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Investigating the Long Non-Coding RNA Expression Profiles in the development of esophageal cancer: Insights from genomic Analysis

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Abstract

Background: Esophageal carcinoma (ESCA) is one of the most common types of cancer. ESCA accounted for the sixth leading cause of cancer-related deaths globally. Most patients are diagnosed at late stages of ESCA, with distance metastasis or chemoresistance, which leads 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 a new prognostic and diagnostic biomarker in ESCA. In this study, we comprehensively explored the interaction of lncRNAs with miRNAs and mRNAs of the TCGA database and proposed a novel promising biomarker with good diagnostic and prognostic values.
Methods: The public data of RNA-seq, miR-seq, and related clinical data were downloaded from the TCGA database. Differential expression analysis was conducted by "limma" in R. GO, and KEGG signaling pathways were used for enrichments. STRING database was used for PPI analysis. CE-network was constructed by the STAR database in R. Kaplan-Meier survival analysis (log-rank test), and ROC curve analysis was used to indicate the diagnostic and prognostic values of the biomarkers.
Results: Differentially expressed data illustrated that 45.8% of the total mRNAs in the data related to ESCA patients showed increased expression and 54.2% decreased expression. The GO and KEGG pathway analysis showed that the differentially expressed mRNAs were enriched in critical biological processes. Important protein-protein interaction hubs were identified. The ceRNA network data demonstrated critical lncRNAs essential in ESCA development, including *TMEM16B-AS1, AC093010.3, SNHG3*, and *PVT1*. The data revealed that the lncRNA *WDFY3-AS2, AC108449.2, DLEU2, AC007128.1,* and *AP003356.1* are potential diagnostic and prognostic biomarkers in ESCA patients.
Conclusion: Altogether, this study demonstrates lncRNA, miRNA, and mRNA interaction and mentions regulatory networks which can be considered as a therapeutic option in ESCA. In addition, we proposed potential diagnostic and prognostic biomarkers for ESCA patients.
Keywords: Esophageal carcinoma; Tumorigenesis; Long non-coding RNAs; MicroRNA

Background

Esophageal carcinoma, also known as esophageal cancer, affects the esophagus, the muscular tube that connects the throat to the stomach. Esophageal carcinoma can be classified into different subtypes based on their pathological features. The two main subtypes are esophageal adenocarcinoma and esophageal squamous cell carcinoma. [1]. ESCA is a significant global health concern and is currently the sixth leading cause of cancer-related deaths worldwide [2]. Unfortunately, most patients with esophageal carcinoma are diagnosed at later stages, which can lead to distant metastasis and chemoresistance, resulting in a poor prognosis [3]. Based on previous reports, the overall 5-year survival rate is so frustrating, around 15-25% in ESCA patients [4]. 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 the advanced stages of the patients, but treatment outcomes remain dismal in ESCA patients [6]. Therefore, there is an urgent need to find novel biomarkers for early diagnosis of ESCA patients to promote therapeutic approaches efficacy and outcomes in the patients.

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Researchers are actively developing new diagnostic tools and therapies, including precision medicine approaches targeting specific molecular and genetic abnormalities in cancer cells.

Recently, it has been demonstrated that the central part of the human genome is transcribed to RNA and not capable of coding proteins which is attributed to non-coding RNAs [7]. Non-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), circular RNAs (circRNAs) [8]. Numerous investigations highlighted the crucial role of LncRNAs in cancer development and progression. LncRNAs is a group of non-coding RNAs with more than 200 nt in length and with no or little capability of coding proteins LncRNA has been explained that it plays different canonical roles not only in diverse biological processes such as cell proliferation, differentiation, and cellular development but also in carcinogenesis and metastasis through regulating cornerstone gene expression [9]. 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 STAT3 signaling pathway through sponging miRNA-124 in the esophageal squamous cell carcinoma cell [10]. 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 [11]. However, the exact mechanisms of lncRNA function in ESCA are poorly 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 to discover novel promising biomarkers with good 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. 185 ESCA were enrolled in this study. Eighty-nine participants had ages> 61 years, 96 patients had ages ≤ 61, and 158 and 27 patients were male and female, respectively. Among 185 patients, only five were Black or African American, 46 were Asian, and 114 were white. Pathological stages 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 ± SD)		62.45±11.90	
	Age > 61	89	48.10
	Age ≤ 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
	NA	17	9.19
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.

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RNA-seq and miR-seq data analysis

ESCA's molecular data (RNA-Seq and miR-Seq Level 3) were downloaded from the TCGA database. Voom and TMM normalization methods normalized the raw count of the reads of RNA-Seq and miR-Seq data. 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 |log2 fold change (FC)| > 1 for DEmRNA, DElncRNA, and DEmiRNA. P-value < 0.05 and false discovery rate (FDR) < 0.05 were considered 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. [9]. The nodes and edges were virtualized by Cytoscape 3.7.2.

Statistical Analysis

All the differentially expressed data were analyzed 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 the patient’s survival time. ROC curve analysis was conducted by SPSS v21. A P-value < 0.05 was considered 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 deferentially expressed lncRNA in the patients. One hundred and one miRNAs, 62 upregulated and 39 down-regulated, demonstrated differential expression in the ESCA samples. The data are shown in Figs. 1, and Tables 2, 3.

