Comprehensive Analysis of LncRNAs Role in Tumorigenesis of Colon Adenocarcinoma

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Abstract

Background Colon adenocarcinoma (COAD) is one of the most common gastrointestinal cancers globally. Molecular aberrations of tumor suppressors and/or oncogenes are the main contributors to its tumorigenesis. However, the exact underlying mechanisms of COAD pathogenesis are not clear yet. Thus, there is an urgent need to indicate promising potential diagnostic and prognostic biomarkers in COAD patients. In the current study, level 3 RNA-seq and miR-seq data and corresponding clinical data of colon adenocarcinoma (COAD) were retrieved from the TCGA database. The “limma” package in R software was utilized to indicate the differentially expressed genes. For in silico functional analysis, GO and KEGG signaling pathways were conducted. PPI network was constructed based on the STRING online database by Cytoscape 3.7.2. A ceRNA network was also constructed by “GDCRNATools” package in R software. Kaplan-Meier survival analysis (log-rank test) was used to indicate the relation between RNA expressions with patient’s survival time.

Results The differential expression data demonstrated that 1512 mRNAs, 188 lncRNAs, and 329 miRNAs were differentially expressed in COAD. The GO and KEGG pathway analysis indicated that the differentially expressed mRNAs were primarily enriched in canonical processes in cancer. The PPI network showed that the PPARGC1A, ADAMTS5, PTGS2, FGFR2, TBX1, TWIST1, KIT, BDNF and MET proteins were the critical hubs. The Kaplan-Meier analysis revealed that 128 mRNAs, 15 lncRNAs, and 39 miRNAs were associated with overall survival time in the patients. The ceRNA network data demonstrated that three lncRNA including MAGI2-AS3, NEAT1, and SNHG3 genes were involved in the development of COAD.

Conclusions Altogether, we integrated differentially expressed mRNAs, lncRNAs, and miRNAs in COAD bioinformatically. Our data suggested promising lncRNAs in the diagnosis and prognosis of patients with COAD.

Introduction

Colon adenocarcinoma (COAD) is one of the most common gastrointestinal (GI) cancers and is the second leading cause of cancer related death globally [1, 2]. It is demonstrated that COAD occurs in approximately 5% of overall population at any given time in the world [3]. Despite current screenings and therapies such as endoscopic resection and radical surgery, near half of the patients are diagnosed as advanced cases of COAD, experiencing tumor recurrence and relapse [4]. The tumorigenesis of this cancer is a complicated multi-step process composed of epithelial cell proliferation, differentiation, apoptosis, survival, and invasion mechanisms [4]. Molecular aberrations of tumor suppressors and/or oncogenes are one of the main contributors in tumorigenesis [5]. However, the exact underlying pathogenesis of COAD are not clear yet. Thus, there is an urgent need to indicate promising diagnostic and prognostic biomarkers for COAD. Recent investigations have highlighted the role of non-coding RNAs in tumorigenesis in a variety of malignancies. Among different kinds of non-coding RNAs, long non-coding RNA (lncRNA) is a putative class of non-coding RNA with more than 200 nucleotides in length,

without any open-reading-frame (ORF) to encode proteins [5]. A large body of evidence indicated that lncRNAs plays critical roles in a variety of biological processes including cell proliferation, cellular development and differentiation, carcinogenesis, and metastasis through modulating gene expression at chromatin remodeling, transcriptional and posttranscriptional levels [6, 7]. Aberrant expression of lncRNAs has been well-documented in different sorts of cancers [8]. Dysregulation of lncRNA HOTAIR, H19, MALAT1, SNHG7, GAS8-AS, and NEAT1 were extensively well-studied and have been demonstrated to contribute in tumorigenesis and poor prognosis [5, 8–12]. A mounting of investigations have shown that lncRNAs exert their function through competing for endogenous RNA (ceRNA) crosstalk [13]. For instance, it has been shown that lncRNA SCARNA2 was overexpressed in COAD tissues and it remarkably correlated to chemoresistance via targeting miR-342-3p to upregulate EGFR and BCL2 in COAD cells [13]. Furthermore, overexpression of lncRNA SNHG1 has been shown to modify epithelial-mesenchymal transition (EMT) by binding to miR-497/miR-195-5p in COAD cells [14]. Moreover, lncRNA BDNF-AS was downregulated in COAD patients and served as a tumor suppressor gene. Besides, ectopic expression of BDNF-AS suppressed the cell proliferation and migration via epigenetically downregulating GSK-3 β expression through EZH2 [15]. Based on these and similar studies, many researchers have suggested the potency of lncRNAs as biomarkers in blood and serum. lncRNAs have been discovered in microvesicles and exosomes which have been protected and stabilized in circulation [16]. In the current study, we comprehensively investigate lncRNAs, miRNAs, and mRNAs expressions from public database the Cancer Genome Atlas (TCGA) and we constructed a ceRNA network in COAD. Besides, we proposed novel potential biomarkers for COAD.

