European Review for Medical and Pharmacological Sciences 2023; 27: 10462-10471

Screening of m6A gene-related lncRNAs in colon adenocarcinoma and construction of a prognostic prediction model

L.-P. JIN, Y.-W. SUN, T. LIU, J.-D. TAI

Department of Colorectal and Anal Surgery, First Hospital Bethune of Jilin University, Changchun, Jilin, China

Abstract. – OBJECTIVE: We aimed to screen the long non-coding RNAs (lncRNAs) related to N6-methyladenosine (*m6A*) gene and build the prognostic prediction model of colon adenocarcinoma (COAD).

MATERIALS AND METHODS: The RNA sequencing data of 435 COAD cases with clinical survival and prognosis information and the GSE39582 dataset were obtained from TCGA and GEO, respectively. The lncRNAs related to the *m6A* gene with significant independent prognosis were identified. We used Cox regression analyses to acquire the lncRNAs associated with prognosis. Moreover, we built a prognostic prediction model of COAD. The Cox regression analyses were applied to obtain the independent prognostic clinical factors. Furthermore, we built the ceRNA regulation network of COAD, and the gene ontology (GO) and Kyoto Encyclopedia of Genes (KEGG) enrichment analysis for the lncRNAs was applied.

RESULTS: Overall, 5 lncRNAs (*MAGI1-IT1, CSNK1G2-AS1, ALMS1-IT1, LINC01341, LOXL1-AS1*) related to *m6A* gene with significant independent prognosis were acquired. A prognostic prediction model of COAD was built, and 4 correlation-independent prognostic factors were found. In addition, the ceRNA regulation network of COAD was built, and mRNAs were significantly enriched in the 15 GO biological processes (such as regulation of transcription) and in 14 KEGG pathways (such as taurine).

CONCLUSIONS: We identified 5 lncRNAs related to the *m6A* gene with significant independent prognosis. The ceRNA regulation network of COAD was built, which has great significance for identifying the biomarkers associated with *m6A* in COAD.

Key Words:
m6A gene, Colon adenocarcinoma, Prognostic prediction model, ceRNA regulation network.

Introduction

Colon adenocarcinoma (COAD), as a widespread disease of the gastrointestinal tract, ranks at the forefront of the incidence of malignant tumors[1]. More than one million new cases of COAD are reported each year, which accounts for 6.1% of all cancers[2]. With the changes in people's diet and the intake of high-calorie, high-protein, and low-fiber foods, the incidence of COAD is increasing year by year, and the age of onset tends to be younger, which becomes one of the main diseases threatening people's health[3]. Although the application of radiotherapy or chemotherapy in COAD can significantly reduce the recurrence rate, it is of little value in prolonging the survival of patients. Thus, the study of RNAs based on ceRNA network will contribute to finding new prognostic biomarkers of COAD. N6-methyladenosine (m6A) is a universal post-transcriptional modification of RNA[4]. m6A methylation is essential for all major biological processes. The modification of m6A methylation influences the process of cancers by regulating the mRNA expression levels of associated genes[5]. m6A modification has been proven to play a vital role in the progression of colorectal cancer[6]. Therefore, m6A may be a significant way to regulate the occurrence and development of cancer.

A large number of research[7] have proven the mutual regulation between long non-coding RNA (lncRNA) and microRNA (miRNA), and their regulated mRNAs are tightly associated with the development of cancers. Additionally, it can also be used as a diagnostic and prognostic biomarker for diseases.

In this research, we first screened the m6A-related lncRNAs that were significantly correlated with prognostic in COAD samples and built a COAD prognostic risk prediction model through the expression pattern and prognostic influence of m6A-related lncRNAs. Moreover, the characteristic RNA molecules with significant changes in the transcriptome level of COAD disease samples were screened by building a ceRNA regulatory network. The results provide a theoretical basis for the development of biomarkers for COAD.

Corresponding Author: Jian Dong Tai, MD; e-mail: jinwen2046@jlu.edu.cn

Materials and Methods

Data Preprocessing

The RNA sequencing data of 435 cases with COAD were used as the training dataset, these cases contained personal clinical information, which was obtained through The Cancer Genome Atlas (TCGA) based on Illumina HiSeq 2000 RNA Sequencing[8]. Meanwhile, GSE39582 dataset was retrieved from the Gene Expression Omnibus (GEO) repository by searching the keywords "colon adenocarcinoma and homo sapiens". The following criteria were applied to perform the search: 1) solid tissues of COAD; 2) sample size was not lower than 400; 3) detection platform can annotate a large number of lncRNAs. Finally, we acquired the GSE39582 gene expression profile data through the platform GPL570 Affymetrix Human Genome U133 Plus 2.0 Array (USA)[9], which met the above criteria and was downloaded from GEO. There were 585 COAD cases, of which 562 had survival-associated prognostic information, and these 562 cases were used as the validation dataset.

Identification of lncRNAs Associated with m6A

The expression levels of the genes related to m6A from TCGA and GSE39582 dataset samples were screened, and included: methylation genes (*WTAP, VIRMA, RBM15, RBM15B, KIAA1429, ZC3H13, METTL3, METTL14, METTL15*); demethylation genes (*FTO, ALKBH5*) and m6A specific binding gene (*RBMX, YTHDC1, YTHDC2, IGF2BP1, IGF2BP2, IGF2BP3, YTHDF1, YTHDF2, YTHDF3, HNRNPA2B1, HNRNPC*). Then, the cor.test function in R3.6.1 (R Foundation, Vienna, Austria) was applied to calculate the Pearson's correlation coefficient (PPC) of the expression level for m6A-related genes, and the identified lncRNAs. The lncRNAs which significantly related to the *m6A* gene at the expression level were screened with the $|PCC| > 0.5$ and the $p < 0.001$ as the thresholds. Finally, we obtained two sets of lncRNAs for the next analysis.

Screening of lncRNAs Associated with Prognosis

The lncRNAs associated with prognosis were acquired through Univariate Cox regression analysis in R3.6.1 survival package[10] ($p < 0.05$). Next, the lncRNAs that were significantly connected with the prognosis of the two datasets were compared, and the overlapping lncRNAs were maintained for further analysis.