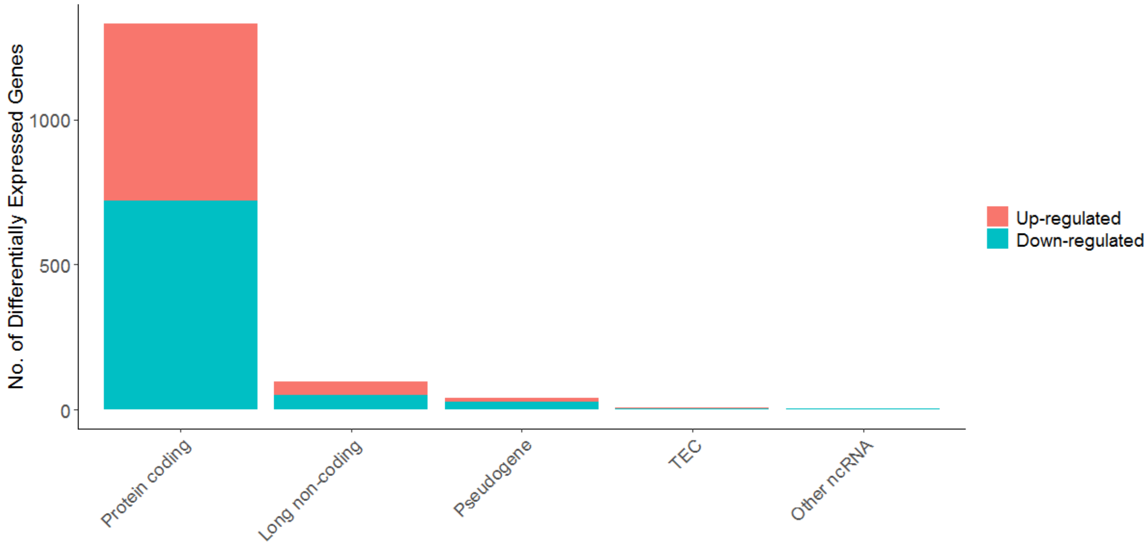

Figure 1. Bar graph of differentially expressed genes in the ESCA samples. TEC: To be Experimentally Confirmed; TR: T cell receptor; IG: Immunoglobulin.

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

mRNA							
	symbol	logFC	AveExpr	t	PValue	FDR	B
ENSG00000128422	*KRT17*	4.77	8.62	3.69	0.00	0.00	-0.08
ENSG00000060718	*COL11A1*	4.66	2.33	3.45	0.00	0.01	-0.60
ENSG00000136231	*IGF2BP3*	4.63	3.32	4.22	0.00	0.00	1.99
ENSG00000123388	*HOXC11*	4.52	1.29	6.73	0.00	0.00	13.01
ENSG00000137745	*MMP13*	4.35	1.30	3.62	0.00	0.00	-0.08
ENSG00000149968	*MMP3*	4.31	3.62	3.69	0.00	0.00	0.16
ENSG00000180818	*HOXC10*	4.31	2.80	4.63	0.00	0.00	3.52
ENSG00000123500	*COL10A1*	4.30	2.90	3.45	0.00	0.01	-0.59
ENSG00000099953	*MMP11*	4.29	5.35	3.86	0.00	0.00	0.68
ENSG00000180806	*HOXC9*	4.29	1.10	8.60	0.00	0.00	22.99
ENSG00000262406	*MMP12*	4.24	4.08	4.16	0.00	0.00	1.75
ENSG00000037965	*HOXC8*	4.18	1.04	7.85	0.00	0.00	18.85
ENSG00000169429	*CXCL8*	4.11	4.99	4.04	0.00	0.00	1.30
ENSG00000123364	*HOXC13*	4.05	0.99	4.19	0.00	0.00	1.86
ENSG00000170373	*CST1*	4.01	3.24	3.22	0.00	0.01	-1.29
ENSG00000131015	*ULBP2*	3.95	2.24	5.91	0.00	0.00	9.01
ENSG00000127928	*GNGT1*	3.94	0.11	3.66	0.00	0.00	0.06
ENSG00000206075	*SERPINB5*	3.83	7.05	4.11	0.00	0.00	1.46
ENSG00000115008	*IL1A*	3.81	1.98	3.57	0.00	0.00	-0.23
ENSG00000164283	*ESM1*	3.80	1.81	5.99	0.00	0.00	9.38
LncRNA							
	symbol	logFC	AveExpr	t	PValue	FDR	B
ENSG00000228742	*AC002384.1*	4.21	0.43	5.23	0.00	0.00	6.00
ENSG00000268621	*IGFL2-AS1*	3.94	0.15	3.69	0.00	0.00	0.15
ENSG00000276850	*AC245041.2*	3.76	1.55	4.17	0.00	0.00	1.79
ENSG00000229970	*AC007128.1*	3.58	-0.70	4.76	0.00	0.00	4.05
ENSG00000281406	*BLACAT1*	3.39	1.85	4.77	0.00	0.00	4.10
ENSG00000204949	*FAM83A-AS1*	3.33	0.27	3.35	0.00	0.01	-0.90
ENSG00000273760	*AC245041.1*	3.31	0.49	3.41	0.00	0.01	-0.71
ENSG00000226476	*LINC01748*	3.24	0.38	4.18	0.00	0.00	1.85
ENSG00000249395	*CASC9*	3.12	2.04	3.37	0.00	0.01	-0.84
ENSG00000206195	*DUXAP8*	3.02	2.03	4.05	0.00	0.00	1.39
ENSG00000230061	*TRPM2-AS*	2.59	0.86	3.23	0.00	0.01	-1.25
ENSG00000259230	*LINC02323*	2.43	0.30	4.02	0.00	0.00	1.27
ENSG00000265415	*AC099850.3*	2.42	1.63	6.74	0.00	0.00	13.04
ENSG00000254560	*BBOX1-AS1*	2.38	1.52	3.94	0.00	0.00	1.00
ENSG00000172965	*MIR4435-2HG*	2.23	4.11	6.84	0.00	0.00	13.72
ENSG00000253669	*AP003356.1*	2.18	0.72	5.20	0.00	0.00	5.88
ENSG00000227403	*LINC01806*	2.17	1.49	3.31	0.00	0.01	-1.01
ENSG00000249859	*PVT1*	2.03	3.97	4.89	0.00	0.00	4.57
ENSG00000222041	*CYTOR*	2.03	3.11	5.99	0.00	0.00	9.49
ENSG00000261116	*AL049555.1*	1.92	4.31	3.45	0.00	0.01	-0.73
miRNA							
		logFC	AveExpr	t	PValue	FDR	B
hsa-miR-196a-5p		5.14	6.47	7.40	0.00	0.00	17.19
hsa-miR-196b-5p		4.16	7.36	7.62	0.00	0.00	18.42
hsa-miR-767-5p		3.94	2.12	2.70	0.01	0.02	-3.02
hsa-miR-944		3.75	3.80	2.46	0.01	0.04	-3.61
hsa-miR-105-5p		3.62	2.31	2.48	0.01	0.03	-3.55
hsa-miR-205-5p		3.40	9.25	2.46	0.01	0.04	-3.95
hsa-miR-1269a		3.21	2.45	2.11	0.04	0.07	-4.37
hsa-miR-135b-5p		2.96	5.08	5.31	0.00	0.00	6.33
hsa-miR-4652-5p		2.89	0.62	4.46	0.00	0.00	2.81
hsa-miR-224-5p		2.48	5.58	3.67	0.00	0.00	-0.37
hsa-miR-615-3p		2.25	0.78	4.69	0.00	0.00	3.72
hsa-miR-205-3p		2.18	0.01	2.83	0.01	0.02	-2.67
hsa-miR-452-3p		2.09	1.75	3.09	0.00	0.01	-1.94
hsa-miR-937-3p		2.03	0.70	4.38	0.00	0.00	2.50
hsa-miR-431-5p		1.99	0.17	4.85	0.00	0.00	4.40
hsa-miR-181b-3p		1.98	2.55	5.92	0.00	0.00	9.32
hsa-miR-4746-5p		1.97	1.96	5.57	0.00	0.00	7.62
hsa-miR-135b-3p		1.94	0.32	4.21	0.00	0.00	1.82
hsa-miR-452-5p		1.79	6.23	3.46	0.00	0.00	-1.08
hsa-miR-675-3p		1.71	3.24	2.34	0.02	0.05	-3.98