Materials And Methods

Sample and data collection

Clinical data of COAD were retrieved from the TCGA database (<https://portal.gdc.cancer.gov/repository>). The inclusion criteria were: (1) the histopathological diagnosis was COAD; (2) having complete demographic data including age, vital status, race, ethnicity, pathological stage, TNM classification, and overall survival time. Totally, 459 COAD were enrolled in this study. Two hundred and thirty participants had age > 68 years and 229 patients had age $\leq$ 68 and 243 and 216 patients were male and female, respectively. Among 459 patients, only 4 patients were Hispanic or Latino and 271 were not Hispanic or Latino. Two hundred and fourteen patients were white, 29 patients were Black or African American, 11 were Asian and 1 American Indian/Alaska Native. Pathological stages of I, II, III, and IV were 76, 178, 129 and 65 respectively. The clinical characteristics are summarized in table 1.

RNA-seq and miR-seq data analysis

RNA-seq and miR-seq Level 3 data were collected from the TCGA database. The raw count of reads of RNA-seq and miR-seq data were normalized by Voom and TMM normalization methods. All the analyses were conducted in R software. The “limma” package in R software was utilized 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.

Functional enrichment analysis and protein-protein interaction (PPI) network

For *in silico* functional enrichment analysis, gene ontology (GO) in three domains including biological processes, cellular components, and molecular functions, besides to Kyoto Encyclopedia of Genes and Genomes (KEGG) signaling pathways were conducted. 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 [13]. 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 used to indicate the relation between over or downregulation of RNA, based on median expression with patient’s survival time. P -value < 0.05 was considered as a significant threshold.

Results

Differentially Expressed Genes

Our data demonstrated that 1512 mRNAs (433 up-regulated and 1079 down-regulated) were differentially expressed in COAD. Moreover, 188 lncRNAs (139 up-regulated and 49 down-regulated) were identified that were differentially expressed in patients. Three hundred and twenty nine miRNAs (169 up-regulated and 160 down-regulated) have been found that were differentially expressed in the COAD samples. The data are presented in Figures 1 and 2 as well as Tables 2 and 3.

GO enrichment and KEGG pathway analysis

GO enrichment analysis demonstrated that the differentially expressed mRNAs were almost enriched in biological processes such as neurogenesis, neuron differentiation, locomotion, biological adhesion and regulation of cell population proliferation. Furthermore, GO analysis in cellular component revealed that the differentially expressed mRNAs predominantly contributed to intrinsic component of plasma membrane, plasma membrane region, extracellular matrix, neuron part, and cell junction. GO molecular function domain indicated that the genes were mostly enriched in calcium ion binding, signaling receptor binding, molecular function regulator, protein dimerization activity, and identical protein binding. GO outputs are presented in Figure 3. In addition, KEGG pathway analysis showed that the differentially expressed genes in the COAD patients were remarkably participated in pathways in cancer, ECM receptor interaction, cell adhesion molecules (CAMs), calcium signaling pathway, WNT signaling pathway, and PPAR signaling pathway (Figure 4).

PPI network construction

The PPI network was constructed based on the STRING database to better understand the roles of the differentially expressed mRNAs. The data demonstrated that PPARGC1A, ADAMTS5, PTGS2, FGFR2, TBX1, TWIST1, KIT, BDNF and MET were the protein-protein interaction (PPI) critical hubs (Figure 5).

