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Bioinformatics analysis of the interactions among lncRNA, miRNA and mRNA expression, genetic mutations and epigenetic modifications in hepatocellular carcinoma

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Abstract. The present study aimed to investigate the regulatory networks involving long noncoding RNA (lncRNA), microRNA (miRNA), mRNA, genetic mutations and epigenetic modifications in hepatocellular carcinoma (HCC) by analyzing datasets from The Cancer Genome Atlas (TCGA) database. TCGA was mined, and miRNAs, lncRNAs and mRNAs that were differentially expressed in HCC were identified using R software. A gene regulatory network was constructed using Cytoscape software. Representative genes were selected for functional enrichment analysis using Gene Ontology and Kyoto Encyclopedia of Genes and Genomes. The associations among various proteins and protein networks were identified using the online software Search Tool for the Retrieval of Interacting Genes/Proteins. The cBioPortal database was used to analyze the association between genetic mutations and epigenetic modification, and the development of HCC. A total of 35 mRNAs were predicted to be targeted by 77 lncRNAs and 16 miRNAs, establishing a lncRNA-miRNA-mRNA regulatory network for HCC. Multivariable Cox regression analysis suggested that long intergenic non-protein coding RNA 200, miRNA-137, PDZ binding kinase and DNA polymerase θ were independent prognostic factors. In a regulatory network centered on miRNA-424, six mRNA target genes were associated with HCC survival rates. Protein-protein interaction analysis suggested that cell division cycle 25A (CDC25A) interacted with centrosomal protein 55 (CEP55), claspin, E2F transcription factor 7 and cyclin E1 (CCNE1. Mutations in CEP55 affected overall survival and disease-free survival in HCC, whereas, mutations in CDC25A affected overall survival, and mutations in E2F7 affected disease-free survival. Decreased methylation levels of CEP55, CDC25A and CCNE1 were associated with vascular invasion. The survival rate of patients with hypermethylation of CCNE1 and CEP55 was significantly associated with the rate of methylation of these loci. The present study provides an integrated bioinformatics analysis of gene expression, genetic mutations and epigenetic modifications that may be associated with the development of HCC.

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Introduction

Primary liver cancer is one of the most common types of malignant tumor. Hepatocellular carcinoma (HCC) is the principal pathological type of liver cancer and is a leading cause of cancer-associated mortalities worldwide (1). HCC is characterized by high malignancy and is generally diagnosed at the middle to late stage. As a result, the prognosis of patients with HCC remains poor. The majority of patients are treated surgically; however, 60-70% of all HCC cases relapse within five years (2). Therefore, it is critical to identify novel molecular markers in order to improve the prognoses of the patients. Additionally, identification of the molecular mechanisms underlying HCC progression may help in developing novel targeted strategies to treat HCC.

In recent years, an increasing number of studies on the roles of microRNAs (miRNAs) and long noncoding RNAs (lncRNAs) have been published. The regulatory network modulating miRNAs, lncRNAs and their target genes represents a popular topic in cancer research. miRNAs are involved in key biological processes, including cell growth, differentiation and apoptosis, and are able to induce mRNA degradation or to inhibit protein translation, altering the expression levels of the downstream proteins (3,4). lncRNAs represent a group of noncoding RNA molecules, that, in contrast to miRNAs, inhibit or activate gene expression by interacting with transcription factors or by binding to specific regions of the target mRNAs, modulating

their translation (5). Via various mechanisms, lncRNAs serve important roles in numerous biological processes, including cell differentiation, cell apoptosis and the heat shock response (6). A previous study demonstrated that lncRNAs are a novel class of regulators involved in multiple human diseases by modulating gene expression at the transcriptional, post-transcriptional or epigenetic level (7). Therefore, the regulatory association between lncRNA, miRNA and mRNA, and tumor pathogenesis requires further investigation.

Genetic mutations are one of the principal factors involved in tumor occurrence and may lead to the activation of proto-oncogenes or the loss of tumor suppressor genes. Furthermore, accumulating evidence demonstrated that genetic modifications are associated with tumorigenesis (8-11). DNA methylation is a mechanism underlying epigenetic modifications, and is able to affect the DNA conformation, the chromatin structure and the stability of DNA, altering the interactions between DNA and proteins regulating gene expression (12). Epigenetic modifications, including DNA methylation and histone modifications, are used as biomarkers to study tumorigenesis and tumor progression, helping clinical diagnosis (11). Furthermore, the investigation of epigenetic modifications may contribute to the development of targeted treatments aimed to inactivate tumor suppressor genes (13). Therefore, examining the associations between genetic mutations and DNA methylation, and the occurrence, development and prognosis of HCC may improve the understanding of HCC pathogenesis and may provide novel strategies to prevent, diagnose and treat this fatal disease.

In the present study, a prognostic model was constructed and the genes involved in HCC were investigated by analyzing biological datasets from online databases. The potential interactions among lncRNAs, miRNAs and mRNAs involved in the development of HCC were investigated. Additionally, the association between RNA expression levels, and the occurrence of genetic mutations and epigenetic modifications were examined in order to identify novel potential biomarkers.

Materials and methods

Database screening. Gene expression data from HCC, relative to 374 tumor samples and 50 normal samples, were downloaded from the The Cancer Genome Atlas (TCGA) database (https://portal.gdc.cancer.gov/) using the key words 'transcriptome profiling', 'HTSeq-Counts' and 'TCGA-LIHC'. The data were combined and the gene identification numbers were converted using Perl (https://www.perl.org/) in order to obtain a gene expression matrix.

