MEDICAL SCIENCE MONITOR

DATABASE ANALYSIS

e-ISSN 1643-3750
© Med Sci Monit, 2020; 26: 921815 e921815
DOI: 10.12659/MSM.921815

Received: 2019.11.29
Accepted: 2020.02.11
Available online: 2020.03.26
Published: 2020.05.21

Bioinformatics Analysis Identifies the Estrogen Receptor 1 (ESR1) Gene and hsa-miR-26a-5p as Potential Prognostic Biomarkers in Patients with Intrahepatic Cholangiocarcinoma

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEFG Xianzheng Qin*
ABDEF Yuning Song*

Queen Mary School of Nanchang University, Nanchang, Jiangxi, P.R. China

* Xianzheng Qin and Yuning Song contributed equally to this work

Corresponding Authors: Xianzheng Qin, e-mail: jp6303416178@qmul.ac.uk, Yuning Song, e-mail jp6303416179@qmul.ac.uk
Source of support: Departmental sources

Background: Intrahepatic cholangiocarcinoma arises from the epithelial cells of the bile ducts and is associated with poor prognosis. This study aimed to use bioinformatics analysis to identify molecular biomarkers of intrahepatic cholangiocarcinoma and their potential mechanisms.

Material/Methods: MicroRNA (miRNA) and mRNA microarrays from GSE53870 and GSE32879 were downloaded from the Gene Expression Omnibus (GEO) database. Differentially expressed miRNAs (DEMs) associated with prognosis were identified using limma software and Kaplan-Meier survival analysis. Predictive target genes of the DEMs were identified using miRWalk, miRTarBase, miRDB, and TargetScan databases of miRNA-binding sites and targets. Target genes underwent Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis. Hub genes were analyzed by constructing the protein-protein interaction (PPI) network using Cytoscape. DEMs validated the hub genes, followed by construction of the miRNA-gene regulatory network.

Results: Twenty-five DEMs were identified. Fifteen DEMs were upregulated, and ten were down-regulated. Kaplan-Meier survival analysis identified seven upregulated DEMs and nine down-regulated DEMs that were associated with the overall survival (OS), and 130 target genes were selected. GO analysis showed that target genes were mainly enriched for metabolism and development processes. KEGG analysis showed that target genes were mainly enriched for cancer processes and some signaling pathways. Fourteen hub genes identified from the PPI network were associated with the regulation of cell proliferation. The overlap between hub genes and DEMs identified the estrogen receptor 1 (ESR1) gene and hsa-miR-26a-5p.

Conclusions: Bioinformatics analysis identified ESR1 and hsa-miR-26a-5p as potential prognostic biomarkers for intrahepatic cholangiocarcinoma.

MeSH Keywords: Biological Markers • Cholangiocarcinoma • Gene Expression Profiling • MicroRNAs

Full-text PDF: https://www.medscimonit.com/abstract/index/idArt/921815

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Background

Intrahepatic cholangiocarcinoma arises from the epithelial cells of the bile ducts and is associated with poor prognosis. Worldwide, intrahepatic cholangiocarcinoma has an increasing incidence and high mortality rate and represents about 15% of cases of primary liver cancer, with hepatocellular carcinoma (HCC) representing about 70% of cases [1–3]. The main risk factors for intrahepatic cholangiocarcinoma include sclerosing cholangitis, biliary anomalies, hepatolithiasis, hepatobiliary flukes, and liver cirrhosis [4]. Patients with intrahepatic cholangiocarcinoma often present with nonspecific symptoms or are asymptomatic. Therefore, without sensitive screening criteria, only a few cases are diagnosed at an early stage [5,6]. Also, most patients are diagnosed with late-stage intrahepatic cholangiocarcinoma with the tumor having invaded into adjacent structures or metastasized to distant sites [7–9]. Even for patients who are diagnosed at an early stage, risk factors such as cirrhosis may increase the difficulty of treatment [6]. Only about 30% of patients with intrahepatic cholangiocarcinoma can undergo surgical resection, and these patients have a high recurrence rate following surgery [5,10]. Despite clinical research on improving the management of patients with intrahepatic cholangiocarcinoma, the prognosis remains poor, with a 30% three-year survival rate and an 18% five-year survival rate [11,12]. Therefore, potential diagnostic and prognostic biomarkers for intrahepatic cholangiocarcinoma remain to be identified.

The microRNAs (miRNAs) are a family of small endogenous non-coding RNA molecules that play an important role in regulating the expression of target genes and proteins through complementary base pairs with mRNAs [13–15]. Recent studies have shown an association between miRNAs and human cancers [16]. Changes in miRNAs affect several cellular processes that include cell proliferation, cell differentiation, and signal transduction [14,17,18]. The progression of intrahepatic cholangiocarcinoma is associated with the abnormal expression of miRNAs [18–20]. Biomarkers of intrahepatic cholangiocarcinoma have included upregulated miR-31, and miR-150 and down-regulated miR-590-3p and miR-424-5p [19–23]. Wang et al. [24] found that increased expression of plasma levels of miR-150 could identify patients with intrahepatic cholangiocarcinoma with high sensitivity, specificity [22,23]. Also, miR41 directly regulates BRCA1-associated protein-1 (BAP-1), which has frequent mutations in intrahepatic cholangiocarcinoma, which is associated with reduced prognosis [20,25,26].

Epithelial-mesenchymal transition (EMT) is a biological developmental process that is considered to be the key mechanism leading to invasion and metastasis of intrahepatic cholangiocarcinoma [27,28]. In 2015, Zhang et al. showed that the expression of miR-590-3p was down-regulated in intrahepatic cholangiocarcinoma and showed that miR-590-3p influenced EMT by inhibiting the expression of the Smad interacting protein 1 (SIP1) [29]. Also, miR-424-5p has been shown to play an important role in promoting cell proliferation and metastasis in intrahepatic cholangiocarcinoma [21,30]. In 2019, Wu et al. [21] proposed that the restoration of miR-424-5p expression may be a promising approach to treat intrahepatic cholangiocarcinoma by targeting the pathway of the binding between miR-424-5p and NUAK family kinase 1 (ARK5) mRNA. Although these previous studies have resulted in the development of drug treatments, the underlying molecular mechanisms in the progression of intrahepatic cholangiocarcinoma remain to be elucidated. Therefore, new diagnostic and prognostic biomarkers in patients with intrahepatic cholangiocarcinoma may also result in new approaches to treatment.

Bioinformatics analysis of microarray data is a high-throughput technology that has been widely used to identify genetic changes in cancer. The analysis of miRNA microarrays can be used to identify potential biomarkers in intrahepatic cholangiocarcinoma [29]. This study aimed to use bioinformatics analysis to identify molecular biomarkers of intrahepatic cholangiocarcinoma and their potential mechanisms. The miRNA and mRNA expression profiles were downloaded to obtain differentially expressed miRNAs (DEMs), and differentially expressed mRNAs. The interactions between DEMs, their target genes, and differentially expressed mRNAs in intrahepatic cholangiocarcinoma were investigated through the microarray profiles of the expression of miRNAs and mRNAs. The construction of the miRNA-gene regulatory network explored the potential molecular prognostic biomarkers for intrahepatic cholangiocarcinoma, which may provide insights into future diagnosis and treatment.

Material and Methods

Microarray data

High-throughput gene expression and microarray data were obtained from the Gene Expression Omnibus (GEO) public genomics online repository (*www.ncbi.nlm.nih.gov/geo*) [31]. The miRNA expression dataset, GSE53870, and the mRNA expression dataset, GSE32879, were downloaded from the GEO database [29,32]. The probes were converted to the corresponding gene symbol using the annotation information in the GEO platform.

Identification of differentially expressed miRNAs (DEMs) and differentially expressed mRNAs

The R (version 1.62.0) Affy package (*www.bioconductor.org/*) was used for the analysis of GSE53870 and GSE32879. The median

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

algorithm performed the data preprocessing and normalization in R (version 3.6.1). The limma package (version 3.40.6) (*http://bioconductor.org/*) was used to screen the DEMs and differentially expressed mRNAs. The adjusted P-value (adj. P-value) and the Benjamini–Hochberg false discovery rate (FDR) were used in the analysis to reduce the rate of false positives. DEMs and differentially expressed mRNAs, which both satisfied the $\log^2$ (fold-change) >2 and the adj. P-value <0.05 were considered to be statistically significant and were selected for further study.

Visualization of DEMs

The HemI Heat map Illustrator (version 1.0) is an open-source bioinformatics toolkit that was used to graphically visualize multi-dimensional and numerical gene expression data as heatmaps [33]. The data of DEMs were visualized with different colors. The volcano plot was performed using the R package ggplot2 version 3.2.1 to visualize the DEMs (*https://cran.r-project.org/web/packages/ggplot2/index.html*).

Kaplan-Meier survival analysis

Data in GSE53870 were processed for statistical analysis to investigate the relationship between DEMs and patients with intrahepatic cholangiocarcinoma. The free R package survival package (version 3.1-7) (*https://cran.r-project.org/web/packages/survival/*) was used for survival analysis of the screened DEMs. The log-rank test was performed to estimate the prognosis of different DEMs. A P<0.05 was considered to be statistically significant.

Prediction and screening of the target genes of DEMs

miRWalk (version 3.0) (*http://mirwalk.umm.uni-heidelberg.de/*) is an open-source website used to predict the target genes [34]. TargetScan (version 7.2) (*http://www.targetscan.org/vert_72/*) is an online database that was used to predict the target genes by searching for the conserved sites on the paired seed region of each DEM [35]. Also, miRDB (*http://mirdb.org/index.html*) and miRTarBase (*http://miRTarBase.mbc.nctu.edu.tw/*) were used to predict the target genes [36,37]. A Venn diagram was produced by the R (venneuler) package (version 1.1-0) (*https://cran.r-project.org/web/packages/venneuler/index.html*) to reduce false positives of data predicted by the online databases.

Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of target genes

The GO resource (*http://geneontology.org/*) is an online database that provides biological information like the function of genes and gene products [38,39]. The GO resource was used to implement functional enrichment analysis of significant target genes with P<0.05 [40]. The KEGG pathway enrichment analysis was performed using KEGG Orthology Based Annotation System (KOBAS) (version 3.0) (*http://kobas.cbi.pku.edu.cn/anno_iden.php*). KOBAS is a web server that can be used to identify significantly enriched pathways by mapping to genes with known annotations [41–43]. P<0.05 was considered as statistically significant.

Construction of the protein-protein interaction (PPI) network of the target genes and centrality analysis

The PPI network was established using the STRING online database (version 11.0) (*https://string-db.org/*), which aims to collect and integrate the interactions between proteins [44]. The PPI network was constructed to analyze the relationships between the screened target genes and the interaction. A combined score >0.400 was regarded as significant PPI node pairs worth further investigation. Cytoscape (version 3.7.1), which is an open software source that integrates biomolecular interaction networks, was used to visualize the data from the STRING [45,46]. The online CentiScape plugin (version 2.2) (*http://apps.cytoscape.org/apps/mcode*) in Cytoscape was used to calculate the centrality parameters to identify the most significant nodes in the PPI network [47].

Hub gene selection and analysis

The hub genes were screened by Cytoscape software, the Molecular Complex Detection (MCODE) (version 1.5.1) (*http//apps.cytoscape.org/apps/mcode*) plugin in Cytoscape was used for detection of the PPI networks with dense connectivity [48]. The selection criteria were the degree of cutoff=2, the node score cutoff=0.2, the K-score=2, and the maximum depth=100. The network degrees >10 were identified as hub genes.

Construction of the miRNA-gene regulatory network

The miRNAs were identified by the online databases that could predict one or more target genes of the DEMs. The miRNA and gene regulatory network was constructed using Cytoscape software to identify the relationship between the target genes and miRNAs.

Results

Identification and visualization of differentially expressed miRNAs (DEMs)

After the processing of the raw data in GSE53870 (Figure 1), a total of 1104 miRNAs were identified, 436 of which were up-regulated, and 668 were down-regulated. Compared with samples of normal intrahepatic bile ducts, patients with intrahepatic

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

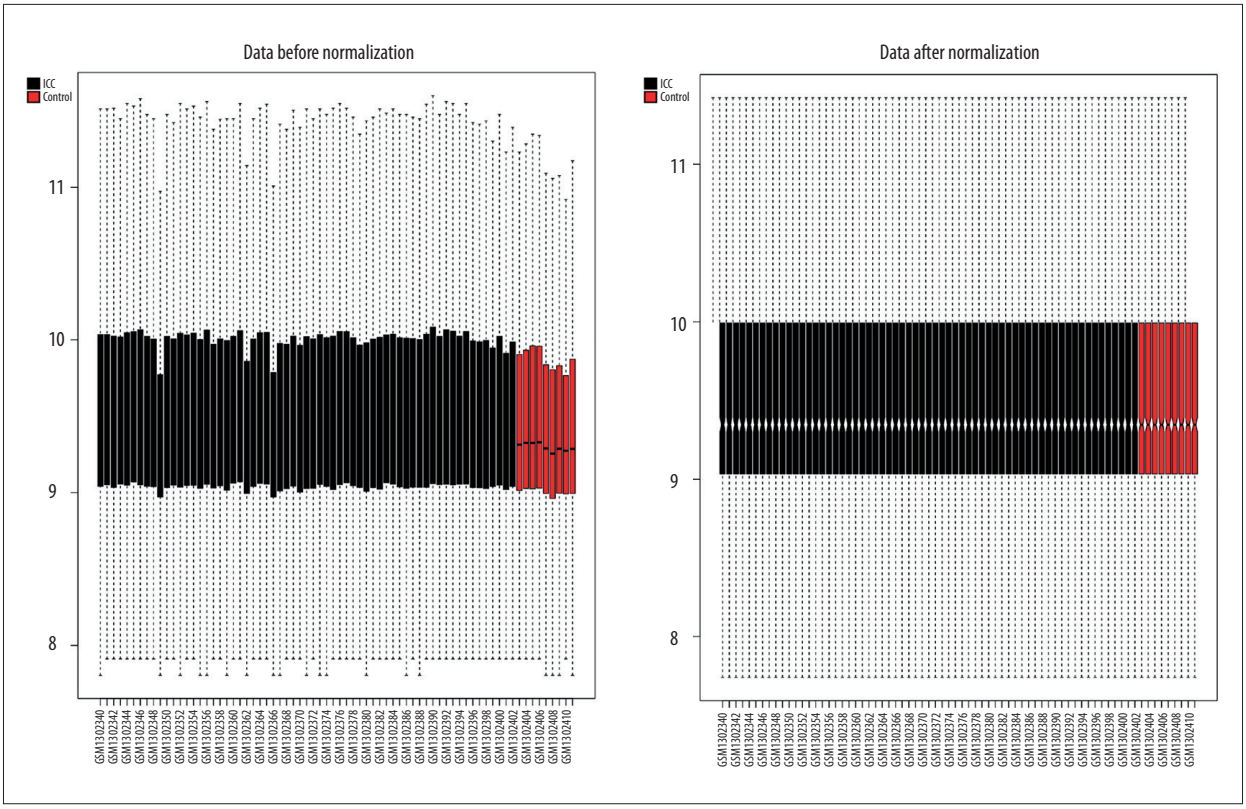

Figure 1. Normalization of the GSE53870 data from the Gene Expression Omnibus (GEO) database. The black boxes represent the microRNA (miRNA) expression values in patients with intrahepatic cholangiocarcinoma. The red boxes represent the miRNA expression values in the normal intrahepatic bile duct control samples.

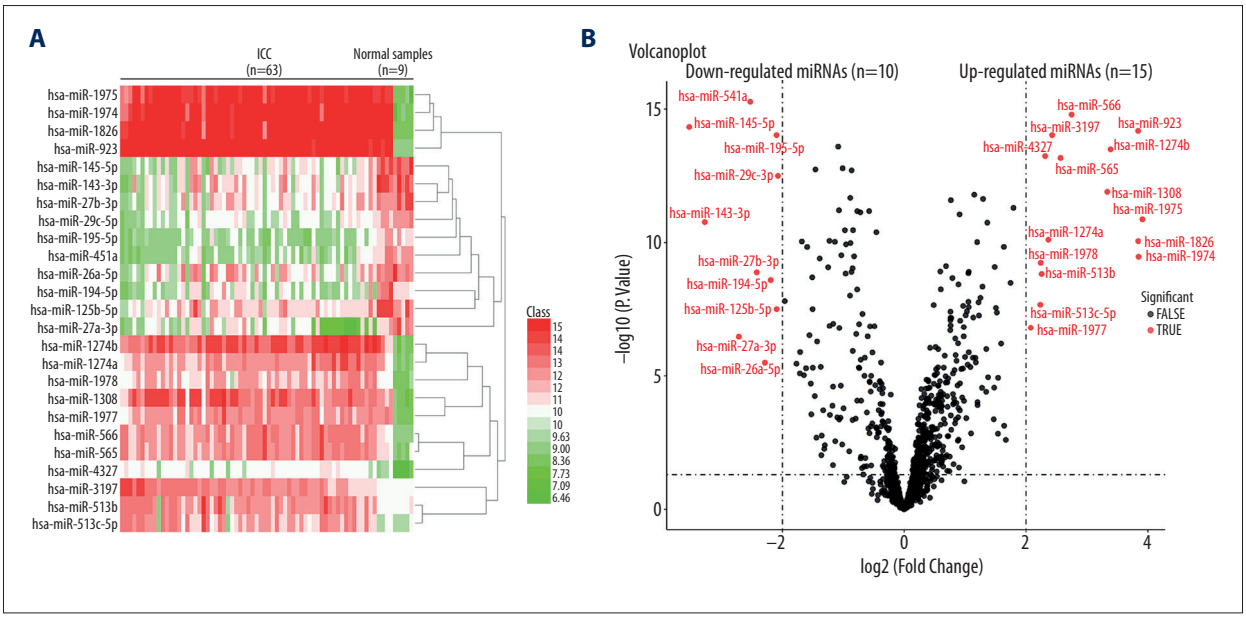

Figure 2. The heatmap and volcano plot of the 25 differentially expressed miRNAs (DEMs) associated with prognosis in intrahepatic cholangiocarcinoma. (**A**) Heatmap of the top 25 DEMs was constructed using HemI. The level of expression is positively correlated with the size of the fluorescence value. The red color indicates high expression. The green color indicates low expression. (**B**) The volcano plot shows DEMs between the samples from patients with intrahepatic cholangiocarcinoma and normal samples. The red color indicates statistical significance.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 1. The top 25 differentially expressed miRNAs (DEMs) in the intrahepatic cholangiocarcinoma samples compared with the normal bile ducts samples.

