Long Non-Coding RNAs Roles in Tumorigenesis of Esophageal Carcinoma

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Abstract

Background: Esophageal carcinoma (ESCA) is one of the most common types of cancer. ESCA is accounted for the sixth leading cause of cancer-related deaths globally. The majority of the patients are diagnosed at late stages of ESCA, with distance metastasis and/or chemoresistance, which lead to a poor prognosis. Previous studies demonstrated lncRNA presentation and roles in ESCA cells and patients tissue. It has been proposed that lncRNAs can be considered as a new prognostic and diagnostic biomarker in ESCA. This study comprehensively explored the interaction of lncRNAs with miRNAs and mRNAs of TCGA database and proposed novel promising biomarker with favorable diagnostic and prognostic values.

Methods: The public data of RNA-seq, miR-seq and related clinical data were downloaded from TCGA database. Differential expression analysis was conducted by “limma” in R. GO and KEGG signaling pathway were used for enrichments. STRING database was used for PPI analysis. CE-network was constructed by STAR database in R. Kaplan-Meier survival analysis (log-rank test) and ROC curve analysis to indicate the biomarkers' diagnostic and prognostic values.

Results: Differentially expressed data illustrated that 1332 mRNA including 610 upregulated and 722 down-regulated were differentially expressed in ESCA. The GO and KEGG pathway analysis showed that the differentially expressed mRNAs were enriched in critical biological processes. The PPI showed that *IGFBP5*, *ACAN*, *ADAMTS12*, *MMP13*, and *CDH2* were the important PPI hubs. The ceRNA network data demonstrated critical lncRNAs including *TMEM16B-AS1*, *AC093010.3*, *SNHG3*, and *PVT1* which have an essential role in ESCA development. The data revealed that the lncRNA *WDFY3-AS2*, *AC108449.2*, *DLEU2*, *AC007128.1*, and *AP003356.1* are potential diagnostic prognostic biomarker in the ESCA patients.

Conclusion: Altogether, in our study, we demonstrated lncRNA, miRNA, and mRNA interaction and mentioned regulatory networks, which can be considered as a therapeutic option in ESCA. In addition, we proposed potential diagnostic and prognostic biomarkers for the patients.

Background

Esophageal carcinoma (ESCA) is one of the most common types of gastrointestinal cancer. According to pathological features, ESCA is mainly classified into esophageal adenocarcinoma and esophageal squamous cell carcinoma(1). ESCA is accounted for the sixth leading cause of malignancy-related deaths in the world(2). The majority of the patients are diagnosed at late stages of ESCA, with distance metastasis and/or chemoresistance, which lead to a poor prognosis (3). Based on previous reports, the overall 5-year survival rate is so frustrating, around 15-25% in the ESCA patients (4). Currently, radical surgery is a favorable option for early-ESCA treatment but is not conclusive in the advanced stages of the disease (5). Furthermore, standard chemotherapies have been implicated in advanced stages of the patients, but treatment outcomes still remain dismal in ESCA patients (6). Therefore, there is an urgent need to find out novel biomarkers for early diagnosis of ESCA patients to promote therapeutic approaches efficacy and outcomes in the patients.

Recently, it has been demonstrated that the main part of the human genome is transcribed to RNA and not capable of coding proteins, which is attributed to non-coding RNAs (7). Non-coding RNA is a class of RNA that includes different types of RNA such as transfer RNAs (tRNAs), ribosomal RNAs (rRNAs), microRNAs (miRNAs), long ncRNAs (lncRNAs), and circular RNAs (circRNAs)(8). Numerous investigations highlighted the crucial role of lncRNAs in cancer development and progression. lncRNAs are a group of non-coding RNAs with more than 200 nt in length and with no or little capability of coding proteins (9). lncRNA has been explained that plays different canonical roles in diverse biological processes such as cell proliferation, differentiation, and cellular development and in carcinogenesis and metastasis through regulating corner stone genes expression (10). Previous studies demonstrated lncRNA presentation and roles in ESCA cells and

patients tissue. For instance, it has been illustrated that lncRNA *ZFAS1* drives tumorigenesis and invasion by regulating the STAT3 signaling pathway through sponging *miRNA-124* in the esophageal squamous cell carcinoma cell(11).

Furthermore, lncRNAs can confer chemoresistance to the ESCA cell by modulating signaling pathways. For instance, lncRNA *TUSC7* overexpression suppressed cell proliferation and chemoresistance by *miR-224/DESC1/EGFR/AKT* axis in the ESCA cells(12). However, the exact mechanisms of lncRNAs function in ESCA are not yet well understood.

In this study, we comprehensively retrieved and explored RNA-seq data of the TCGA (The Cancer Genome Atlas) database to illustrate the interaction of lncRNAs with miRNAs and mRNAs and find a novel promising biomarker favorable diagnostic and prognostic values.

Materials And Methods

Sample and data collection

The ESCA data of the patients were retrieved from the TCGA database (<https://portal.gdc.cancer.gov/repository>). The inclusion criteria were: (1) the histopathological diagnosis was ESCA; (2) having complete demographic data including age, vital status, race, ethnicity, pathological stage, TNM classification, and overall survival time. Totally, 185ESCA were enrolled in this study. Eighty-nine participants had age > 61 years and 96 patients had age ≤ 61 and 158 and 27 patients were male and female, respectively. Among 185 patients, only 5 patients were Black or African American, 46 were Asian, and 114 were white. Pathological stages of I, II, III, and IV were 18, 79, 56, and 9, respectively. The clinical characteristics are presented in Table 1.

