoma [41], suggesting lncRNA may be an important regulator of miRNA and gene expression. In addition, Zhao J, et al. interrogated the differentially expressed miRNAs, lncRNAs and mRNAs between lung adenocarcinoma and normal samples using TCGA (The Cancer Genome Atlas) database, and identified novel prognostic makers including hsa-miR-204, hsa-miR-96, SFTA1P, PGM5P2, RGS9BP, RGS20, FGB and INA in lung adenocarcinoma [30], which will help to accelerate the clinical transform for the judgement of the patients with lung adenocarcinoma. In the current study, 40 lncRNAs, 28 miRNAs and 233 mRNAs were identified to participate in the regulation of ceRNA network, and 677 nodes were produced by lncRNA-miRNA-mRNA. Meanwhile, top 15 of high nodes regarding lncRNA (ZNF667-AS1, AC091563.1, TMPO-AS1, AC092718.4 and MBNL1-AS1), miRNA (hsa-miR-1-3p, hsa-miR-7-5p, hsa-miR-503-5p, hsa-miR-129-5p and hsa-miR-183-5p) and mRNA (SULT1B1, TSPAN12, NEK2, UBE2C and MYH11) were also found in the regulatory network of ceRNA. Among these high node molecules, only hsa-miR-7-5p displayed obvious prognostic value in ESCA. These important node molecules may harbors the important biological significance, which will be under investigation in future.

To confirm the readability of TCGA data, GEO datasets (GSE89102) was used to detect the expression profiles of 10 interest of lncRNA or protein coding genes. We found that 6 of 10 lncRNAs (HNF1A-AS1, MEG3, RAB11B-AS1, SLC2A1-AS1, TMEM161B-AS1 and ZEB1-AS1) in TCGA database were consistent with GEO database, and expressions of 10 mRNAs including FOXA1, SNAI2, TMSB10, CENPQ, SPC25, CCNA2, RFC4, CKS1B, UBE2C and NEK2 in TCGA database all exhibited the same tendency with GEO database. These data indicate suggest that these differential