Kaplan-Meier survival analysis of differentially expressed genes

Kaplan-Meier survival analysis was used to indicate the association of differentially expressed mRNAs, lncRNAs, miRNA, and prognosis of COAD patients. The data showed that 128 mRNAs, 15 lncRNAs, and 39 miRNAs were associated with overall survival time in the patients. Top 10 the each group are presented in Table 4.

LncRNA-miRNA-mRNA ceRNA network construction

According to competing endogenous RNA (ceRNA) hypothesis, which implicates that lncRNAs regulate mRNA expression level by binding and controlling miRNAs in cells, a ceRNA network was built based on lncRNAs, mRNAs, and miRNAs expression in the samples based on starbase online tool in R software. The nodes and edges were drawn by Cytoscape 3.7.2. The ceRNA network data demonstrated three important lncRNAs including *MAGI2-AS3*, *NEAT1*, and *SNHG3* which have implied in development of COAD (Figure 6).

Discussion

LncRNAs regulate critical and canonical biological functions in different types of normal human cells and in a variety of tumor cells [17]. An escalating number of investigations reported lncRNAs function such as tumor proliferation, cell invasion and migration, chemotherapy resistance, and stemness capability in tumorigenesis and progression of COAD [18–20]. However, the exact underlying mechanisms of lncRNAs function in the progression of COAD are still unclear. Several different regulatory roles for lncRNAs have been proposed in biological processes. Some previous studies have demonstrated that lncRNAs regulate mRNA expression via binding and sponging miRNA in cells known as competing endogenous RNA (ceRNA) hypothesis that generate a new aspect in the lncRNA regulatory mechanism [21, 22]. To the best of our knowledge, only a few investigations have indicated ceRNA networks between lncRNAs and miRNAs in COAD so far. Thus, a clear image of lncRNAs-miRNAs links still remains uncharacterized. In this study, we studied the differentially expressed genes including lncRNAs, miRNAs, and mRNAs in the COAD patients based on TCGA database. Gene set enrichment by GO and KEGG signaling pathway mainly illustrated that the differentially expressed genes were significantly enriched in cell proliferation, differentiation, protein phosphorylation, and signaling pathways. Furthermore, KEGG signaling pathway analysis demonstrated several canonical signaling pathways including Wnt signaling pathways and PPAR signaling which have been shown to contribute in tumor progression [23, 24]. A mounting of evidence has emphasized on Wnt/ β -catenin signaling pathway that marvels actions in tumor growth, invasion and metastasis, and chemoresistance in COAD [25, 26]. For instance, it has been demonstrated that lncRNA H19 overexpression evoked Epithelial-Mesenchymal Transition (EMT) of colorectal cancer (CRC) cells by sponging miR-29b-3p in order to directly upregulate PGRN and activate Wnt axis [27]. Moreover, the up-regulation of lncRNA colorectal cancer-associated lncRNA (CCAL) promotes CRC progression through suppressing the activator protein 2 α (AP-2 α) to ignite Wnt/ β -catenin signaling pathway in the cells [28]. In the present study, the KEGG analysis indicated peroxisome proliferator-activated receptor (PPAR) pathway contribute with Wnt signaling. It has been discovered that the PPAR signaling pathway reduces cell proliferation and inhibits tumorigenesis in different types of cancer. Down-regulation of PPAR- α has been correlated to poor clinicopathological features of CRC that was remarkably higher in well to moderately differentiated adenocarcinoma than in mucinous adenocarcinoma [29]. In addition, lncRNA TINCR modulate PPAR signaling pathway through binding to miR-107 to up-regulate CD36 in CRC [30]. In a recent review article, the PPAR aberration expression and its important roles in gastrointestinal tract has been extensively reviewed [31]. Our ceRNA network data demonstrated three important lncRNA including MAGI2-AS3, NEAT1, and SNHG3 which have implied in the development of COAD. LncRNA MAGI2-AS3 have been discovered to play a crucial role as a tumor suppressor in breast cancer by targeting Fas/FasL in the tumor cells [32]. Moreover, MAGI2-AS3 hampers hepatocellular carcinoma cell growth and its invasion through sponging miR-374b-5p to up-regulate SMG1 axis [33]. On the other hand, overexpression of MAGI2-AS3 has been explained to straighten tumor progression by absorbing miR-141/200a and consequently, up-regulate ZEB1 in the gastric cancer cells [34]. In addition, MAGI2-AS3 up-regulation has been shown to exacerbated CRC proliferation and migration by modulating miR-3163 through upregulating TMEM106B [35]. LncRNA NEAT1 is a prominent