Identification of differentially expressed (DE) genes. The expression matrix of miRNAs, lncRNAs and mRNAs was analyzed using the edgeR package (version 3.22.3; http://www.bioconductor.org/packages/release/bioc/html/edgeR.html) of R software (version 3.4.2) (http://www.r-project.org/). The correlations between DElncRNA-DEmiRNA pairs and DEmiRNA-DEmRNA pairs were matched using Perl. The pairs used the Spearman's test with a coefficient >0.95 were considered as co-expressed. The lncRNAs-miRNAs-mRNAs regulatory interactions were identified based on the DElncRNA-DEmiRNA and DEmiRNA-DEmRNA regulation pairs. The regulatory associations between DElncRNAs and DEmiRNAs were investigated using miRcode (http://www.mircode.org). The regulatory associations between DEmiRNAs and DEmRNAs were investigated using miRDB (http://www.mirdb.org/), miRTarBase (release 7.0) (http://mirtarbase.mbc.nctu.edu.tw/php/index.php) and TargetScan (release 7.2) (http://www.targetscan.org/). The negatively associated DEmiRNA-DEmRNA and DElncRNA-DEmiRNA regulatory pairs were selected to construct regulatory networks. The VennDiagram package (version 1.6.20; https://cran.r-project.org/web/packages/VennDiagram/index.html) of R software was used to draw Venn diagrams.

Multivariable Cox regression analysis of independent prognostic factors in HCC. Univariate analysis was performed for DElncRNAs, DEmRNAs and DEmiRNAs. The DE-RNAs with $P<0.001$ were selected for further multivariate Cox regression analysis. A prognostic model of HCC was established, and Receiver Operating Characteristic (ROC) curves were used to test the reliability of the constructed model.

Construction of a competing endogenous (ceRNA) regulatory network and survival analysis. The integrated co-expression network of DElncRNA-DEmiRNA-DEmRNA regulatory associations was visualized using Cytoscape software (version 3.6.1) (https://cytoscape.org/). Furthermore, prognostic DEmRNAs, DElncRNAs and DEmiRNAs in the ceRNA network were identified, and Kaplan-Meier survival plots were drawn using R survival package (version 2.42-6) (https://cran.r-project.org/web/packages/survival/index.html).

Functional enrichment analysis. The BINGO plugin (version 3.0.3) (https://www.psb.ugent.be/cbd/papers/BiNGO/Tutorial.html) of Cytoscape software was used for Gene Ontology (GO) analysis (https://david.ncifcrf.gov/). KOBAS 3.0 (http://kobas.cbi.pku.edu.cn/) was used for Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis.

Construction of protein-protein interaction (PPI) networks. PPI networks were constructed using Search Tool for the Retrieval of Interacting Genes/Proteins (https://string-db.org/). In order to model the network, the minimum required interaction score was set to 0.4. In analyzing the six genes associated with miRNA-424, the minimum required interaction score was set to 0.4.

Association between genetic mutations and DNA methylation. cBioPortal database (http://www.cbioportal.org/) was used to investigate the association between the genetic mutations and DNA methylation of six target genes regulated by miRNA-424 in HCC. Their association with the overall survival of the patients with HCC was further assessed by Kaplan-Meier analysis. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Identification of DElncRNAs, DEmiRNAs and DEmRNAs associated with HCC survival. A total of 1,077 DElncRNAs, 122 DEmiRNAs and 1,985 DEmRNAs were identified by analyzing RNA-sequencing (seq) and miRNA-seq data

Table I. Multivariate Cox regression analysis for differentially expressed RNAs.

Type of RNA	Gene name	Coefficient	exp(coef)	se(coef)	Z score	P-value
lncRNA	AC114489.1	0.1136	1.1203	0.0725	1.57	0.1170
lncRNA	AP002478.1	0.0938	1.0984	0.0605	1.55	0.1210
lncRNA	C10orf91	0.0742	1.0770	0.0482	1.54	0.1237
lncRNA	LINC00200	0.1568	1.1698	0.0497	3.16	0.0016[a]
lncRNA	LINC00462	0.1377	1.1477	0.0731	1.88	0.0595
miRNA	miRNA-137	0.1135	1.1202	0.0571	1.99	0.0467[a]
mRNA	CBX2	0.1408	1.1512	0.0770	1.83	0.0675
mRNA	CLSPN	0.1861	1.2046	0.1113	1.67	0.0945
mRNA	E2F2	-0.2127	0.8084	0.1295	-1.64	0.1004
mRNA	EZH2	0.3413	1.4068	0.1907	1.79	0.0734
mRNA	PBK	0.2564	1.2923	0.1205	2.13	0.0333[a]
mRNA	POLQ	-0.3420	0.7104	0.1503	-2.28	0.0228[a]

[a]P<0.05. Cox regression equation for prognosis: Y=0.1136x AC114489.1 +0.0938x AP002478.1 +0.0742x C10orf91 +0.1568x LINC00200 +0.1377x LINC00462 +0.1135x miRNA-137 +0.1408x CBX2 +0.1861x CLSPN -0.2127x E2F2 +0.3413x EZH2 +0.2564x PBK -0.3420x POLQ. lncRNA, long noncoding RNA; miRNA, microRNA; C10orf91, chromosome 10 open reading frame 91; LINC, long intergenic non-protein coding RNA; CBX2, chromobox 2; CLSPN, claspin; E2F2, E2F transcription factor 2; EZH2, enhancer of zeste 2 polycomb repressive complex 2 subunit; PBK, PDZ binding kinase; POLQ, DNA polymerase θ; se, standard error; coef, coefficient; exp, exponential.

downloaded from TCGA, and derived from 374 HCC and 50 normal liver samples. Target prediction analysis, performed using miRDB, miRTarBase and TargetScan, suggested that 747 of the DEmRNAs were potential targets of lncRNAs and miRNAs. Among the 747 DEmRNAs, 35 were identified as potential targets of 77 DElncRNAs and 16 DEmiRNAs (data not shown).