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

mRNA							
	symbol	**logFC**	**AveExpr**	**t**	**PValue**	**FDR**	**B**
ENSG00000096088	*PGC*	-9.90	1.58	-7.95	0.00	0.00	19.92
ENSG00000168631	*DPCR1*	-6.40	1.02	-7.60	0.00	0.00	17.91
ENSG00000184956	*MUC6*	-6.08	2.13	-6.17	0.00	0.00	10.30
ENSG00000167653	*PSCA*	-5.90	3.12	-8.23	0.00	0.00	21.56
ENSG00000019102	*VSIG2*	-5.25	1.84	-8.64	0.00	0.00	23.99
ENSG00000196188	*CTSE*	-5.07	2.69	-4.47	0.00	0.00	2.60
ENSG00000215182	*MUC5AC*	-5.03	2.86	-4.65	0.00	0.00	3.34
ENSG00000115386	*REG1A*	-4.81	1.67	-4.14	0.00	0.00	1.35
ENSG00000160182	*TFF1*	-4.81	1.65	-4.73	0.00	0.00	3.70
ENSG00000134240	*HMGCS2*	-4.80	0.95	-5.25	0.00	0.00	5.97
ENSG00000112936	*C7*	-4.54	1.11	-7.29	0.00	0.00	16.22
ENSG00000109906	*ZBTB16*	-4.45	0.54	-8.59	0.00	0.00	23.63
ENSG00000174514	*MFSD4A*	-4.43	2.79	-8.94	0.00	0.00	25.79
ENSG00000168079	*SCARA5*	-4.30	0.20	-7.44	0.00	0.00	17.04
ENSG00000066405	*CLDN18*	-4.24	3.86	-3.84	0.00	0.00	0.24
ENSG00000125144	*MT1G*	-4.23	3.14	-8.73	0.00	0.00	24.52
ENSG00000163884	*KLF15*	-4.18	0.38	-9.84	0.00	0.00	31.17
ENSG00000170011	*MYRIP*	-4.16	0.14	-9.12	0.00	0.00	26.77
ENSG00000180875	*GREM2*	-4.14	0.10	-7.52	0.00	0.00	17.47
ENSG00000139874	*SSTR1*	-4.07	0.26	-4.82	0.00	0.00	4.19
LncRNA							
	symbol	**logFC**	**AveExpr**	**t**	**PValue**	**FDR**	**B**
ENSG00000241388	*HNF1A-AS1*	-3.02	1.24	-3.24	0.00	0.01	-1.51
ENSG00000254343	*AC091563.1*	-2.97	0.09	-6.23	0.00	0.00	10.67
ENSG00000259291	*ZNF710-AS1*	-2.97	2.31	-11.47	0.00	0.00	41.54
ENSG00000203709	*C1orf132*	-2.93	1.77	-9.43	0.00	0.00	28.67
ENSG00000250742	*LINC02381*	-2.68	2.18	-6.90	0.00	0.00	14.14
ENSG00000260912	*AL158206.1*	-2.24	2.63	-6.94	0.00	0.00	14.31
ENSG00000268388	*FENDRR*	-2.17	2.25	-4.87	0.00	0.00	4.40
ENSG00000272894	*AC004982.2*	-2.12	0.66	-4.56	0.00	0.00	3.21
ENSG00000227218	*AL157935.1*	-2.05	0.28	-4.92	0.00	0.00	4.69
ENSG00000196167	*COLCA1*	-1.98	2.77	-3.42	0.00	0.01	-1.03
ENSG00000224078	*SNHG14*	-1.96	3.00	-4.53	0.00	0.00	2.95
ENSG00000249669	*CARMN*	-1.76	1.78	-3.75	0.00	0.00	0.22
ENSG00000180769	*WDFY3-AS2*	-1.70	0.29	-6.59	0.00	0.00	12.44
ENSG00000188242	*PP7080*	-1.64	4.99	-3.81	0.00	0.00	0.12
ENSG00000277496	*AL357033.4*	-1.59	1.04	-3.73	0.00	0.00	0.20
ENSG00000260461	*AL133355.1*	-1.54	1.14	-6.53	0.00	0.00	12.16
ENSG00000180139	*ACTA2-AS1*	-1.49	0.34	-3.72	0.00	0.00	0.19
ENSG00000251615	*AC104825.2*	-1.48	1.56	-4.48	0.00	0.00	2.91
ENSG00000261338	*AC021016.2*	-1.48	0.14	-6.76	0.00	0.00	13.28
ENSG00000225302	*AC023283.1*	-1.48	1.22	-3.57	0.00	0.00	-0.34
miRNA							
		logFC	**AveExpr**	**t**	**PValue**	**FDR**	**B**
hsa-miR-204-5p		-3.91	0.51	-8.58	0.00	0.00	24.23
hsa-miR-375		-3.71	11.09	-3.90	0.00	0.00	0.35
hsa-miR-133a-3p		-2.98	4.07	-5.24	0.00	0.00	5.86
hsa-miR-1-3p		-2.63	4.72	-4.30	0.00	0.00	1.87
hsa-miR-133b		-2.39	0.64	-4.59	0.00	0.00	3.17
hsa-miR-129-5p		-2.38	1.30	-5.62	0.00	0.00	7.72
hsa-miR-1468-5p		-2.25	1.67	-7.11	0.00	0.00	15.49
hsa-miR-139-5p		-2.03	5.22	-7.48	0.00	0.00	17.60
hsa-miR-29b-2-5p		-2.02	4.15	-8.54	0.00	0.00	23.98
hsa-miR-148a-3p		-1.95	14.33	-6.52	0.00	0.00	12.20
hsa-miR-30a-3p		-1.93	10.62	-6.16	0.00	0.00	10.35
hsa-miR-29c-3p		-1.89	10.36	-6.05	0.00	0.00	9.79
hsa-miR-30a-5p		-1.87	13.23	-5.98	0.00	0.00	9.41
hsa-miR-145-5p		-1.78	11.00	-4.35	0.00	0.00	2.06
hsa-miR-338-5p		-1.75	2.04	-4.92	0.00	0.00	4.52
hsa-miR-378c		-1.74	3.04	-6.49	0.00	0.00	12.09
hsa-miR-145-3p		-1.62	5.36	-4.17	0.00	0.00	1.36
hsa-miR-338-3p		-1.56	9.42	-4.19	0.00	0.00	1.43
hsa-miR-29c-5p		-1.55	3.70	-7.52	0.00	0.00	17.83
hsa-miR-139-3p		-1.47	3.00	-4.79	0.00	0.00	3.90