miRNA ID	log2 FC	B	t	P-value	adj. P-value	Expression
hsa-miR-1975	3.91	16.1352	8.02	1.33E-11	5.66E-10	Upregulated
hsa-miR-1974	3.849	12.9719	7.27	3.38E-10	8.47E-09	Upregulated
hsa-miR-1826	3.844	14.2929	7.58	8.75E-11	2.84E-09	Upregulated
hsa-miR-923	3.842	23.6002	9.78	6.54E-15	1.73E-12	Upregulated
hsa-miR-1274b	3.386	22.0409	9.41	3.21E-14	4.43E-12	Upregulated
hsa-miR-1308	3.335	18.4515	8.56	1.25E-12	9.22E-11	Upregulated
hsa-miR-566	2.749	24.9821	10.11	1.60E-15	8.81E-13	Upregulated
hsa-miR-565	2.567	21.3078	9.24	6.79E-14	7.50E-12	Upregulated
hsa-miR-3197	2.429	23.2466	9.7	9.39E-15	1.73E-12	Upregulated
hsa-miR-1274a	2.367	14.4059	7.61	7.79E-11	2.69E-09	Upregulated
hsa-miR-4327	2.314	21.4746	9.28	5.73E-14	7.03E-12	Upregulated
hsa-miR-513b	2.256	11.5083	6.91	1.51E-09	2.92E-08	Upregulated
hsa-miR-1978	2.242	12.4543	7.14	5.73E-10	1.38E-08	Upregulated
hsa-miR-513c-5p	2.235	8.9229	6.29	2.13E-08	3.32E-07	Upregulated
hsa-miR-1977	2.078	6.98	5.8	1.57E-07	1.81E-06	Upregulated
hsa-miR-145-5p	–3.527	23.9275	–9.86	4.68E-15	1.72E-12	Down-regulated
hsa-miR-143-3p	–3.272	15.8895	–7.96	1.71E-11	7.00E-10	Down-regulated
hsa-miR-27a-3p	–2.715	6.2213	–5.61	3.44E-07	3.68E-06	Down-regulated
hsa-miR-451a	–2.526	26.0606	–10.37	5.30E-16	5.86E-13	Down-regulated
hsa-miR-27b-3p	–2.42	11.6593	–6.95	1.29E-09	2.64E-08	Down-regulated
hsa-miR-26a-5p	–2.287	4.0601	–5.05	3.21E-06	2.81E-05	Down-regulated
hsa-miR-194-5p	–2.189	10.9978	–6.79	2.54E-09	4.68E-08	Down-regulated
hsa-miR-195-5p	–2.094	23.2455	–9.7	9.40E-15	1.73E-12	Down-regulated
hsa-miR-125b-5p	–2.093	8.5621	–6.2	3.09E-08	4.45E-07	Down-regulated
hsa-miR-29c-3p	–2.072	19.7967	–8.88	3.17E-13	2.50E-11	Down-regulated

log^2 FC – log^2 (fold-change); adj. P-value – adjusted P-value; B – B-value; t – t-statistics.

cholangiocarcinoma had 25 DEMs that satisfied log^2 (fold-change) >2 and adj. P <0.05, consisting of ten down-regulated miRNAs and 15 upregulated miRNAs (Figure 2). The top 25 DEMs are listed in Table 1.

Kaplan-Meier survival analysis

Based on the data in GSE53870, Kaplan-Meier survival analysis identified 16 DEMs that were associated with overall survival (OS), which included seven upregulated DEMs and nine down-regulated DEMs. In patients with intrahepatic cholangiocarcinoma, high expression of hsa-miR-1308 (P=4.59E-2), hsa-miR-566 (P=4.40E-2), hsa-miR-565 (P=4.53E-2), hsa-miR-3197 (P=1.98E-3), hsa-miR-4327 (P=1.72E-2), hsa-miR-513b (P=4.45E-2), hsa-miR-513c-5p (P=2.52E-2) and low expression of hsa-miR-145-5p (P=2.94E-2), hsa-miR-143-3p (P=1.46E-2), hsa-miR-451a (P=6.69E-3), hsa-miR-27b-3p (P=3.38E-3), hsa-miR-26a-5p (P=2.67E-2), hsa-miR-194-5p (P=2.53E-2), hsa-miR-195-5p (P=8.18E-3), hsa-miR-125b-5p (P=3.53E-2) and hsa-miR-29c-3p (P=1.19E-3) were significantly associated with reduced OS. The remaining DEMs were not significant survival biomarkers. Survival

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

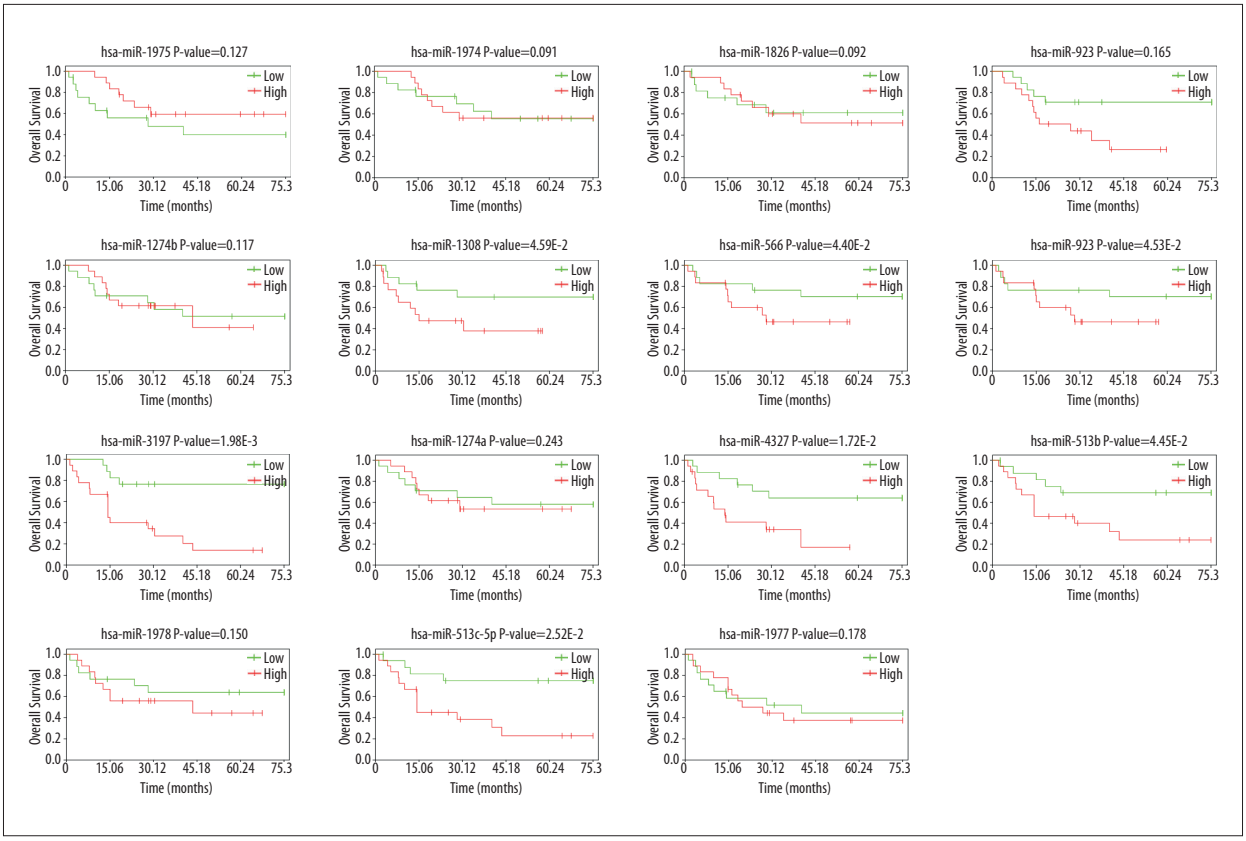

Figure 3. Kaplan-Meier survival analysis of upregulated differentially expressed microRNAs (miRNAs) (DEMs). The red lines show individuals with high expression of DEMs. The green lines show individuals with low expression of DEMs.

analysis of all screened miRNAs associated with OS are shown in Figures 3 and 4.

Prediction and screening of target genes of DEMs associated with patient survival

Different databases used in this study had their own algorithms to predict the target genes. After matching the overlap of the results of miRWalk between the online databases, TargetScan, miRDB, and miRTarBase, 130 target genes were predicted from eight DEMs, TargetScan identified 990 target genes, 1183 target genes were identified in miRDB, and 392 target genes were identified in miRTarBase. The overlap of target genes between the three datasets is shown in the Venn diagram (Figure 5).

Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of target genes

GO and KEGG functional and pathway enrichment analysis of 130 target genes was performed to understand the screened target genes better. The results of GO biological process (BP) analysis identified target genes that were significantly enriched for the processes of substance metabolism, development, and the regulation of gene expression. GO molecular function (MF) showed that target genes were mainly enriched in protein binding, cyclic compound binding, sequence-specific DNA binding, and transcription regulator activity. GO cellular component (CC) showed that target genes were mainly involved in the nucleus, cytosol, intracellular organelles, and membrane-enclosed lumen. Also, KEGG pathway analysis showed that target genes were significantly enriched in cancer processes, including pathways in cancer, miRNA in cancer, proteoglycans in cancer, Ras, FoxO, and PI3K-Akt signaling pathways, and resistance to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors. Pathways in cancers were the most significantly enriched (P=5.42E-13). The most significant findings from GO and KEGG enrichment analysis are shown in Figure 6 and Table 2.

Construction of the protein-protein interaction (PPI) network and centrality analysis

Based on the information of target genes from the STRING database, the PPI network, including the combined score >0.400, with 130 nodes and 231 edges (Figure 7), was constructed by Cytoscape. CentiScape was used to calculate the value of the degree of centrality, which was used in the selection of hub genes.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

