肝细胞癌进展过程中的关键基因ATP1B3和ENAH的筛选与鉴定:基于数据挖掘和临床验证

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摘要:目的 筛选与肝细胞癌(HCC)预后、进展和临床诊断相关标记物。**方法** 分析来自癌症基因组图谱数据库(TCGA)和基因表达综合(GEO)的TCGA-LIHC、GSE84432、GSE14323和GSE63898数据集。利用GEO2R和edge R包获得各疾病类型之间共同差异基因(DEG),并对DEG进行基因本体论(GO)和京都基因与基因组百科全书(KEGG)富集分析。将DEG在TCGA-LIHC中正常和癌组织进行差异表达验证分析,挑选在HCC中上调基因。利用R语言进行生存分析、受试者工作特征(ROC)曲线分析、基因与患者临床特征关系分析。再通过RT-qPCR验证15对临床HCC和癌旁组织中基因的差异表达。**结果** 数据库挖掘共获得118个共同DEG,挑选出2个基因:ATP酶Na+/K+转运亚基Beta 3(ATP1B3)和肌动蛋白调节器(ENAH),其表达随疾病进展升高。结合TCGA-LIHC数据集生存分析发现两者高表达与HCC患者不良预后显著相关(P<0.05)。ROC曲线分析ATP1B3和ENAH的AUC值分别是0.821和0.933。ATP1B3高表达与晚期病理T分期、Stage和Grade相关(P<0.05),而ENAH高表达仅与晚期病理Grade有关(P<0.05)。RT-qPCR结果发现,ATP1B3和ENAH在临床HCC组织中表达上调(P<0.05)。**结论** ATPIB3和ENAH有望成为肝脏疾病恶化和肝细胞癌的不良预后标志物。

关键词:肝纤维化;肝硬化;肝细胞癌;ATP1B3;ENAH

Screening and identification of key genes ATP1B3 and ENAH in the progression of hepatocellular carcinoma: based on data mining and clinical validation

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Abstract: Objective To explore the marker genes correlated with the prognosis, progression and clinical diagnosis of hepatocellular carcinoma (HCC) based on bioinformatics methods. **Methods** The TCGA-LIHC, GSE84432, GSE143233 and GSE63898 datasets from The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) were analyzed. The differentially expressed genes (DEGs) shared by different disease types were obtained using GEO2R and edge R packages, and Gene Ontology (GO) and Kyoto Gene and Genome Encyclopedia (KEGG) enrichment analyses of the DEGs were performed. The expression levels of these DEGs in normal and cancerous tissues were verified in TCGA-LIHC to identify the upregulated genes in HCC. Survival analysis, receiver-operating characteristic (ROC) curve analysis, and correlation analysis between the key genes and the clinical features of the patients were carried out using the R language. The differential expressions of 15 key genes were verified in clinical samples of HCC and adjacent tissues using RT-qPCR. **Results** A total of 118 common DEGs were obtained in the database, and among them two genes, namely ATPase Na+/K+ transport subunit beta 3 (ATP1B3) and actin regulator (ENAH), showed increased expressions with disease progression. Survival analysis combined with the TCGA-LIHC dataset suggested that high expressions of ATP1B3 and ENAH were both significantly correlated with a poor prognosis of HCC patients (P<0.05), and their AUC values were 0.821 and 0.933, respectively. A high expression of ATP1B3 was correlated with T stage, pathological stage and pathological grade of the tumors (P<0.05), while that of ENAH was associated only with an advanced tumor grade (P<0.05). The results of RT-qPCR showed that ATP1B3 and ENAH were both significantly upregulated in clinical HCC tissues (P<0.05). **Conclusion** ATPIB3 and ENAH are both upregulated in HCC, and their high expressions may serve as biomarkers of progression of liver diseases and a poor prognosis of HCC.

Keyword: liver fibrosis; liver cirrhosis; hepatocellular carcinoma; ATP1B3; ENAH

肝细胞癌(HCC)是一个主要的全球健康问题和死亡原因。据世界卫生组织估计,到2030年,将有超过100万人死于HCC,与其他癌症相比,HCC发病率在2020年继续以更快的速度上升。这种癌症的主要病因包括代谢紊乱、肝炎病毒的慢性感染、吸烟和过量饮酒等[1]。不管肝脏的病因如何,HCC与肝纤维化和肝硬化密切相关[2,3]。HCC预后的一个重要部分取决于潜在的慢性肝病,这也是其发生的危险因素。肝硬化死亡率的