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GO enrichment and KEGG pathway analysis

Thereby GO enrichment analysis, we indicated several prominent roles of the DEmRNAs. The 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 the Cell cycle, DNA replication, p53 signaling pathway, AGE-RAGE signaling pathway in diabetics, and PPAR signaling pathway (fig 3).

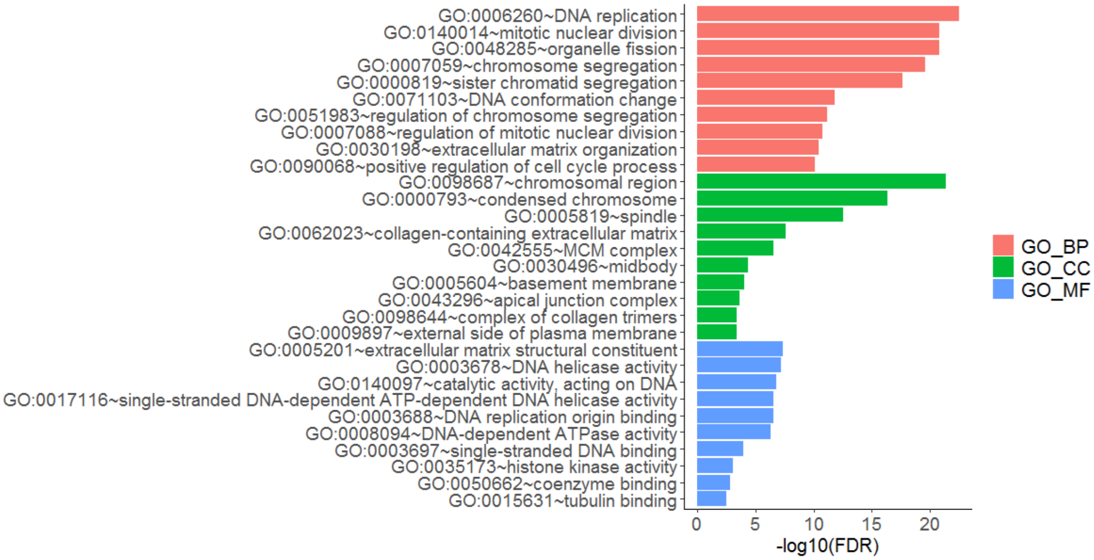

Figure 2. GO enrichment analysis of the differentially expressed mRNAs in ESCA (Top 10 GO enrichment are presented).

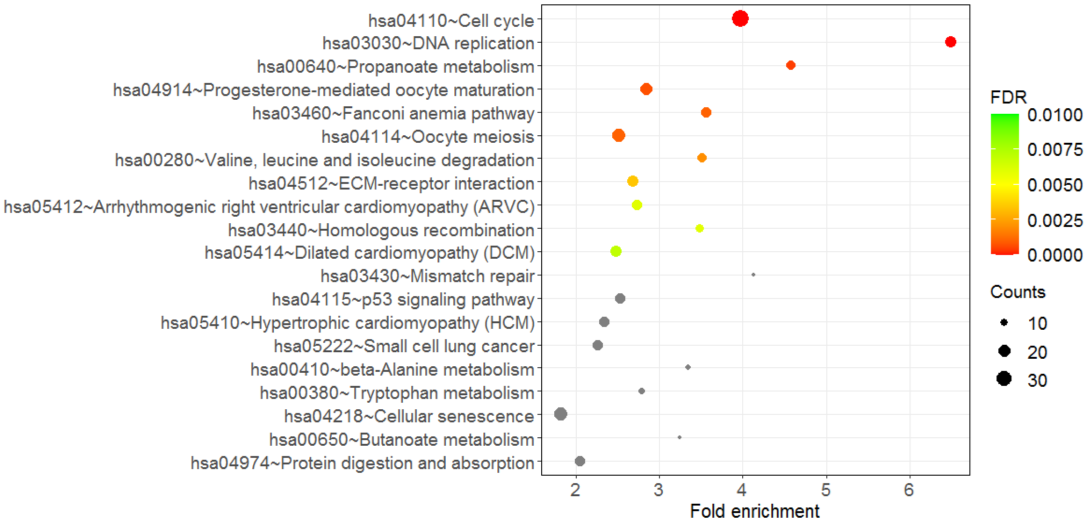

Figure 3. KEGG signaling pathway analysis of differentially expressed mRNAs in ESCA. (Top 20 KEGG terms are presented).

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Protein-protein interaction (PPI) network construction

To better understand 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).

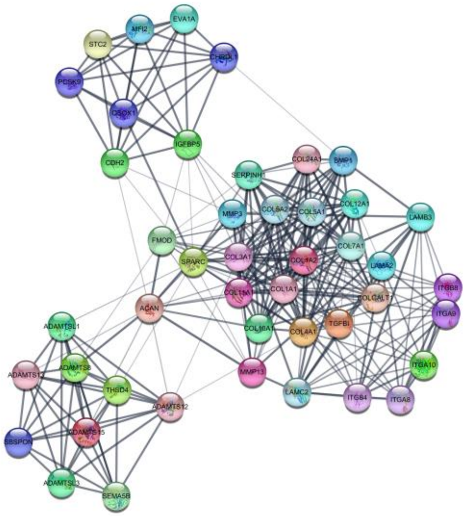

Figure 4. PPI network of the DE mRNAs in ESCA (score > 0.4) with Node:45, eadge:281, MCADE score: 12.773.