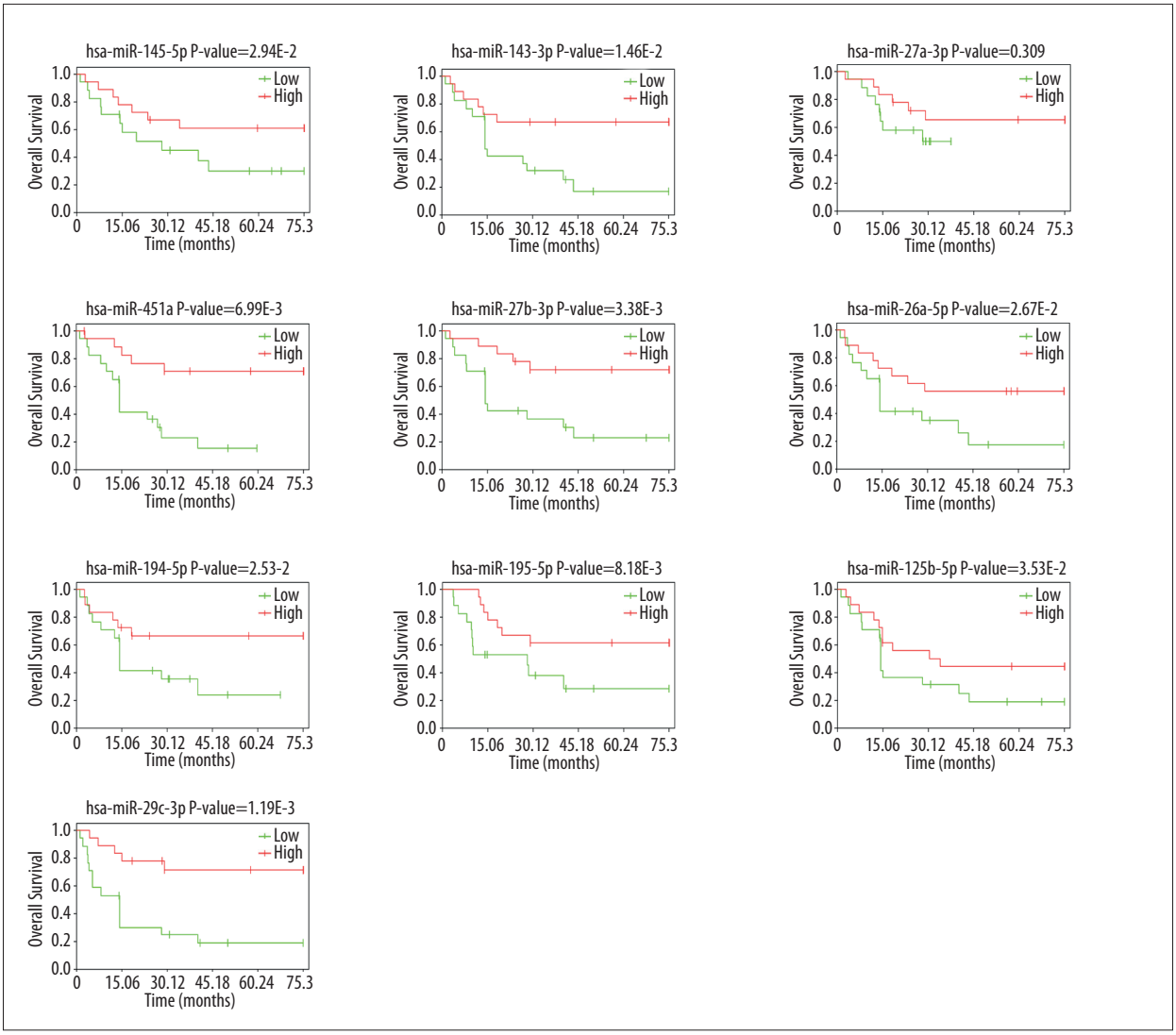

Figure 4. Kaplan-Meier survival analysis of down-regulated differentially expressed microRNAs (miRNAs) (DEMs). The red lines show individuals with high expression of DEMs. The green lines show individuals with low expression of DEMs.

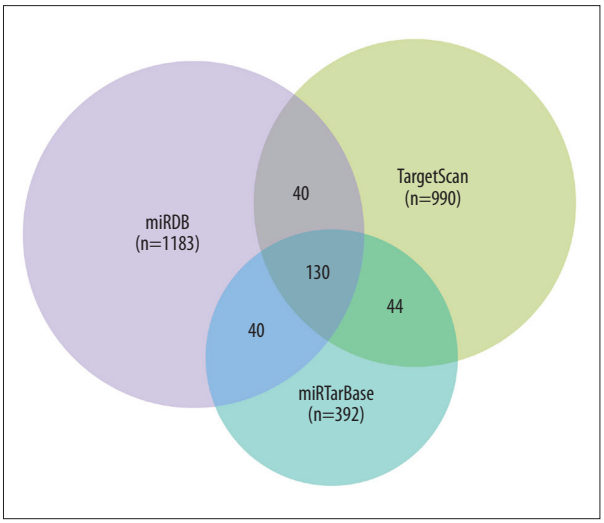

Figure 5. Venn diagram of the four datasets of 130 target genes. The target genes identified from the miRWalk database were screened again using the miRWalk, TargetScan, miRDB, and miRTarBase databases. The four datasets show an overlap of 130 target genes.

Selection and analysis of the hub genes

Hub genes were identified by the Molecular Complex Detection (MCODE) plugin in Cytoscape, and a total of 14 genes were screened from 130 target genes (Figure 8). The results were sorted by degree scores and identified the following 14 genes: KRAS, ESR1, STAT3, VEGFA, IGF1R, SMAD2, FGF2, DICER1, ACTB, CDK6, MET, FOXO1, ETS1, and HBEGF (Table 3). These 14 hub genes were used to process the GO and KEGG enrichment analysis. GO biologic process (BP) and KEGG enrichment analysis

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

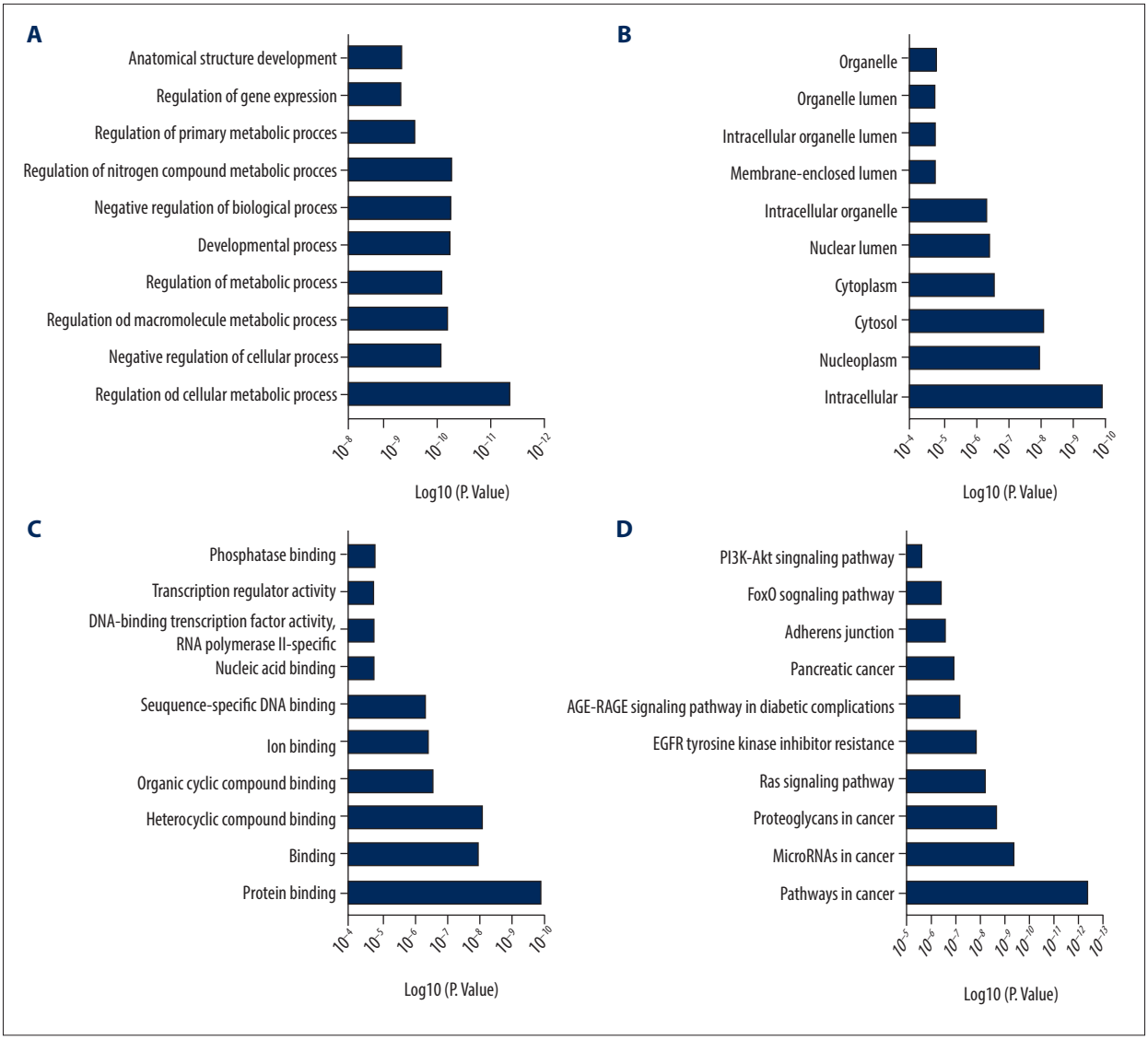

Figure 6. Gene Ontology (GO) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of the most significant target genes in intrahepatic cholangiocarcinoma. (**A**) The biological process of the top ten genes. (**B**) Cellular components of the top ten genes. (**C**) Molecular function of the top ten genes (**D**) The KEGG pathway of the top ten genes.

showed that hub genes were primarily enriched for the regulation of cell proliferation, anatomical structure and tube morphogenesis, and some receptor protein signaling pathways, proteoglycans, and pathways in cancer (Table 4).

Construction of the miRNA and gene regulatory network

According to the data from the results of 130 predicted target genes and eight corresponding miRNAs, Cytoscape was used to construct miRNA and gene regulatory network, to identify the regulatory association between the miRNAs and hub genes. The relationships were visualized with the miRNA and gene regulatory network (Figure 9).

Validation of the hub genes using the Gene Expression Omnibus (GEO) mRNA expression dataset

Data in the GSE32879 dataset from GEO were analyzed to validate the identity of the hub genes found in this study. A total of 766 differentially expressed mRNAs were identified, 173 of which were upregulated, and 593 were down-regulated. The overlap between hub genes from GSE53870 and differentially expressed mRNAs from GSE32879 showed that ESR1 was the only gene that occurred in both GEO datasets. Finally, hsa-miR-26a-5p and the corresponding miRNA of ESR1 were identified in the miRNA and gene regulatory network.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 2. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis of target genes for differentially expressed miRNAs (DEMs) in intrahepatic cholangiocarcinoma.