Table 1. Clinicopathological characteristics of ESCA patients.

Characteristics	N	(%)
Age (year) (mean $\pm$ SD)	62.45 $\pm$ 11.90	
Age > 61	89	48.10
Age $\leq$ 61	96	51.89
Sex		
Male	158	85.41
Female	27	14.59
Race		
Asian	46	24.86
Black or African American	5	2.70
White	114	61.62
NA	20	10.81
Vital status		
Alive	108	58.38
Dead	77	41.62
Pathologic (stage)		
Stage I	18	9.73
Stage II	79	42.70
Stage III	56	30.27
Stage IV	9	4.86
NA	23	12.43
Pathologic (T)		
T0	1	0.54
T1	31	16.76
T2	43	23.24
T3	88	47.57
T4	5	2.70
NA	17	9.19
Pathologic (M)		
M0	136	73.51
M1	9	4.86
MX	18	9.73
NA	22	11.89
Pathologic (N)		

N0	77	41.62
N1	69	37.30
N2	12	6.49
N3	8	4.32
NX	2	1.08
NA	17	9.19

NA: Not Available.

RNA-seq and miR-seq data analysis

The molecular data (RNA-Seq and miR-Seq Level 3) of ESCA were downloaded from the TCGA database. The raw count of the reads of RNA-Seq and miR-Seq data was normalized by Voom and TMM normalization methods. The “limma” package was used to indicate the differentially expressed mRNAs (DEmRNAs), lncRNAs (DElncRNAs), and miRNAs (DEmiRNAs) between normal solid tissues and primary tumors. The concluded data were filtered based on the $|\log_2 \text{fold change (FC)}| > 1$ for DEmRNA, DElncRNA, and DEmiRNA. P-value < 0.05 and false discovery rate (FDR) < 0.05 were considered as significant thresholds. All the analyses were accomplished in R software.

In Silico functional enrichment analysis and protein-protein interaction (PPI) network

Gene ontology (GO) in three domains, including biological processes, cellular components, and molecular functions, and Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathways were used for functional enrichment analysis. The GO and KEGG outputs were visualized by R software (ggplot2 package). The PPI network was constructed based on the STRING online database by Cytoscape 3.7.2. Molecular Complex Detection (MCODE) was used to analyze and predict the interactions (score value > 0.4).

LncRNA-miRNA-mRNA ceRNA network construction

LncRNA-miRNA-mRNA ceRNA network was constructed by “GDCRNATools” (<http://bioconductor.org/packages/devel/bioc/html/GDCRNATools.html>) package in R software based on starbase database (10). The nodes and edges were virtualized by Cytoscape 3.7.2.

Statistical Analysis

All the differentially expressed data were analyzed by using R software (3.5.2) through the “GDCRNATools” package. Kaplan-Meier survival analysis (log-rank test) was utilized to indicate the relationship between over or downregulation of the RNA, based on median expression with patient’s survival time. ROC curve analysis was conducted by SPSS v21. P-value < 0.05 was considered as a significant threshold.

Results

Differentially Expressed Genes

Differentially expressed data illustrated that 1332 mRNA including 610 upregulated and 722 down-regulated were differentially expressed in ESCA. Furthermore, 98 lncRNAs including 49 upregulated and 49 down-regulated were indicated as differentially expressed lncRNA in the patients. One hundred and one miRNAs including 62 up-regulated and 39 down-regulated demonstrated differential expression in the ESCA samples. The data are shown in Figs. 1, and Tables 2, 3.

Table 2. Top 20 up-regulated mRNAs, lncRNAs, and miRNAs

mRNA

	symbol	logFC	AveExpr	t	PValue	FDR	B
ENSG00000128422	<i>KRT17</i>	4.77	8.62	3.69	0.00	0.00	-0.08
ENSG00000060718	<i>COL11A1</i>	4.66	2.33	3.45	0.00	0.01	-0.60
ENSG00000136231	<i>IGF2BP3</i>	4.63	3.32	4.22	0.00	0.00	1.99
ENSG00000123388	<i>HOXC11</i>	4.52	1.29	6.73	0.00	0.00	13.01
ENSG00000137745	<i>MMP13</i>	4.35	1.30	3.62	0.00	0.00	-0.08
ENSG00000149968	<i>MMP3</i>	4.31	3.62	3.69	0.00	0.00	0.16
ENSG00000180818	<i>HOXC10</i>	4.31	2.80	4.63	0.00	0.00	3.52
ENSG00000123500	<i>COL10A1</i>	4.30	2.90	3.45	0.00	0.01	-0.59
ENSG00000099953	<i>MMP11</i>	4.29	5.35	3.86	0.00	0.00	0.68
ENSG00000180806	<i>HOXC9</i>	4.29	1.10	8.60	0.00	0.00	22.99
ENSG00000262406	<i>MMP12</i>	4.24	4.08	4.16	0.00	0.00	1.75
ENSG00000037965	<i>HOXC8</i>	4.18	1.04	7.85	0.00	0.00	18.85
ENSG00000169429	<i>CXCL8</i>	4.11	4.99	4.04	0.00	0.00	1.30
ENSG00000123364	<i>HOXC13</i>	4.05	0.99	4.19	0.00	0.00	1.86
ENSG00000170373	<i>CST1</i>	4.01	3.24	3.22	0.00	0.01	-1.29
ENSG00000131015	<i>ULBP2</i>	3.95	2.24	5.91	0.00	0.00	9.01
ENSG00000127928	<i>GNGT1</i>	3.94	0.11	3.66	0.00	0.00	0.06
ENSG00000206075	<i>SERPINB5</i>	3.83	7.05	4.11	0.00	0.00	1.46
ENSG00000115008	<i>IL1A</i>	3.81	1.98	3.57	0.00	0.00	-0.23
ENSG00000164283	<i>ESM1</i>	3.80	1.81	5.99	0.00	0.00	9.38