Construction of Prognostic Risk Prediction Model

Multivariate Cox regression analysis in R3.6.1 survival package[11] was applied to acquire lncRNAs that were linked to prognosis in the TCGA dataset ($p < 0.05$). On the basis of the prognostic coefficient of each element in the lncRNAs combination obtained by the multivariate Cox regression algorithm, a risk prediction model was built on the basis of lncRNA expression levels in TCGA dataset. The risk score (RS) of each sample was calculated according to the following formula:

$$RS = \sum Coef_{lncRNAs} \times Exp_{lncRNAs}$$

where $\sum Coef_{lncRNAs}$ indicates the prognostic coefficient of lncRNAs in multivariate Cox regression analysis, and $Exp_{lncRNAs}$ represents the expression levels of lncRNAs in the TCGA dataset.

With the median RS as the critical value, the cases at the training dataset were separated into high- and low-risk group; and the association between the prognosis and risk model was measured by applying the Kaplan-Meier (KM) curve in the R3.6.1 language survival package[12].

Screening of Independent Prognostic Clinical Factors

The univariate and multivariate Cox regression analyses were performed by applying the R3.6.1 language survival package[12]. Then, the independent prognostic clinical factors of COAD cases in the TCGA dataset were acquired ($p < 0.05$).

According to RS value, the cases in the TCGA dataset were separated into high- and low-risk groups. Afterward, we used the R3.6.1 limma package12 to study the differentially expressed genes (DEGs) between the mRNA expression matrices of the samples in these two groups [False Discovery Rate (FDR) < 0.05, |logFold Change (FC)| > 0.5]. Gene set enrichment analysis (GSEA)[13] was applied to obtain the significant Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways ($p < 0.05$).

Construction and Evaluation of ceRNA Regulation Network

First, we downloaded the miRNAs directly connected with COAD through the Human MicroRNA Disease Database (HMDD)[14]. Then, to build the lncRNA-miRNA regulation network, the DIANA-LncBasev2 database was used to identify the regulatory interactions

between the RS prognostic model-related lncRNAs and COAD-related miRNAs. Target genes regulated by COAD-related miRNAs were revealed via the starBase database[15]. We collected at least one database (targetScan, picTar, RNA22, PITA, and miRanda databases) to predict regulatory interactions, and DEGs were mapped to mRNAs for miRNA-mRNA regulation network construction. Combining lncRNA-miRNA and miRNA-mRNA regulatory interactions, a ceRNA regulation network was built. The GO and KEGG pathways on the regulated mRNAs of the ceRNA regulatory network were analyzed through the DAVID 6.8 (https://david.ncifcrf.gov/home.jsp)[16] ($p < 0.05$). The flow chart of this research is displayed in Figure 1.

Statistical Analysis

We used R language (version 3.6.1, R Foundation, Vienna, Austria) for statistical analysis. Univariate Cox regression analysis was conducted to acquire the prognostic-related lncRNAs, KM curve was performed to show the survival time of patients in high- or low-risk groups, and the log-rank test was carried out to compare the difference in survival time between the two groups. We used ROC analysis to assess the predictive capacity of the prognostic model. $p < 0.05$ was set as the statistical significance level.

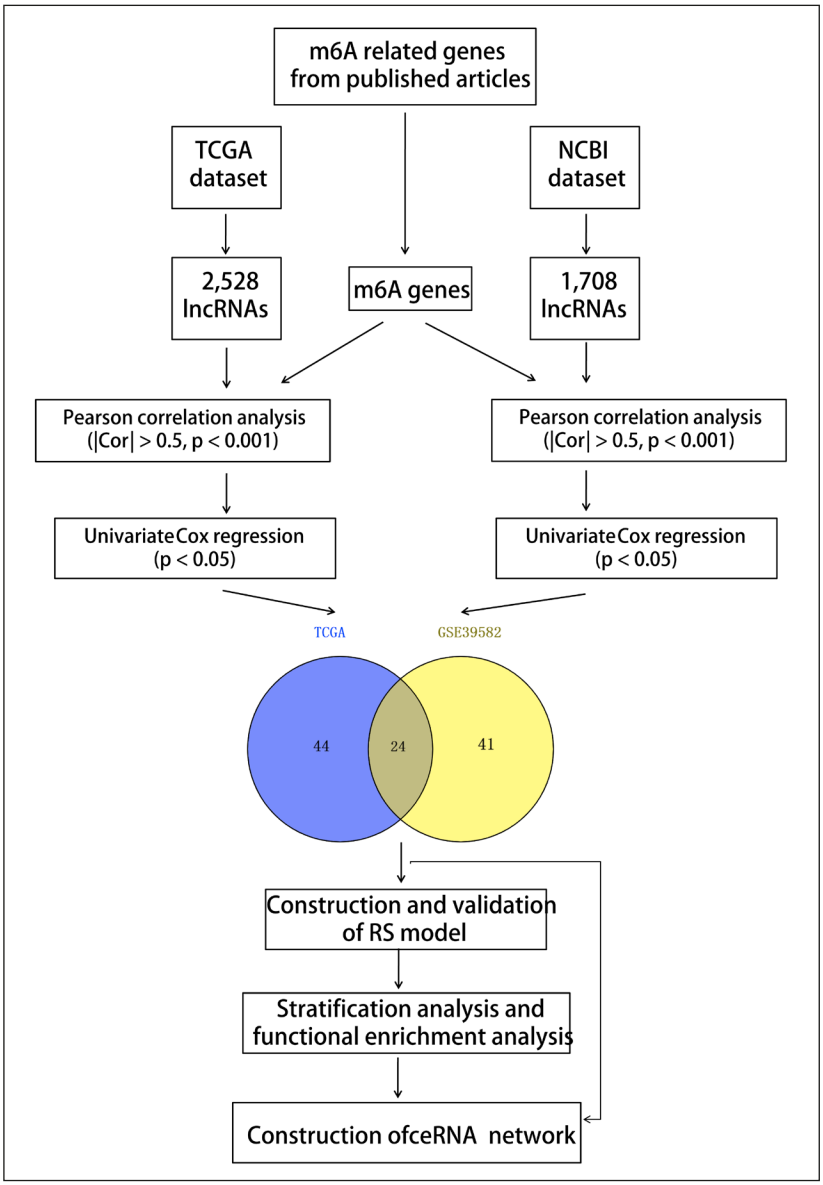

Figure 1. The flowchart of ceRNA regulatory network. TCGA, The Cancer Genome Atlas; NCBI, National Center for Biotechnology Information; RS, risk score.