Kaplan-Meier survival analysis suggested that 13 DElncRNAs and 19 DEmRNAs were associated with the 5-year survival rate of patients with HCC (data not shown). In contrast, no miRNAs were associated with the 5-year survival rate.

Univariate analysis of DERNAs suggested that nine DElncRNAs, one DEmiRNA and 14 DEmRNAs were risk factors of HCC development (data not shown. Multivariable Cox regression analysis suggested that 12 DERNAs were associated with HCC, whereas, long intergenic non-protein coding RNA 200, miRNA-137, PDZ binding kinase (PBK) and DNA polymerase θ (POLQ) were independent prognostic factors (Table I). These 12 DERNAs were used to construct a prognostic model, and the overall survival rate of patients with a low risk score was significantly increased compared with patients with a high risk score. Furthermore, the ROC analysis performed resulted in an area under the curve of 0.756, indicating the reliability of the model (data not shown). A heat map of the 12 DERNAs suggested that their expression patterns exhibited consistency among 374 tumor samples compared with 50 normal liver samples (data not shown).

Construction of a ceRNA regulatory network for HCC. The regulatory associations among DElncRNAs, DEmiRNAs and DEmRNAs were analyzed by constructing a ceRNA network. A total of 290 lncRNA-miRNA pairs, 16 DEmiRNAs and 35 DEmRNAs were used to construct the ceRNA network (data not shown). The lncRNA-miRNA-mRNA regulatory network included 128 nodes and 331 edges. Among the DEmiRNAs, only miRNA-424 was downregulated. In a secondary regulatory network centered on miRNA-424, 13 DEmRNAs were identified: One was downregulated and 12 were upregulated (data not shown). Among these 13 DEmRNAs, the following six genes were associated with the overall survival of patients with HCC: E2F transcription factor 7 (E2F7), cytoplasmic polyadenylation element binding protein 3 (CPEB3), claspin (CLSPN), centrosomal protein 55 (CEP55), cell division cycle 25A (CDC25A) and cyclin E1 (CCNE1; Fig. 1). Furthermore, 29 DElncRNAS were identified, and five of them, including TSPEAR antisense RNA 1, MYLK antisense RNA 1, CLRN1 antisense RNA 1, chromosome 2 open reading frame 48 and AP002478.1, were associated with the survival rate of patients with HCC (Fig. 2). Therefore, the regulatory network of miRNA-424 was further investigated in the present study.

GO and KEGG functional enrichment analyses in HCC. Functional enrichment analysis of the mRNAs regulated by miRNA-424 resulted in five enriched GO terms. The GO terms are categorized into 'biological process', 'molecular function' and 'cellular component'. A total of 108 nodes and 166 edges constituted a functional enrichment regulatory network (data not shown). Additionally, 13 enriched signaling pathways were identified by KEGG analysis and the most significant enriched pathways were 'progesterone-mediated oocyte maturation', 'oocyte meiosis', 'cell cycle' and 'microRNAs in cancer' (Fig. 3A).

PPI network analysis in HCC. The functional interactions were examined at the protein level by constructing PPI networks based on the DEmRNAs (Fig. 3B) and the six target genes of miRNA-424 (Fig. 3C). CDC25A presented multiple

