Identification of The Critical Genes and miRNAs in Hepatocellular Carcinoma by Integrated Bioinformatics Analysis

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Abstract

Hepatocellular carcinoma (HCC) is a global health problem with a complex etiology and pathogenesis. Microarray data is increasingly being used as a novel and effective method for cancer pathogenesis analysis. To unravel the potential prognosis of HCC, an integrative study of mRNA and miRNA for HCC was conducted. Two microarray datasets (GSE89377 and GSE101685) and two miRNAs expression profiles (GSE112264 and GSE113740) were obtained from Gene Expression Omnibus (GEO) database. A total of 177 DEGs and 80 DEMs were screened out. Functional enrichment of DEGs was proceeded by Clue GO, including GO and KEGG pathway analysis. These genes were significantly enriched in chemical carcinogenesis. PPI network was then established on the STRING platform, and ten hub genes (CDC20, TOP2A, ASPM, NCAPG, AURKA, CYP2E1, HMMR, PRC1, TYMS, and CYP4A11) were visualized via Cytoscape software. Then, a miRNA-target network was established to identify the dysregulated miRNA. A key miRNA (hsa-miR-124-3p) was filtered. Finally, the miRNA-target- transcription factor networks were constructed for hsa-miR-124-3p. The network for hsa-miR-124-3p included two transcription factors (TFs) and five targets. These identified DEGs and DEMs, TFs, targets, and regulatory networks may help advance our understanding the underlying pathogenesis of HCC.

Introduction

Hepatocellular carcinoma (HCC), a highly prevalent malignant tumor, is the major type of primary liver cancers (PLCs), accounting for nearly 75-85% of all cases [1]. HCC is generally ranked as one of the most leading causes of cancer-associated deaths based on the WHO classification [2]. Virus infection, hepatotoxins, alcohol, and tobacco are potential risk factors for HCC [3]. Many HCC patients are diagnosed at advanced stages with poor 5-year survival rates regarding the unobvious early symptoms and highly invasive character [4]. Although surgical resection, radiotherapy, and giving sorafenib are the mainstay treatments, the prognosis of HCC is still dismal due to its high postoperative recurrence and drug resistance [5]. Hence, a deep exploration of the molecular mechanisms of HCC is urgently needed, which may contribute to excavate therapeutic targets for effective therapies.

Previously, many papers have demonstrated the function of mRNAs and miRNAs participating in the carcinogenesis of HCC. For instance, ATF3, an affiliate of the ATF/CREB family, is an inhibitor of the tumorigenesis and progression for HCC [6]. CXCL8, one of the CXC chemokines, the dysregulated of CXCL8 accelerates the malignant of HCC [7]. As for miRNAs, a recent study demonstrated that miR-133b regulates the HCC cell progression [8]. miR-1-3p governed the develop of HCC though the inhibition of cancer cell proliferation and invasion [9]. Although many studies regarding dysregulated genes and miRNAs have been conducted, the detailed mechanisms of HCC remained poorly understood because of the limitation on the comparative analysis of the genes and miRNAs.

Recently, microarray bioinformatics analysis has been booming as an eye-catching strategy to investigate the interactions of genes and miRNAs during tumorigenesis [10]. Hence, we downloaded two gene datasets (GSE89377 and GSE101685) and two miRNA datasets (GSE112264 and GSE113740)

from the GEO database, to identify DEGs and DEMs between HCC samples and healthy samples. Then, GO annotation, KEGG pathway analysis, and network construction were then applied to spot DEGs, which were integrated with miRNA interaction analysis to screen out hub genes and miRNAs and their potential molecular mechanisms in HCC.

Materials And Methods

Microarray acquirement

We explored the microarray profiles of HCC-related genes and miRNAs expression in the GEO database. Only the profiles derived from human HCC patients and healthy samples were selected. Animal and cell line source datasets were not included. Finally, the profiles of GSE89377, GSE101685, GSE112264, and GSE113740 met the abovementioned rules and were chosen for the downstream analysis. The two gene datasets (GSE89377 and GSE101685) contained 40 HCC samples with 13 healthy samples and 24 HCC samples with eight healthy samples individually. The two miRNA datasets (GSE112264 and GSE113740) contained 50 HCC samples with 41 healthy samples and 40 HCC samples with ten healthy samples individually.

The screening of DEGs and DEMs

The "LIMMA" package in the R software was utilized to identify DEGs and DEMs. The genes that GSE101685 and GSE89377 shared were finally filtered out for further analysis. |log2FC| > 1 and adj p-value < 0.05 were set as the criterion for identifying DEGs. The miRNAs common in GSE112264 and GSE113740 were screened, and DEMs were identified with the thresholds of |log2FC| > 2 and adj p-value < 0.05. Then the screening results were displayed by the volcano plots and Venn diagrams using the OmicStudio tools (https://www.omicstudio.cn/tool).

GO and KEGG Enrichment Analyses

To further explore the biological functions of DEGs, a plug-in named ClueGO v2.5.1 from the Cytoscape platform was utilized to perform Gene Ontology (GO) annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis. The GO annotation including three major functional classifications: biological process (BP), molecular function (MF), and cellular component (CC). The top results are filtered on the grounds of p-value, with p-value < 0:05 considered statistically significant.

PPI network and module analysis

STRING v11.5 established functional networks for PPI as a bioinformatic database to integrate and score protein-protein interactions through co-expression and co-occurrence. In this study, the identified DEGs were key into the STRING database to identify significant node pairs. The PPI node pairs were filtered with a combined score ≥0.4, and the disconnected nodes in the network were deleted. In addition, the hub genes in the PPI network were identified by cytoHubba through calculating gene node scores based on three different centrality parameters (degree centrality, betweenness centrality, and closeness centrality).