LncRNA-miRNA-mRNA ceRNA network construction

Based on the competing endogens RNA (ceRNA) hypothesis, which explains that lncRNAs regulate mRNA expression levels by competing with the shared miRNAs in cells, a ceRNA network was built based on the differentially expressed genes data via the 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 essential role in the development of ESCA (Fig 5).

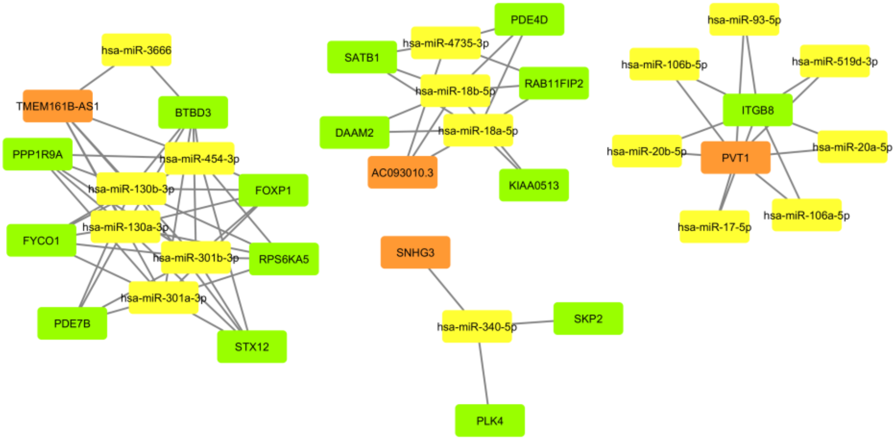

Figure 5. LncRNA-miRNA-mRNA ceRNA network construction of ESCA. (Orange: LncRNA, Yellow: miRNA, and Green: mRNA)

Kaplan-Meier survival analysis of differentially expressed genes

Kaplan-Meier survival analysis was conducted over the differentially expressed genes to explore the association of differential expression and the ESCA patient's prognosis. The data indicated that 41 mRNAs, five 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.

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

mRNA					
	symbol	**HR**	**lower95**	**upper95**	**pValue**
ENSG00000091879	*ANGPT2*	2.10	1.28	3.46	0.00
ENSG00000146386	*ABRACL*	2.10	1.27	3.45	0.00
ENSG00000168298	*HIST1H1E*	1.90	1.15	3.13	0.01
ENSG00000130208	*APOC1*	1.89	1.15	3.11	0.01
ENSG00000121769	*FABP3*	1.76	1.08	2.88	0.02
ENSG00000164283	*ESM1*	1.72	1.05	2.82	0.03
ENSG00000130826	*DKC1*	1.66	1.01	2.72	0.04
ENSG00000180818	*HOXC10*	1.66	1.02	2.71	0.04
ENSG00000040275	*SPDL1*	1.64	1.00	2.69	0.04
ENSG00000105486	*LIG1*	1.64	1.00	2.70	0.04
ENSG00000153310	*FAM49B*	1.64	1.00	2.68	0.04
ENSG00000124731	*TREM1*	1.61	0.97	2.67	0.05
ENSG00000126709	*IFI6*	0.62	0.38	1.01	0.05
ENSG00000148180	*GSN*	0.61	0.37	1.00	0.05
ENSG00000175287	*PHYHD1*	0.61	0.37	1.00	0.05
ENSG00000149582	*TMEM25*	0.61	0.37	0.99	0.05
ENSG00000128340	*RAC2*	0.61	0.37	0.99	0.05
ENSG00000137198	*GMPR*	0.61	0.37	0.99	0.04
ENSG00000182568	*SATB1*	0.60	0.36	1.00	0.04
ENSG00000090006	*LTBP4*	0.60	0.37	0.98	0.04
LncRNA					
	symbol	**HR**	**lower95**	**upper95**	**pValue**
ENSG00000180769	*WDFY3-AS2*	0.51	0.31	0.85	0.01
ENSG00000253669	*AP003356.1*	1.66	1.01	2.74	0.03
ENSG00000229970	*AC007128.1*	1.65	1.01	2.70	0.05
ENSG00000259366	*AC108449.2*	0.52	0.32	0.86	0.01
ENSG00000231607	*DLEU2*	1.70	1.04	2.80	0.03
miRNA					
		HR	**lower95**	**upper95**	**pValue**
hsa-miR-29c-3p		0.56	0.36	0.88	0.01
hsa-miR-181b-3p		1.61	1.03	2.51	0.04
hsa-miR-550a-3p		1.73	1.10	2.70	0.02
hsa-miR-3682-3p		1.71	1.09	2.68	0.02
hsa-miR-101-3p		0.61	0.39	0.97	0.03
hsa-miR-27a-3p		0.59	0.38	0.92	0.02
hsa-miR-23a-3p		0.59	0.38	0.92	0.02
hsa-miR-99a-5p		0.58	0.37	0.91	0.02
hsa-miR-1249-3p		0.64	0.41	1.00	0.05
hsa-miR-425-5p		1.96	1.25	3.08	0.00
hsa-miR-323b-3p		1.72	1.09	2.69	0.02
hsa-miR-1269a		1.56	0.99	2.47	0.04
hsa-miR-6842-3p		0.62	0.40	0.97	0.04
hsa-miR-151a-3p		0.63	0.40	0.98	0.04
hsa-let-7b-3p		0.56	0.36	0.88	0.01
hsa-let-7a-5p		0.55	0.35	0.87	0.01
hsa-miR-412-5p		0.60	0.39	0.95	0.03
hsa-let-7a-3p		0.57	0.37	0.89	0.01
hsa-miR-33a-3p		0.58	0.37	0.91	0.02
hsa-miR-31-3p		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.