lncRNA that has been described in a variety of cancers [36]. NEAT1 expression is associated with unfavorable overall survival and tumor recurrence in patients with COAD [37]. Besides, NEAT1 can activate Wnt/ β -catenin signaling pathway by decreasing miRNA-34a expression to upregulate SIRT1 axis in COAD [38]. Moreover, NEAT1 promote cell growth and cell migration via upregulating glial cell-derived neurotrophic factor (GDNF) through binding to miR-196a-5p in COAD [39]. lncRNA SNHG3 has been demonstrated to promote tumor progression and invasion and it have been associated with poor prognosis in a wide range of cancers [40–42]. A previous study showed that SNHG3 was remarkably upregulated in COAD cells. SNHG3 can promote tumorigenesis of COAD via sponging miR-182-5p to upregulate c-Myc in tumor cells [43]. According to the aforementioned data, the proposed lncRNAs can be considered as potential promising biomarkers that dive tumorigenesis through hijacking canonical signaling pathways in COAD.

Conclusions

Altogether, in our study, we demonstrated the differentially expressed mRNAs, lncRNAs, and miRNAs in COAD. GO and KEGG pathway analysis demonstrated canonical biological processes and signaling pathways, which have been well described previously in tumorigenesis. Moreover, our Kaplan-Meier analysis results suggested several prominent prognostic differentially expressed genes associated with poor overall survival in the patients. Last but not least, our ceRNA network presented important lncRNAs-miRNAs-mRNAs links between the differentially expressed genes. The data provided might pave the way for further investigation and clinical application in COPD patients.

Declarations

Acknowledgments

This study was part of a Ph.D. dissertation (AP).

Author's contributions

AP, MRA, NN and MAK were all participated in study design, data analysis, and preparation of the drafted manuscript. All authors read 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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Tables

Table 1. Clinicopathological characteristics of COAD patients.

Characteristics	N	(%)
Age (year) (mean ± SD)	66.92 (13)	
Age > 68	230	50.1
Age ≤ 68	229	49.9
Sex		
Male	243	52.9
Female	216	47.1
Ethnicity		
Hispanic or Latino	4	0.9
Not Hispanic or Latino	271	59
NA	184	40.1
Race		
American Indian or Alaska Native	1	0.2
Asian	11	2.4
Black or African American	59	12.9
White	214	46.6
NA	174	37.9
Vital status		
Alive	357	77.8
Dead	102	22.2
Pathologic (stage)		
Stage I	76	16.5
Stage II	178	38.7
Stage III	129	28.1
Stage IV	65	14.1
Pathologic (T)		
T1	11	2.4
T2	78	17
T3	313	68.2
T4	56	12.2
Tis	1	0.2
Pathologic (M)		
M0	337	73.4
M1	65	14.2
MX	50	10.9
NA	7	1.5
Pathologic (N)		
N0	270	58.8
N1	106	23.1
N2	83	18

NA: Not Applicable.