Prediction of Target Genes for DEMs

The candidate targets of DEMs were predicted by Funrich, a software to conduct functional enrichment and build an interaction network. The intersections between the candidate targets and DEGs were identified as the targets for DEMs. Then, the miRNA-target network was structured, which was visualized by Cytoscape. Moreover, the network topology was analyzed using CytoNCA with the same centrality parameters above to identify key DEM.

The TF-miRNA-targets network construction

miRNAs and TFs are two major trans-regulators in gene regulatory networks [11]. In this study, the iRegulon plug-in v1.3 in Cytoscape can predict critical regulators and construct the TF-miRNA-targets network [12]. The minimum identity between orthologous genes was set to 0.05, the maximum FDR on motif similarity was set to 0.001, the enrichment score threshold was set to 5.0, the ROC threshold for AUC calculation was set to 0.05, and the rank threshold was set to 5.0.

Results

DEGs and DEMs Screening

A sum of two gene and two miRNA profiles were obtained from the GEO database. Two gene expression profiles (GSE101685 and GSE89377) identified 364 upregulated genes, 618 downregulated genes, and 74 upregulated genes, 240 downregulated genes individually (Fig. 1A). Two miRNA expression profiles (GSE112264 and GSE113740) identified 65 upregulated miRNAs, 148 downregulated miRNAs, and 25 upregulated miRNAs, 105 downregulated miRNAs individually (Fig. 1B). The overlapping genes in the mRNA expression profile datasets were considered as common DEGs. The DEMs commonly appeared in the two miRNAs sets were chosen as candidate DEMs. Finally, 177 common DEGs were screened, including 34 increasing genes and 143 decreasing genes in HCC samples compared to healthy samples (Fig. 1C). 80 candidate DEMs were filtered, including 11 increasing miRNAs and 69 decreasing miRNAs (Fig. 1D).

Functional and Pathway Enrichment Analysis

The ClueGO plug-in was utilized to conduct GO annotation and KEGG pathway analysis of the previously common DEGs. With threshold p-value < 0.05, the significantly enriched GO terms were present in Fig. 2. In the BP group, the most significantly enriched GO terms were monocarboxylic acid metabolic process (GO: 0032787) with p-value = 1.30E-12; drug metabolic process (GO: 0017144) with p-value = 3.61E-12; fatty acid metabolic process (GO: 0006631) with p-value= 7.41E-12; cellular response to xenobiotic stimulus (GO: 0071466) with p-value= 7.51E-10 (Fig. 2A). Moreover, the most significantly enriched GO terms were steroid hydroxylase activity (GO: 0008395) with p-value= 7.13E-08; aromatase activity (GO: 0070330) with p-value= 2.12E-07; alcohol dehydrogenase activity, zinc-dependentc (GO: 0004024) with p-value= 2.68E-07 and caffeine oxidase activity (GO: 0034875) with p-value= 3.34E-06 in the MF group

(Fig. 2B). Additionally, these identified DEGs were significantly enriched in high-density lipoprotein particle (GO: 0034364) with p-value= 7.43E-06; blood microparticle (GO: 0072562) with p-value= 1.62E-05; lipoprotein particle (GO: 1990777) with p-value= 3.19E-05 and plasma lipoprotein particle (GO: 0034358) with p-value= 3.08E-05 in the CC group (Fig. 2C). Moreover, the identified DEGs were mainly enriched in complement and coagulation cascades (KEGG:04610) with p-value= 2.60E-08; chemical carcinogenesis (KEGG: 05204) with p-value= 2.31E-07 and drug metabolism (KEGG: 00982) with p-value= 6.94E-07 (Fig. 3).

PPI Network Structure and Analysis of Modules

The STRING database was applied to structure a PPI network with 177 DEGs (34 increasing genes and 143 decreasing genes). The result was downloaded and analyzed using Cytoscape software. A PPI network was constructed after removing disconnected nodes. As shown in Fig. 4, the network contains 91 nodes (genes) and 336 edges (interactions). The top 20 nodes with high topology scores of degrees, betweenness and closeness centralities were identified (Table 1). Then, ten common genes among these top 20 nodes were screened as hub genes including CDC20, TOP2A, ASPM, NCAPG, AURKA, CYP2E1, HMMR, PRC1, TYMS, and CYP4A11.

Table 1
Top 20 nodes of DEGs in PPI network based on 177 DEGs

Name	Degree	Name	Betweenness	Name	Closeness
CDC20	36	TBL1X	284	CDC20	21.5
TOP2A	30	CYP4A11	264	TOP2A	20.33
ASPM	28	NCOR1	252	ASPM	19.83
NCAPG	28	CYP2E1	214	NCAPG	19.83
AURKA	26	CDC20	184.53	AURKA	19.33
CYP2E1	22	ASPM	149.45	CENPF	18.17
CENPF	22	SIRT1	144	PTTG1	18.17
PTTG1	22	FOXO1	123	KIF20A	18.17
KIF20A	22	HMMR	96	NUSAP1	18.17
NUSAP1	22	PPARGC1A	93	MELK	17.33
MELK	20	TOP2A	91.28	PRC1	17.33
HMMR	20	FOS	84	HMMR	17
PRC1	20	TYMS	54.23	CDKN3	16.33
CDKN3	16	KNTC1	50	TYMS	15.58
TYMS	14	NCAPG	43.45	MCM2	15.08
MCM5	12	AURKA	22.55	MCM5	14.58
MCM2	12	CYP1A2	16	CDC6	14.25
CYP1A2	12	PRC1	13.02	CYP2E1	13.92
CYP4A11	12	CETP	10	MCM6	13.42
SIRT1	10	C6	8	CYP4A11	12.2

Targets Prediction and TF-miRNA-Target Network Structure

A total of 2600 candidate target genes of DEMs were predicted with Funrich software. Then, the intersections between the candidate targets and the common DEGs were screened out to structure the miRNA-mRNA network. As depicted in Fig. 5A, the miRNA-mRNA network constituted 18 nodes and 15 edges, including four increasing miRNAs and 14 decreased mRNAs. From the top nodes with the highest degrees, a key DEM (hsa-miR-124-3p) was identified, which regulates seven mRNAs.