LncRNA

	symbol	logFC	AveExpr	t	PValue	FDR	B
ENSG00000228742	<i>AC002384.1</i>	4.21	0.43	5.23	0.00	0.00	6.00
ENSG00000268621	<i>IGFL2-AS1</i>	3.94	0.15	3.69	0.00	0.00	0.15
ENSG00000276850	<i>AC245041.2</i>	3.76	1.55	4.17	0.00	0.00	1.79
ENSG00000229970	<i>AC007128.1</i>	3.58	-0.70	4.76	0.00	0.00	4.05
ENSG00000281406	<i>BLACAT1</i>	3.39	1.85	4.77	0.00	0.00	4.10
ENSG00000204949	<i>FAM83A-AS1</i>	3.33	0.27	3.35	0.00	0.01	-0.90
ENSG00000273760	<i>AC245041.1</i>	3.31	0.49	3.41	0.00	0.01	-0.71
ENSG00000226476	<i>LINC01748</i>	3.24	0.38	4.18	0.00	0.00	1.85
ENSG00000249395	<i>CASC9</i>	3.12	2.04	3.37	0.00	0.01	-0.84
ENSG00000206195	<i>DUXAP8</i>	3.02	2.03	4.05	0.00	0.00	1.39

ENSG00000230061	<i>TRPM2-AS</i>	2.59	0.86	3.23	0.00	0.01	-1.25
ENSG00000259230	<i>LINC02323</i>	2.43	0.30	4.02	0.00	0.00	1.27
ENSG00000265415	<i>AC099850.3</i>	2.42	1.63	6.74	0.00	0.00	13.04
ENSG00000254560	<i>BBOX1-AS1</i>	2.38	1.52	3.94	0.00	0.00	1.00
ENSG00000172965	<i>MIR4435-2HG</i>	2.23	4.11	6.84	0.00	0.00	13.72
ENSG00000253669	<i>AP003356.1</i>	2.18	0.72	5.20	0.00	0.00	5.88
ENSG00000227403	<i>LINC01806</i>	2.17	1.49	3.31	0.00	0.01	-1.01
ENSG00000249859	<i>PVT1</i>	2.03	3.97	4.89	0.00	0.00	4.57
ENSG00000222041	<i>CYTOR</i>	2.03	3.11	5.99	0.00	0.00	9.49
ENSG00000261116	<i>AL049555.1</i>	1.92	4.31	3.45	0.00	0.01	-0.73

miRNA

	logFC	AveExpr	t	PValue	FDR	B
<i>hsa-miR-196a-5p</i>	5.14	6.47	7.40	0.00	0.00	17.19
<i>hsa-miR-196b-5p</i>	4.16	7.36	7.62	0.00	0.00	18.42
<i>hsa-miR-767-5p</i>	3.94	2.12	2.70	0.01	0.02	-3.02
<i>hsa-miR-944</i>	3.75	3.80	2.46	0.01	0.04	-3.61
<i>hsa-miR-105-5p</i>	3.62	2.31	2.48	0.01	0.03	-3.55
<i>hsa-miR-205-5p</i>	3.40	9.25	2.46	0.01	0.04	-3.95
<i>hsa-miR-1269a</i>	3.21	2.45	2.11	0.04	0.07	-4.37
<i>hsa-miR-135b-5p</i>	2.96	5.08	5.31	0.00	0.00	6.33
<i>hsa-miR-4652-5p</i>	2.89	0.62	4.46	0.00	0.00	2.81
<i>hsa-miR-224-5p</i>	2.48	5.58	3.67	0.00	0.00	-0.37
<i>hsa-miR-615-3p</i>	2.25	0.78	4.69	0.00	0.00	3.72
<i>hsa-miR-205-3p</i>	2.18	0.01	2.83	0.01	0.02	-2.67
<i>hsa-miR-452-3p</i>	2.09	1.75	3.09	0.00	0.01	-1.94
<i>hsa-miR-937-3p</i>	2.03	0.70	4.38	0.00	0.00	2.50
<i>hsa-miR-431-5p</i>	1.99	0.17	4.85	0.00	0.00	4.40
<i>hsa-miR-181b-3p</i>	1.98	2.55	5.92	0.00	0.00	9.32
<i>hsa-miR-4746-5p</i>	1.97	1.96	5.57	0.00	0.00	7.62
<i>hsa-miR-135b-3p</i>	1.94	0.32	4.21	0.00	0.00	1.82
<i>hsa-miR-452-5p</i>	1.79	6.23	3.46	0.00	0.00	-1.08
<i>hsa-miR-675-3p</i>	1.71	3.24	2.34	0.02	0.05	-3.98