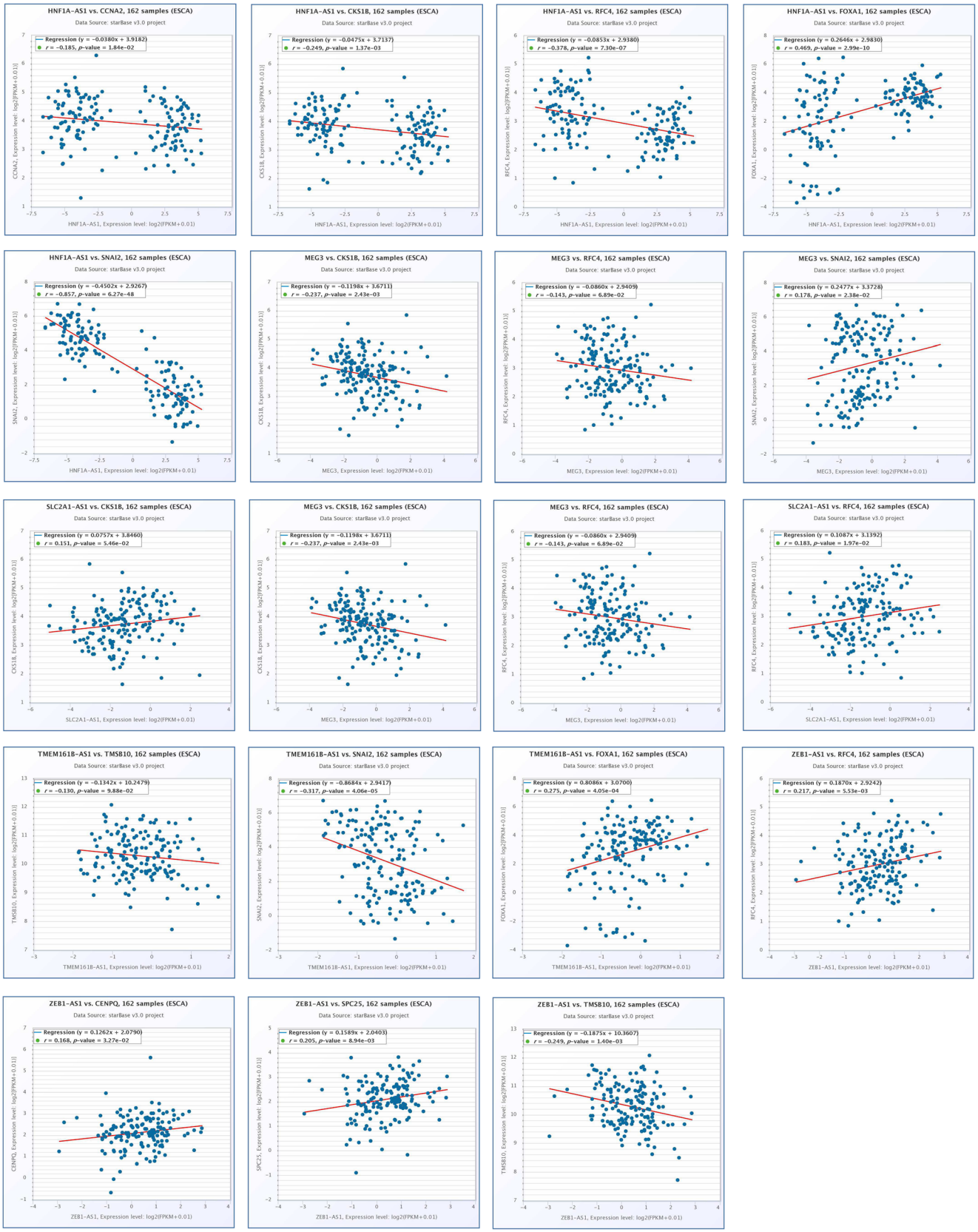

Fig. 6 Coexpression assay of lncRNAs and mRNAs in ESCA. LncRNAs and mRNAs selected for consistency assay in TCGA and GEO database were used to construct the coexpression relations. LncRNAs and mRNAs were submitted to Starbase online software as described in online manufacture's instructions

lncRNA or protein coding gene obtained by TCGA or GEO may harbor the important biological functions, however, these data remains to be verified in future experiments.

Increasing evidence has demonstrated that lncRNAs may be the important prognostic factor for many different tumors [42–45]. Mao, et al. reported 7 lncRNAs (RP5-1172 N10.2, RP11-579D7.4, RP11-89 N17.4, LA16c-325D7.2, RP1-251 M9.2, RP11-259O2.2 and LINC00173) predicted overall survival of the patients with ESCC [46]. Besides, lncRNA FOXD2-AS1 was upregulated in ESCC tissues, and high FOXD2-AS1 level predicted poor prognosis of the patients with ESCC [47]. In the current study, we found 8 prognosis-related lncRNAs (WDFY3-AS2, CASC8, UGDH-AS1, RAP2C-AS1, AC007128.1, AC016205.1, AC092803.2 and AC079949.2), in which WDFY3-AS2, UGDH-AS1 and RAP2C-AS1 were displayed in low expression level in ESCA samples, whereas CASC8, AC007128.1, AC016205.1, AC092803.2 and AC079949.2 were exhibited in high expression level in ESCA samples. These data suggest these lncRNAs may be a novel prognostic factor for the patients with ESCA, however, the precise functions for these lncRNA remain under investigation.

In conclusion, in the current study, some differential expression genes of lncRNA, miRNA and protein coding gene were found, and their function is tightly involved in cell cycle, apoptosis and corresponding signaling pathway. Meanwhile, ceRNA network will provide the novel idea to seek for novel molecular therapeutic target and prognostic marker in ESCA. Most notably, 8 lncRNAs is tightly associated with the prognosis of the patients with ESCA. All data presented herein may provide new strategy for further development of tumor targeted drug and early diagnosis in patients with ESCA.

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Compliance with Ethical Standards

Declaration of Conflicting Interests The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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