Results

Identification of lncRNAs Associated with m6A

After the annotation of lncRNAs in the TCGA dataset and GSE39582 expression profile, 2,528 and 1,708 lncRNAs were obtained, respectively. The expression values of all genes related to m6A from the two datasets were extracted. The lncRNAs that were significantly related to the *m6A* gene at the expression level were screened. A total of 1,279 connection pairs were acquired, involving 534 and 423 lncRNAs, respectively ($|PCC| > 0.5$, $p < 0.001$).

Screening of lncRNAs Associated with Prognosis

Based on the two sets of lncRNAs related to m6A, 68 and 65 lncRNAs associated with the prognosis were acquired through a Univariate Cox regression analysis on the TCGA and GSE39582 datasets, respectively. After comparing two sets of lncRNAs associated with the prognosis, there were a total of 24 overlapping lncRNAs.

Construction of Prognostic Risk Prediction Model

Based on the acquired 24 overlapping lncRNAs, 5 independent lncRNAs (*MAGI1-IT1, CSNK1G2-AS1, ALMS1-IT1, LINC01341, LOXL1-AS1*) that were connected with prognostic were identified by regression analysis in the TCGA dataset (Table I). According to the prognostic regression coefficient of these lncRNAs, a risk prediction model was built. First, we calculated the RS value of each sample at the TCGA and GSE39582 datasets based on the RS formula. The RS distribution of the two data sets is shown in Figure 2. We used the KM curves of TCGA and GSE39582 datasets to assess the relationship between these two groups and actual prognostic information for COAD (Figure 2). It indicated that the low-risk samples at the TCGA dataset had a better survival prognosis [p = 2.516e-04, HR: 2.129 (1.402-3.233)], and the GSE39582 dataset also had the same trend [p = 1.765e-02, HR: 1.413 (1.061-1.883)]. The results suggested that there was an evident relationship between the actual prognosis and the different risk groups acquired according to the RS prediction model. The ROC curve of 1, 3 and 5-year survival prediction based on RS are shown in the right panel of Figure 2.

Screening of Independent Prognostic Clinical Factors

Four independent prognostic factors (age, pathologic stage, recurrence and RS model status) were found to have a significant correlation according to Cox regression analyses (Table II). We found that the RS risk prediction model was independently prognostic related to other clinical factors.

After that, a total of 798 DEGs were identified using the limma package. We analyzed the KEGG pathway of the screened mRNA through GSEA, and finally, we obtained 8 KEGG pathways, such as drug metabolism and other enzymes (Figure 3).

Construction and Evaluation of ceRNA Regulation Network

First, a total of 24 COAD-related miRNAs were obtained from the HMDD database. Then, a total of 25 connection pairs were acquired to build the lncRNA-miRNA regulation network based on the 5 lncRNAs and COAD–related miRNAs. After that, to build the miRNA-mRNA regulation network, we used the starBase database to search for target genes for miRNAs that have a connection relationship with lncRNAs. Then, we compared the target genes with the significant DEGs in different risk groups and kept the intersections, in total, 195 connection pairs were acquired.

Combining the above two steps, the lncRNA-miRNA-mRNA ceRNA regulation network was established (Figure 4). Finally, we identified the regulated mRNAs of the ceRNA network through GO and KEGG pathway analysis. In total, 15 significantly related GO and 14 KEGG

Table I. List of the lncRNAs related to prognosis.

Symbol	Coef	*p*-value	Hazard Ratio	95% CI
MAGI1-IT1	-5.631192	2.540E-03	0.004	0.0000926-0.139
CSNK1G2-AS1	3.284068	7.400E-03	26.684	2.410-295.144
ALMS1-IT1	0.902828	1.588E-02	2.467	1.180-5.138
LINC01341	1.589652	3.090E-02	4.902	1.160-20.763
LOXL1-AS1	0.50267	3.649E-02	1.653	1.030-2.648

LncRNA: long non-coding RNA; CI: confidence interval.