Pathway ID	Pathway description	Count	P-value
GO: 0031323	regulation of cellular metabolic process	78	5.34E-12
GO: 0048523	negative regulation of cellular process	64	1.33E-10
GO: 0060255	regulation of macromolecule metabolic process	75	1.01E-10
GO: 0019222	regulation of metabolic process	79	1.23E-10
GO: 0032502	developmental process	73	8.71E-11
GO: 0048519	negative regulation of biological process	69	8.51E-11
GO: 0051171	regulation of nitrogen compound metabolic process	73	7.74E-11
GO: 0080090	regulation of primary metabolic process	73	4.64E-10
GO: 0010468	regulation of gene expression	60	8.99E-10
GO: 0048856	anatomical structure development	68	8.44E-10
GO: 0005622	intracellular	123	1.41E-10
GO: 0005654	nucleoplasm	54	1.10E-08
GO: 0005829	cytosol	64	7.77E-09
GO: 0005737	cytoplasm	102	2.57E-07
GO: 0031981	nuclear lumen	57	3.57E-07
GO: 0043229	intracellular organelle	108	4.34E-07
GO: 0031974	membrane-enclosed lumen	61	1.58E-05
GO: 0070013	intracellular organelle lumen	61	1.58E-05
GO: 0043233	organelle lumen	61	1.58E-05
GO: 0043226	organelle	110	1.41E-05
GO: 0005515	protein binding	109	1.41E-10
GO: 0005488	binding	122	1.10E-08
GO: 1901363	heterocyclic compound binding	65	7.77E-09
GO: 0097159	organic cyclic compound binding	65	2.57E-07
GO: 0043167	ion binding	65	3.57E-07
GO: 0043565	sequence-specific DNA binding	22	4.34E-07
GO: 0003676	nucleic acid binding	47	1.58E-05
GO: 0000981	DNA-binding transcription factor activity, RNA polymerase II-specific	20	1.58E-05
GO: 0140110	transcription regulator activity	27	1.58E-05
GO: 0019902	phosphatase binding	8	1.41E-05
hsa05200	Pathways in cancer	16	5.42E-13
hsa05206	MicroRNAs in cancer	12	5.18E-10
hsa05205	Proteoglycans in cancer	10	2.54E-09
hsa04014	Ras signaling pathway	10	6.74E-09
hsa01521	EGFR tyrosine kinase inhibitor resistance	7	1.69E-08
hsa04933	AGE-RAGE signaling pathway in diabetic complications	7	7.04E-08
hsa05212	Pancreatic cancer	6	1.40E-07
hsa04520	Adherens junction	6	2.63E-07
hsa04068	FoxO signaling pathway	7	4.36E-07
hsa04151	PI3K-Akt signaling pathway	9	2.47E-06

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

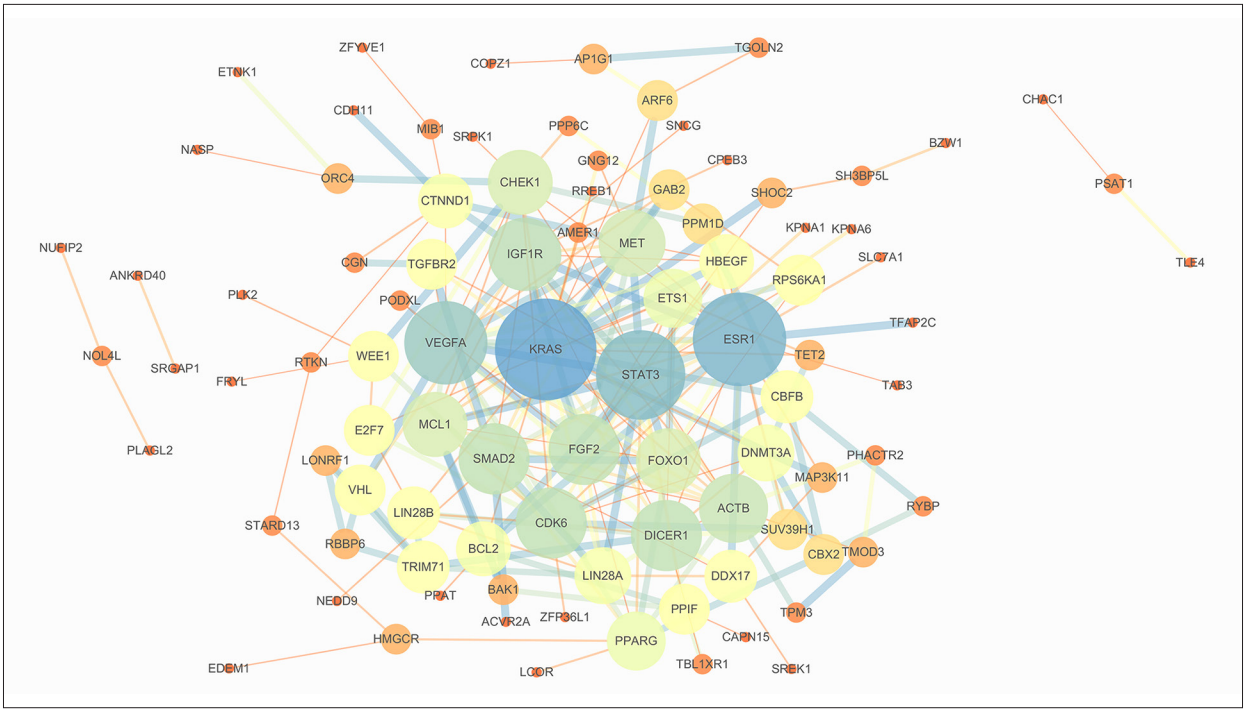

Figure 7. Construction of the protein-protein interaction (PPI) network of the target genes. The PPI network of target genes was visualized using Cytoscape.

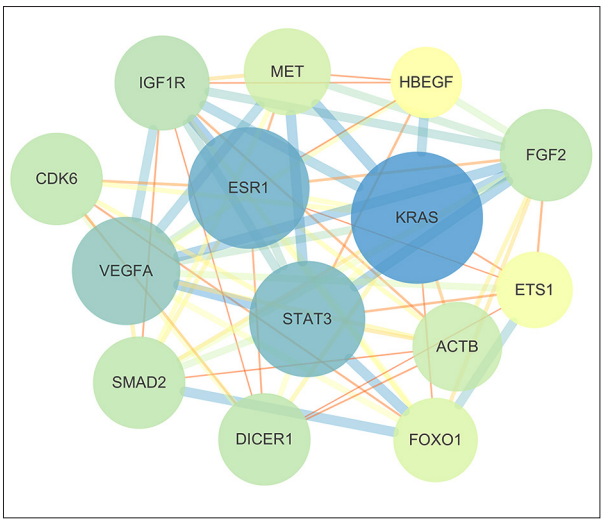

Figure 8. Selection of the hub genes from the protein-protein interaction (PPI) network. The hub genes were selected from the PPI network with 14 nodes and 68 edges. The lines represent the relationships between the nodes.

Discussion

Intrahepatic cholangiocarcinoma arises from bile duct epithelial cells and has high morbidity and mortality [10,22,49]. Due to the lack of effective methods for early diagnosis, the majority of patients with intrahepatic cholangiocarcinoma do not have symptoms in the early stages, and present with late-stage disease. Despite clinical studies to improve patient management, the molecular mechanisms remain unclear, and there are no prognostic molecular biomarkers. Therefore, the identification of molecular biomarkers associated with intrahepatic cholangiocarcinoma, their biological significance, and biological functions may provide insight into the pathogenesis of intrahepatic cholangiocarcinoma at the molecular level.

In the present study, microRNA (miRNA) and mRNA microarrays from GSE53870 and GSE32879 were downloaded from the Gene Expression Omnibus (GEO) database for intrahepatic cholangiocarcinoma and were used to identify differentially expressed miRNAs (DEMs) in comparison with normal intrahepatic bile ducts. This study identified 25 DEMs from the dataset, including 15 upregulated miRNAs and ten down-regulated miRNAs. Kaplan-Meier survival analysis showed that seven upregulated miRNAs (hsa-miR-1308, hsa-miR-566, hsa-miR-565, hsa-miR-3197, hsa-miR-4327, hsa-miR-513b, and hsa-miR-513c-5p) and nine down-regulated DEMs (hsa-miR-145-5p, hsa-miR-143-3p, hsa-miR-451a, hsa-miR-27b-3p, hsa-miR-26a-5p, hsa-miR-194-5p, hsa-miR-195-5p, hsa-miR-125b-5p, and hsa-miR-29c-3p) were associated with the overall survival (OS) of patients with intrahepatic cholangiocarcinoma. The associations between some of the identified DEMs and intrahepatic cholangiocarcinoma have also been identified in previous studies. Specifically, miR-145 has been reported as a tumor suppressor, and the levels are reduced in intrahepatic cholangiocarcinoma, which affects Akt/FoxO1

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

Table 3. The top 14 hub genes with the degree score.

Gene symbol	Gene description	Degree
KRAS	KRAS proto-oncogene, GTPase	30
ESR1	Estrogen receptor 1	26
STAT3	Signal transducer and activator of transcription 3	24
VEGFA	Vascular endothelial growth factor A	21
IGF1R	Insulin like growth factor 1 receptor	16
SMAD2	Smad family member 2	15
FGF2	Fibroblast growth factor 2	15
DICER1	Dicer 1, ribonuclease III	15
ACTB	Actin beta	15
CDK6	Cyclin dependent kinase 6	15
MET	MET proto-oncogene, receptor tyrosine kinase	13
FOXO1	Forkhead box O1	12
ETS1	ETS proto-oncogene 1, transcription factor	9
HBEGF	Heparin binding EGF like growth factor	7

Table 4. Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) biologic process pathway enrichment analysis of the hub genes.