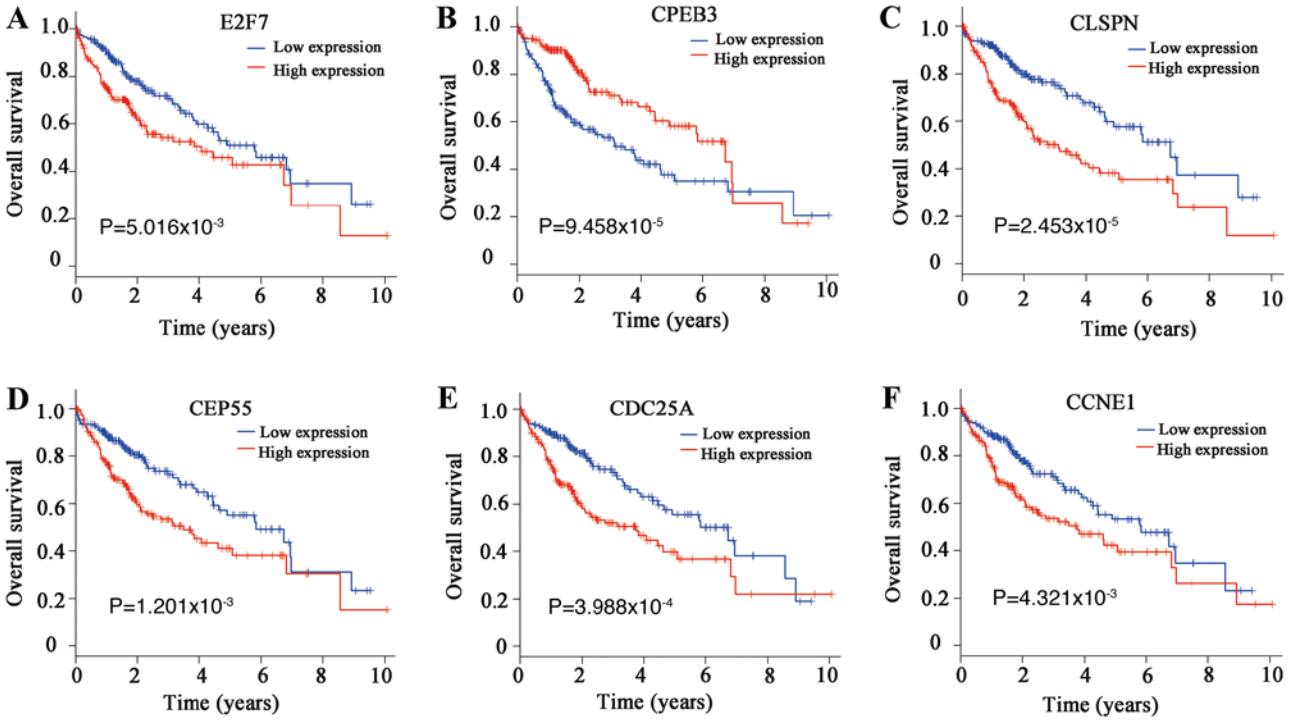

Figure 1. Survival analysis of differentially expressed mRNAs in a competing endogenous RNA regulatory network in hepatocellular carcinoma. Association between the overall survival rate and the expression levels of (A) E2F7, (B) CPEB3, (C) CLSPN, (D) CEP55, (E) CDC25A and (F) CCNE1. CCNE1, cyclin E1; CDC25A, cell division cycle 25A; CEP55, centrosomal protein 55; CLSPN, claspin; CPEB3, cytoplasmic polyadenylation element binding protein 3; E2F7, E2F transcription factor 7.

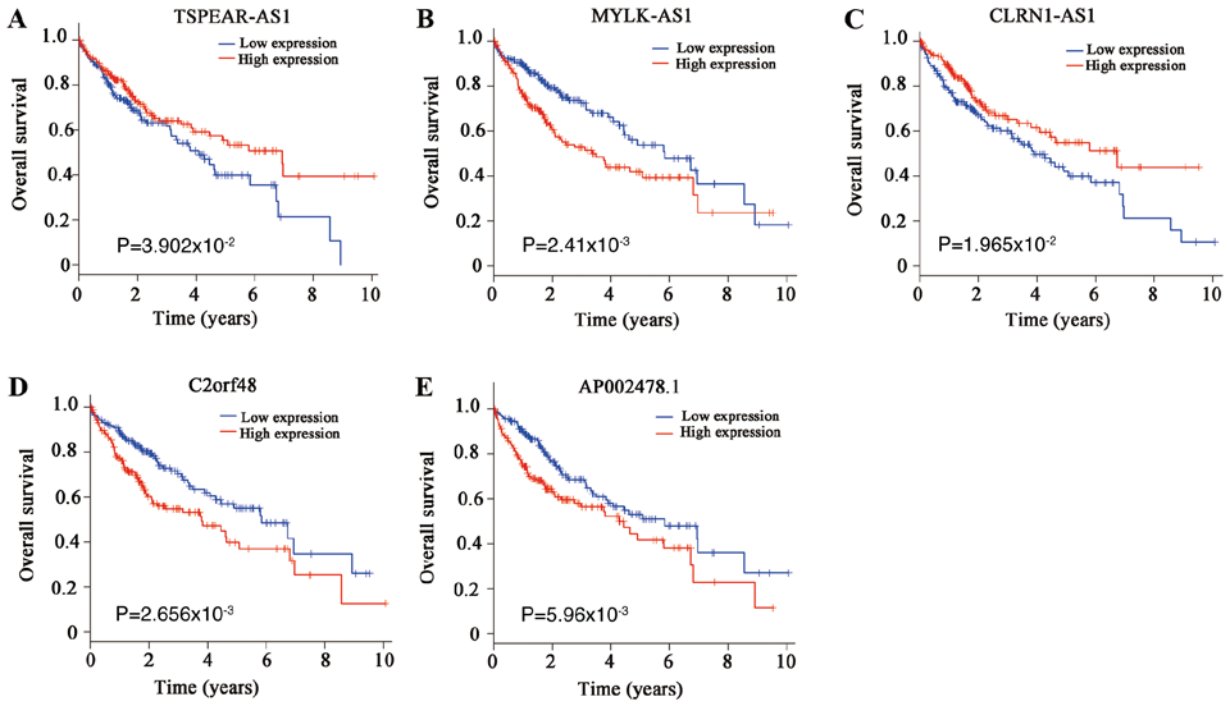

Figure 2. Survival analysis of differentially expressed long noncoding RNAs in a competing endogenous RNA regulatory network in hepatocellular carcinoma. Association between the overall survival rate and the expression levels of (A) TSPEAR-AS1, (B) MYLK-AS1, (C) CLRN1-AS1, (D) C2orf48 and (E) AP002478.1. C2orf48, chromosome 2 open reading frame 48; CLRN1-AS1, CLRN1 antisense RNA 1; MYLK-AS1, MYLK antisense RNA 1; TSPEAR-AS1, TSPEAR antisense RNA 1.

interactions in the two networks and was the central node in the miRNA-424-regulated network (Fig. 3C). Furthermore, CEP55, CLSPN, E2F7 and CCNE1 were associated with CDC25A, although no interaction with CPEB3 was identified (Fig. 3C). The interaction between CDC25A and CCNE1 presented the highest combined score, 0.981.