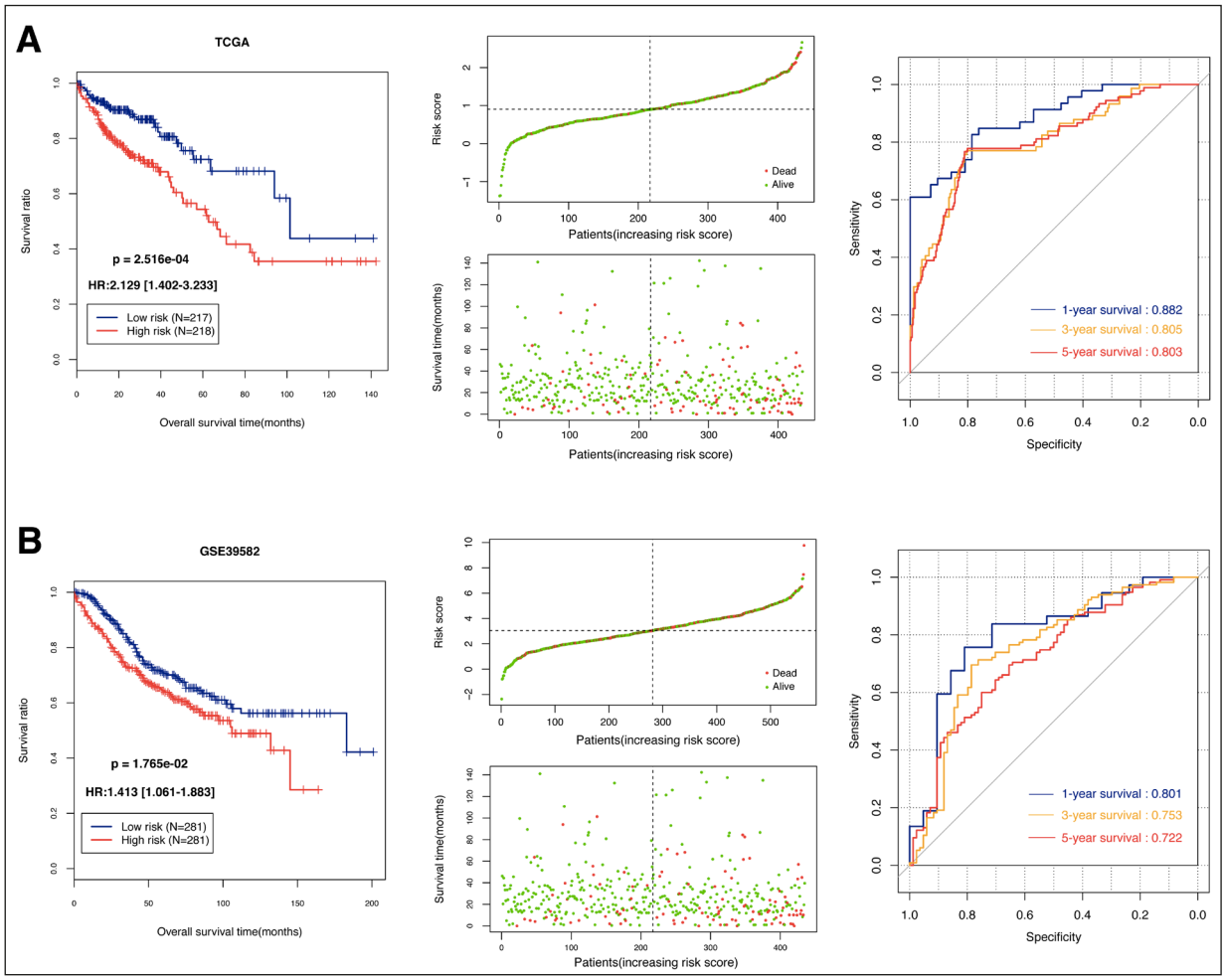

Figure 2. Left: TCGA (**A**) and GSE39582 (**B**) samples are based on the KM curve of the RS prediction model and prognosis. Left: The blue and red curves indicate the low- and high-risk samples, respectively. Middle: Distribution of RS and survival time. Right: ROC curve of survival prediction based on RS, the blue, orange and red curves represent the ROC curve of 1-year, 3-year and 5-year, respectively. KM, Kaplan-Meier; ROC, receiver operating characteristic.

Table II. The screening information of the clinical factors.

		Uni-variable cox		Multi-variable cox	
Clinical characteristics	**TCGA (N=435)**	**HR (95% CI)**	***p*-value**	**HR (95% CI)**	***p*-value**
Age (years, mean±sd)	66.95±12.91	1.021 [1.005-1.039]	1.015E-02	1.039 [1.018-1.060]	2.930E-04
Gender (Male/Female)	323/203	1.064 [0.717-1.578]	7.574E-01	-	-
Pathologic stage (I / II / III / IV /-)	74/168/124/59/10	2.052 [1.629-2.586]	3.692E-10	1.812 [1.297-2.531]	4.970E-04
Colon polyps' history (Yes/No/-)	130/240/65	0.775 [0.462-1.300]	3.322E-01	-	-
Lymphatic invasion (Yes/No/-)	154/240/41	2.322 [1.517-3.553]	6.663E-05	1.225 [0.696-2.155]	4.816E-01
Recurrence (Yes/No/-)	78/291/66	2.562 [1.633-4.02]	2.202E-05	2.128 [1.262-3.588]	4.603E-03
RS model status (High/Low)	217/218	2.129 [1.402-3.233]	2.516E-04	1.723 [1.028-2.887]	3.896E-02
Vital status (Dead/Alive)	100/335	-	-	-	-
Overall survival time (months,mean±sd)	29.20±25.49	-	-	-	-

N: number; sd: Standard Deviation; HR: hazard ratio; CI: confidence interval; RS: risk score; TCGA: The Cancer Genome Atlas.

pathways were acquired (Figure 5), and the GO analysis suggested that the DEGs in the ceRNA regulatory network mainly participated in anterior/posterior pattern specification (6 genes, such as