It was assumed that miRNAs with high degrees in the regulatory network might play a significant role in cancer pathogenesis [11]. Thus, the TF-miRNA- target gene regulatory networks for the key miRNA were

constructed. As shown in Figure 5B, the regulatory network for hsa-miR-124-3p including two TFs and seven target genes.

Discussion

Recently, liver cancer has emerged as a global health challenge, and its incidence is booming worldwide. It is estimated that more than 1 million individuals will have liver cancer by 2025 [13]. HCC serves as the major form of primary liver cancer. Integrated miRNA and mRNA microarray analysis provide a new approach to explore the mechanism of HCC. Previous researchers have conducted plenty of expression profiles about HCC to explore dysregulated genes and miRNAs. However, because of different platforms and control selection, the results are always discrepant and unsatisfying. Hence, there are a lot of unexplained mysteries between DEGs and DEMs involved in HCC owing to the lack of integrated analysis. Furthermore, the synergistic effects of TFs and miRNAs regulating genes in the network remain to be observed. We are convinced that this study is a significant trial to reveal interactions between DEGs and DEMs.

In this study, 177 common DEGs were identified, involving 34 increasing genes and 143 decreasing genes. To further understand the function of these DEGs, the GO and KEGG enrichment analyses were conducted by ClueGO. For GO enrichment analysis, most of the genes were enriched in the GO terms related to the cellular metabolic processes, which have previously been essential to cancer progression. And the KEGG analysis indicated that these identified genes were significantly enriched in the chemical carcinogenesis pathway. It is well known that the chemical carcinogens is highly relevent to the imitation and procession of cancers [14]. Various investigations have revealed that chemical carcinogenesis played a crucial role in the etiology of HCC. Ten DEGs (ADH1B, ADH1C, ADH4, ADH6, CYP1A2, CYP2C19, CYP2C9, CYP2E1, CYP3A4, NAT2) were associated with this pathway. ADH1B and ADH1C are involved in alcohol metabolism and strongly impact carcinogenic acetaldehyde accumulation [15]. ADH4 served as an essential member of the ADH family and was suggested as a potential prognostic marker for HCC patients [16]. CYP1A2, predominantly expressed in the liver tissue, could inhibit cell viability and clonogenicity and reduce cell migration and invasion in HCC [17]. CYP2C19 played a crucial role in detoxifying or inactivating potential carcinogens through activating DNA-binding metabolites. The expression of CYP2C19 has been proved to be associated with the development of various cancers such as hepatocellular carcinoma, lung cancer, and gastric cancer [18]. CYP2E1 is one of the carcinogen metabolism genes, and it is reported to activate chemical carcinogenesis [19].

Additionally, ten hub genes (CDC20, TOP2A, ASPM, NCAPG, AURKA, CYP2E1, HMMR, PRC1, TYMS, and CYP4A11) were identified by construction of the PPI network. Of these DEGs, specific genes have previously been reported to be related to the genesis and development in HCC. CDC20 is proved to be an essential cell-cycle regulator and the overexpression of CDC20 accelerates the progression of HCC [20]. TOP2A plays a critical regulatory role in cancers and is closely related to the prognosis of HCC. In hepatocarcinogenesis, the overexpressed TOP2A is correlated with early age onset, poor survival rates, and chemoresistance [21]. ASPM regulated cell proliferation and is identified as a biomarker for early

recurrence and poor prognosis of HCC [22]. NCAPG is performed as an essential oncogene for HCC growth [23]. AURKA has been proved to promote cancer metastasis in HCC [24]. CYP2E1 regulate the metabolism of various toxicants and activate chemical procarcinogens. And the overexpression of CYP2E1 is proved to accelerate the procession of hepatocarcinogenesis [25]. Previous research showed that in HCC tissues, the expression of HMMR was more elevated than healthy liver tissues, which was consistent with the results of this study. And the increased expression of HMMR in liver hepatocellular carcinoma patients was identified as an adverse prognostic factor [26]. High PRC1 activity is a risk factor for early HCC recurrence and poor patient outcomes. In HCC, PRC1 perfomed as an oncogenic gene to accelerate cancer cell proliferaton, metastasis and stemness [27]. TYMS plays an essential role in the development of various malignancies, including HCC [28]. CYP4A11 has been linked to the aggravation of various cancers and affects different regulated metabolites. And for HCC patients, the CYP4A11 is a favorable prognostic factor, and its expression is positively correlated with good prognostic factors [29].