Table 2. Top 20 upregulated mRNAs, lncRNAs, and miRNAs

Top 20 up-regulated mRNAs							
	symbol	logFC	AveExpr	t	PValue	FDR	B
ENSG00000144355	<i>DLX1</i>	5.79	3.61	8.31	0.00	0.00	25.33
ENSG00000119919	<i>NKX2-3</i>	4.89	-0.33	15.20	0.00	0.00	87.83
ENSG00000043355	<i>ZIC2</i>	4.85	0.17	10.80	0.00	0.00	45.29
ENSG00000115844	<i>DLX2</i>	4.50	0.25	13.01	0.00	0.00	65.77
ENSG00000164175	<i>SLC45A2</i>	4.34	0.67	10.33	0.00	0.00	41.30
ENSG00000197757	<i>HOXC6</i>	3.92	2.92	11.17	0.00	0.00	48.68
ENSG00000095627	<i>TDRD1</i>	3.80	2.36	6.49	0.00	0.00	13.35
ENSG00000251493	<i>FOXD1</i>	3.46	0.11	8.17	0.00	0.00	24.34
ENSG00000167346	<i>MMP26</i>	3.38	0.00	10.15	0.00	0.00	39.72
ENSG00000130675	<i>MNX1</i>	3.37	0.25	9.21	0.00	0.00	32.10
ENSG00000233041	<i>PHGR1</i>	3.33	-0.05	8.91	0.00	0.00	29.79
ENSG00000123500	<i>COL10A1</i>	3.29	1.41	7.73	0.00	0.00	21.29
ENSG00000198353	<i>HOXC4</i>	3.22	1.82	10.44	0.00	0.00	42.21
ENSG00000119547	<i>ONECUT2</i>	3.09	1.95	8.74	0.00	0.00	28.55
ENSG00000242110	<i>AMACR</i>	2.97	6.13	9.43	0.00	0.00	33.78
ENSG00000159263	<i>SIM2</i>	2.89	5.87	14.52	0.00	0.00	81.57
ENSG00000167332	<i>OR51E2</i>	2.87	7.93	8.88	0.00	0.00	29.29
ENSG00000136944	<i>LMX1B</i>	2.81	0.18	10.63	0.00	0.00	43.85
ENSG00000105707	<i>HPN</i>	2.79	7.54	16.56	0.00	0.00	103.52
ENSG00000187122	<i>SLIT1</i>	2.78	3.08	7.78	0.00	0.00	21.61
Top 20 up-regulated lncRNAs							
	symbol	logFC	AveExpr	t	PValue	FDR	B
ENSG00000225937	<i>PCA3</i>	4.68	6.65	7.31	0.00	0.00	18.28
ENSG00000223400	<i>AP006748.1</i>	4.06	-0.07	11.33	0.00	0.00	49.99
ENSG00000260896	<i>LINC02170</i>	3.31	3.70	5.04	0.00	0.00	5.48
ENSG00000280623	<i>PCAT14</i>	3.11	6.84	7.96	0.00	0.00	22.57
ENSG00000260228	<i>AC009119.1</i>	3.03	1.86	9.57	0.00	0.00	35.02
ENSG00000255545	<i>AP004608.1</i>	3.00	2.91	7.88	0.00	0.00	22.30
ENSG00000231324	<i>AP000696.1</i>	2.83	1.00	11.20	0.00	0.00	48.86
ENSG00000273179	<i>AC092535.4</i>	2.81	2.42	6.71	0.00	0.00	14.62
ENSG00000231806	<i>PCAT7</i>	2.79	0.84	12.79	0.00	0.00	63.66
ENSG00000203635	<i>AC144450.1</i>	2.78	0.00	8.26	0.00	0.00	24.96
ENSG00000228613	<i>AC141930.1</i>	2.71	0.08	8.39	0.00	0.00	25.92
ENSG00000233056	<i>ERVH48-1</i>	2.66	1.19	6.01	0.00	0.00	10.61
ENSG00000259641	<i>PCAT29</i>	2.62	0.53	12.62	0.00	0.00	62.00
ENSG00000234949	<i>AC104667.2</i>	2.50	1.38	11.40	0.00	0.00	50.68
ENSG00000253438	<i>PCAT1</i>	2.49	1.27	8.54	0.00	0.00	27.08
ENSG00000245750	<i>DRAIC</i>	2.39	4.14	9.26	0.00	0.00	32.56
ENSG00000261211	<i>AL031123.2</i>	2.36	0.59	13.17	0.00	0.00	67.31
ENSG00000258314	<i>AL589182.1</i>	2.29	-0.05	6.47	0.00	0.00	13.26
ENSG00000234753	<i>FOXP4-AS1</i>	2.25	0.14	13.59	0.00	0.00	71.50
ENSG00000259457	<i>AC100826.1</i>	2.25	1.55	5.15	0.00	0.00	6.11
Top 20 up-regulated miRNAs							
	symbol	logFC	AveExpr	t	PValue	FDR	B
hsa-miR-374a-3p		7.69	9.55	15.73	0.00	0.00	90.87
hsa-miR-135b-5p		6.45	5.85	8.48	0.00	0.00	26.20
hsa-miR-21-5p		6.32	17.44	27.96	0.00	0.00	219.16
hsa-miR-19b-3p		6.31	7.48	13.27	0.00	0.00	66.88