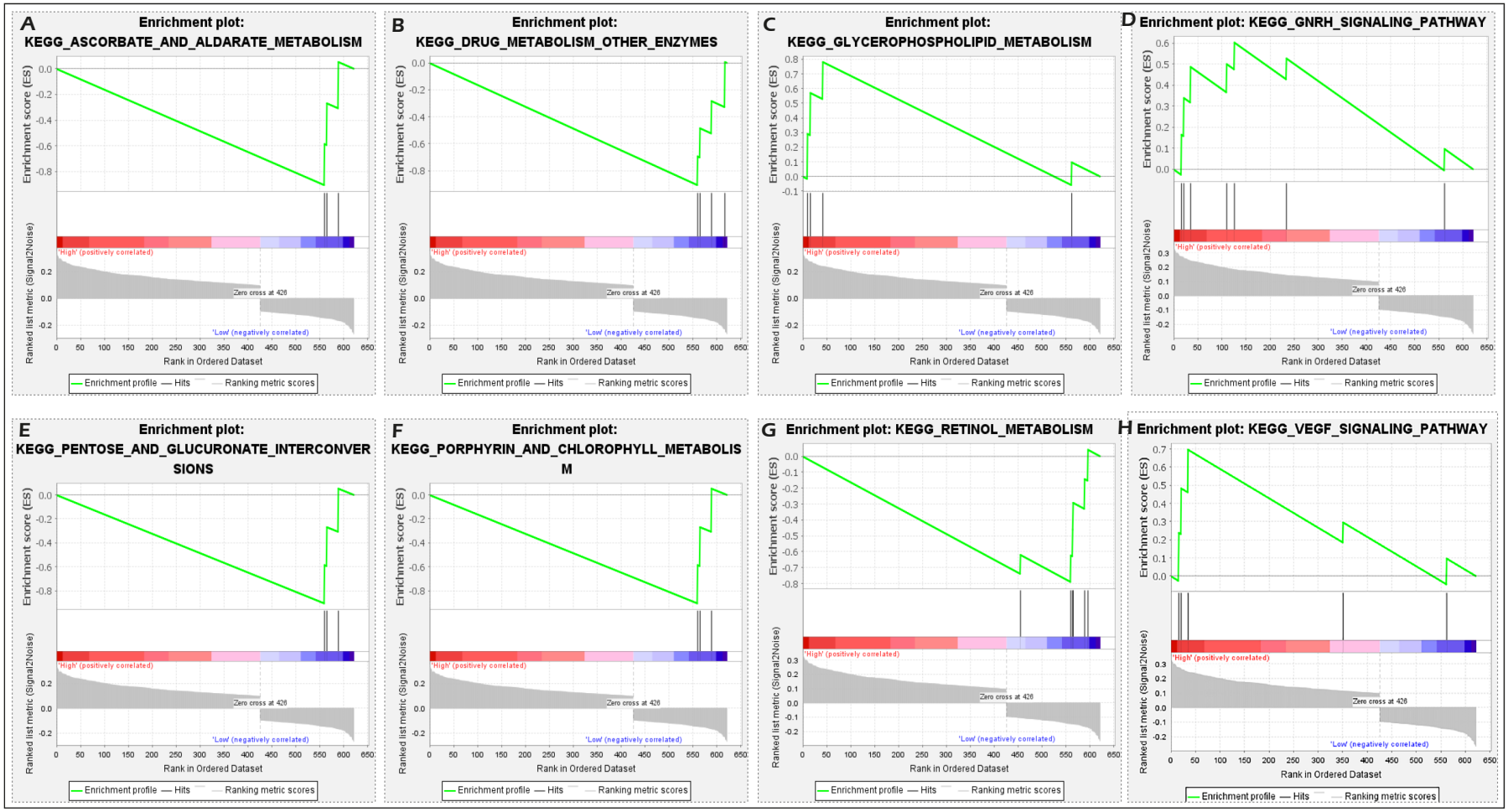

Figure 3. Significantly correlated ES plots of the KEGG pathway. GSEA was used to determine the KEGG pathway (**A-H**) of significant enrichment in different groups. ES, enrichment score; KEGG, Kyoto Encyclopedia of Genes and Genomes; GSEA, Gene set enrichment analysis. Abbreviations: ES, enrichment score; KEGG, Kyoto Encyclopedia of Genes and Genomes.

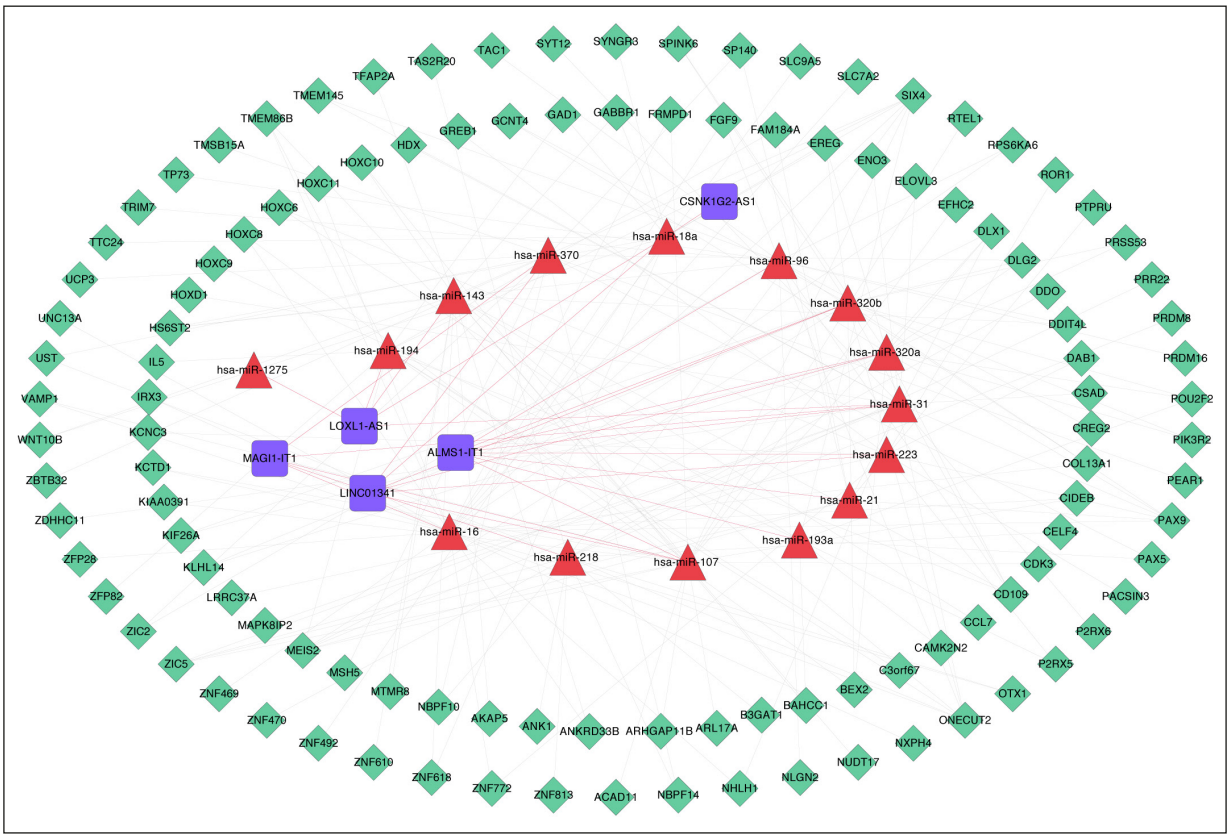

Figure 4. The construction of ceRNA regulation network. Squares indicate lncRNA, triangles indicate miRNA, and circles indicate mRNA. The red and gray connecting lines indicate the lncRNA-miRNA and the miRNA-mRNA network, respectively.