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

lncRNA	AUC	SE	p-value	Lower (95%CI)	Upper (95%CI)	expression
MIR4435-2HG	0.99	0.007	0	0.977	1	Up
CYTOR	0.977	0.013	0	0.951	1	Up
AP003356.1	0.955	0.033	0	0.891	1	Up
PVT1	0.951	0.024	0	0.905	0.997	Up
C1orf132	0.941	0.031	0	0	0.12	Down
MAFG-AS1	0.94	0.035	0	0.872	1	Up
AL158212.3	0.936	0.046	0	0	0.155	Down
DLEU2	0.928	0.026	0	0.877	0.98	Up
ZNF710-AS1	0.926	0.039	0	0	0.151	Down
AC021016.2	0.924	0.04	0	0	0.155	Down
AL133355.1	0.919	0.039	0	0.004	0.159	Down
MELTF-AS1	0.916	0.035	0	0.848	0.984	Up
BLACAT1	0.911	0.029	0	0.854	0.969	Up
AC002384.1	0.909	0.035	0	0.84	0.978	Up
AC099850.3	0.906	0.053	0	0.802	1	Up
AC092718.4	0.903	0.052	0	0.8	1	Up
TYMSOS	0.901	0.041	0	0.82	0.981	Up
AC091563.1	0.901	0.044	0	0.014	0.185	Down
TMPO-AS1	0.899	0.031	0	0.839	0.96	Up
AC026401.3	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 biomarkers in 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
WDFY3-AS2	0.514	0.313	0.846	0.006	0.885	0.062	0.000	0.000	0.236	Down
AC108449.2	0.524	0.318	0.863	0.007	0.842	0.051	0.000	0.058	0.257	Down
DLEU2	1.702	1.035	2.799	0.029	0.928	0.026	0.000	0.877	0.980	Up
AP003356.1	1.661	1.005	2.744	0.034	0.955	0.033	0.000	0.891	1.000	Up
AC007128.1	1.654	1.013	2.700	0.046	0.854	0.063	0.000	0.731	0.977	Up
UGDH-AS1	0.645	0.395	1.053	0.077	0.827	0.083	0.000	0.011	0.335	Down
TMEM161B-AS1	1.478	0.905	2.416	0.114	0.862	0.061	0.000	0.019	0.257	Down
CD44-AS1	1.412	0.853	2.337	0.153	0.804	0.067	0.001	0.672	0.936	Up
AGAP2-AS1	0.707	0.433	1.154	0.164	0.853	0.060	0.000	0.734	0.971	Up
LINC00511	1.396	0.852	2.290	0.173	0.843	0.064	0.000	0.717	0.969	Up
AC122129.1	0.721	0.440	1.179	0.182	0.824	0.060	0.000	0.058	0.294	Down
AL357033.4	1.387	0.849	2.266	0.193	0.804	0.059	0.001	0.081	0.311	Down
AL133355.1	0.731	0.447	1.196	0.202	0.919	0.039	0.000	0.004	0.159	Down
FOXD2-AS1	1.374	0.841	2.243	0.205	0.827	0.091	0.000	0.648	1.000	Up
AC004803.1	0.728	0.446	1.190	0.208	0.780	0.070	0.002	0.084	0.357	Down
AC099850.3	0.735	0.450	1.201	0.210	0.906	0.053	0.000	0.802	1.000	Up
AC022211.2	0.735	0.450	1.203	0.214	0.863	0.048	0.000	0.769	0.956	Up
CASC9	1.338	0.818	2.187	0.239	0.822	0.064	0.000	0.695	0.948	Up
LINC01572	1.325	0.811	2.164	0.255	0.785	0.097	0.002	0.595	0.974	Up
TSC22D1-AS1	0.754	0.462	1.231	0.255	0.862	0.044	0.000	0.053	0.224	Down

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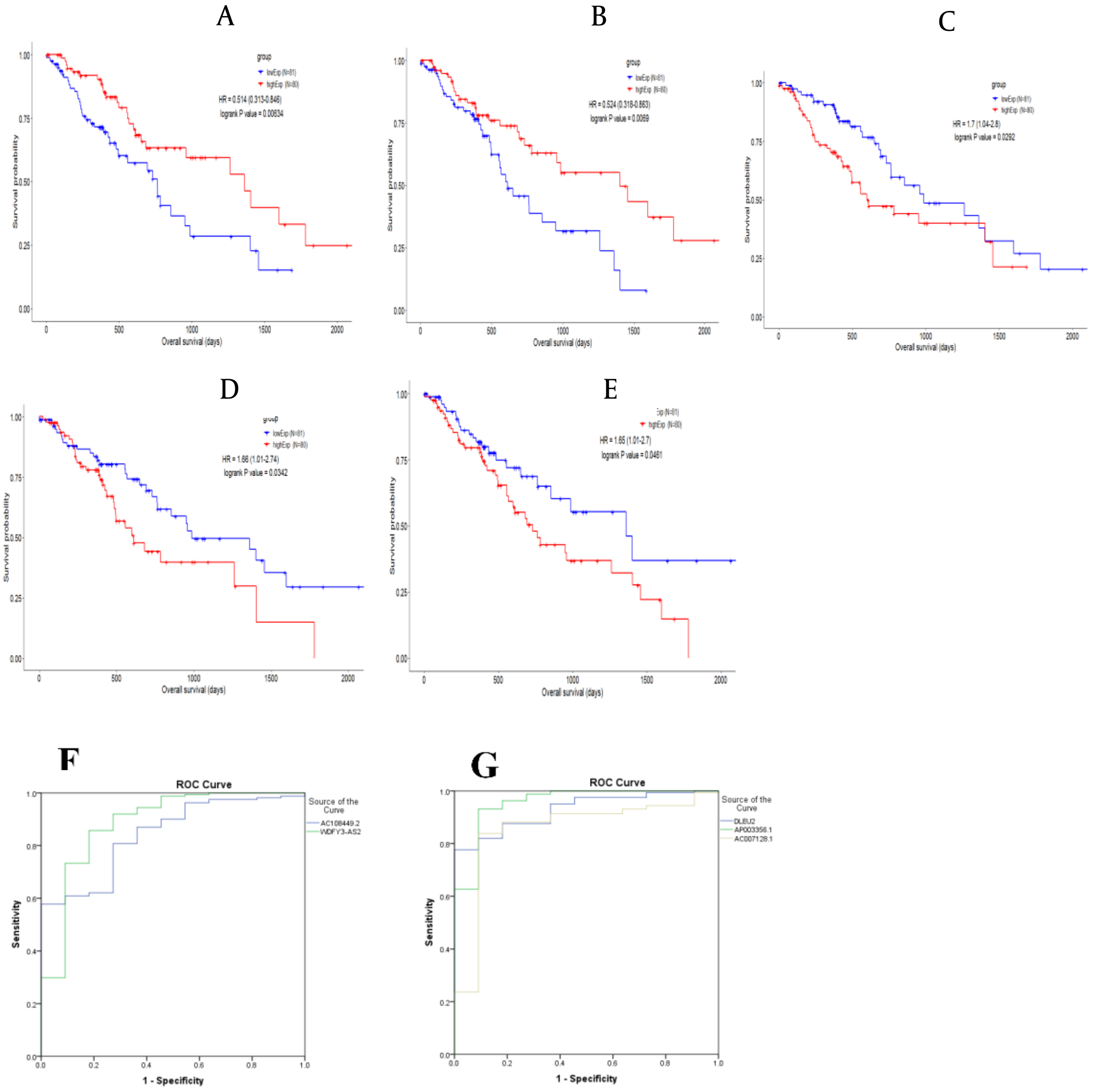