hsa-miR-142-3p	6.20	10.81	11.47	0.00	0.00	50.44
hsa-miR-19a-3p	6.16	5.36	10.03	0.00	0.00	38.20
hsa-miR-424-5p	6.07	6.75	12.54	0.00	0.00	59.99
hsa-miR-142-5p	6.04	6.12	12.36	0.00	0.00	58.34
hsa-miR-542-3p	5.74	7.00	14.22	0.00	0.00	75.91
hsa-miR-577	5.59	5.62	5.91	0.00	0.00	9.83
hsa-miR-29b-3p	5.40	9.40	13.88	0.00	0.00	72.73
hsa-miR-126-5p	5.24	7.06	13.04	0.00	0.00	64.66
hsa-miR-32-5p	5.23	4.63	14.04	0.00	0.00	74.18
hsa-miR-33a-5p	5.13	5.14	7.48	0.00	0.00	19.30
hsa-miR-582-3p	5.08	8.28	11.93	0.00	0.00	54.45
hsa-miR-203b-3p	5.05	7.07	7.03	0.00	0.00	16.42
hsa-miR-101-3p	5.01	12.47	21.16	0.00	0.00	148.02
hsa-miR-18a-5p	4.99	4.50	9.33	0.00	0.00	32.60
hsa-miR-429	4.93	8.33	11.45	0.00	0.00	50.22
hsa-miR-374a-5p	4.91	4.77	12.57	0.00	0.00	60.33