Table 3. Top 20 down-regulated mRNAs, lncRNAs, and miRNAs

mRNA

	symbol	logFC	AveExpr	t	PValue	FDR	B
ENSG00000096088	<i>PGC</i>	-9.90	1.58	-7.95	0.00	0.00	19.92
ENSG00000168631	<i>DPCR1</i>	-6.40	1.02	-7.60	0.00	0.00	17.91
ENSG00000184956	<i>MUC6</i>	-6.08	2.13	-6.17	0.00	0.00	10.30
ENSG00000167653	<i>PSCA</i>	-5.90	3.12	-8.23	0.00	0.00	21.56
ENSG00000019102	<i>VSIG2</i>	-5.25	1.84	-8.64	0.00	0.00	23.99
ENSG00000196188	<i>CTSE</i>	-5.07	2.69	-4.47	0.00	0.00	2.60
ENSG00000215182	<i>MUC5AC</i>	-5.03	2.86	-4.65	0.00	0.00	3.34
ENSG00000115386	<i>REG1A</i>	-4.81	1.67	-4.14	0.00	0.00	1.35
ENSG00000160182	<i>TFF1</i>	-4.81	1.65	-4.73	0.00	0.00	3.70
ENSG00000134240	<i>HMGCS2</i>	-4.80	0.95	-5.25	0.00	0.00	5.97
ENSG00000112936	<i>C7</i>	-4.54	1.11	-7.29	0.00	0.00	16.22
ENSG00000109906	<i>ZBTB16</i>	-4.45	0.54	-8.59	0.00	0.00	23.63
ENSG00000174514	<i>MFSD4A</i>	-4.43	2.79	-8.94	0.00	0.00	25.79
ENSG00000168079	<i>SCARA5</i>	-4.30	0.20	-7.44	0.00	0.00	17.04
ENSG00000066405	<i>CLDN18</i>	-4.24	3.86	-3.84	0.00	0.00	0.24
ENSG00000125144	<i>MT1G</i>	-4.23	3.14	-8.73	0.00	0.00	24.52
ENSG00000163884	<i>KLF15</i>	-4.18	0.38	-9.84	0.00	0.00	31.17
ENSG00000170011	<i>MYRIP</i>	-4.16	0.14	-9.12	0.00	0.00	26.77
ENSG00000180875	<i>GREM2</i>	-4.14	0.10	-7.52	0.00	0.00	17.47
ENSG00000139874	<i>SSTR1</i>	-4.07	0.26	-4.82	0.00	0.00	4.19

LncRNA

	symbol	logFC	AveExpr	t	PValue	FDR	B
ENSG00000241388	<i>HNF1A-AS1</i>	-3.02	1.24	-3.24	0.00	0.01	-1.51
ENSG00000254343	<i>AC091563.1</i>	-2.97	0.09	-6.23	0.00	0.00	10.67
ENSG00000259291	<i>ZNF710-AS1</i>	-2.97	2.31	-11.47	0.00	0.00	41.54
ENSG00000203709	<i>C1orf132</i>	-2.93	1.77	-9.43	0.00	0.00	28.67
ENSG00000250742	<i>LINC02381</i>	-2.68	2.18	-6.90	0.00	0.00	14.14
ENSG00000260912	<i>AL158206.1</i>	-2.24	2.63	-6.94	0.00	0.00	14.31
ENSG00000268388	<i>FENDRR</i>	-2.17	2.25	-4.87	0.00	0.00	4.40
ENSG00000272894	<i>AC004982.2</i>	-2.12	0.66	-4.56	0.00	0.00	3.21

ENSG00000227218	<i>AL157935.1</i>	-2.05	0.28	-4.92	0.00	0.00	4.69
ENSG00000196167	<i>COLCA1</i>	-1.98	2.77	-3.42	0.00	0.01	-1.03
ENSG00000224078	<i>SNHG14</i>	-1.96	3.00	-4.53	0.00	0.00	2.95
ENSG00000249669	<i>CARMN</i>	-1.76	1.78	-3.75	0.00	0.00	0.22
ENSG00000180769	<i>WDFY3-AS2</i>	-1.70	0.29	-6.59	0.00	0.00	12.44
ENSG00000188242	<i>PP7080</i>	-1.64	4.99	-3.81	0.00	0.00	0.12
ENSG00000277496	<i>AL357033.4</i>	-1.59	1.04	-3.73	0.00	0.00	0.20
ENSG00000260461	<i>AL133355.1</i>	-1.54	1.14	-6.53	0.00	0.00	12.16
ENSG00000180139	<i>ACTA2-AS1</i>	-1.49	0.34	-3.72	0.00	0.00	0.19
ENSG00000251615	<i>AC104825.2</i>	-1.48	1.56	-4.48	0.00	0.00	2.91
ENSG00000261338	<i>AC021016.2</i>	-1.48	0.14	-6.76	0.00	0.00	13.28
ENSG00000225302	<i>AC023283.1</i>	-1.48	1.22	-3.57	0.00	0.00	-0.34