miRNAs are a kind of small RNAs that regulate gene expression via binding miRNAs to the 3' untranslated region of mRNAs, thereby inducing mRNA-silencing [30]. Mounting evidence has shown that the aberrant expression of miRNA may trigger carcinogenesis [31]. This study screened DEMs, and the potential candidate target genes of the DEMs were predicted. Then a miRNA-mRNA network was created based on the DEMs and their targets. A key DEM (hsa-miR-124-3p) was identified according to the network topology. And the miRNA-target gene-TF regulatory networks for hsa-miR-124-3p were constructed. hsa-miR-124-3p and two TFs such as GTF2A1 and POU5F1 were identified as co-regulators of EGR1 and SLITRK3. Lately, many researches have verified that aberrant expression of miR-124-3p is associated with various cancers. For triple-negative breast cancer cells, miR-124-3p can regulate cell proliferation by interaction with TNBC [32]. And in gastric cancer, miR-124-3p enhanced cell metastasis and proliferation by targeting the DNMT3B. GTF2A1, performed as a transcriptional factor, is associated with the susceptibility to gastric cancer. POU5F1 functions as a transcription factor that can activate their downstream genes through binding to the octameric sequence motif. Previous studies showed that the POU5F1 is a pan-cancer gene for various cancers and indicated that POU5F1 is functionally carcinogenic in HCC [33]. EGR1 can be rapidly and transiently induced in response to several stimuli, including growth factors, cytokines, and mechanical stresses. It has been proved that EGR1 can bind with miR-365a-3p promoted by CBX8 to exhibit oncogenic activity in HCC [34]. SLITRK3 is a prognostic molecular biomarker for gastrointestinal stromal tumors, and the expression of SLITRK3 correlates to gastrointestinal stromal tumor risk rating and prognosis [35]. However, given the complication of miRNA and TF crosstalk, these regulatory interactions need to be scanned thoroughly. In any case, these potential regulatory patterns may cast light to discover new molecular targets for HCC diagnosis and treatment.

Conclusion

In brief, our study identified 177 DEGs and 80 DEMs between HCC tissues and healthy ones. DEGs were significantly enriched in the chemical carcinogenesis, which played a crucial role in regulating HCC. Ten hub genes, including CDC20, TOP2A, ASPM, NCAPG, AURKA, CYP2E1, HMMR, PRC1, TYMS, CYP4A11, and a key DEM, namely hsa-miR-124-3p, were identified. Besides, network analysis revealed the co-

regulatory associations among the key miRNA, corresponding targeted genes, and TFs in HCC. These findings may help to develop promising biomarkers and novel strategies for HCC.

Declarations

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Declarations

The authors declare that they have no conflict of interest.

References

1. Dai X, Guo Y, Hu Y, et al. Immunotherapy for targeting cancer stem cells in hepatocellular carcinoma. Theranostics. 2021; 11:3489–3501.
2. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Ca-Cancer J. Clin.2020; 68:394–424.
3. Chen T, Dai X, Dai J, et al. AFP promotes HCC progression by suppressing the HuR-mediated Fas/FADD apoptotic pathway. Cell Death Dis.2020;11(10):822.
4. Wang J, Wang CY. Integrated miRNA and mRNA omics reveal the anti-cancerous mechanism of Licochalcone B on Human Hepatoma Cell HepG2. Food Chem Toxicol. 2021; 15:112096.
5. Zhang J, Song J, Wu D, et al. Hesperetin induces the apoptosis of hepatocellular carcinoma cells via mitochondrial pathway mediated by the increased intracellular reactive oxygen species, ATP and calcium. Med. Oncol. 2015;32(4):101.
6. Chen C, Ge C, Liu Z, et al. ATF3 inhibits the tumorigenesis and progression of hepatocellular carcinoma cells via upregulation of CYR61 expression. J. Exp. Clin. Cancer Res.2018;37:263.
7. Yang S, Wang H, Qin C, et al. Up-regulation of CXCL8 expression is associated with a poor prognosis and enhances tumor cell malignant behaviors in liver cancer. Biosci. Rep.2020; 40: BSR20201169.
8. Tian Z, Jiang H, Liu Y, et al. MicroRNA-133b inhibits hepatocellular carcinoma cell progression by targeting Sirt1. Exp. Cell Res.2016;343:135–147.
9. Chen H, Bao L, Hu J, et al. ORC6, Negatively Regulated by miR-1-3p, Promotes Proliferation, Migration, and Invasion of Hepatocellular Carcinoma Cells. Front Cell Dev Biol.2021;9: 652292.

10. Long W, Li Q, Zhang J, et al. Identification of key genes in the tumor microenvironment of lung adenocarcinoma. Med Oncol.2021;38(7):83.
11. Gao Y, Zhang S, Zhang Y, et al. Identification of MicroRNA-Target Gene-Transcription Factor Regulatory Networks in Colorectal Adenoma Using Microarray Expression Data. Front Genet.2020;11:463.
12. Janky R, Verfaillie A, Imrichova H, et al. iRegulon: from a gene list to a gene regulatory network using large motif and track collections. PLoS Comput Biol.2014;10: e1003731.
13. Llovet J M, Kelley R K, Villanueva A, et al. Hepatocellular carcinoma.2021;7:6.
14. Stults W P, Wei Y. Ambient air emissions of polycyclic aromatic hydrocarbons and female breast cancer incidence in US. Med Oncol. 2018;35:88.
15. Shih S, Huang Y T, Yang H I. A multiple mediator analysis approach to quantify the effects of the ADH1B and ALDH2 genes on hepatocellular carcinoma risk. Genet Epidemiol.2018;42: 394–404.
16. Wei R R, Zhang M Y, Rao H L, et al. Identification of ADH4 as a novel and potential prognostic marker in hepatocellular carcinoma. Med Oncol.2012;29:2737:2743.
17. Yu J, Xia X, Dong Y, et al. CYP1A2 suppresses hepatocellular carcinoma through antagonizing HGF/MET signaling. Theranostics. 2021;11:2123–2136.
18. Sugimoto M, Furuta T, Shirai N, et al. Poor metabolizer genotype status of CYP2C19 is a risk factor for developing gastric cancer in Japanese patients with helicobacter pylori infection. Aliment. Pharmacol. Ther. 2005;22:1033–1040.
19. Kang J S, Wanibuchi H, Morimura K, et al. Role of CYP2E1 in diethylnitrosamine-induced hepatocarcinogenesis in vivo. Cancer Res. 2007;67:11141–11146.
20. Li J, Gao J Z, Du J L, et al. Increased CDC20 expression is associated with development and progression of hepatocellular carcinoma. Int. J. Oncol. 2014;45:1547–1555.
21. Wong N, Yeo W, Wong W L, et al. TOP2A overexpression in hepatocellular carcinoma correlates with early age onset, shorter patients survival and chemoresistance. Int J Cancer. 2009;124:644–652.
22. Lin S Y, Pan H W, Liu S H, et al. ASPM is a novel marker for vascular invasion, early recurrence, and poor prognosis of hepatocellular carcinoma. Clin Cancer Res. 2008;14:4814:4820.
23. Wang Y, Gao B, Tan P Y, et al. Genome-wide CRISPR knockout screens identify NCAPG as an essential oncogene for hepatocellular carcinoma tumor growth. FASEB J.2019;33:8759–8770.
24. Dauch D, Rudalska R, Cossa G, et al. A MYC-aurora kinase A protein complex represents an actionable drug target in p53-altered liver cancer. Nat Med. 2016;22:744–753.
25. Gao J, Wang Z, Wang G J, et al. From hepatofibrosis to hepatocarcinogenesis: Higher cytochrome P450 2E1 activity is a potential risk factor. Mol. Carcinog. 2018;57:1371–1382.
26. Lei X, Zhang M, Guan B, et al. Identification of hub genes associated with prognosis, diagnosis, immune infiltration and therapeutic drug in liver cancer by integrated analysis. Hum Genomics.2021;15:39.