Figure 6. Kaplan-Meier and ROC curve analysis of the WDFY3-AS2, AC108449.2, DLEU2, AP003356.1, AC007128.1. A. Kaplan-Meier curve of WDFY3-AS2. B. Kaplan- Meier curve of AC108449.2. C. Kaplan-Meier curve of DLEU2. D. Kaplan-Meier curve of AP003356.1. E. Kaplan-Meier curve of AC007128.1. F and G. ROC curve of the lncRNAs.

Discussion

Esophageal cancer is one of the most aggressive types, with an increasing death rate and dismal prognosis. Previous investigations highlighted non-coding RNA, particularly lncRNA's roles in cancer development, progression, and clinicopathological features of the patients [12-14]. Many studies considered lncRNAs as a significant contributor to ESCA development and showed the lncRNAs' prognostic and diagnostic values for ESCA patients [15]. 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 patient 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 [16]. Furthermore, it has been shown that PRDX2 develops ESCA by instigating Wnt/β-catenin and AKT pathways in the cells [17]. P53 is one of the well-known tumor suppressor genes, which is dysregulated in the number of malignancies. Many examples depicted lncRNAs' role in p53 regulation in different 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. [18]. LncRNA SNHG1 increases liver cancer progression by recruiting DNMT1 to suppress p53 expression epigenetically [19].

Recently, the cross-talk between metabolism and cancer has been vastly explained in various cancer. It has been demonstrated that PPAR Signaling Pathway is one of the important signaling hubs between lipid metabolism and carcinogenesis [20]. LncRNA Ftx has been shown that promotes tumorigenesis by increasing glucose uptake, lactate production, and relative glycolytic enzyme through controlling the PPARγ pathway in hepatocellular carcinoma (HCC) [21].

Our protein-protein interaction data demonstrated that IGFBP5, ACAN, ADAMTS12, MMP13, and CDH2 had a central role in the signaling hubs through the PPI network. IGFBP5 has been discovered that act as an oncogene in the cells and drive tumorigenesis in different kinds of cancer. LncRNA UCA1 promotes carcinogenesis by upregulating IGFBP5 through sponging miR-204 in papillary thyroid carcinoma (PTC) cells [22]. ADAMTS12 has been reported that have 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 [23]. The previous investigation depicted that MMP13 (Matrix Metalloproteases 13) had vital roles in embryogenic development and cancerogenesis, such as proliferation and migration [24]. LncRNA LINC00511 promotes tumor growth, migration, and invasion by directly binding to miR-150 to upregulate MMP13 in breast cancer cells [25]. Cadherin-2 (CDH2) is a member of the cadherin family, which regulates crucial biological functions and tumorigenesis in various cancers [26]. 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 [27].

Furthermore, we demonstrated that lncRNA TMEM16B-AS1, AC093010.3, SNHG3, and PVT1 participated in CE networks and regulated 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 tumorigeneses by epigenetically suppressing MED18 through recruiting EZH2 to methylate the MED18 neighboring region in gastric cancer [28]. Recently, in a study, lncRNA SNHG3 has been shown that elevate the m6A level by binding to miR-186-5p to increase METTL3 expression in the ESCA cells [29]. 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 [30]. Furthermore, in vitro studies showed that PVT1 can induce invasion and metastasis by instigating epithelial-to-mesenchymal transition (EMT) in ESCA cells [31]. Interestingly, PVT1 has been indicated that induced tumorigenesis through sponging miR-203 and LASP1, which have tumor suppressive impact in the ESCA cells [32].

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 and ESCA. Overexpression of *lncRNA WDFY3-AS2* has been demonstrated that remarkably associated with clinical and molecular characteristics of glioma in patients and involve in the TNF signaling pathway [33]. 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 [34]. Previous studies have illustrated that lncRNA *DLEU2* expression correlates to poor prognosis in ESCA patients [35]. Furthermore, it has been shown that *DLEU2* can induce tumor growth, cell proliferation, invasion, and metastasis by upregulating E2F7 by directly inhibiting *miR-30e-5p* in ESCA cells [36].

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

Conflict of interest

The authors declare that there is no conflict of interest.

References

1. Liu, J., et al., Identification of a TLR-induced four-lncRNA signature as a novel prognostic biomarker in Esophageal Carcinoma. Frontiers in Cell and Developmental Biology, 2020. 8: p. 649.
2. Kang, M., et al., Exosome-mediated transfer of lncRNA PART1 induces gefitinib resistance in esophageal squamous cell carcinoma via functioning as a competing endogenous RNA. Journal of Experimental & Clinical Cancer Research, 2018. 37(1): p. 1-16.
3. Chu, J., et al., LncRNA MNX1-AS1 promotes progression of esophageal squamous cell carcinoma by regulating miR-34a/SIRT1 axis. Biomedicine & Pharmacotherapy, 2019. 116: p. 109029.
4. Liang, Y., et al., LncRNA CASC9 promotes esophageal squamous cell carcinoma metastasis through upregulating LAMC2 expression by interacting with the CREB-binding protein. Cell Death & Differentiation, 2018. 25(11): p. 1980-1995.
5. Chen, S., et al., LncRNA CASC11 promotes the development of esophageal carcinoma by regulating KLF6. Eur. Rev. Med. Pharmacol. Sci, 2019. 23: p. 8878-8887.
6. Wang, M., et al., lncRNA CCAT2 promotes radiotherapy resistance for human esophageal carcinoma cells via the miR-145/p70S6K1 and p53 pathway. International journal of oncology, 2020. 56(1): p. 327-336.
7. Poursheikhani, A., et al., Non-coding RNAs underlying chemoresistance in gastric cancer. Cellular Oncology, 2020: p. 1-28.
8. Yan, Y., et al., LncRNA Snhg1, a non-degradable sponge for miR-338, promotes expression of proto-oncogene CST3 in primary esophageal cancer cells. Oncotarget, 2017. 8(22): p. 35750.
9. Poursheikhani, A., et al., Integration analysis of long non-coding RNA (lncRNA) role in tumorigenesis of colon adenocarcinoma. BMC Medical Genomics, 2020. 13(1): p. 1-16.