miRNA

	logFC	AveExpr	t	PValue	FDR	B
<i>hsa-miR-204-5p</i>	-3.91	0.51	-8.58	0.00	0.00	24.23
<i>hsa-miR-375</i>	-3.71	11.09	-3.90	0.00	0.00	0.35
<i>hsa-miR-133a-3p</i>	-2.98	4.07	-5.24	0.00	0.00	5.86
<i>hsa-miR-1-3p</i>	-2.63	4.72	-4.30	0.00	0.00	1.87
<i>hsa-miR-133b</i>	-2.39	0.64	-4.59	0.00	0.00	3.17
<i>hsa-miR-129-5p</i>	-2.38	1.30	-5.62	0.00	0.00	7.72
<i>hsa-miR-1468-5p</i>	-2.25	1.67	-7.11	0.00	0.00	15.49
<i>hsa-miR-139-5p</i>	-2.03	5.22	-7.48	0.00	0.00	17.60
<i>hsa-miR-29b-2-5p</i>	-2.02	4.15	-8.54	0.00	0.00	23.98
<i>hsa-miR-148a-3p</i>	-1.95	14.33	-6.52	0.00	0.00	12.20
<i>hsa-miR-30a-3p</i>	-1.93	10.62	-6.16	0.00	0.00	10.35
<i>hsa-miR-29c-3p</i>	-1.89	10.36	-6.05	0.00	0.00	9.79
<i>hsa-miR-30a-5p</i>	-1.87	13.23	-5.98	0.00	0.00	9.41
<i>hsa-miR-145-5p</i>	-1.78	11.00	-4.35	0.00	0.00	2.06
<i>hsa-miR-338-5p</i>	-1.75	2.04	-4.92	0.00	0.00	4.52
<i>hsa-miR-378c</i>	-1.74	3.04	-6.49	0.00	0.00	12.09
<i>hsa-miR-145-3p</i>	-1.62	5.36	-4.17	0.00	0.00	1.36
<i>hsa-miR-338-3p</i>	-1.56	9.42	-4.19	0.00	0.00	1.43

<i>hsa-miR-29c-5p</i>	-1.55	3.70	-7.52	0.00	0.00	17.83
<i>hsa-miR-139-3p</i>	-1.47	3.00	-4.79	0.00	0.00	3.90

GO enrichment and KEGG pathway analysis

Thereby GO enrichment analysis, we indicated several prominent roles of the DEmRNAs, Biological process of GO illustrated that the DEmRNAs are majorly assigned to DNA replication, mitotic nuclear division, organelle fission, chromosome segregation, and sister chromatid segregation. Also, the cellular component of GO depicted that the genes were significantly classified in the chromosomal region, condensed chromosome, spindle, collagen-containing, and extracellular matrix. Moreover, the GO molecular function part showed that the DEmRNAs dominantly enriched in extracellular matrix structural constituent, DNA helicase activity, catalytic activity, acting on DNA, single-stranded DNA-dependent ATP-dependent DNA helicase activity, and DNA replication origin binding (Fig 2). Furthermore, KEGG pathway analysis showed that the DEmRNAs remarkably attributed to Cell cycle, DNA replication, p53 signaling pathway, AGE-RAGE signaling pathway in diabetic, and PPAR signaling pathway (fig 3).

protein-protein interaction (PPI) network construction

For better understanding of the protein-protein interactions, we constructed a PPI network of the DEmRNAs via the STRING database. The data showed that *IGFBP5*, *ACAN*, *ADAMTS12*, *MMP13*, and *CDH2* were the important PPI hubs (Fig 4)

LncRNA-miRNA-mRNA ceRNA network construction

Based on the competing endogenous RNA (ceRNA) hypothesis, which explains that lncRNAs regulate mRNA expression level by competing the shared miRNAs in cells, a ceRNA network was built based on the differentially expressed genes data via starbase database in R software. The nodes and edges were visualized by Cytoscape 3.7.2. The ceRNA network data demonstrated critical lncRNAs including *TMEM16B-AS1*, *AC093010.3*, *SNHG3*, and *PVT1* which have an important role in the development of ESCA (Fig. 5).

Kaplan-Meier survival analysis of differentially expressed genes

To explore the association of differential expression of the genes and the ESCA patient's prognosis, Kaplan-Meier survival analysis was conducted over the differentially expressed genes. The data indicated that 41 mRNAs, 5 lncRNAs, and 23 miRNAs were associated with the overall survival rate in the patients. The top 20 hits of each group are presented in Table 4.

Table 4. Top 20 mRNAs, lncRNAs, and miRNAs that were associated with overall survival.

mRNA

	symbol	HR	lower95	upper95	pValue
ENSG00000091879	<i>ANGPT2</i>	2.10	1.28	3.46	0.00
ENSG00000146386	<i>ABRACL</i>	2.10	1.27	3.45	0.00
ENSG00000168298	<i>HIST1H1E</i>	1.90	1.15	3.13	0.01
ENSG00000130208	<i>APOC1</i>	1.89	1.15	3.11	0.01
ENSG00000121769	<i>FABP3</i>	1.76	1.08	2.88	0.02
ENSG00000164283	<i>ESM1</i>	1.72	1.05	2.82	0.03
ENSG00000130826	<i>DKC1</i>	1.66	1.01	2.72	0.04
ENSG00000180818	<i>HOXC10</i>	1.66	1.02	2.71	0.04
ENSG00000040275	<i>SPDL1</i>	1.64	1.00	2.69	0.04
ENSG00000105486	<i>LIG1</i>	1.64	1.00	2.70	0.04
ENSG00000153310	<i>FAM49B</i>	1.64	1.00	2.68	0.04
ENSG00000124731	<i>TREM1</i>	1.61	0.97	2.67	0.05
ENSG00000126709	<i>IFI6</i>	0.62	0.38	1.01	0.05
ENSG00000148180	<i>GSN</i>	0.61	0.37	1.00	0.05
ENSG00000175287	<i>PHYHD1</i>	0.61	0.37	1.00	0.05
ENSG00000149582	<i>TMEM25</i>	0.61	0.37	0.99	0.05
ENSG00000128340	<i>RAC2</i>	0.61	0.37	0.99	0.05
ENSG00000137198	<i>GMPR</i>	0.61	0.37	0.99	0.04
ENSG00000182568	<i>SATB1</i>	0.60	0.36	1.00	0.04
ENSG00000090006	<i>LTBP4</i>	0.60	0.37	0.98	0.04