27. Chen J, Rajasekaran M, Xia H, et al. The microtubule-associated protein PRC1 promotes early recurrence of hepatocellular carcinoma in association with the Wnt/beta-catenin signalling pathway. Gut.2016;65:1522–1534.
28. Wang X, Sun X, Du X, et al. Thymidylate synthase gene polymorphisms as important contributors affecting hepatocellular carcinoma prognosis. Clin Res Hepatol Gastroenterol.2017;41:319–326.
29. Eun H S, Cho S Y, Lee B S, et al. Cytochrome P450 4A11 expression in tumor cells: A favorable prognostic factor for hepatocellular carcinoma patients. J. Gastroenterol. Hepatol. 2019;34:224–233.
30. Gu J, Zhu X, Li Y, et al. miRNA-21 regulates arsenic-induced anti-leukemia activity in myelogenous cell lines.Med. Oncol. 2011;28:211–218.
31. Paydas S, Acikalin A, Ergin M, et al. Micro-RNA (miRNA) profile in Hodgkin lymphoma: association between clinical and pathological variables. Medical Oncology. 2016;33(4):34.
32. Zhang J., Zhang X., Li Z., et al. The miR-124-3p/neuropilin-1 axis contributes to the proliferation and metastasis of triple-negative breast cancer cells and co-activates the TGF-β pathway. Front Oncol.2021;11:654672.
33. He D, Zhang X, Tu J. Diagnostic significance and carcinogenic mechanism of pan-cancer gene POU5F1 in liver hepatocellular carcinoma. Cancer Med.2020;9:8782–8800.
34. Zhang C Z, Chen S L, Wang C H, et al. CBX8 exhibits oncogenic activity via akt/β-catenin activation in hepatocellular carcinoma. Cancer Res.2018;78:51–63.
35. Wang C. J., Zhang Z. Z., Xu J., et al. SLITRK3 expression correlation to gastrointestinal stromal tumor risk rating and prognosis. World J Gastroenterol. 2015;21:8398–8407.