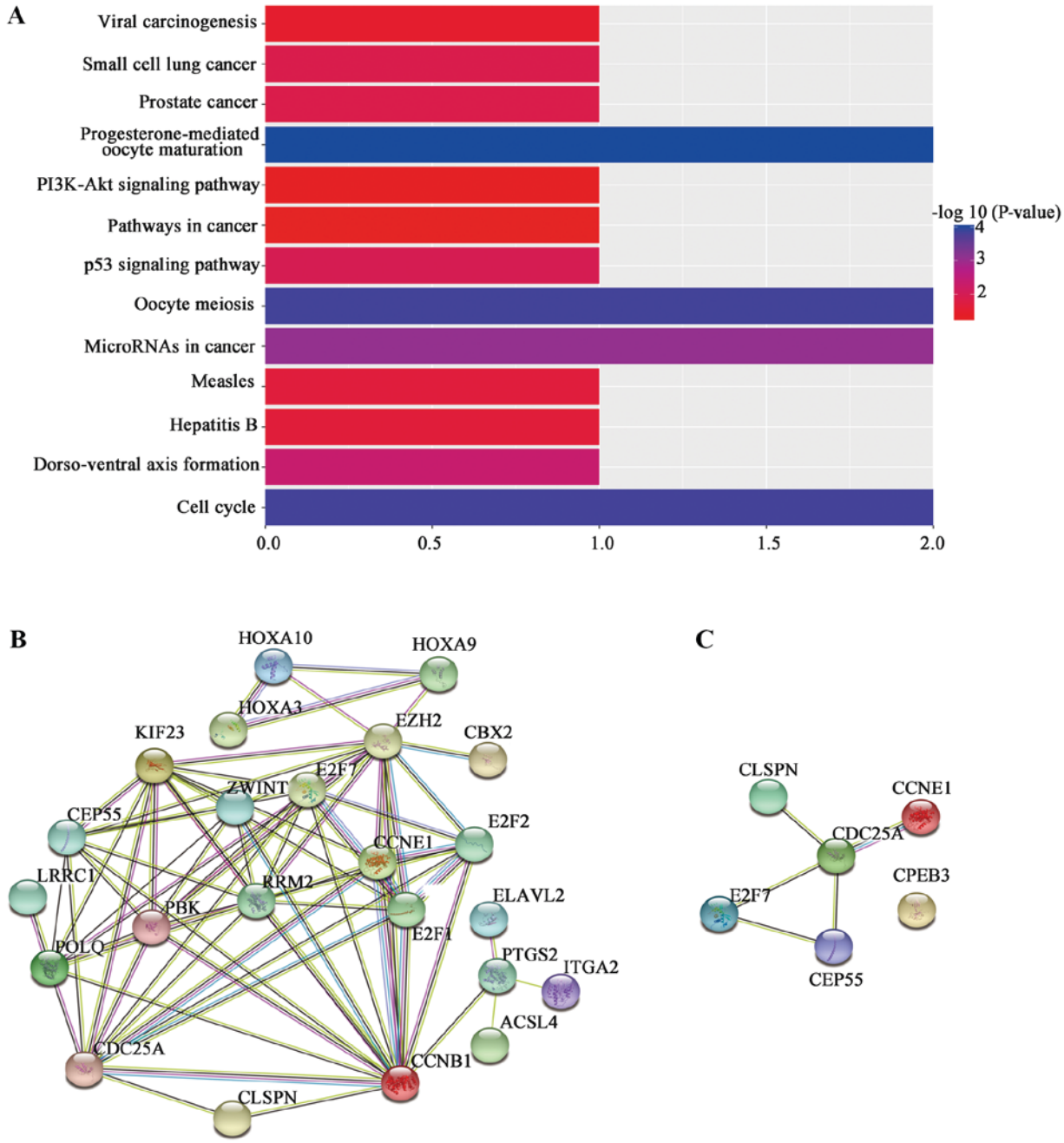

Figure 3. KEGG and PPI analyses. (A) KEGG analysis of genes regulated by microRNA-424. (B) PPI analysis of genes that are differentially expressed in HCC. (C) PPI analysis of the predicted target genes of microRNA-424 that are associated with the survival rate of patients with HCC. The nodes represent proteins and each color corresponds to a cluster. The edges indicate the functional associations and are colored according to the type of functional association. Red, green, blue, purple, yellow, light blue and black lines indicate fusion evidence, neighborhood evidence, co-occurrence evidence, experimental evidence, text-mining evidence, database evidence and co-expression evidence, respectively. The line thickness indicates the degree of confidence for the prediction of the interaction. KEGG, Kyoto Encyclopedia of Genes and Genomes; PPI, protein-protein interaction; HCC, hepatocellular carcinoma.