收稿日期:2021-12-07
基金项目:广西科技基地和人才专项(桂科AD18126021);国家自然科学基金(81872491)
Supported by National Natural Science Foundation of China (81872491).
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Child-Pugh评分对于患者分层至关重要[4]。大约80%~90%的HCC有潜在肝纤维化，大约三分之一的肝硬化患者会发展成HCC[5]。由于早期临床症状不明显，发病机制不明，HCC确诊时通常为晚期或已发生远处转移，增加治疗难度，且预后较差[6]。目前，常用的HCC诊断方法，如血清肿瘤标志物、影像学技术等临床效果并不理想[7]。筛选与HCC早期诊断和不良预后相关的标志物，对于提高HCC的治疗效果和预后至关重要。随着多组学分析方法的应用，大量重要分子被发现并成功应用于临床诊断和治疗[8]。但是，从肝纤维化进展到HCC是一个漫长的过程，其机制非常复杂，目前尚未发现能作为HCC进程的标记物[9, 10]。因此，我们通过生物信息学数据挖掘，着眼于HCC进程中各疾病表型之间的基因变化，找到与HCC进展相关的新基因，以期作为HCC的诊断和预后的新靶点。

1 材料和方法

1.1 数据集选择

从TCGA数据库（https://portal.gdc.cancer.gov/）下载374个HCC和50个正常组织的表达谱，从GEO（https://www.ncbi.nlm.nih.gov/geo/database）获取HBV相关肝纤维化患者基因表达谱数据集GSE84044、正常与肝硬化相关数据集GSE14323、肝硬化与HCC相关数据集GSE63898。其中GSE84044数据集基于GPL570平台［HG-U133_Plus_2］Affymetrix Human Genome U133 Plus 2.0 Array、GSE14323数据集基于GPL571平台［HG-U133A_2］Affymetrix Human Genome U133A 2.0 Array、GSE63898数据集基于GPL13667平台［HG-U219］Affymetrix Human Genome U219 Array。

1.2 DEG的筛选

TCGA-LIHC数据集的DEG使用edge R包分析，GSE84044、GSE14323和GSE63898数据集的DEG使用在线工具GEO2R来识别。选择$P<0.05$，$|FC|>2$作为TCGA-LIHC、GSE14323和GSE63898数据集DEG的截断值，$P<0.05$，$|FC|>1.3$作为GSE84044数据集DEG的截断值。

1.3 DEG的火山图、韦恩图绘制

使用ggplot2 R包绘制火山图和韦恩图。

1.4 DEG的富集分析

使用clusterProfiler R包对差异基因绘制GO分析，包括生物过程(BP)、细胞成分(CC)和分子功能(MF)以及KEGG通路分析。$P<0.05$被认为差异具有统计学意义。

1.5 基因的差异表达和临床参数相关性分析

使用Wilcoxon秩和检验对基因在各不同疾病类型中进行差异表达分析；基因与临床特征关系分析，计数资料采用卡方检验或Fisher's精准检验，连续资料采用t检验；使用logrank检验对基因在TCGA-LIHC数据集中进行生存分析，使用survminer包对生存结果进行可视化；使用pROC包绘制基因关于预测正常和肿瘤结局的ROC曲线图；使用单因素方差分析分析基因表达量与T分期、病理Stage和病理Grade关系，使用ggplot2 R包进行可视化。

1.6 RT-qPCR

收集来自广西医科大学附属肿瘤医院15对HCC组织和邻近癌旁组织。采样时间为2018年6月~2020年9月。本研究获得广西医科大学伦理委员会批准（20200035）。所有患者均提供知情同意。Trizol试剂提取HCC组织和癌旁组织中的总RNA。用Takala反转录试剂盒（RR047A）将总RNA逆转录为cDNA，然后用Takala试剂盒（RR820A）进行RT-qPCR扩增。引物序列：ATP1B3正向5'-TCATCTACAACCCGACCACCG-3'，反向5'-GAGTCTGAAGCATAACCCACATC-3'；ENAH正向5'-TGACTATTGTGCTGTCTGCTGC-3'，反向5'-AAAGGTTCACTGCTGACTCCC-3'；ACTB正向5'-AGATCAAGATCATTGCTCCTCCTG-3'，反向5'-AGTCATAGTCCGCCTAGAAGCAT-3'。ATP1B3和ENAH的检测以β-Actin作为内参。$2^{-\Delta\Delta Ct}$法计算ATP1B3和ENAH的相对表达水平。

2 结果

2.1 不同肝脏相关疾病组别共同DEG筛选

本研究使用GEO2R分别对GSE84044数据集中肝纤维化组织、GSE14323数据集中正常与肝硬化组织、GSE63898数据集中肝硬化与HCC组织；使用edgeR包对TCGA-LIHC中正常与HCC组织按筛选标准（FC=1.3/2，$P<0.05$）进行差异基因分析（表1），分别得到了1972，912，1107，8975个DEG。使用火山图对各数据集差异基因进行可视化（图1A~D），取交集后得到了118个共同DEG（图1E）。

2.2 DEG的富集分析

GO富集分析显示差异基因主要富集在细胞外结构组织，含细胞外基质的胶原蛋白，细胞外基质成分等方面。KEGG富集显示主要富集在黏着斑途径中（图2）。

2.3 筛选DEG的核心基因

对118个DEG在TCGA-LIHC数据集表达情况进行验证。发现除9个无明显差异外，95个DEG在HCC组织下调（$P<0.05$），14个DEG在HCC组织上调（$P<0.05$，表2）。为了能筛选出与HCC进展相关并且能成为疾病恶化程度的预后指标，结合Pubmed文献检索，在上调基因中选择ATP1B3和ENAH这2个研究很少的基因。接着对ATP1B3和ENAH分别做了在不同肝脏疾病类型数据集中的表达差异分析，发现ATP1B3在恶