Figures

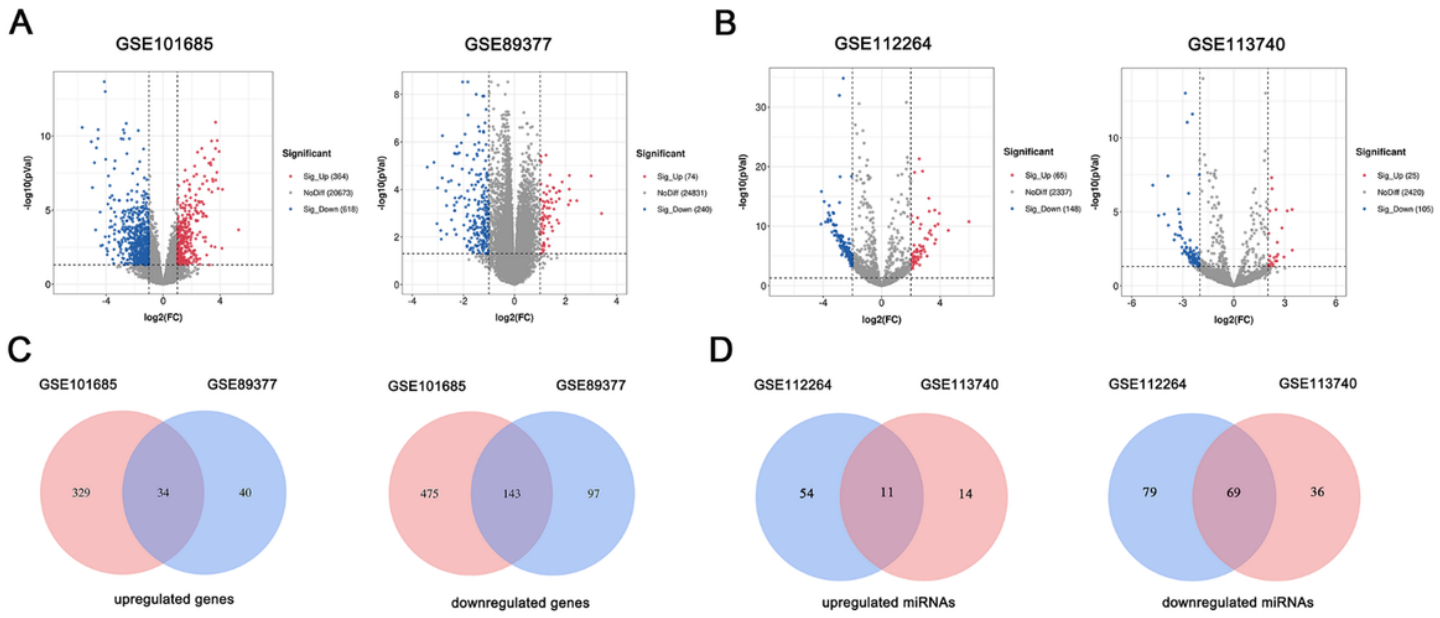

Figure 1

Identification of DEGs and DEMs in the four profile datasets. (A) Volcano plots of DEGs in profile datasets (GSE101685 and GSE89377). (B) Volcano plots of DEGs in profile datasets (GSE112264, and GSE113740). (C) The overlapping of genes in the two gene expression profiles. (D) The overlapping of miRNAs in the two miRNA expression profiles.

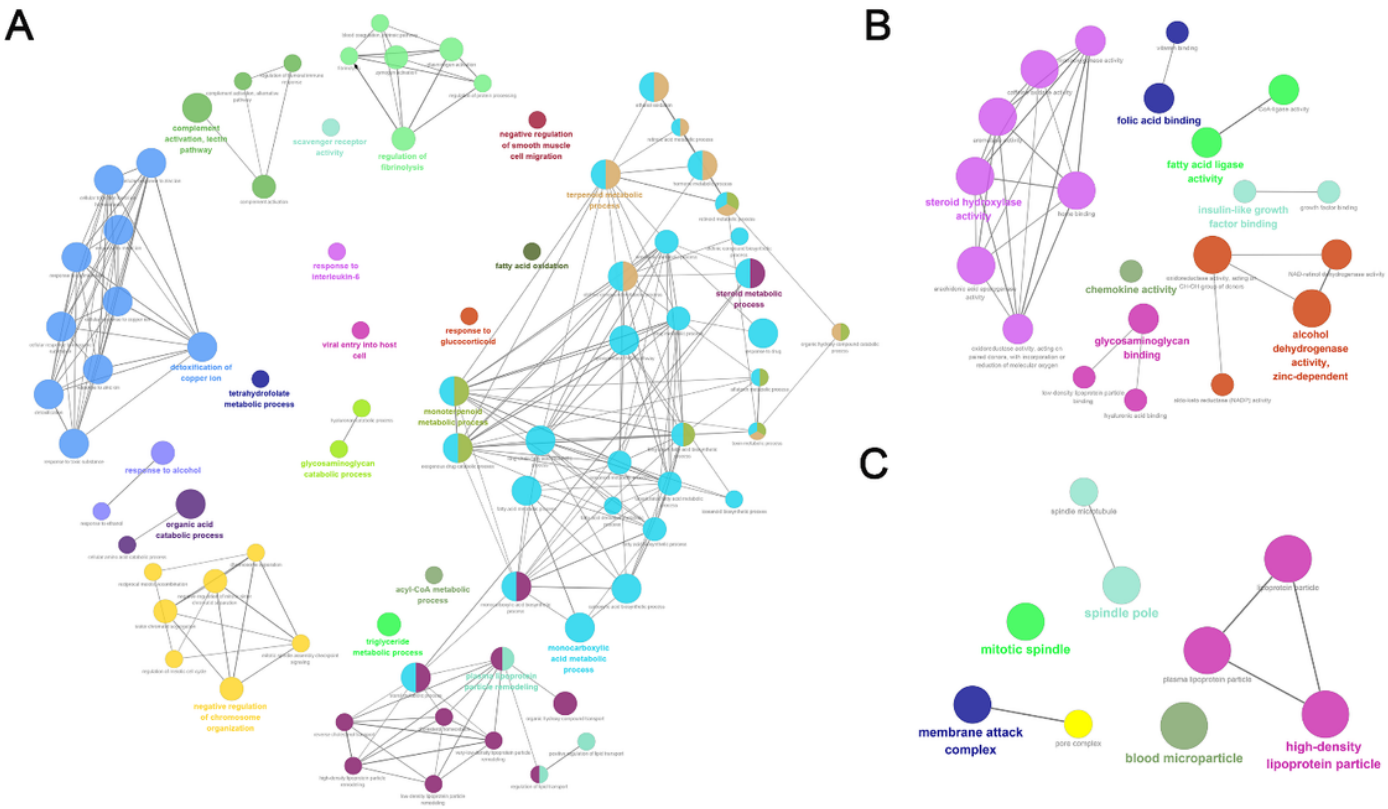

Figure 2

GO enrichment analyses of DEGs. The node size designates the enrichment significance. Functionally associated groups partially overlap. (A) BP-associated group. (B) CC-associated group. (C) MF-associated group.