Analysis of genetic mutations of miRNA-424 target genes in HCC. The mutation rate in HCC was 5% for CDC25A, CEP55, CCNE1 and CPEB3, and 6% for CLSPN and E2F7, and mutations in these genes were significantly associated with vascular invasion (data not shown). The associations among the genetic mutations of the six genes were analyzed using the cBioPortal database. The results suggested that mutations in CDC25A were positively associated with mutations in CEP55, CLSPN and E2F7; however, no significant association was identified with mutations in CCNE1 or CPEB3 (Table II). Furthermore, the associations between the mutations of the six genes and the survival rates of patients with HCC were assessed by Kaplan-Meier analysis (Fig. 4). Mutations in CEP55 were associated with the overall survival and the disease-free survival rates of patients with HCC (Fig. 4B and D). CDC25A mutations were associated with the overall survival rate (Fig. 4A); however, they were not significantly associated with the disease-free survival rate (data not shown). In contrast, mutations in E2F7 were associated with the disease-free survival (Fig. 4C) and not with the overall survival rate (data not shown). Mutations in CCNE1, CLSPN and CPEB3 were not significantly associated with the survival rates (data not shown).

Table II. Association between genetic mutations.

A, Tendency toward co-occurrence

Gene A	Gene B	P-value
CLSPN	E2F7	<0.001
CLSPN	CEP55	<0.001
CDC25A	CLSPN	<0.001
E2F7	CEP55	<0.001
CDC25A	CEP55	<0.001
CDC25A	E2F7	0.002
CDC25A	CCNE1	0.087
CLSPN	CCNE1	0.115
E2F7	CCNE1	0.115
CCNE1	CPEB3	0.612
CEP55	CCNE1	0.631

B, Tendency toward mutual exclusivity

Gene A	Gene B	P-value
CLSPN	CPEB3	0.275
CDC25A	CPEB3	0.319
CEP55	CPEB3	0.369
E2F7	CPEB3	0.651

CCNE1, cyclin E1; CDC25A, cell division cycle 25A; CEP55, centrosomal protein 55; CLSPN, claspin; CPEB3, cytoplasmic polyadenylation element binding protein 3; E2F7, E2F transcription factor 7.

DNA methylation affects the biological features of HCC. The association between the levels of DNA methylation of the miRNA-424 target genes and the biological features of HCC samples were investigated (Fig. 5). The DNA methylation levels of CEP55, CDC25A and CCNE1 were negatively associated with vascular invasion. HCC samples presenting decreased levels of methylation exhibited increased vascular invasion (Fig. 5A-C). The DNA methylation levels of E2F7, CLSPN and CPEB3 were not associated with vascular invasion (data not shown). To investigate the association between DNA methylation levels and prognosis of patients with HCC, a Kaplan-Meier analysis was performed. The overall survival was increased when the methylation level of CEP55 was increased (Fig. 5D). The overall survival was decreased when the methylation level of CCNE1 was high (Fig. 5E). The disease-free survival was higher when CEP55 methylation was higher (Fig. 5F). Disease-free survival relative to CCNE1 methylation is not presented. No significant associations were identified between the DNA methylation levels of the other four genes and survival rates (data not shown).

Discussion

HCC is one of the most common types of tumor, exhibiting high morbidity, malignancy and mortality rates. The occurrence and development of HCC are regulated by complex multi-factorial processes involving multiple genes, and gene regulation may be influenced by lncRNAs, miRNAs, genetic mutations and epigenetic modifications (14,15). Therefore, investigating a single factor associated with gene regulation is not sufficient to assess the prognosis of patients with HCC. Previous studies have identified genes that regulate HCC progression; however, the majority of these studies focused on single genes or investigated only one of the factors influencing gene regulation (16-18), without considering their combinatorial effect. To understand the complex molecular biological characteristics of HCC, differentially expressed lncRNAs, miRNAs and mRNAs were identified by analyzing biological datasets from TCGA database. Subsequently, lncRNA-miRNA-mRNA gene regulatory networks were constructed. To identify the independent prognostic factors that affected the biological features of HCC, the functions and the signaling pathways associated with the genes identified were investigated. Additionally, genetic mutations and epigenetic modifications were examined, and an association between DNA methylation level and tumor vascularization was identified. Furthermore, the epigenetic states of the genes examined were identified to be associated with patient survival.

Accumulating evidence has demonstrated the regulatory association between miRNAs and mRNAs, although the roles of lncRNAs have frequently been ignored. lncRNAs serve numerous roles in tumor occurrence and progression (15,19,20), whereas, miRNAs have been identified to exert their biological functions primarily by regulating gene expression. Furthermore, previous studies demonstrated that lncRNAs, miRNAs and mRNAs are able to serve important roles in tumor progression by regulating each other (21-23). Zhang *et al* (24) performed a comprehensive analysis of the lncRNA-miRNA-mRNA regulatory network in breast cancer by analyzing the TCGA database. The previous study by Zhang *et al* (24) provided important insight for future breast cancer studies, and laid the foundation for the development of novel strategies for targeting breast cancer. Previous studies investigated the role of lncRNA and miRNA regulation in HCC (14,25); however, to the best of the authors' knowledge, the lncRNA-miRNA-mRNA network in HCC has not been examined. Therefore, the present systematic analysis of the lncRNA-miRNA-mRNA regulatory network provides novel insight into the complex regulation of HCC development and progression. In the present study, univariate and multivariate Cox regression analysis based on genomic datasets from TCGA database suggested that miRNA-137, PBK, LINC00200 and POLQ are independent prognostic factors of HCC. Furthermore, Kaplan-Meier survival analyses suggested that PBK and POLQ were associated with the 5-year survival rate of patients with HCC (data not shown), and that these two genes may serve a role in carcinogenesis. He *et al* (26) demonstrated, by analyzing the Gene Expression Omnibus database, that PBK was associated with the prognosis of patients with HCC; however, to the best of the authors' knowledge, experimental studies of POLQ in HCC have not been conducted. Therefore, the present results provide an *in silico* analysis that may represent the basis for future experimental and theoretical studies aiming to investigate the prognosis of HCC. The present prognosis model of HCC may help in performing individualized diagnoses and developing targeted treatments for HCC.