Table 3. Top 20 downregulated mRNAs, lncRNAs, and miRNAs

Top 20 down-regulated mRNAs							
	symbol	logFC	AveExpr	t	PValue	FDR	B
ENSG00000171401	<i>KRT13</i>	-3.36	1.73	-10.69	0.00	0.00	44.42
ENSG00000188488	<i>SERPINA5</i>	-3.36	0.24	-16.46	0.00	0.00	102.35
ENSG00000159251	<i>ACTC1</i>	-3.36	2.91	-13.89	0.00	0.00	75.07
ENSG00000100884	<i>CPNE6</i>	-3.07	0.06	-12.83	0.00	0.00	64.46
ENSG00000157551	<i>KCNJ15</i>	-2.95	0.44	-16.90	0.00	0.00	107.15
ENSG00000176153	<i>GPX2</i>	-2.90	1.02	-16.22	0.00	0.00	99.73
ENSG00000147255	<i>IGSF1</i>	-2.88	0.01	-14.40	0.00	0.00	80.29
ENSG00000149021	<i>SCGB1A1</i>	-2.87	1.22	-8.98	0.00	0.00	30.27
ENSG00000161055	<i>SCGB3A1</i>	-2.84	0.26	-12.32	0.00	0.00	59.50
ENSG00000134184	<i>GSTM1</i>	-2.83	0.51	-7.55	0.00	0.00	19.85
ENSG00000133067	<i>LGR6</i>	-2.82	1.13	-15.73	0.00	0.00	94.44
ENSG00000156076	<i>WIF1</i>	-2.79	0.68	-11.96	0.00	0.00	56.10
ENSG00000181195	<i>PENK</i>	-2.77	0.70	-13.08	0.00	0.00	66.94
ENSG00000002726	<i>AOC1</i>	-2.75	1.93	-8.99	0.00	0.00	30.30
ENSG00000104879	<i>CKM</i>	-2.73	0.79	-9.58	0.00	0.00	35.13
ENSG00000140279	<i>DUOX2</i>	-2.71	0.85	-13.29	0.00	0.00	69.01
ENSG00000248485	<i>PCP4L1</i>	-2.69	0.86	-16.62	0.00	0.00	104.05
ENSG00000162989	<i>KCNJ3</i>	-2.68	0.86	-13.05	0.00	0.00	66.67
ENSG00000142973	<i>CYP4B1</i>	-2.68	2.62	-14.18	0.00	0.00	78.03
ENSG00000181778	<i>TMEM252</i>	-2.67	-0.21	-12.94	0.00	0.00	65.54
Top 20 down-regulated lncRNAs							
	symbol	logFC	AveExpr	t	PValue	FDR	B
ENSG00000241158	<i>ADAMTS9-AS1</i>	-2.57	1.21	-14.25	0.00	0.00	78.78
ENSG00000224958	<i>PGM5-AS1</i>	-2.49	1.59	-11.29	0.00	0.00	49.89
ENSG00000261116	<i>AL049555.1</i>	-2.36	-0.23	-11.49	0.00	0.00	51.73
ENSG00000267405	<i>AC005180.1</i>	-2.23	0.67	-12.93	0.00	0.00	65.41
ENSG00000230937	<i>MIR205HG</i>	-2.20	3.46	-7.39	0.00	0.00	18.53
ENSG00000267505	<i>AC005180.2</i>	-2.16	1.27	-13.55	0.00	0.00	71.58
ENSG00000250056	<i>LINC01018</i>	-1.99	0.26	-11.06	0.00	0.00	47.85
ENSG00000226237	<i>GAS1RR</i>	-1.99	-0.09	-13.18	0.00	0.00	67.81
ENSG00000250508	<i>AP000808.1</i>	-1.93	0.49	-8.29	0.00	0.00	25.19
ENSG00000232855	<i>AF165147.1</i>	-1.91	2.55	-11.10	0.00	0.00	48.07
ENSG00000254510	<i>AP001107.5</i>	-1.89	0.05	-10.82	0.00	0.00	45.69
ENSG00000237949	<i>LINC00844</i>	-1.86	1.40	-8.27	0.00	0.00	24.94
ENSG00000241684	<i>ADAMTS9-AS2</i>	-1.83	0.48	-13.23	0.00	0.00	68.39
ENSG00000250786	<i>SNHG18</i>	-1.81	2.50	-11.70	0.00	0.00	53.59
ENSG00000174403	<i>C20orf166-AS1</i>	-1.72	0.61	-9.40	0.00	0.00	33.65
ENSG00000260265	<i>LINC02562</i>	-1.68	0.12	-5.89	0.00	0.00	9.77
ENSG00000237989	<i>LINC01679</i>	-1.66	0.00	-11.59	0.00	0.00	52.58
ENSG00000272143	<i>FGF14-AS2</i>	-1.59	0.03	-10.46	0.00	0.00	42.49
ENSG00000261616	<i>AC036108.3</i>	-1.57	0.58	-11.15	0.00	0.00	48.64
ENSG00000234456	<i>MAGI2-AS3</i>	-1.56	3.10	-14.43	0.00	0.00	80.60
Top 20 down-regulated miRNAs							
	symbol	logFC	AveExpr	t	PValue	FDR	B
hsa-miR-486-5p		-6.12	5.62	-14.35	0.00	0.00	77.43
hsa-miR-139-3p		-6.10	1.99	-14.44	0.00	0.00	78.35
hsa-miR-129-5p		-5.76	0.92	-13.35	0.00	0.00	67.73
hsa-miR-328-3p		-5.63	3.77	-21.46	0.00	0.00	151.16

hsa-miR-139-5p	-5.38	4.32	-17.29	0.00	0.00	107.26
hsa-miR-6511b-3p	-5.37	0.31	-17.29	0.00	0.00	107.15
hsa-miR-642a-5p	-5.07	1.39	-11.47	0.00	0.00	50.39
hsa-miR-197-3p	-4.97	7.87	-22.64	0.00	0.00	163.76
hsa-miR-133a-3p	-4.59	3.71	-8.32	0.00	0.00	24.94
hsa-miR-194-3p	-4.51	7.68	-13.59	0.00	0.00	70.05
hsa-miR-766-3p	-4.50	2.50	-18.10	0.00	0.00	115.63
hsa-miR-149-5p	-4.49	3.27	-13.05	0.00	0.00	64.85
hsa-miR-1306-5p	-4.46	2.46	-17.48	0.00	0.00	109.21
hsa-miR-1976	-4.44	2.84	-18.10	0.00	0.00	115.64
hsa-miR-125a-5p	-4.39	7.80	-16.53	0.00	0.00	99.38
hsa-let-7d-3p	-4.34	8.50	-18.59	0.00	0.00	120.89
hsa-miR-671-3p	-4.28	1.31	-16.20	0.00	0.00	95.92
hsa-miR-150-3p	-4.27	0.56	-9.24	0.00	0.00	31.83
hsa-miR-1180-3p	-4.23	3.03	-11.14	0.00	0.00	47.49
hsa-miR-378a-5p	-4.20	6.76	-15.80	0.00	0.00	91.92