Pathway ID	Pathway description	Count	P-value
GO: 0042127	regulation of cell population proliferation	12	3.90E-12
GO: 0009653	anatomical structure morphogenesis	12	1.04E-10
GO: 0007167	enzyme linked receptor protein signaling pathway	9	1.20E-10
GO: 0007169	transmembrane receptor protein tyrosine kinase signaling pathway	8	3.74E-10
GO: 0010604	positive regulation of macromolecule metabolic process	13	5.82E-10
GO: 0008284	positive regulation of cell population proliferation	9	1.04E-09
GO: 0009893	positive regulation of metabolic process (GO: 0009893)	13	1.59E-09
GO: 0010628	positive regulation of gene expression	11	1.77E-09
GO: 0035239	tube morphogenesis	8	2.51E-09
GO: 0080134	regulation of response to stress	10	2.88E-09
hsa05205	Proteoglycans in cancer	10	1.69E-20
hsa05200	Pathways in cancer	10	1.09E-17
hsa01521	EGFR tyrosine kinase inhibitor resistance	6	2.72E-13
hsa05212	Pancreatic cancer	5	3.10E-11
hsa05218	Melanoma	5	4.40E-11
hsa04015	Rap1 signaling pathway	6	7.12E-11
hsa04014	Ras signaling pathway	6	1.12E-10
hsa04933	AGE-RAGE signaling pathway in diabetic complications	5	2.40E-10
hsa05206	MicroRNAs in cancer	6	5.52E-10
hsa04068	FoxO signaling pathway	5	9.46E-10

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

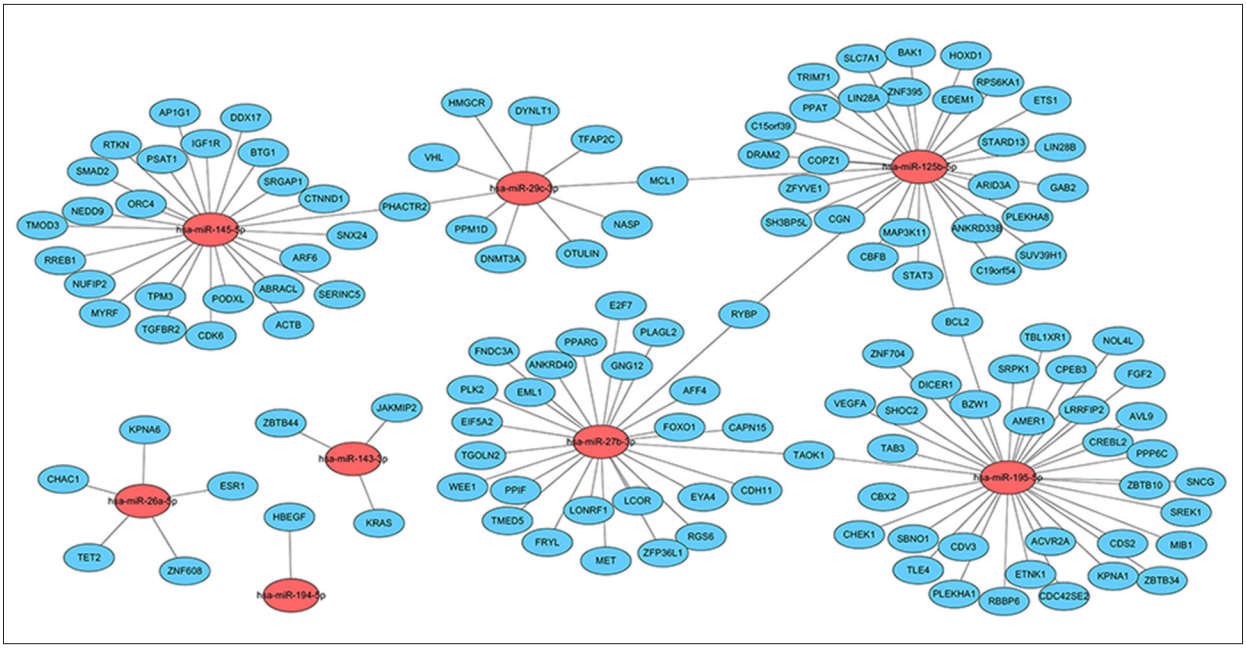

Figure 9. The microRNA (miRNA) and gene regulation network. The network was constructed using Cytoscape software. The red color represents the miRNA. The blue color represents the corresponding target gene.

signaling [50–52]. Increased expression of miR-145 is associated with inhibition of the growth of intrahepatic cholangiocarcinoma by inhibiting cancer cell proliferation, growth, and invasion [53,54]. Also, miR-26a was previously shown to be significantly down-regulated in cholangiocarcinoma cells *in vitro*, and miR-195 expression was reduced in cholangiocarcinoma cells [50–52]. A miRNA expression profile in intrahepatic cholangiocarcinoma previously reported the aberrant expression of some miRNAs, which included upregulated hsa-miR-566, while hsa-miR-29c-3p, hsa-miR-26a-5p, hsa-miR-451a, and hsa-miR-143-3p were down-regulated, which supports the findings of the DEMs identified in the present study [53,55].

However, miRNAs have different functional roles in the regulation of specific genes [56]. Therefore, target gene prediction of miRNAs is of importance. Several online databases are currently used to predict target genes of miRNAs, and each miRNA may predict a large number of target genes with the help of the algorithms from online databases. However, many gene target databases do not fully understand the relationships between miRNAs and target genes, which may result in false positives [56]. The miRWalk database predicts target genes by integrating six conventional features and seven new features [34,57]. TargetScan considers site type and searches for the conserved sites that pair the seed region of each DEM and then considers another 14 features to predict the target genes [35]. By using the support vector machine framework, miRDB may be used to predict target genes [36,58]. The online database, miRTarBase, predicts target genes by collecting and organizing the relationship between miRNAs and target genes from published studies [37]. Based on different computational methods of the online databases miRWalk, TargetScan, miRDB, and miRTarBase, the overlap of target genes in all datasets may reduce the false positives of the predicted results of miRWalk and make the identification of target genes more credible, as in the present study.

In this study, there were 130 genes selected. Gene Ontology (GO) functional enrichment analysis showed that these 130 genes were significantly enriched in the substance metabolism, development process, and regulation of gene expression. There are several previous studies have shown that regulation of cell proliferation and cellular metabolic processes are associated with cancer progression [59–61]. The findings from the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis identified target genes that were enriched for resistance to epidermal growth factor (EGFR) tyrosine kinase inhibitors, hormone resistance, and other signaling pathways, including Ras and FoxO, and cancer development pathways. Several previously published studies have shown that miRNAs are associated with cancer [62–64]. Kim et al. identified the role of signal transduction in cancer, which is consistent with the findings from the present study [65]. Tyrosine kinase inhibitors specific for EGFR1 could affect the role of EGFR and contribute to the progression of cholangiocarcinoma, as shown in a previous study by Lee et al. [66], which is consistent with the finding of EGFR tyrosine kinase inhibitor resistance identified in the present study. Rizvi et al. [22] showed that the Ras pathway was involved in malignancy. Other studies have shown that activation of the FoxO1 signaling pathway

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

could inhibit cell proliferation in malignant cells, including intrahepatic cholangiocarcinoma [67,68]. The findings from these previous studies support the findings from the present study.

The construction of the protein-protein interaction (PPI) network and the measurement of the degree of centrality identified a total of 14 hub genes in this study. Among these hub genes, KRAS and ESR1 showed the highest degree of centrality and directly interacted with several other genes. KRAS has previously been reported to be expressed in cholangiocarcinoma, and the activation of KRAS might also be involved in the progression of intrahepatic cholangiocarcinoma [69,70]. KRAS affects the expression of glucose transporter-1 (GLUT-1), which is a major glucose transporter in intrahepatic cholangiocarcinoma, and KRAS has also been identified as a negative prognostic molecular biomarker [70,71]. Also, KRAS is a molecular biomarker for ovarian mucinous tumors and pancreatic ductal adenocarcinoma [72,73]. The ESR1 gene is a specific diagnostic biomarker for breast cancer and is expressed by several types of cancer. Mutations of the ESR1 gene have been reported as prognostic factors associated with poor survival [74–77]. Previous studies have reported that mutation of ESR1 could affect hormone resistance and reduce the response to treatment [78,79]. Carausu et al. showed that the use of CDK4/6 inhibitors reduced the prevalence of ESR1 mutations [80]. Both KRAS and ESR1 are involved in the development and progression of several cancers and may be regarded as valuable biomarkers for diagnosis and treatment.

In the present study, the miRNA and gene regulatory network demonstrated the regulatory association between the miRNAs and genes. By overlapping the results from the hub genes in GSE53870 and the differentially expressed mRNAs in GSE32879, ESR1 was the only gene in both Gene Expression Omnibus (GEO) datasets. These findings indicated that ESR1 and its corresponding miRNA, hsa-miR-26a-5p, might be novel biomarkers for intrahepatic cholangiocarcinoma. Also, the expression of ESR1 was down-regulated in intrahepatic cholangiocarcinoma. Kuper et al. showed that patients cholangiocarcinoma with a higher estrogen level [81]. Previous studies showed that ESRs are expressed in the hepatobiliary epithelium, and estrogen produces its effect through specific integration with ESRs, which include ESR1 and ESR2 [81,82]. Therefore, estrogen might have a role in oncogenesis in cholangiocarcinoma. ESR1 has been reported as a tumor suppressor in colorectal cancer, and the genetic variation of ESR1 might increase the risk for hepatocellular carcinoma and prostate cancer [83,84]. Given these associations, ESR1 could be regarded as a potential tumor suppressor for intrahepatic cholangiocarcinoma. The results of KEGG pathway enrichment analysis showed that ESR1 was mainly enriched in proteoglycans in cancer. Proteoglycans are components of the extracellular matrix (ECM), which has been shown in tumorigenesis of leiomyomas by VCAN by down-regulating ESR1 [85]. Also, changes in ECM are associated with the development of hepatocellular carcinoma (HCC) and liver cirrhosis [86,87]. The roles of proteoglycan and ESR1 in intrahepatic cholangiocarcinoma require further study, as liver cirrhosis is also a risk factor for intrahepatic cholangiocarcinoma.