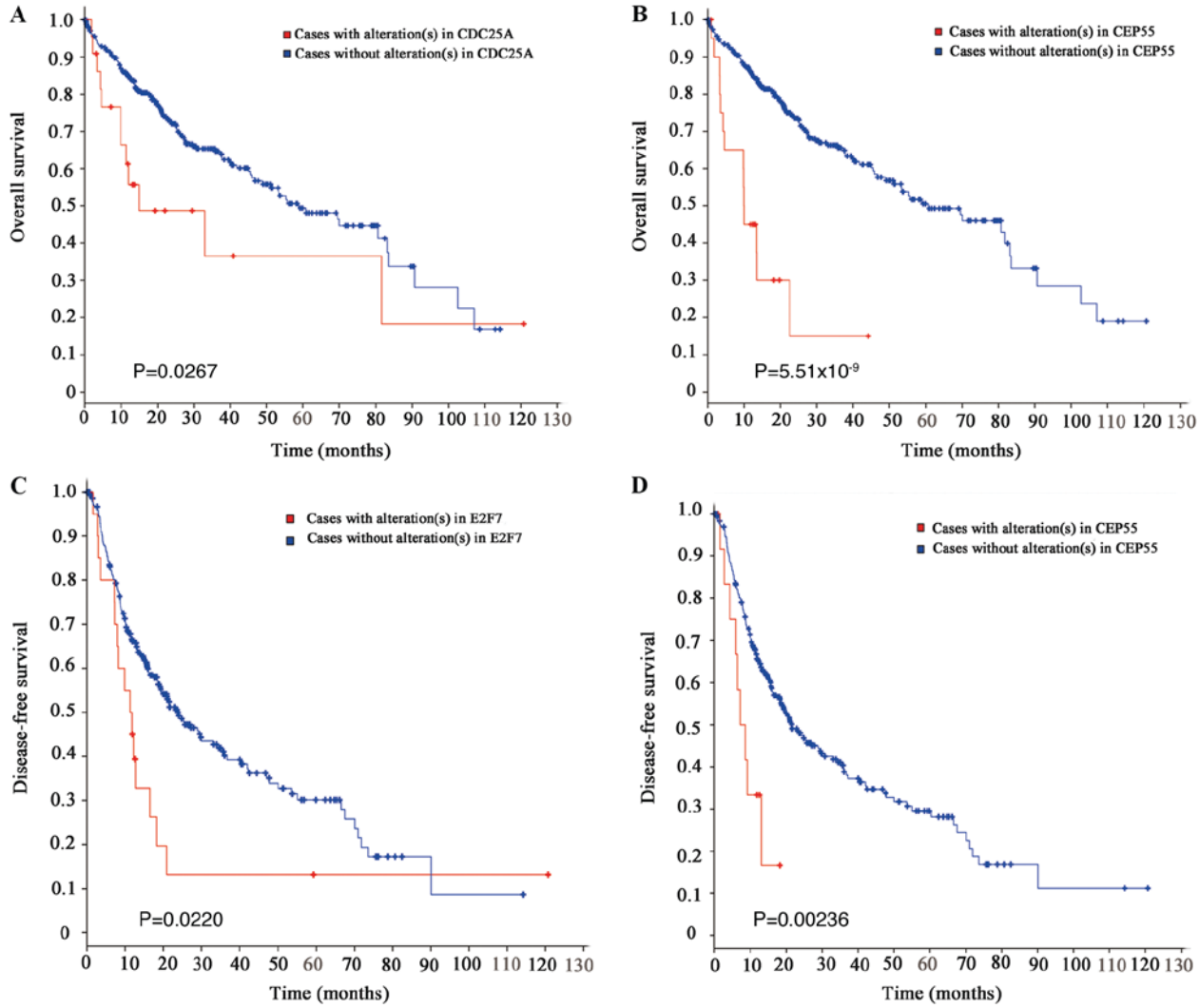

Figure 4. Association between survival rates and genetic mutations in genes differentially expressed in HCC that are targets of microRNA-424. The association between the genetic mutations in (A) CDC25A and (B) CEP55, and overall survival rate of patients with HCC. The association between the genetic mutations in (C) E2F7 and (D) CEP55, and disease-free survival rate of patients with HCC. HCC, hepatocellular carcinoma; CDC25A, cell division cycle 25A; CEP55, centrosomal protein 55; E2F7, E2F transcription factor 7.