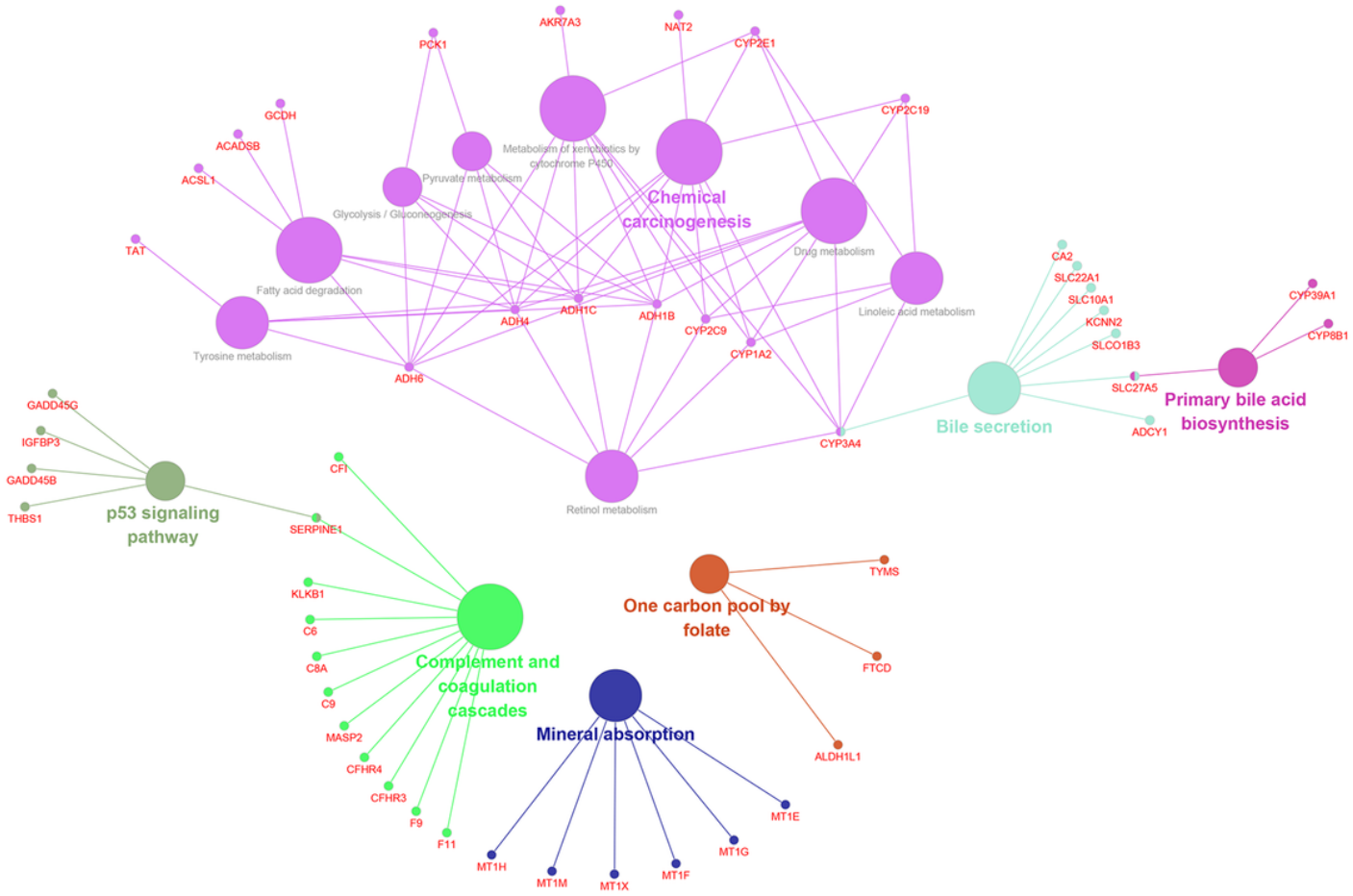

Figure 3

KEGG pathways analysis of DEGs. The node size designates the enrichment significance.