LncRNA

	symbol	HR	lower95	upper95	pValue
ENSG00000180769	<i>WDFY3-AS2</i>	0.51	0.31	0.85	0.01
ENSG00000253669	<i>AP003356.1</i>	1.66	1.01	2.74	0.03
ENSG00000229970	<i>AC007128.1</i>	1.65	1.01	2.70	0.05
ENSG00000259366	<i>AC108449.2</i>	0.52	0.32	0.86	0.01
ENSG00000231607	<i>DLEU2</i>	1.70	1.04	2.80	0.03

miRNA

	symbol	HR	lower95	upper95	pValue
	<i>hsa-miR-29c-3p</i>	0.56	0.36	0.88	0.01
	<i>hsa-miR-181b-3p</i>	1.61	1.03	2.51	0.04
	<i>hsa-miR-550a-3p</i>	1.73	1.10	2.70	0.02

<i>hsa-miR-3682-3p</i>	1.71	1.09	2.68	0.02
<i>hsa-miR-101-3p</i>	0.61	0.39	0.97	0.03
<i>hsa-miR-27a-3p</i>	0.59	0.38	0.92	0.02
<i>hsa-miR-23a-3p</i>	0.59	0.38	0.92	0.02
<i>hsa-miR-99a-5p</i>	0.58	0.37	0.91	0.02
<i>hsa-miR-1249-3p</i>	0.64	0.41	1.00	0.05
<i>hsa-miR-425-5p</i>	1.96	1.25	3.08	0.00
<i>hsa-miR-323b-3p</i>	1.72	1.09	2.69	0.02
<i>hsa-miR-1269a</i>	1.56	0.99	2.47	0.04
<i>hsa-miR-6842-3p</i>	0.62	0.40	0.97	0.04
<i>hsa-miR-151a-3p</i>	0.63	0.40	0.98	0.04
<i>hsa-let-7b-3p</i>	0.56	0.36	0.88	0.01
<i>hsa-let-7a-5p</i>	0.55	0.35	0.87	0.01
<i>hsa-miR-412-5p</i>	0.60	0.39	0.95	0.03
<i>hsa-let-7a-3p</i>	0.57	0.37	0.89	0.01
<i>hsa-miR-33a-3p</i>	0.58	0.37	0.91	0.02
<i>hsa-miR-31-3p</i>	0.63	0.40	0.99	0.04

Diagnostic value analysis of differentially expressed lncRNAs

For demonstrating the diagnostic value of each DElncRNAs, AUC curve analysis was accomplished in the ESCA samples. All 98 DElncRNAs indicated remarkable diagnostic values in the patients. The top 30 hits of the lncRNAs are presented in Table 5.

Table 5. Top 20 lncRNAs that had remarkable diagnostic value.

lncRNA	AUC	SE	p-value	Lower (95%CI)	Upper (95%CI)	expression
<i>MIR4435-2HG</i>	0.99	0.007	0	0.977	1	Up
<i>CYTOR</i>	0.977	0.013	0	0.951	1	Up
<i>AP003356.1</i>	0.955	0.033	0	0.891	1	Up
<i>PVT1</i>	0.951	0.024	0	0.905	0.997	Up
<i>C1orf132</i>	0.941	0.031	0	0	0.12	Down
<i>MAFG-AS1</i>	0.94	0.035	0	0.872	1	Up
<i>AL158212.3</i>	0.936	0.046	0	0	0.155	Down
<i>DLEU2</i>	0.928	0.026	0	0.877	0.98	Up
<i>ZNF710-AS1</i>	0.926	0.039	0	0	0.151	Down
<i>AC021016.2</i>	0.924	0.04	0	0	0.155	Down
<i>AL133355.1</i>	0.919	0.039	0	0.004	0.159	Down
<i>MELTF-AS1</i>	0.916	0.035	0	0.848	0.984	Up
<i>BLACAT1</i>	0.911	0.029	0	0.854	0.969	Up
<i>AC002384.1</i>	0.909	0.035	0	0.84	0.978	Up
<i>AC099850.3</i>	0.906	0.053	0	0.802	1	Up
<i>AC092718.4</i>	0.903	0.052	0	0.8	1	Up
<i>TYMSOS</i>	0.901	0.041	0	0.82	0.981	Up
<i>AC091563.1</i>	0.901	0.044	0	0.014	0.185	Down
<i>TMPO-AS1</i>	0.899	0.031	0	0.839	0.96	Up
<i>AC026401.3</i>	0.898	0.04	0	0.82	0.977	Up

Potential diagnostic and prognostic lncRNA

Thereby merging the diagnostic (AUC value) and prognostic (HR) values of the lncRNAs in the ESCA patients, potential novel lncRNA biomarkers were retrieved. The summary of the data is presented in table 6. The data demonstrated that the lncRNA *WDFY3-AS2*, *AC108449.2*, *DLEU2*, *AC007128.1*, and *AP003356.1* as potential diagnostic and prognostic biomarker in the ESCA patients (Figure 6).

Table 6. Merge diagnostic and prognostic data of the lncRNAs.

symbol	HR	lower95	upper95	pValue	AUC	SE	p-value	Lower (95%CI)	Upper (95%CI)	expression
<i>WDFY3-AS2</i>	0.514	0.313	0.846	0.006	0.885	0.062	0.000	0.000	0.236	Down
<i>AC108449.2</i>	0.524	0.318	0.863	0.007	0.842	0.051	0.000	0.058	0.257	Down
<i>DLEU2</i>	1.702	1.035	2.799	0.029	0.928	0.026	0.000	0.877	0.980	Up
<i>AP003356.1</i>	1.661	1.005	2.744	0.034	0.955	0.033	0.000	0.891	1.000	Up
<i>AC007128.1</i>	1.654	1.013	2.700	0.046	0.854	0.063	0.000	0.731	0.977	Up
<i>UGDH-AS1</i>	0.645	0.395	1.053	0.077	0.827	0.083	0.000	0.011	0.335	Down
<i>TMEM161B-AS1</i>	1.478	0.905	2.416	0.114	0.862	0.061	0.000	0.019	0.257	Down
<i>CD44-AS1</i>	1.412	0.853	2.337	0.153	0.804	0.067	0.001	0.672	0.936	Up
<i>AGAP2-AS1</i>	0.707	0.433	1.154	0.164	0.853	0.060	0.000	0.734	0.971	Up
<i>LINC00511</i>	1.396	0.852	2.290	0.173	0.843	0.064	0.000	0.717	0.969	Up
<i>AC122129.1</i>	0.721	0.440	1.179	0.182	0.824	0.060	0.000	0.058	0.294	Down
<i>AL357033.4</i>	1.387	0.849	2.266	0.193	0.804	0.059	0.001	0.081	0.311	Down
<i>AL133355.1</i>	0.731	0.447	1.196	0.202	0.919	0.039	0.000	0.004	0.159	Down
<i>FOXD2-AS1</i>	1.374	0.841	2.243	0.205	0.827	0.091	0.000	0.648	1.000	Up
<i>AC004803.1</i>	0.728	0.446	1.190	0.208	0.780	0.070	0.002	0.084	0.357	Down
<i>AC099850.3</i>	0.735	0.450	1.201	0.210	0.906	0.053	0.000	0.802	1.000	Up
<i>AC022211.2</i>	0.735	0.450	1.203	0.214	0.863	0.048	0.000	0.769	0.956	Up
<i>CASC9</i>	1.338	0.818	2.187	0.239	0.822	0.064	0.000	0.695	0.948	Up
<i>LINC01572</i>	1.325	0.811	2.164	0.255	0.785	0.097	0.002	0.595	0.974	Up
<i>TSC22D1-AS1</i>	0.754	0.462	1.231	0.255	0.862	0.044	0.000	0.053	0.224	Down

Discussion

Esophageal cancer is one of the most aggressive types of cancer with an increasing rate of death and dismal prognosis. Previous investigations highlighted non-coding RNA particularly lncRNA's roles in cancer development, progression, and clinicopathological features of the patients (13-15). A large body of studies considered lncRNAs as a major contributor to ESCA development and showed the lncRNAs prognostic and diagnostic values for the ESCA patients (16). Our study comprehensively considered the expression and interaction of protein-coding RNAs (mRNAs), miRNAs, and lncRNAs. Furthermore, our data presented the CE-network of lncRNA-miRNA-mRNA in ESCA patients specimens. GO and KEGG pathway analysis demonstrated that several crucial signaling pathways such as cell cycle and replication, p53, AGE-RAGE, and PPAR (peroxisome proliferator-activated receptor) signaling pathways have the main contribution to tumorigenesis of ESCA patients. Accumulating evidence illustrated that cell cycle regulatory proteins dysregulation such as *cyclin-dependent kinase inhibitor 3 (CDKN3)* can drive tumorigenesis and chemoresistance of ESCA cells (17). Furthermore, it has been shown that *PRDX2* develops ESCA by instigating Wnt/ β -catenin and AKT pathways in the cells (18). P53 is one of the well-known tumor suppressor genes, which is dysregulated in the number of malignancies. There are many examples that depicted lncRNAs role in p53 regulation in different types of cancers. For instance, it has been demonstrated that lncRNA

AK001796 had an invention in ESCA tumorigenesis by regulating MDM2 to suppress p53 in the cells (19). LncRNA *SNHG1* increases liver cancer progression by recruiting DNMT1 to epigenetically suppress *p53* expression (20).

Recently, the cross-talk between metabolism and cancer are vastly explained in various cancer. It has been demonstrated that PPAR Signaling Pathway is one of the most important signaling hubs between lipid metabolism and carcinogenesis (21). LncRNA *Ftx* has been shown that promotes tumorigenesis, by increasing glucose uptake, lactate production, and relative glycolytic enzyme through controlling the PPAR γ pathway in hepatocellular carcinoma (HCC) (22).