Table 4. Top 10 mRNAs, lncRNAs, and miRNAs that were associated with overall survival.

mRNA					
	symbol	HR	lower95	upper95	pValue
ENSG00000108852	<i>MPP2</i>	2.08	1.41	3.07	0.00
ENSG00000184988	<i>TMEM106A</i>	1.99	1.35	2.94	0.00
ENSG00000099822	<i>HCN2</i>	1.92	1.30	2.84	0.00
ENSG00000128714	<i>HOXD13</i>	1.90	1.28	2.81	0.00
ENSG00000157168	<i>NRG1</i>	0.51	0.35	0.76	0.00
ENSG00000198944	<i>SOWAHA</i>	0.53	0.36	0.78	0.00
ENSG00000137960	<i>GIPC2</i>	0.53	0.36	0.78	0.00
ENSG00000188818	<i>ZDHHC11</i>	1.89	1.28	2.79	0.00
ENSG00000087077	<i>TRIP6</i>	1.88	1.28	2.77	0.00
ENSG00000087253	<i>LPCAT2</i>	0.54	0.37	0.80	0.00
lncRNA					
	symbol	HR	lower95	upper95	pValue
ENSG00000227953	<i>LINC01341</i>	1.81	1.23	2.67	0.00
ENSG00000263272	<i>AC004148.2</i>	1.78	1.21	2.63	0.00
ENSG00000259793	<i>AC013726.1</i>	1.78	1.21	2.62	0.00
ENSG00000268388	<i>FENDRR</i>	0.60	0.41	0.88	0.01
ENSG00000226237	<i>GAS1RR</i>	1.61	1.09	2.38	0.01
ENSG00000206195	<i>DUXAP8</i>	1.58	1.07	2.33	0.02
ENSG00000272695	<i>GAS6-AS2</i>	1.57	1.07	2.32	0.02
ENSG00000260920	<i>AL031985.3</i>	0.64	0.44	0.95	0.03
ENSG00000228613	<i>AC141930.1</i>	1.54	1.04	2.27	0.03
ENSG00000258727	<i>AL135999.1</i>	1.52	1.03	2.24	0.04
miRNA					
		HR	lower95	upper95	pValue
hsa-miR-128-1-5p		0.40	0.27	0.60	0.00
hsa-miR-21-3p		0.51	0.35	0.76	0.00
hsa-miR-130a-3p		1.84	1.24	2.72	0.00
hsa-miR-210-3p		1.79	1.21	2.65	0.00
hsa-miR-3614-5p		0.56	0.38	0.82	0.00
hsa-miR-194-3p		0.57	0.38	0.84	0.00
hsa-miR-193a-3p		1.78	1.21	2.63	0.00
hsa-miR-200a-5p		0.57	0.39	0.85	0.00
hsa-miR-887-3p		1.76	1.19	2.59	0.01
hsa-miR-486-5p		0.58	0.39	0.86	0.01

Figures

Figure 1

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

Figure 2

Volcano plot of differentially expressed genes and miRNAs. A. Volcano plot of differentially expressed lncRNAs and mRNAs. Overexpressed genes are demonstrated in red and down-regulated genes are demonstrated in green. B. Volcano plot of differentially expressed miRNA. Overexpressed and down-regulated genes are demonstrated in red and green, respectively.

Figure 3

GO enrichment analysis of the differentially expressed mRNAs in COAD (Top 20 GO enrichment are presented). A. Biological processes (BP), B. Cellular component (CC), C. Molecular function (MF).

Figure 4

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

Figure 5

Protein-protein interaction (PPI) network of the differentially mRNAs in COAD (score > 0.4).

Figure 6

LncRNA-miRNA-mRNA ceRNA network construction of COAD differentially expressed genes.