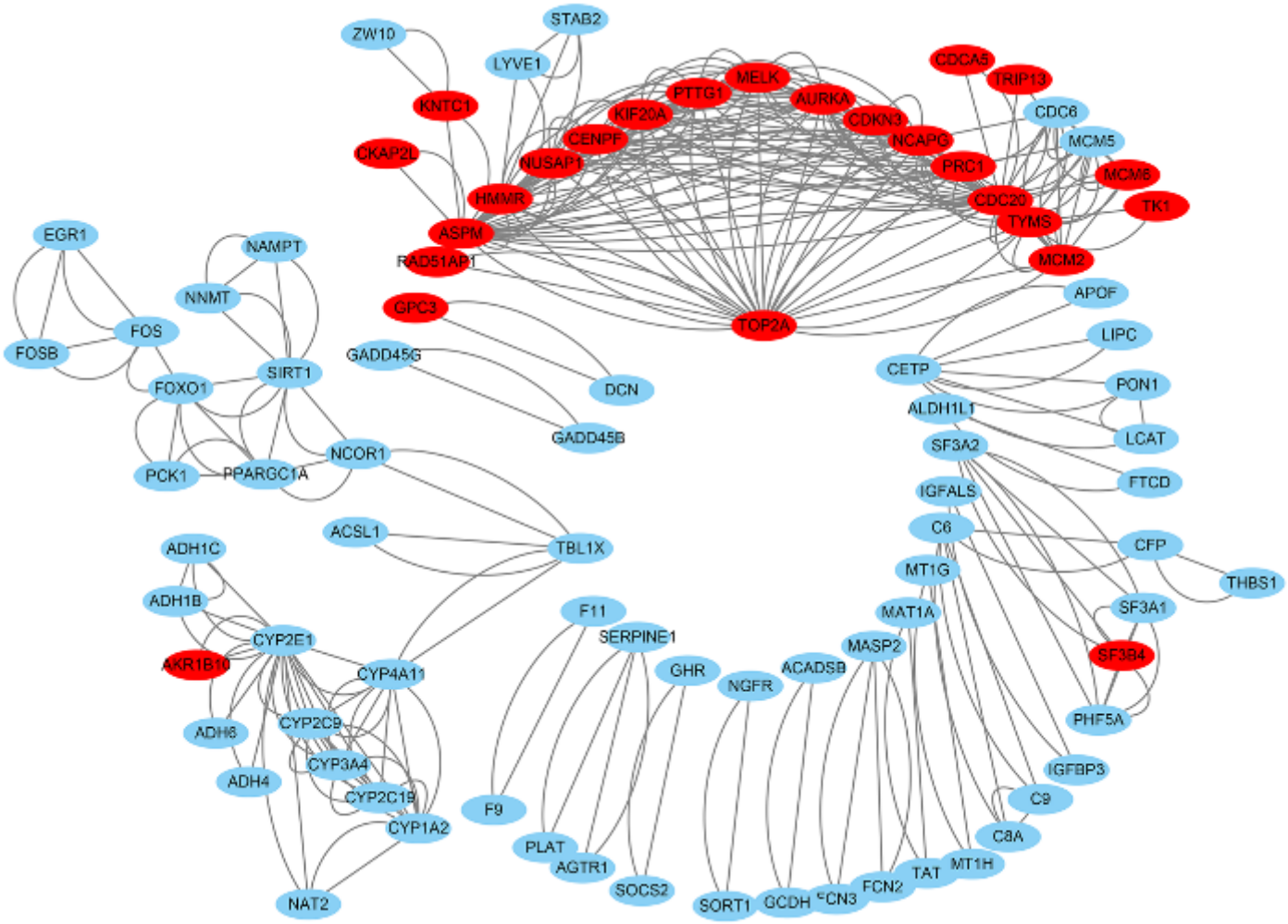

Figure 4

The PPI network of DEGs. Red nodes represent upregulated genes and blue nodes represent downregulated genes, respectively.

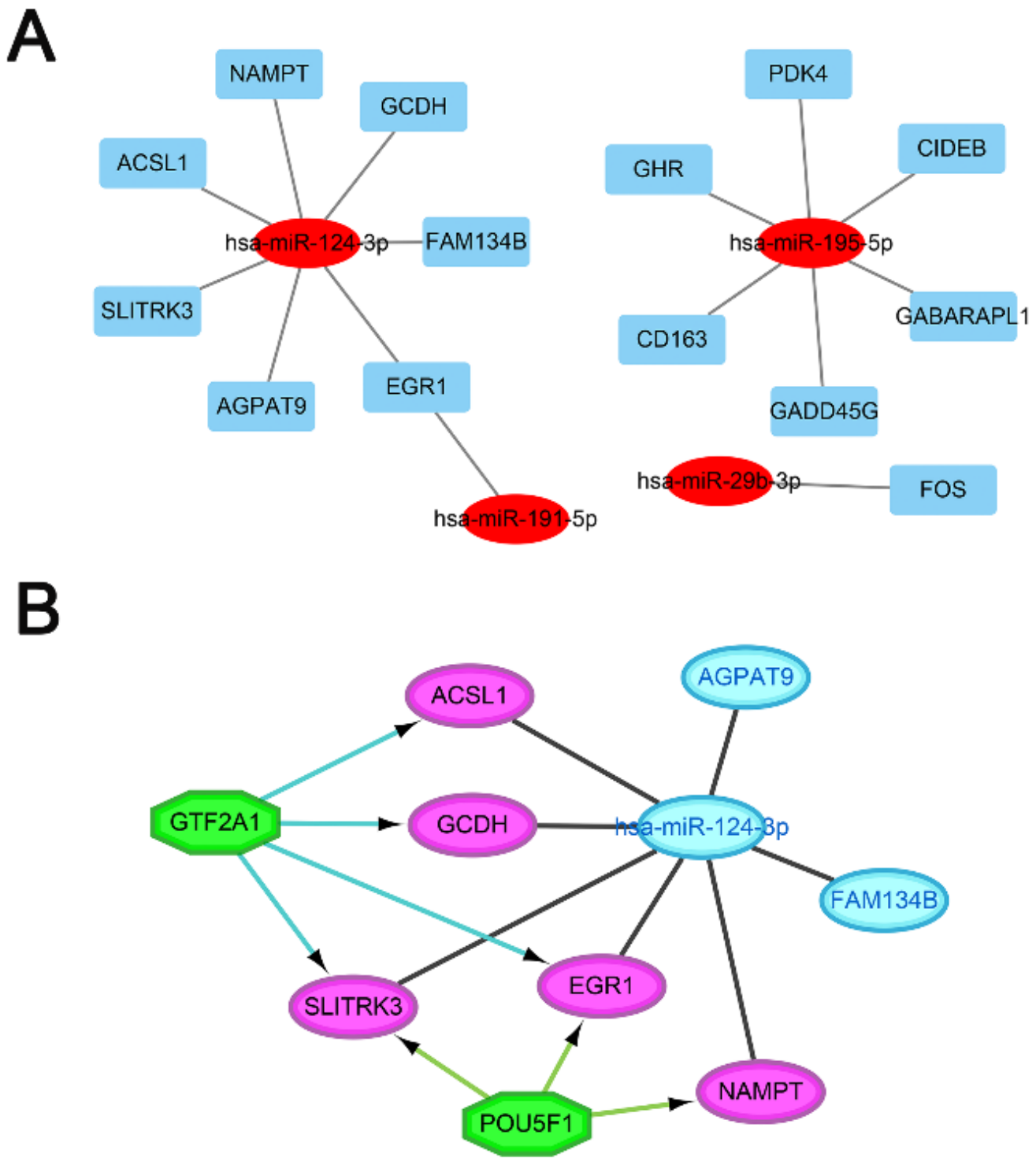

Figure 5

Networks of DEMs and their interaction factors. (A) The network of DEMs and their targets. (B) The networks of the key miRNA, target genes and TFs. Green octagons symbolize TFs, purple circles symbolize target genes regulated by key miRNAs and TFs, blue circles symbolize targets regulated by key miRNAs, and triangles symbolize key miRNAs.