[DOI: 10.52547/jhgg.6.2.84]

10. Li, Z., et al., Exosomal lncRNA ZFAS1 regulates esophageal squamous cell carcinoma cell proliferation, invasion, migration and apoptosis via microRNA-124/STAT3 axis. Journal of Experimental & Clinical Cancer Research, 2019. 38(1): p. 1-13.
11. Chang, Z.-w., et al., LncRNA-TUSC7/miR-224 affected chemotherapy resistance of esophageal squamous cell carcinoma by competitively regulating DESC1. Journal of Experimental & Clinical Cancer Research, 2018. 37(1): p. 1-12.
12. Rafieenia, F., et al., In silico evidence of high frequency of miRNA-related SNPs in Esophageal Squamous Cell Carcinoma. Journal of cellular physiology, 2020. 235(2): p. 966-978.
13. Poursheikhani, A., M.R. Abbaszadegan, and M.A. Kerachian, Mechanisms of long non-coding RNA function in colorectal cancer tumorigenesis. Asia-Pacific Journal of Clinical Oncology, 2021. 17(1): p. 7-23.
14. Poursheikhani, A., et al., Clinicopathological Significance of Long Non-Coding RNA GHET1 in Human Cancers: A Meta-Analysis. Current Pharmaceutical Biotechnology, 2020. 21(14): p. 1422-1432.
15. Song, W. and S.-b. Zou, Prognostic role of lncRNA HOTAIR in esophageal squamous cell carcinoma. Clinica chimica acta, 2016. 463: p. 169-173.
16. Wang, J., et al., CDKN3 promotes tumor progression and confers cisplatin resistance via RAD51 in esophageal cancer. Cancer management and research, 2019. 11: p. 3253.
17. Feng, A., et al., PRDX2 plays an oncogenic role in esophageal squamous cell carcinoma via Wnt/β-catenin and AKT pathways. Clinical and Translational Oncology, 2020. 22(10): p. 1838-1848.
18. Liu, B., et al., The long non-coding RNA AK001796 contributes to tumor growth via regulating expression of p53 in esophageal squamous cell carcinoma. Cancer cell international, 2018. 18(1): p. 1-8.
19. Li, S., et al., LncRNA SNHG1 promotes liver cancer development through inhibiting p53 expression via binding to DNMT1. Eur Rev Med Pharmacol Sci, 2019. 23(7): p. 2768-2776.
20. La Paglia, L., et al., Potential role of ANGPTL4 in the cross talk between metabolism and cancer through PPAR signaling pathway. PPAR research, 2017. 2017.
21. Li, X., et al., lncRNA Ftx promotes aerobic glycolysis and tumor progression through the PPARγ pathway in hepatocellular carcinoma. International journal of oncology, 2018. 53(2): p. 551-566.
22. Liu, H., et al., Knockdown of lncRNA UCA1 inhibits proliferation and invasion of papillary thyroid carcinoma through regulating miR-204/IGFBP5 axis. OncoTargets and therapy, 2018. 11: p. 7197.
23. Zheng, S., et al., Long non-coding RNA AK001058 regulates tumor growth and angiogenesis in colorectal cancer via methylation of ADAMTS12. American journal of translational research, 2019. 11(9): p. 6117.
24. Dumortier, M., et al., ETV4 transcription factor and MMP13 metalloprotease are interplaying actors of breast tumorigenesis. Breast Cancer Research, 2018. 20(1): p. 1-18.
25. Shi, G., et al., Long non-coding RNA LINC00511/miR-150/MMP13 axis promotes breast cancer proliferation, migration and invasion. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 2021. 1867(3): p. 165957.
26. Gao, S., et al., MicroRNA-194 regulates cell viability and apoptosis by targeting CDH2 in prostatic cancer. OncoTargets and therapy, 2018. 11: p. 4837.
27. Yao, Y., et al., LncRNA JPX overexpressed in oral squamous cell carcinoma drives malignancy via miR-944/CDH2 axis. Oral Diseases, 2021. 27(4): p. 924-933.
28. Xuan, Y. and Y. Wang, Long non-coding RNA SNHG3 promotes progression of gastric cancer by regulating neighboring MED18 gene methylation. Cell death & disease, 2019. 10(10): p. 1-12.
29. Zhang, M., et al., Targeting SNHG3/miR-186-5p reverses the increased m6A level caused by platinum treatment through regulating METTL3 in esophageal cancer. Cancer Cell International, 2021. 21(1): p. 1-11.
30. Xu, Y., et al., LncRNA PVT1 up-regulation is a poor prognosticator and serves as a therapeutic target in esophageal adenocarcinoma. Molecular cancer, 2019. 18(1): p. 1-15.
31. Zheng, X., H. Hu, and S. Li, High expression of lncRNA PVT1 promotes invasion by inducing epithelial-to-mesenchymal transition in esophageal cancer. Oncology letters, 2016. 12(4): p. 2357-2362.
32. Li, P.-D., et al., Upregulation of the long non-coding RNA PVT1 promotes esophageal squamous cell carcinoma progression by acting as a molecular sponge of miR-203 and LASP1. Oncotarget, 2017. 8(21): p. 34164.
33. Wu, Z., et al., Upregulation of microRNA-96 and its oncogenic functions by targeting CDKN1A in bladder cancer. Cancer cell international, 2015. 15(1): p. 1-9.
34. Rodrigues de Bastos, D. and M.A. Nagai, In silico analyses identify lncRNAs: WDFY3-AS2, BDNF-AS and AFAP1-AS1 as potential prognostic factors for patients with triple-negative breast tumors. PloS one, 2020. 15(5): p. e0232284.
35. Ma, W., et al., Upregulated long-non-coding RNA DLEU2 exon 9 expressions was an independent indicator of unfavorable overall survival in patients with esophageal adenocarcinoma. Biomedicine & Pharmacotherapy, 2019. 113: p. 108655.
36. Lu, T., et al., Long non-coding RNA DLEU2 promotes the progression of esophageal cancer through miR-30e-5p/E2F7 axis. Biomedicine & Pharmacotherapy, 2020. 123: p. 109650.

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