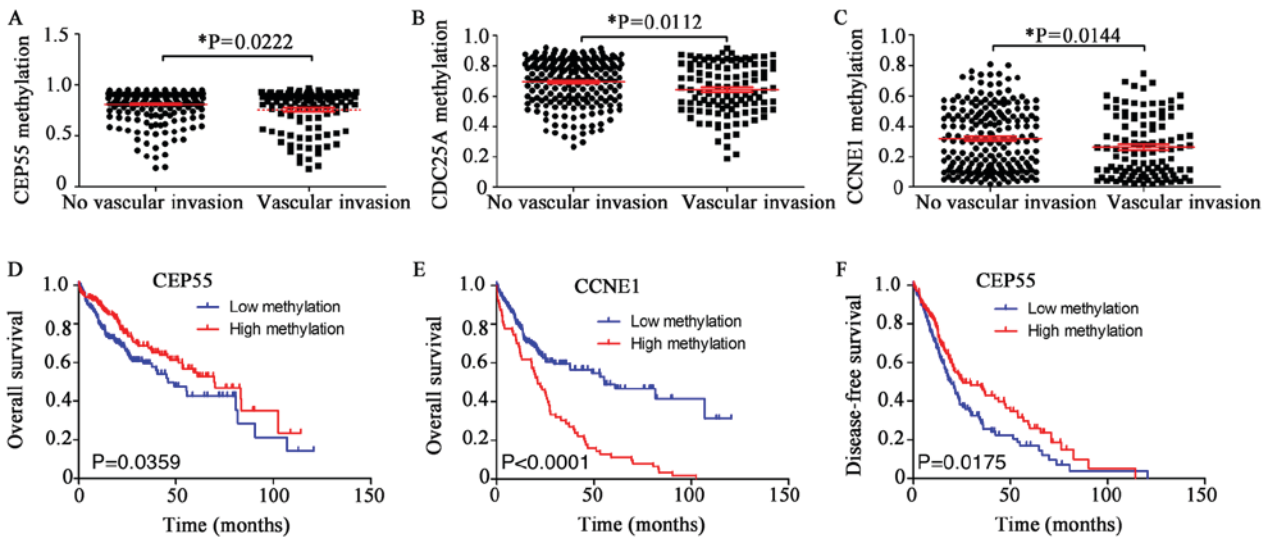

Figure 5. Role of DNA methylation in HCC. Association between methylation levels of (A) CEP55, (B) CDC25A and (C) CCNE1, and vascular invasion. Association between the methylation levels of (D) CEP55 and (E) CCNE1, and the overall survival rate of patients with HCC. (F) Association between the methylation levels of CEP55 and the disease-free survival rate of patients with HCC. *P<0.05. HCC, hepatocellular carcinoma; CDC25A, cell division cycle 25A; CEP55, centrosomal protein 55; CCNE1, cyclin E1.

A total of 77 lncRNAs, 16 DEmiRNAs and 35 DEmRNAs were identified to be involved in the constructed lncRNA-miRNA-mRNA regulation network. In total, 12 DEmiRNAs were upregulated and only one DEmiRNA, miRNA-424, was downregulated. The majority of the predicted target genes of miRNA-424, which were additionally differentially expressed in HCC, were associated with the 5-year survival rate of patients with HCC. Therefore, the miRNA-424 regulation network was selected for subsequent analyses. To assess the biological effects of the miRNA-424 regulatory network in HCC, functional enrichment analyses using GO and KEGG were performed. GO analysis suggested that the examined genes were associated with a number of 'biological process', 'molecular function' and 'cellular component' terms, whereas, KEGG analysis suggested that the signaling pathways 'progesterone-mediated oocyte maturation', 'oocyte meiosis', 'cell cycle' and 'microRNAs in cancer' were significantly enriched. Notably, these pathways have been previously identified to be associated with the occurrence and development of cancer (27-29). The present results suggested that the prognosis of HCC may be improved by testing the identified regulatory genes, although further experimental studies are required to verify this hypothesis.

PPI analysis identified that CDC25A, CEP55, CLSPN, CCNE1 and E2F7 were associated with the prognosis of HCC, further suggesting that the occurrence and development of HCC is a multi-factor process involving numerous genes and proteins. CDC25A was identified to be associated with CCNE1, in agreement with previous studies (30-33). Notably, CDC25A and CCNE1 were identified to be associated with the development of HCC.

Accumulating evidence demonstrated that genetic mutations are associated with the occurrence and prognosis of tumors (34-36), including HCC (37-39). In the present study, it was identified that mutations in miRNA-424 target genes were associated with biological features of HCC. Mutations in CEP55, CDC25A and E2F7 were identified to affect overall survival and disease-free survival. Notably, these genes may represent potential novel biomarkers for assessing the prognosis of patients with HCC, and may be considered potential targets for treating HCC. Epigenetic analysis suggested that differential methylation states of CDC25A, CCNE1 and CEP55 were associated with the occurrence, prognosis and biological features of HCC. Previous studies demonstrated that DNA methylation serves an important role in cancer (10,11,40). In the present study, the level of DNA methylation of CDC25A, CCNE1 and CEP55 was negatively associated with vascular invasion in HCC. Furthermore, the methylation state of CCNE1 and CEP55 was associated with the prognosis of HCC. The present findings identified a number of novel genes potentially involved in HCC development and progression that may represent novel biomarkers and novel targets for gene therapy, resulting in an improved prognosis of HCC.

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Availability of data and materials

The genetic transcriptome of hepatocellular carcinoma and the methylation data were downloaded from the TCGA data portal (https://portal.gdc.cancer.gov/). All datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

SH designed and conducted the study. CL performed all bioinformatics analysis and wrote the manuscript. GY analyzed the data and performed the statistical analyses. ZH and YZ participated in literature retrieval. XQ and HY made contributions to the design of this study and revised the manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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