Several previous studies have reported that hsa-miR-26a-5p acts as a tumor suppressor and in cancer [88]. The expression of hsa-miR-26a-5p was reduced in bladder cancer, colorectal cancer, and HCC [89–91]. In this study, the result also demonstrated hsa-miR-26a-5p were down-regulated. Chang et al. showed that patients with HCC who had increased expression of hsa-miR-26a-5p had an increase in overall survival (OS) rates and a reduced the risk of tumor recurrence [92]. Also, hsa-miR-26a-5p was shown to be associated with the expression of E-cadherin and vimentin, which are involved in epithelial-mesenchymal transition (EMT) [93]. EMT has been identified as an important factor in tumor metastasis in intrahepatic cholangiocarcinoma [27,28]. By targeting EMT, hsa-miR-26a-5p might interfere with tumor development to improve the prognosis of patients. Therefore, hsa-miR-26a-5p should be regarded as a potential molecular biomarker in patients with intrahepatic cholangiocarcinoma.

Conclusions

This study aimed to use bioinformatics analysis to identify molecular biomarkers of intrahepatic cholangiocarcinoma and their potential mechanisms and identified down-regulated hsa-miR-26a-5p and ESR1. The findings from the present study, combined with the findings from previous studies, support the importance of hsa-miR-145-5p, KRAS, and hsa-miR-143-3p in intrahepatic cholangiocarcinoma. Further clinical studies are required to verify the findings from this preliminary study.

Conflict of interests

None.

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

References:

1. Rahnemai-Azar AA, Weisbrod A, Dillhoff M et al: Intrahepatic cholangiocarcinoma: Molecular markers for diagnosis and prognosis. Surg Oncol, 2017; 26(2): 125–37
2. Altekruse SF, Devesa SS, Dickie LA et al: Histological classification of liver and intrahepatic bile duct cancers in SEER registries. J Registry Manag, 2011; 38(4): 201–5
3. Global Burden of Disease Cancer Collaboration, Fitzmaurice C, Dicker D, Pain A et al: The Global Burden of Cancer 2013. JAMA Oncol, 2015; 1(4): 505–27
4. Zhang H, Yang T, Wu M, Shen F: Intrahepatic cholangiocarcinoma: Epidemiology, risk factors, diagnosis and surgical management. Cancer Lett, 2016; 379(2): 198–205
5. Chun YS, Javle M: Systemic and adjuvant therapies for intrahepatic cholangiocarcinoma. Cancer Control, 2017; 24(3): 1073274817729241
6. Massarweh NN, El-Serag HB: Epidemiology of hepatocellular carcinoma and intrahepatic cholangiocarcinoma. Cancer Control, 2017; 24(3): 1073274817729245
7. Banales JM, Cardinale V, Carpino G et al: Expert consensus document: Cholangiocarcinoma: current knowledge and future perspectives consensus statement from the European Network for the Study of Cholangiocarcinoma (ENS-CCA). Nat Rev Gastroenterol Hepatol, 2016; 13(5): 261–80
8. Weber SM, Ribero D, O'Reilly EM et al: Intrahepatic cholangiocarcinoma: expert consensus statement. HPB (Oxford), 2015; 17(8): 669–80
9. Blechacz B, Komuta M, Roskams T, Gores GJ: Clinical diagnosis and staging of cholangiocarcinoma. Nat Rev Gastroenterol Hepatol, 2011; 8(9): 512–22
10. Rahnemai-Azar AA, Weisbrod AB, Dillhoff M et al: Intrahepatic cholangiocarcinoma: Current management and emerging therapies. Expert Rev Gastroenterol Hepatol, 2017; 11(5): 439–49
11. Bartella I, Dufour JF: Clinical diagnosis and staging of intrahepatic cholangiocarcinoma. J Gastrointestin Liver Dis, 2015; 24(4): 481–89
12. Khan SA, Davidson BR, Goldin R et al: Guidelines for the diagnosis and treatment of cholangiocarcinoma: Consensus document. Gut, 2002; 51(Suppl. 6): VI1–9
13. Lou W, Liu J, Gao Y et al: MicroRNA regulation of liver cancer stem cells. Am J Cancer Res, 2018; 8(7): 1126–41
14. Li J, Tan S, Kooger R et al: MicroRNAs as novel biological targets for detection and regulation. Chem Soc Rev, 2014; 43(2): 506–17
15. Lou W, Ding B, Xu L, Fan W: Construction of potential glioblastoma multiforme-related miRNA-mRNA regulatory network. Front Mol Neurosci, 2019; 12: 66
16. Tutar Y: miRNA and cancer; Computational and experimental approaches. Curr Pharm Biotechnol, 2014; 15(5): 429
17. Zhang RX, Zheng Z, Li K et al: Both plasma and tumor tissue miR-146a high expression correlates with prolonged overall survival of surgical patients with intrahepatic cholangiocarcinoma. Medicine 2017; 96(44): e8267
18. Haga H, Patel T: Molecular diagnosis of intrahepatic cholangiocarcinoma. J Hepatobiliary Pancreat Sci, 2015; 22(2): 114–23
19. Zu C, Liu S, Cao W et al: MiR-590-3p suppresses epithelial-mesenchymal transition in intrahepatic cholangiocarcinoma by inhibiting SIP1 expression. Oncotarget, 2017; 8(21): 34698–708
20. Sarcognato S, Gringeri E, Fassan M et al: Prognostic role of BAP-1 and PBRM-1 expression in intrahepatic cholangiocarcinoma. Virchows Arch, 2019; 474(1): 29–37
21. Wu J, Yang B, Zhang Y et al: miR-424-5p represses the metastasis and invasion of intrahepatic cholangiocarcinoma by targeting ARK5. Int J Biol Sci, 2019; 15(8): 1591–99
22. Rizvi S, Gores GJ: Emerging molecular therapeutic targets for cholangiocarcinoma. J Hepatol, 2017; 67(3): 632–44
23. Kayhanian H, Smyth EC, Braconi C: Emerging molecular targets and therapy for cholangiocarcinoma. World J Gastrointest Oncol, 2017; 15(9): 268–80
24. Wang S, Yin J, Li T et al: Upregulated circulating miR-150 is associated with the risk of intrahepatic cholangiocarcinoma. Oncol Rep, 2015; 33(2): 819–25
25. Misumi K, Hayashi A, Shibahara J et al: Intrahepatic cholangiocarcinoma frequently shows loss of BAP1 and PBRM1 expression, and demonstrates specific clinicopathological and genetic characteristics with BAP1 loss. Histopathology, 2017; 70(5): 766–74
26. Carbone M, Yang H, Pass HI et al: BAP1 and cancer. Nat Rev Cancer, 2013; 13(3): 153–59
27. Thiery JP: Epithelial-mesenchymal transitions in tumour progression. Nat Rev Cancer, 2002; 2(6): 442–54
28. Huang JY, Zhang K, Chen DQ et al: MicroRNA-451: Epithelial-mesenchymal transition inhibitor and pro gnostic biomarker of hepatocelluar carcinoma. Oncotarget, 2015; 6(21): 18613–30
29. Zhang MY, Li SH, Huang GL et al: Identification of a novel microRNA signature associated with intrahepatic cholangiocarcinoma (ICC) patient prognosis. BMC Cancer, 2015; 15: 64
30. Wang J, Wang S, Zhou J, Qian Q: miR-424-5p regulates cell proliferation, migration and invasion by targeting doublecortin-like kinase 1 in basal-like breast cancer. Biomed Pharmacother, 2018; 102: 147–52
31. Clough E, Barrett T: The Gene Expression Omnibus Database. Methods Mol Biol, 2016; 1418: 93–110
32. Oishi N, Kumar MR, Roessler S et al: Transcriptomic profiling reveals hepatic stem-like gene signatures and interplay of miR-200c and epithelial-mesenchymal transition in intrahepatic cholangiocarcinoma. Hepatology, 2012; 56(5): 1792–803
33. Deng W, Wang Y, Liu Z et al: HemI: A toolkit for illustrating heatmaps. PLoS One, 2014; 9(11): e111988
34. Sticht C, De La Torre C, Parveen A, Gretz N: miRWalk: An online resource for prediction of microRNA binding sites. PLoS One, 2018; 13(10): e0206239
35. Agarwal V, Bell GW, Nam JW, Bartel DP: Predicting effective microRNA target sites in mammalian mRNAs. Elife, 2015; 4
36. Wong N, Wang X: miRDB: An online resource for microRNA target prediction and functional annotations. Nucleic Acids Res, 2015; 43(Database issue): D146–52
37. Hsu SD, Lin FM, Wu WY et al: miRTarBase: A database curates experimentally validated microRNA-target interactions. Nucleic Acids Res, 2011; 39(Database issue): D163–69
38. Ashburner M, Ball CA, Blake JA et al: Gene ontology: Tool for the unification of biology. The Gene Ontology Consortium. Nat Genet, 2000; 25(1): 25–29
39. The Gene Ontology Comsortium: The Gene Ontology Resource: 20 years and still GOing strong. Nucleic Acids Res, 2019; 47(D1): D330–38
40. Mi H, Muruganujan A, Ebert D et al: PANTHER version 14: More genomes, a new PANTHER GO-slim and improvements in enrichment analysis tools. Nucleic Acids Res, 2019; 47(D1): D419–26
41. Xie C, Mao X, Huang J et al: KOBAS 2.0: a web server for annotation and identification of enriched pathways and diseases. Nucleic Acids Res, 2011; 39(Web Server issue): W316–22
42. Wu J, Mao X, Cai T et al: KOBAS server: A web-based platform for automated annotation and pathway identification. Nucleic Acids Res, 2006; 34(Web Server issue): W720–24
43. Ai C, Kong L: CGPS: A machine learning-based approach integrating multiple gene set analysis tools for better prioritization of biologically relevant pathways. J Genet Genomics, 2018; 45(9): 489–504
44. Szklarczyk D, Gable AL, Lyon D et al: STRING v11: Protein-protein association networks with increased coverage, supporting functional discovery in genome-wide experimental datasets. Nucleic Acids Res, 2019; 47(D1): D607–13
45. Shannon P, Markiel A, Ozier O et al: Cytoscape: A software environment for integrated models of biomolecular interaction networks. Genome Res, 2003; 13(11): 2498–504
46. Doncheva NT, Morris JH, Gorodkin J, Jensen LJ: Cytoscape StringApp: Network analysis and visualization of proteomics data. J Proteome Res, 2019; 18(2): 623–32
47. Scardoni G, Tosadori G, Faizan M et al: Biological network analysis with CentiScaPe: centralities and experimental dataset integration. F1000Res, 2014; 3: 139
48. Bader GD, Hogue CW: An automated method for finding molecular complexes in large protein interaction networks. BMC Bioinformatics, 2003; 4: 2
49. Shaib YH, Davila JA, McGlynn K, El-Serag HB: Rising incidence of intrahepatic cholangiocarcinoma in the United States: A true increase? J Hepatol, 2004; 40(3): 472–77