OTX1, HOXC9), transcription from RNA polymerase II promoter (12 genes, such as *TFAP2A, ONECUT2*) and regulation of transcription, DNA-templated (21 genes, such as *PRDM8, DLX1*). The KEGG pathway analysis suggested that the significant genes of the ceRNA regulatory network linked to taurine and hypotaurine metabolism (*GAD1, CSAD*), neurotrophin signaling pathway, and alanine (*RPS6KA6, PIK3R2, TP73*).

Discussion

COAD ranks third and fourth in global cancer incidence and mortality rankings, respectively, and more than 1,000,000 new cases of COAD each year, approximately 700,000 COAD-related deaths[17]. Although we have reached some achievements in the therapy of COAD, the patients' long-term survival rate is still very low, and the prognosis of patients with advanced COAD is severe[18]. Thus, it is important to determine the specific biomarkers of COAD for early diagnosis and evaluation of patient prognosis.

m6A is the most universal internal transcription modification for lncRNA, miRNA and mRNA, and participates in massive biological processes[19]. Up to now, massive evidence[20-22] has shown that m6A levels in RNAs may affect the process of some cancers; therefore, *m6A* modification has attracted a lot of attention. LncRNAs have been shown[23-25] to be involved in many major biological functions and can be used as prognostic biomarkers in the field of cancer, such as cervical cancer and lung cancer. However, there are few reports on the lncRNAs related to m6A of COAD.

Therefore, in this research, the bioinformatics analysis was performed to screen lncRNAs related to m6A in COAD patients. Moreover, to further distinguish the lncRNA-based model that was linked to prognosis, we identified 5 independent m6A-related lncRNAs (*MAGI1-IT1, CSNK1G2-AS1, ALMS1-IT1, LINC01341, LOXL1-AS1*) that were associated with prognostic factors by Cox regression analysis at the TCGA dataset. *LOXL1-AS1* has been proven to be involved in many cancers, like lung cancer, and breast cancer[26,27]. *LOXL1-AS1* can participate in the regulation of tumor occurrence, development,

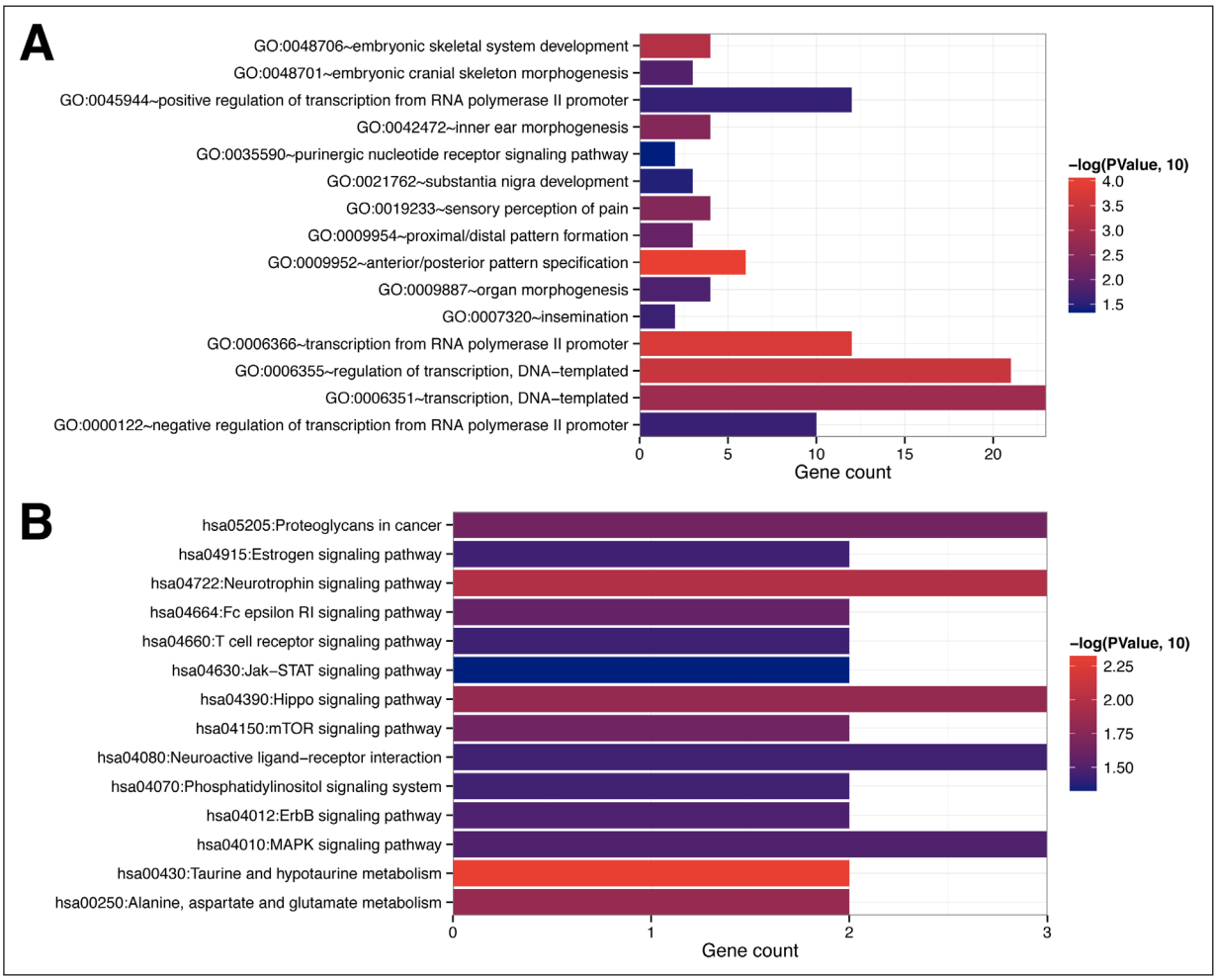

Figure 5. Enrichment analysis of GO biological process (**A**) and KEGG pathway (**B**) associated with significant genes of the ceRNA regulatory network. The horizontal axis indicates genes' count, the vertical axis indicates the GO and KEGG entry name; and the color indicates significance, the closer the color is to red, the greater the significance. Abbreviations: GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes.

and metastasis, as a new tumor biomarker. Many reports proved[25,28] that *ALMS1-IT1* could regulate the biological process of cancer and was associated with the metastasis and invasion of cancer, including squamous cell carcinoma, and lung cancer. However, there is little research about the biological process of *MAGI1-IT1, CSNK1G2-AS1, ALMS1-IT1, LINC01341 and LOXL1-AS1 in the COAD.*

Thus, based on these 5 lncRNAs (*MAGI1-IT1, CSNK1G2-AS1, ALMS1-IT1, LINC01341, LOXL1-AS1*), a prediction model was built that was related to the prognosis of COAD patients. The KM curves and the ROC curve were used to assess the effectiveness of this model, and we found that the prognostic risk prediction model for COAD had satisfactory predictive ability.

LncRNAs have been shown[29] to be involved in many major biological functions, like targeting miRNA. In this research, we built the lncRNA-miRNA-mRNA-ceRNA regulation network. Then, GO and KEGG pathways analysis were performed. Similar to the results of the GO analysis, previous studies[30-32] have reported that *OTX1*[30], *HOXC9*[31] and *TFAP2A*[32] can be used as the predictive model marker for COAD. *RPS6KA6* has been confirmed[33] to relate to the development process of COAD. *PIK3R2* has been reported[34] to participate in the metastasis and progression of COAD.

Conclusions

In summary, we identified 5 independent m6A-related lncRNAs (*MAGI1-IT1, CSNK1G2-AS1, ALMS1-IT1, LINC01341, LOXL1-AS1*) that were associated with prognostic factors, and

an RS model of COAD was built. Four independent prognostic factors (age, pathologic stage, recurrence and RS model status) were identified to have a significant correlation. Moreover, lncRNA-miRNA-mRNA regulatory network has been built to better understand the underlying molecular mechanism of COAD.

Conflict of Interest
The authors declare no conflict of interest.

Ethics Approval
Not applicable.

Informed Consent
Not applicable.

Availability of Data and Materials
The raw data were collected and analyzed by the Authors, named Jian Dong Tai and Li Peng Jin, and are not ready to share their data because the data have not been published.

Funding
None.

Authors' Contributions
LPJ and YWS participated in the design of this study, and they both performed the statistical analysis. TL carried out the study and collected important background information. JDT drafted the manuscript. All authors read and approved the final manuscript.

References

1) Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. CA Cancer J Clin 2020; 70: 7-30.
2) Iqbal A, George TJ. Randomized clinical trials in colon and rectal cancer. Surg Oncol Clin N Am 2017; 26: 689-704.
3) Li H, Liu L, Huang T, Jin M, Zheng Z, Zhang H, Ye M, Liu K. Establishment of a novel ferroptosis-related lncrna pair prognostic model in colon adenocarcinoma. Aging 2021; 13: 23072-23095.
4) Zhao Q, Zhao Y, Hu W, Zhang Y, Wu X, Lu J, Li M, Li W, Wu W, Wang J, Du F, Ji H, Yang X, Xu Z, Wan L, Wen Q, Li X, Cho CH, Zou C, Shen J, Xiao Z. M(6)a rna modification modulates pi3k/akt/mtor signal pathway in gastrointestinal cancer. Theranostics 2020; 10: 9528-9543.
5) Chen XY, Zhang J, Zhu JS. The role of m(6)a rna methylation in human cancer. Mol Cancer 2019; 18: 103.
6) Li J, Liang L, Yang Y, Li X, Ma Y. N(6)-methyladenosine as a biological and clinical determinant in colorectal cancer: Progression and future direction. Theranostics 2021; 11: 2581-2593.
7) Liu F, Xing L, Zhang X, Zhang X. A four-pseudogene classifier identified by machine learning serves as a novel prognostic marker for survival of osteosarcoma. Genes 2019; 10: 414.
8) Zhao G, Fu Y, Su Z, Wu R. How long non-coding rnas and micrornas mediate the endogenous rna network of head and neck squamous cell carcinoma: A comprehensive analysis. Cell Physiol Biochem 2018; 50: 332-341.
9) Barrett T, Wilhite SE, Ledoux P, Evangelista C, Kim IF, Tomashevsky M, Marshall KA, Phillippy KH, Sherman PM, Holko M, Yefanov A, Lee H, Zhang N, Robertson CL, Serova N, Davis S, Soboleva A. Ncbi geo: Archive for functional genomics data sets--update. Nucleic Acids Res 2013; 41: D991-D995.
10) Marisa L, de Reyniès A, Duval A, Selves J, Gaub MP, Vescovo L, Etienne-Grimaldi MC, Schiappa R, Guenot D, Ayadi M, Kirzin S, Chazal M, Fléjou JF, Benchimol D, Berger A, Lagarde A, Pencreach E, Piard F, Elias D, Parc Y, Olschwang S, Milano G, Laurent-Puig P, Boige V. Gene expression classification of colon cancer into molecular subtypes: Characterization, validation, and prognostic value. PLoS Med 2013; 10: e1001453.
11) Wang P, Wang Y, Hang B, Zou X, Mao JH. A novel gene expression-based prognostic scoring system to predict survival in gastric cancer. Oncotarget 2016; 7: 55343-55351.
12) Ritchie ME, Phipson B, Wu D, Hu Y, Law CW, Shi W, Smyth GK. Limma powers differential expression analyses for rna-sequencing and microarray studies. Nucleic acids Res 2015; 43: e47.
13) Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP. Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A 2005; 102: 15545-15550.
14) Huang Z, Shi J, Gao Y, Cui C, Zhang S, Li J, Zhou Y, Cui Q. Hmdd v3.0: A database for experimentally supported human microrna-disease associations. Nucleic acids Res 2019; 47: D1013-d1017.
15) Paraskevopoulou MD, Vlachos IS, Karagkouni D, Georgakilas G, Kanellos I, Vergoulis T, Zagganas K, Tsanakas P, Floros E, Dalamagas T, Hatzigeorgiou AG. Diana-lncbase v2: Indexing microrna targets on non-coding transcripts. Nucleic acids Res 2016; 44: D231-D238.
16) Li JH, Liu S, Zhou H, Qu LH, Yang JH. Starbase v2.0: Decoding mirna-cerna, mirna-ncrna and protein-rna interaction networks from large-scale clipseq data. Nucleic acids Res 2014; 42: D92-D97.
17) Huang da W, Sherman BT, Lempicki RA. Bioinformatics enrichment tools: Paths toward the com-

prehensive functional analysis of large gene lists. Nucleic acids Res 2009; 37: 1-13.

18) Ouyang R, Li Z, Peng P, Zhang J, Liu J, Qin M, Huang J. Exploration of the relationship between tumor mutation burden and immune infiltrates in colon adenocarcinoma. Int J Med Sci 2021; 18: 685-694.

19) Jiang F, Ren S, Chen Y, Zhang A, Zhu Y, Zhang Z, Li Z, Piao D. Fangchinoline exerts antitumour activity by suppressing the egfr-pi3k/akt signalling pathway in colon adenocarcinoma. Oncol Rep 2021; 45: 139-150.

20) Lin Y, Wang S, Liu S, Lv S, Wang H, Li F. Identification and verification of molecular subtypes with enhanced immune infiltration based on m6a regulators in cutaneous melanoma. BioMed Res Int 2021; 2021: 2769689.

21) Han J, Wang JZ, Yang X, Yu H, Zhou R, Lu HC, Yuan WB, Lu JC, Zhou ZJ, Lu Q, Wei JF, Yang H. Mettl3 promote tumor proliferation of bladder cancer by accelerating pri-mir221/222 maturation in m6a-dependent manner. Mol Cancer 2019; 18: 110.

22) Gong D, Zhang J, Chen Y, Xu Y, Ma J, Hu G, Huang Y, Zheng J, Zhai W, Xue W. The m(6)a-suppressed p2rx6 activation promotes renal cancer cells migration and invasion through atp-induced ca(2+) influx modulating erk1/2 phosphorylation and mmp9 signaling pathway. J Exp Clin Cancer Res 2019; 38: 233.

23) Ianniello Z, Paiardini A, Fatica A. N(6)-methyladenosine (m(6)a): A promising new molecular target in acute myeloid leukemia. Front Oncol 2019; 9: 251.

24) Mao X, Qin X, Li L, Zhou J, Zhou M, Li X, Xu Y, Yuan L, Liu QN, Xing H. A 15-long non-coding rna signature to improve prognosis prediction of cervical squamous cell carcinoma. Gynecol Oncol 2018; 149: 181-187.

25) Xing L, Zhang X, Chen A. Prognostic 4-lncrna-based risk model predicts survival time of patients with head and neck squamous cell carcinoma. Oncol Lett 2019; 18: 3304-3316.

26) Dong HT, Liu Q, Zhao T, Yao F, Xu Y, Chen B, Wu Y, Zheng X, Jin F, Li J, Xing P. Long non-coding rna loxl1-as1 drives breast cancer invasion and metastasis by antagonizing mir-708-5p expression and activity. Molecular therapy. Nucleic Acids 2020; 19: 696-705.

27) Zhao L, Zhang X, Guo H, Liu M, Wang L. Loxl1-as1 contributes to non-small cell lung cancer progression by regulating mir-3128/rhoxf2 axis. Onco Targets Ther 2020; 13: 6063-6071.

28) Lei Y, Shi Y, Duan J, Liu Y, Lv G, Shi R, Zhang F, Yang Q, Zhao W. Identification of alternative splicing and lncrna genes in pathogenesis of small cell lung cancer based on their rna sequencing. Adv Clin Exp Med 2019; 28: 1043-1050.

29) Taniue K, Akimitsu N. The functions and unique features of lncrnas in cancer development and tumorigenesis. Int J Mol Sci 2021; 22: 632.

30) Yu K, Cai XY, Li Q, Yang ZB, Xiong W, Shen T, Wang WY, Li YF. Otx1 promotes colorectal cancer progression through epithelial-mesenchymal transition. Biochem Bio Res Co 2014; 444: 1-5.

31) Liu J, Dong C, Jiang G, Lu X, Liu Y, Wu H. Transcription factor expression as a predictor of colon cancer prognosis: A machine learning practice. BMC Med Genomics 2020; 13: 135.

32) Sun YL, Zhang Y, Guo YC, Yang ZH, Xu YC. A prognostic model based on the immune-related genes in colon adenocarcinoma. Int J Med Sci 2020; 17: 1879-1896.

33) ME LL, Vidal F, Gallardo D, Diaz-Fuertes M, Rojo F, Cuatrecasas M, López-Vicente L, Kondoh H, Blanco C, Carnero A, Ramón y Cajal S. New p53 related genes in human tumors: Significant downregulation in colon and lung carcinomas. Oncol Rep 2006; 16: 603-608.

34) Zamame Ramirez JA, Romagnoli GG, Falasco BF, Gorgulho CM, Sanzochi Fogolin C, Dos Santos DC, Junior JPA, Lotze MT, Ureshino RP, Kaneno R. Blocking drug-induced autophagy with chloroquine in hct-116 colon cancer cells enhances dc maturation and t cell responses induced by tumor cell lysate. Int Immunopharmacol 2020; 84: 106495.