表1 数据集样本信息及差异基因筛选

Tab.1 Dataset sample information and differential gene screening

Dataset name	Sample information	Threshold	Differential genes
GEO-GSE84044	Low fibrosis F0-F1:63; High fibrosis F3-F4:28	FC=1.3, P<0.05	up:1658;down:314
GEO-GSE14323	Normal:19; Cirrhosis:41	FC=2, P<0.05	up:739;down:173
GEO-GSE63898	Cirrhosis:168; HCC:228	FC=2, P<0.05	up:323;down:784
TCGA-LIHC	Normal:50; HCC:374	FC=2, P<0.05	up:6519;down:2456

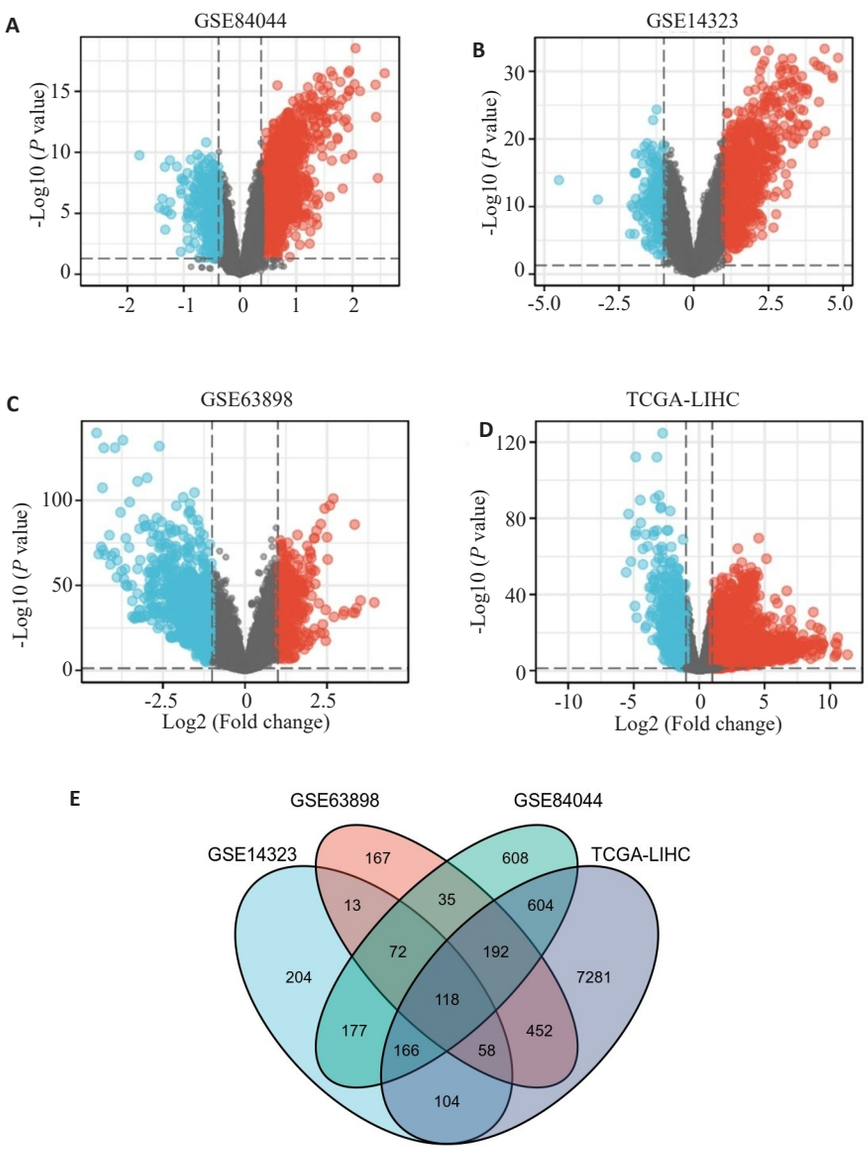

图1 筛选共同差异表达基因

Fig.1 Screening of common differentially expressed genes (DEGs). **A**: Visualization of GSE84044 differential genes in volcanic maps. **B**: Visualization of GSE14323 differential genes in volcanic maps. **C**: Visualization of GSE63898 differential genes in volcanic maps. **D**: Visualization of TCGA-LIHC differential genes in volcanic maps. E: Venn diagram visualizing the common DEGs.

性疾病中表达较高，特别在癌前病变肝硬化时期表达更高（图3A），ENAH随着疾病进展加剧，其表达逐渐增高（图3B）。

2.3 核心基因与临床特征关系

ATP1B3表达与T分期和临床病理分期相关（P<0.05），而ENAH表达与病理分级相关（P<0.05，表3）。

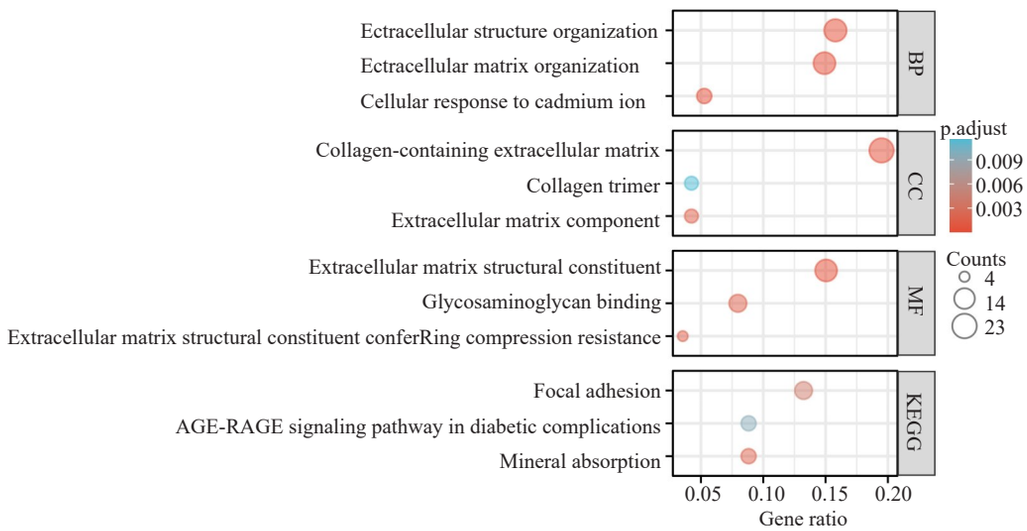

图2　DEG的GO和KEGG富集分析

Fig.2　GO and KEGG enrichment analysis of DEGs. BP: Biological process. CC: Cellular component. MF: Molecular function.

表2　118个DEG在TCGA-LIHC数据集中正常和HCC组织表达情况

Tab.2　Expression of 118 DEGs in normal and HCC tissues in the TCGA-LIHC dataset

TCGA-LIHC	Gene name
Low expression	DCN/C7/CYTIP/IL7R/EPHA3/TACSTD2/PDGFRA/ASPN/TRIM22/FBLN5/ANK3/PTPRC/GZMK/EPCAM/PTGDS/KLF6/LAMA2/CD69/MFAP4/ID4/LCP2/BGN/SRGN/F3/FGL2/OLFML3/FXYD2/CHST4/GEM/CRISPLD2/EVI2B/PTGIS/CXCL12/ZFPM2/DPT/ITGA4/SFRP5/PRELP/GATA6/FILIP1L/FHL2/CLEC7A/PPP1R1A/PZP/ADAMTSL2/SRPX/SLA/IRF8/CDH19/MMP7/PRKCB/EDNRB/DHODH/JUN/RUNX3/PAIP2B/MAFF/HCLS1/C1QB/PFKFB3/THBS1/KCNN2/RND3/MT1G/LPA/MFAP3L/PDE4B/SAMSN1/ADAMTS1/THBD/ADRA1A/SERPINB9/MT1X/DNAJC12/ATF5/MT1F/HGF/CCL2/RGS4/MT1M/MXRA5/CXCL1/FMO2/BBOX1/PHLDA1/APOF/CPEB3/FCN2/IL1RAP/CYP2A6/HAO2/CYP2C19/BCHE/CYP1A2/CYP3A4
High expression	MGP/KRT23/ROBO1/RFX5/LAMC1/ATP1B3/SPP1/GMNN/AKR1B10/ACSL4/ENAH/COL15A1/PDZK1IP1/SULT1C2
No significant	EFEMP1/SEL1L3/CD53/PTGER4/EMP1/ITK/COL3A1/DHRS2/MMP19

生存分析发现，ATP1B3和ENAH高表达与HCC患者不良预后有关（P<0.05，图4A、B）。ROC曲线分析表明ATP1B3和ENAH，曲线下面积AUC分别是0.821和0.933，在预测患者为正常或肿瘤结局检验效能方面具有较高准确性（图4C、D）。接着我们分析了ATP1B3和ENAH的表达量与临床各分期和分级关系。从T分期、病理Stage和病理Grade来看，ATP1B3表达随着HCC疾病进展表达量逐渐升高。T3、T2分期ATP1B3表达量显著高于T1分期，Stage III、Stage II高于Stage I分期，Grade3分级高于Grade1（P<0.05，图4E~G）。ENAH高表达与HCC晚期病理Grade相关，Grade3、Grade2分级ENAH表达量显著高于Grade1（P<0.05，图4H~J）。

2.4　ATP1B3和ENAH在临床样本中的验证

收集来自广西医科大学附属肿瘤医院15对HCC及邻近癌旁组织样本，提取总RNA，接着对ATP1B3和ENAH在HCC组织及癌旁组织的表达进行了RT-qPCR实验。可能由于样本数较少，暂未发现这两个基因与HCC临床特征有显著关系（表4）。RT-qPCR实验发现，ATP1B3和ENAH在HCC组织表达高于癌旁组织（P<0.05，图5），可作为诊断HCC的良好指标。

3　讨论

HCC是一种严重的全球健康负担，其发生和发展的潜在机制很复杂，是全球癌症相关死亡的常见原因[11]。目前，尽管HCC在系统治疗方面取得了进展，但由于诊断晚和频繁潜在的肝脏疾病，患者的生存率仍然很低[12]。尚不清楚不同形式的肝炎和肝损害是如何引发HCC的。因此，确定共同的致病基因对早期HCC的检测和预防非常重要[13]。随着多组学技术的应用，生物标志物已成为诊断、预后和治疗的有力工具[14]。尽管，许多与

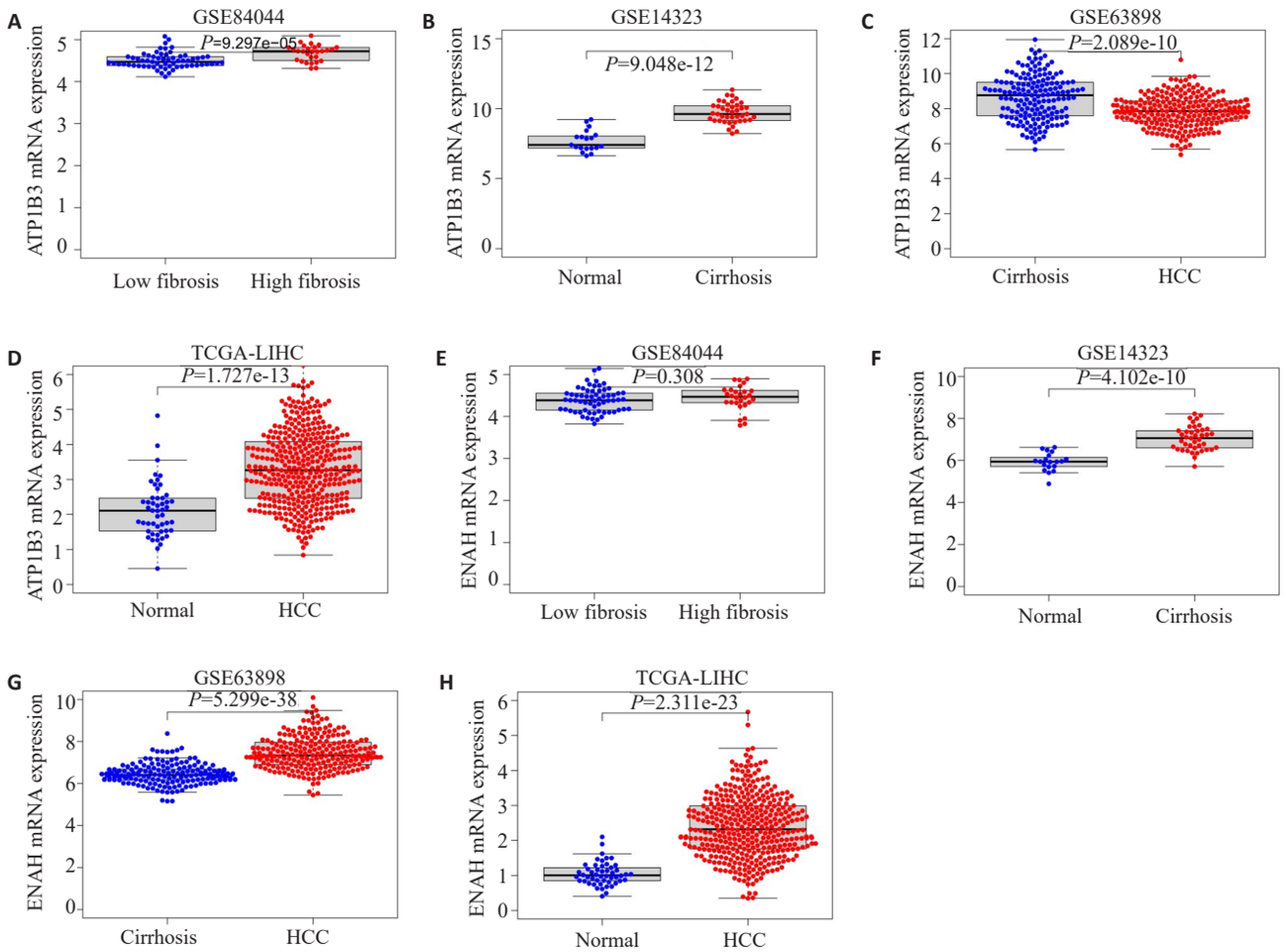

图3 ATP1B3和ENAH在各数据集不同疾病状态表达差异情况

Fig.3 Differential expressions of ATP1B3 (**A**-**D**) and ENAH (**E**-**H**) in different disease states in each dataset.

HCC不同发生阶段相关的关键分子被筛选出来，成为HCC早期筛查和治疗的靶点[15]。但是，肝细胞癌发生过程中的共同基因的研究，尚未见报道。

在本研究中，我们筛选了GEO数据库中3个数据集：肝纤维化数据集GSE84044、肝硬化相关数据集GSE14323、肝硬化与HCC相关数据集GSE63898和TCGA中肝细胞癌TCGA-LIHC数据集。取四个数据集DEG交集，我们获得了118个共同DEG。对DEG做GO和KEGG分析，发现DEG在细胞外基质组织、胶原蛋白三聚体、糖胺聚糖结合、黏着斑和AGE-RAGE通路中起重要作用。我们发现在上调基因中发现ROBO1、LAMC1、ATP1B3、SPP1、AKR1B10、ENAH等基因在TCGA数据库中，其高表达与HCC患者不良预后相关（P<0.05）。通过Pubmed文献检索，发现ROBO1作为分泌蛋白Slit2的受体，通过Slit2-Robo1信号通路激活肝星状细胞，从而促进肝损伤和纤维化[16]。Song等[17]在HCC组织和细胞中检测Robo1的水平，发现Robo1被上调。Qin等[18]发现层粘连蛋白γ1（Lamc1）在肝细胞癌组织中上调，体外实验推测Lamc1可能通过PTEN/AKT途径调节PKM2表达而参与HCC的进展。分泌型磷蛋白1（SPP1）参与编码骨桥蛋白（OPN），OPN在肝纤维化和肝硬化等慢性肝脏疾病中起关键作用表达上调[19]。Zhu等[20]检测170例HCC患者和120例健康体检者血清醛酮还原酶1B10（AKR1B10）水平，发现HCC患者血清AKR1B10水平显著高于健康对照组，联合血清AFP检测可提高肝癌的诊断率。AKR1B10被认为是一种新的HCC血清学生物标志物。

目前，ATP1B3和ENAH与HCC进程相关的研究非常少。ATP1B3编码Na+/K+-ATPase的β亚基，Na+/K+-ATPase不仅参与正常的细胞活动，在致癌过程中也至关重要[21]。Li等[22]发现ATP1B3在胃癌组织中高表达，可通过PI3K/AKT通路调节胃癌细胞的进展和预后。Zhang等[23]通过综合数据集多转录组学、蛋白质组学分析，发现ATP1B3在HCC中高表达，且与生存和免疫浸润相关。体内功能分析发现ATP1B3参与HCC的增殖、迁移和转化。预测ATP1B3可作为HCC诊断和潜在治疗靶点。ENAH是一种肌动蛋白调节蛋白，参与控制细胞运动和细胞-细胞黏附[24]。ENAH在多种癌症

表3 ATP1B3和ENAH与临床特征关系

Tab.3 Relationship of ATP1B3 and ENAH with clinical characteristics of HCC patients [*n* (%)]

Characteristic	Low expression of ATP1B3	High expression of ATP1B3	*P*	Low expression of ENAH	High expression of ENAH	*P*
Case (*n*)	187	187		187	187	
Age (year)			0.380			0.567
≤60	84 (22.5%)	93 (24.9%)		85 (22.8%)	92 (24.7%)	
>60	103 (27.6%)	93 (24.9%)		101 (27.1%)	95 (25.5%)	
Gender			0.825			0.825
Female	59 (15.8%)	62 (16.6%)		59 (15.8%)	62 (16.6%)	
Male	128 (34.2%)	125 (33.4%)		128 (34.2%)	125 (33.4%)	
T stage			<0.001			0.094
T1	110 (29.6%)	73 (19.7%)		100 (27%)	83 (22.4%)	
T2	37 (10%)	58 (15.6%)		45 (12.1%)	50 (13.5%)	
T3	33 (8.9%)	47 (12.7%)		36 (9.7%)	44 (11.9%)	
T4	4 (1.1%)	9 (2.4%)		3 (0.8%)	10 (2.7%)	
N stage			0.364			0.624
N0	130 (50.4%)	124 (48.1%)		122 (47.3%)	132 (51.2%)	
N1	1 (0.4%)	3 (1.2%)		1 (0.4%)	3 (1.2%)	
M stage			1.000			1.000
M0	135 (49.6%)	133 (48.9%)		128 (47.1%)	140 (51.5%)	
M1	2 (0.7%)	2 (0.7%)		2 (0.7%)	2 (0.7%)	
Stage			0.004			0.208
Stage I	103 (29.4%)	70 (20%)		95 (27.1%)	78 (22.3%)	
Stage II	35 (10%)	52 (14.9%)		43 (12.3%)	44 (12.6%)	
Stage III	35 (10%)	50 (14.3%)		35 (10%)	50 (14.3%)	
Stage IV	2 (0.6%)	3 (0.9%)		3 (0.9%)	2 (0.6%)	
Grade			0.083			<0.001
G1	34 (9.2%)	21 (5.7%)		41 (11.1%)	14 (3.8%)	
G2	92 (24.9%)	86 (23.3%)		89 (24.1%)	89 (24.1%)	
G3	54 (14.6%)	70 (19%)		50 (13.6%)	74 (20.1%)	
G4	4 (1.1%)	8 (2.2%)		5 (1.4%)	7 (1.9%)	

中包括食管癌、胃癌和乳腺癌中过表达[25-28]，Deng等[29]结合数据库分析和体外细胞实验发现ENAH可通过Notch信号通路促进肝癌细胞的增殖、侵袭和迁移。ENAH可逆转miR-139-5P对肝癌细胞的抑制功能作用，诱导肝细胞癌的恶性进展[30]。本研究通过收集各肝脏疾病相关数据集分析，发现ATP1B3和ENAH在正常组织、低纤维化、高纤维化、肝硬化、HCC组织中其表达量随着疾病进展大体上调，但ATP1B3在肝硬化阶段表达最高。

生存分析发现，ATP1B3和ENAH在TCGA-LIHC数据集中，基因的高表达HCC患者生存率低，有不良预后。ROC曲线分析ATP1B3和ENAH在诊断正常或肿瘤结局检验效能方面，ENAH AUC高达0.933，ATP1B3为0.821，具有较高检验效能。接着我们分析了ATP1B3和ENAH与HCC患者各临床参数之间关系，发现ATP1B3随着T分期、病理Stage和Grade进展，其表达量逐渐增高。ENAH在晚期病理Grade中，其表达量更高。以上说明ATP1B3和ENAH密切参与肝细胞癌的进展过程，可作为肝细胞癌预后和诊断标记物。最后我们收集到15对肝细胞癌和邻近癌旁组织，对ATP1B3和ENAH做RT-qPCR验证。研究发现ATP1B3和ENAH mRNA在癌组织表达上调。

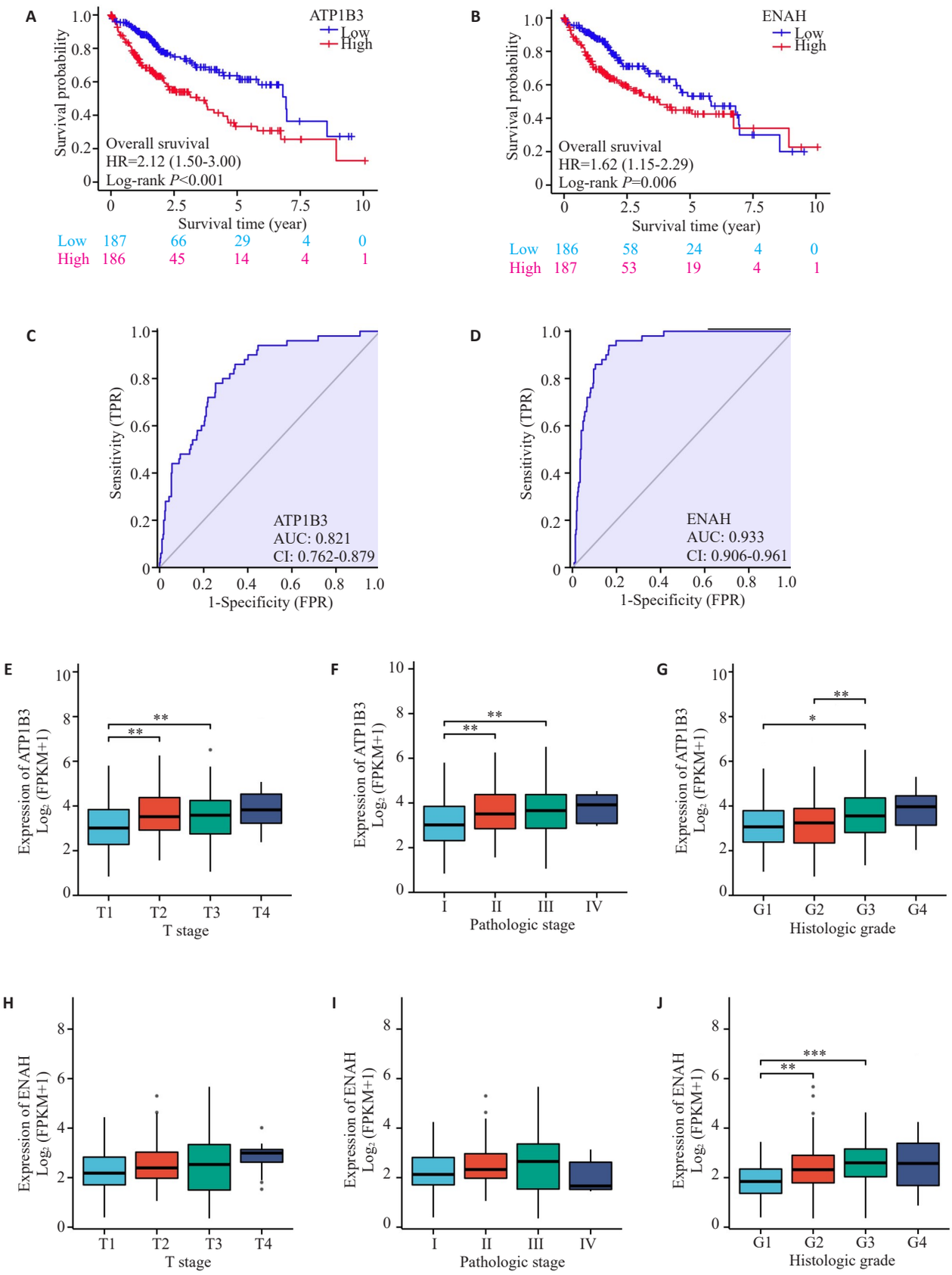

图4 ATP1B3和ENAH与临床参数关系

Fig.4 Relationship of ATP1B3 and ENAH with clinical parameters of HCC patients. **A**, **B**: K-M survival curve of ATPIB3/ENAH. **C**, **D**: ROC curve plot of ATPIB3/ENAH prediction of normal or tumor outcome. **E**, **G**: Relationship between ATPIB3 expression and T stage, pathologic stage and pathologic grade. **H**, **J**: Relationship between ENAH expression and T stage, pathologic stage and pathologic grade. **P*<0.05, ***P*<0.01, ****P*<0.001.

表4 临床样本中基因与临床特征关系分析

Tab.4 Relationship between the expressions of the genes and clinical characteristics in clinical samples [*n* (%)]

Characteristic	High expression of ATP1B3	Low expression of ATP1B3	*P*	High expression of ENAH	Low expression of ENAH	*P*
Case (*n*)	7	8		7	8	
Gender			0.467			0.467
Female	0 (0%)	2 (13.3%)		0 (0%)	2 (13.3%)	
Male	7 (46.7%)	6 (40%)		7 (46.7%)	6 (40%)	
Smoking			0.608			0.608
0	2 (13.3%)	4 (26.7%)		2 (13.3%)	4 (26.7%)	
1	5 (33.3%)	4 (26.7%)		5 (33.3%)	4 (26.7%)	
Alcohol			0.619			0.132
0	3 (20%)	5 (33.3%)		2 (13.3%)	6 (40%)	
1	4 (26.7%)	3 (20%)		5 (33.3%)	2 (13.3%)	
BCLC			0.073			1.000
A	2 (13.3%)	7 (46.7%)		4 (26.7%)	5 (33.3%)	
B	3 (20%)	1 (6.7%)		2 (13.3%)	2 (13.3%)	
C	2 (13.3%)	0 (0%)		1 (6.7%)	1 (6.7%)	
Edmondson grade			0.128			0.200
I	0 (0%)	1 (6.7%)		1 (6.7%)	0 (0%)	
II	4 (26.7%)	7 (46.7%)		6 (40%)	5 (33.3%)	
III	3 (20%)	0 (0%)		0 (0%)	3 (20%)	
Age (year, *Mean*±*SD*)	47±14.59	53.25±11.96	0.378	48.14±13.68	52.25±13.31	0.566

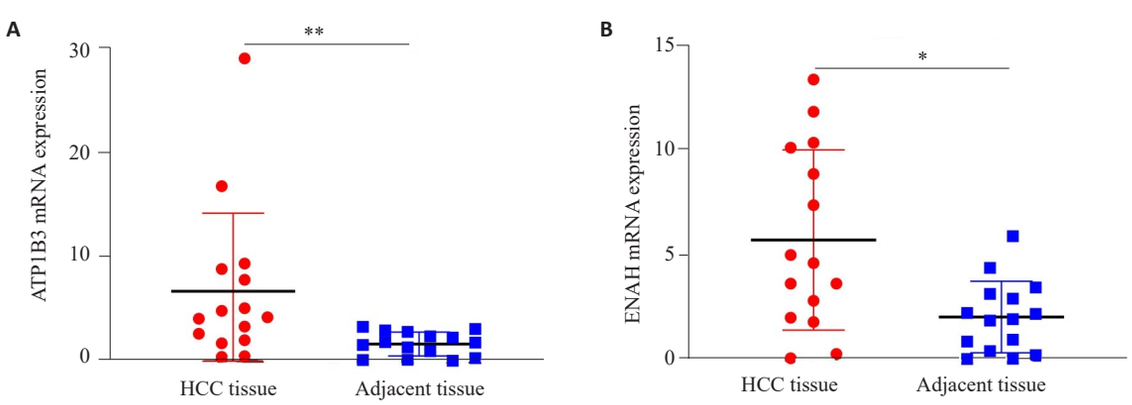

图5 临床样本验证ATPIB3和ENAH表达

Fig.5 Validation of ATPIB3 (**A**) and ENAH (**B**) expressions in clinical samples. *P<0.05, **P<0.01.

综上所述，我们通过生物信息学方法挖掘出从肝纤维化、肝硬化到HCC疾病进程中，表达逐渐上调且与HCC患者生存、预后、进展和诊断密切相关基因：ATP1B3和ENAH，并通过收集临床样本验证了其在肝癌组织的高表达，可以为HCC诊断和预后提供新的靶点和治疗方向。

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