Our protein-protein interaction data demonstrated that *IGFBP5*, *ACAN*, *ADAMTS12*, *MMP13*, and *CDH2* had a main role in the signaling hubs through the PPI network. *IGFBP5* has been discovered to act as an oncogene in the cells and drive tumorigenesis in different cancer types. LncRNA *UCA1* promotes carcinogenesis by upregulating *IGFBP5* through sponging *miR-204* in papillary thyroid carcinoma (PTC) cells (23). *ADAMTS12* has been reported that had an anti-tumorigenic effect in various cancer. LncRNA *AK001058* can regulate tumor development, progression, and invasion by suppressing *ADAMTS12* expression via methylation of its promoter (24). The previous investigation depicted that *MMP13* (Matrix Metalloproteases 13) had key roles in embryogenic development and cancerogenesis such as proliferation, migration (25). LncRNA *LINC00511* promotes tumor growth, migration and invasion by directly binding to *miR-150* to upregulate *MMP13* in the breast cancer cells (26). *Cadherin-2* (*CDH2*) is a member of the cadherin family that regulates crucial biological functions and tumorigenesis in a variety of cancers (27). Overexpression of lncRNA *JPX* has been reported that elevates cell proliferation and tumor growth by upregulating *CDH2* through sponging *miR-944* in Oral squamous cell carcinoma (OSCC) cells (28).

Furthermore, we demonstrated that lncRNA *TMEM16B-AS1*, *AC093010.3*, *SNHG3*, and *PVT1* participated in CE-networks and regulate several mRNAs expression by sponging various miRNAs.

A large body of evidence indicated that overexpression of lncRNA *SNHG3* is associated with tumorigenesis, invasion and metastasis, and poor prognosis in patients. It can promote tumorigenesis by epigenetically suppressing *MED18* through recruiting EZH2 to methylate the *MED18* neighboring region in gastric cancer (29). Recently, in a study, lncRNA *SNHG3* has been shown that elevated the m6A level by binding to *miR-186-5p* to increase *METTL3* expression in the ESCA cells (30).

LncRNA *PVT1* has presented oncogenic effects in various tumor types. The last investigation demonstrated that overexpression of *PVT1* is associated with poor clinicopathological characteristics and overall survival rate in ESCA patients (31). Furthermore, in vitro studies showed that *PVT1* could induce invasion and metastasis by instigating epithelial-to-mesenchymal transition (EMT) in ESCA cells (32). Interestingly, *PVT1* has been indicated that induced tumorigenesis through sponging *miR-203* and *LASP1* which have tumor suppressive impact in the ESCA cells (33).

Finally, in the last part of our work, our results proposed potential diagnostic and prognostic lncRNAs, including *WDFY3-AS2*, *AC108449.2*, *DLEU2*, *AC007128.1*, and *AP003356.1* which showed promising outcomes. To the best of our knowledge, lncRNA *AC108449.2*, *AC007128.1*, and *AP003356.1* were presented for the first time reviewed in the studies as new novel biomarkers in ESCA. While, lncRNA *WDFY3-AS2* and *DLEU2* have been considered different types of cancer as well as ESCA. Overexpression of lncRNA *WDFY3-AS2* has been demonstrated remarkably associated with clinical and molecular characteristics of glioma in the patients and involved in the TNF signaling pathway (34). Furthermore, *WDFY3-AS2* expression was showed that significantly associated with a dismal overall survival rate in patients with triple-negative breast cancer (TNBC), which is consistent with our results (35). Previous studies have illustrated that lncRNA *DLEU2* expression correlates to poor prognosis in ESCA patients (36). Furthermore, it has been shown that *DLEU2* can induce tumor growth, cell proliferation, invasion, and metastasis by upregulating E2F7 through directly inhibiting *miR-30e-5p* in ESCA cells (37).

Conclusion

Numerous reports explained lncRNA roles in ESCA, but in this work, we thoroughly presented lncRNA, miRNA, and mRNA networks. Altogether, in our study, we demonstrated lncRNA, miRNA, and mRNA interaction and mentioned regulatory networks, which can be considered as a therapeutic option in ESCA. In addition, we proposed potential diagnostic and prognostic biomarkers for the patients.

Declarations

Acknowledgments

Not applicable.

Author's contributions

S.T. M.K. M.J. and M.G. conducted the research; S.T. M.K. M.J. and M.G. analyzed the data; S.T. M.K. M.J. and M.G. wrote the paper; M.G. had primary responsibility for the final content. All authors have reviewed and approved the final manuscript.

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Availability of data and materials

The authors declare that the datasets on which the conclusions of this manuscript rely are deposited in publicly available repositories.

Ethics approval and consent to participate

The authors declare that there is no conflict of interest.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

Figure 1

Bar graph of differentially expressed genes in the ESCA samples. TEC: To be Experimentally Confirmed; TR: T cell receptor; IG: Immunoglobulin

Figure 2

GO enrichment analysis of the differentially expressed mRNAs in ESCA(Top 10 GO enrichment are presented).

Figure 5

LncRNA-miRNA-mRNA ceRNA network construction of ESCA. (Orange: LncRNA, Yellow: miRNA, and Green: mRNA)

Figure 6

Kaplan-Meier and ROC curve analysis of the WDFY3-AS2, AC108449.2, DLEU2, AP003356.1, AC007128.1. A. Kaplan-Meier curve of WDFY3-AS2. B. Kaplan-Meier curve of AC108449.2. C. Kaplan-Meier curve of DLEU2. D. Kaplan-Meier curve of AP003356.1. E. Kaplan-Meier curve of AC007128.1. F and G. ROC curve of the lncRNAs.