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]

50. Voigtlander T, Gupta SK, Thum S et al: MicroRNAs in serum and bile of patients with primary sclerosing cholangitis and/or cholangiocarcinoma. PLoS One, 2015; 10(10): e0139305
51. Li L, Piontek K, Ishida M et al: Extracellular vesicles carry microRNA-195 to intrahepatic cholangiocarcinoma and improve survival in a rat model. Hepatology, 2017; 65(2): 501–14
52. Lin KY, Ye H, Han BW et al: Genome-wide screen identified let-7c/miR-99a/miR-125b regulating tumor progression and stem-like properties in cholangiocarcinoma. Oncogene, 2016; 35(26): 3376–86
53. Karakatsanis A, Papaconstantinou I, Gazouli M et al: Expression of microRNAs, miR-21, miR-31, miR-122, miR-145, miR-146a, miR-200c, miR-221, miR-222, and miR-223 in patients with hepatocellular carcinoma or intrahepatic cholangiocarcinoma and its prognostic significance. Mol Carcinog, 2013; 52(4): 297–303
54. Xiong X, Sun D, Chai H et al: MiR-145 functions as a tumor suppressor targeting NUAK1 in human intrahepatic cholangiocarcinoma. Biochem Biophys Res Commun, 2015; 465(2): 262–69
55. Li Z, Shen J, Chan MT, Wu WK: The role of microRNAs in intrahepatic cholangiocarcinoma. J Cell Mol Med, 2017; 21(1): 177–84
56. Oh M, Rhee S, Moon JH et al: Literature-based condition-specific miRNA-mRNA target prediction. PLoS One, 2017; 12(3): e0174999
57. Ding J, Li X, Hu H: TarPmiR: A new approach for microRNA target site prediction. Bioinformatics, 2016; 32(18): 2768–75
58. Wang X, El Naqa IM: Prediction of both conserved and nonconserved microRNA targets in animals. Bioinformatics, 2008; 24(3): 325–32
59. Sawai H, Okada Y, Funahashi H et al: Activation of focal adhesion kinase enhances the adhesion and invasion of pancreatic cancer cells via extracellular signal-regulated kinase-1/2 signaling pathway activation. Mol Cancer, 2005; 4: 37
60. Gionfra F, De Vito P, Pallottini V et al: The role of thyroid hormones in hepatocyte proliferation and liver cancer. Front Endocrinol (Lausanne), 2019; 10: 532
61. Anania MC, Miranda C, Vizioli MG et al: S100A11 overexpression contributes to the malignant phenotype of papillary thyroid carcinoma. J Clin Endocrinol Metab, 2013; 98(10): E1591–600
62. Zhang Y, Huang W, Ran Y et al: miR-582-5p inhibits proliferation of hepatocellular carcinoma by targeting CDK1 and AKT3. Tumour Biol, 2015; 36(11): 8309–16
63. Nassirpour R, Mehta PP, Yin MJ: miR-122 regulates tumorigenesis in hepatocellular carcinoma by targeting AKT3. PLoS One, 2013; 8(11): e79655
64. Gramantieri L, Fornari F, Ferracin M et al: MicroRNA-221 targets Bmf in hepatocellular carcinoma and correlates with tumor multifocality. Clin Cancer Res, 2009; 15(16): 5073–81
65. Kim A, Pratilas CA: The promise of signal transduction in genetically driven sarcomas of the nerve. Exp Neurol, 2018; 299(Pt B): 317–25
66. Lee JI, Campbell JS: Role of desmoplasia in cholangiocarcinoma and hepatocellular carcinoma. J Hepatol, 2014; 61(2): 432–34
67. Xing YQ, Li A, Yang Y et al: The regulation of FOXO1 and its role in disease progression. Life Sci, 2018; 193: 124–31
68. Chen B, Zhou W, Zhao W et al: Oxaliplatin reverses the GLP-1R-mediated promotion of intrahepatic cholangiocarcinoma by altering FoxO1 signaling. Oncol Lett, 2019; 18(2): 1989–98
69. O'Dell MR, Huang JL, Whitney-Miller CL et al: Kras(G12D) and p53 mutation cause primary intrahepatic cholangiocarcinoma. Cancer Res, 2012; 72(6): 1557–67
70. Churi CR, Shroff R, Wang Y et al: Mutation profiling in cholangiocarcinoma: Prognostic and therapeutic implications. PLoS One, 2014; 9(12): e115383
71. Ikeno Y, Seo S, Iwaisako K et al: Preoperative metabolic tumor volume of intrahepatic cholangiocarcinoma measured by (18)F-FDG-PET is associated with the KRAS mutation status and prognosis. J Transl Med, 2018; 16(1): 95
72. Mann KM, Ying H, Juan J et al: KRAS-related proteins in pancreatic cancer. Pharmacol Ther, 2016; 168: 29–42
73. Li XS, Sun J, He XL: Expression of c-myc and mutation of the KRAS gene in patients with ovarian mucinous tumors. Genet Mol Res, 2015; 14(3): 10752–59
74. Clatot F, Augusto L, Di Fiore F: ESR1 mutations in breast cancer. Aging (Albany NY), 2017; 9(1): 3–4
75. Varga Z, Lebeau A, Bu H et al: An international reproducibility study validating quantitative determination of ERBB2, ESR1, PGR, and MKI67 mRNA in breast cancer using MammaTyper(R). Breast Cancer Res, 2017; 19(1): 55
76. Wang YM, Liu ZW, Guo JB et al: ESR1 gene polymorphisms and prostate cancer risk: A HuGE Review and Meta-Analysis. PLoS One, 2013; 8(6): e66999
77. Hishida M, Nomoto S, Inokawa Y et al: Estrogen receptor 1 gene as a tumor suppressor gene in hepatocellular carcinoma detected by triple-combination array analysis. Int J Oncol, 2013; 43(1): 88–94
78. Augusto TV, Correia-da-Silva G, Rodrigues CMP et al: Acquired resistance to aromatase inhibitors: where we stand! Endocr Relat Cancer, 2018; 25(5): R283–301
79. Cardoso F, Senkus E, Costa A et al: 4th ESO-ESMO International Consensus Guidelines for Advanced Breast Cancer (ABC 4) dagger. Ann Oncol, 2018; 29(8): 1634–57
80. Carausu M, Bidard FC, Callens C et al: ESR1 mutations: A new biomarker in breast cancer. Expert Rev Mol Diagn, 2019; 19(7): 599–611
81. Huang YJ, Wu AT, Chiou HY et al: Interactive role of diabetes mellitus and female sex in the risk of cholangiocarcinoma: A population-based nested case-control study. Oncotarget, 2017; 8(4): 6642–51
82. Curtis Hewitt S, Couse JF, Korach KS: Estrogen receptor transcription and transactivation: Estrogen receptor knockout mice: What their phenotypes reveal about mechanisms of estrogen action. Breast Cancer Res, 2000; 2(5): 345–52
83. Evdokimov AA, Netesova NA, Smetannikova NA et al: [Application of GLAD-PCR analysis for the methylation sites detection in the regulatory areas of tumor-suppressor genes ELMo1 and EsR1 in colorectal cancer]. Vopr Onkol, 2016; 62(1): 117–21 [in Russian]
84. Sun H, Deng Q, Pan Y et al: Association between estrogen receptor 1 (ESR1) genetic variations and cancer risk: A meta-analysis. J BUON, 2015; 20(1): 296–308
85. Gueye NA, Mead TJ, Koch CD et al: Versican proteolysis by ADAMTS proteases and its influence on sex steroid receptor expression in uterine leiomyoma. J Clin Endocrinol Metab, 2017; 102(5): 1631–41
86. Sevic I, Spinelli FM, Cantero MJ et al: The role of the tumor microenvironment in the development and progression of hepatocellular carcinoma. In: Tirnitz-Parker JEE (ed.), Hepatocellular Carcinoma. Brisbane, 2019
87. Baghy K, Tatrai P, Regos E, Kovalszky I: Proteoglycans in liver cancer. World J Gastroenterol, 2016; 22(1): 379–93
88. Gasparini P, Cascione L, Landi L et al: microRNA classifiers are powerful diagnostic/prognostic tools in ALK-, EGFR-, and KRAS-driven lung cancers. Proc Natl Acad Sci USA, 2015; 112(48): 14924–29
89. Canturk KM, Ozdemir M, Can C et al: Investigation of key miRNAs and target genes in bladder cancer using miRNA profiling and bioinformatic tools. Mol Biol Rep, 2014; 41(12): 8127–35
90. Liang L, Zeng JH, Wang JY et al: Down-regulation of miR-26a-5p in hepatocellular carcinoma: A qRT-PCR and bioinformatics study. Pathol Res Pract, 2017; 213(12): 1494–509
91. Vishnubalaji R, Hamam R, Abdulla MH et al: Genome-wide mRNA and miRNA expression profiling reveal multiple regulatory networks in colorectal cancer. Cell Death Dis, 2015; 6: e1614
92. Chang L, Li K, Guo T: miR-26a-5p suppresses tumor metastasis by regulating EMT and is associated with prognosis in HCC. Clin Transl Oncol, 2017; 19(6): 695–703
93. Zhou J, Tao D, Xu Q et al: Expression of E-cadherin and vimentin in oral squamous cell carcinoma. Int J Clin Exp Pathol, 2015; 8(3): 3150–54

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS]