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·临床研究·

# 基于生物信息学筛选肝细胞癌年轻患者特有关键枢纽基因及其临床意义

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[摘 要] **目的**:运用生物信息学方法筛选年轻肝细胞癌(hepatocellular carcinoma,HCC)患者特有的关键枢纽基因(Hub gene),并探索其生物学和临床意义。**方法**:从GEO芯片数据集GSE45267获取年轻(确诊HCC时年龄≤40岁)和年老(确诊HCC时年龄>40岁)组的HCC组织及正常肝组织数据信息,通过GEO2R和Venn图工具筛选两组的HCC组织相对正常肝组织差异表达基因(differentially expressed gene,DEG),运用STRING和Cytoscape软件构建年轻组特有差异表达基因的蛋白互作网络并筛选关键Hub基因及显著模块。利用GEPIA数据库对关键基因进行验证,并通过Kaplan-Meier分析相关HCC患者总生存期。最后应用DAVID对年轻组特有基因及年轻与年老组共有DEGs进行GO富集分析和KEGG通路分析比较。**结果**:筛选出年轻组特有117个上调、179个下调DEGs,构建PPI网络选取出10个连接度最高的基因为Hub基因,其中7个Hub基因集中于第一模块。GEPIA验证与Kaplan-Meier生存分析提示TYMS、CDC6、BUB1、TPX2、OIP5、KIF23等6个表达上调的Hub基因可能与年轻HCC癌患者的不良预后相关。功能富集分析显示年轻HCC特有DEGs主要参与ATP结合等生物学过程,并主要富集到了细胞周期S期;年轻与年老组共有DEGs主要参与环氧酶P450、细胞分裂等生物学过程,并主要富集到细胞周期G2/M期。**结论**:本研究鉴定出6个在年轻HCC患者肿瘤组织中特有的显著上调且提示预后不良的Hub基因,可能成为年轻HCC患者潜在的治疗和预后预测靶点。

[关键词] 年轻;肝细胞癌;生物信息学分析;枢纽基因;GSE45267
[中图分类号] R735.7;R730.43 [文献标识码] A [文章编号] 1007-385X(2020)02-0161-09

# Identification of specific key Hub genes in young hepatocellular carcinoma patients based on bioinformatical analysis and its clinical significance

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**[Abstract] Objective:** To identify the specific Hub genes in young hepatocellular carcinoma (HCC) patients, and to explore their biological and clinical significance by using bioinformatic methods. **Methods:** The data information of HCC and normal tissues of young (≤40 years old at diagnosis) and old (>40 years old at diagnosis) HCC patients were obtained from GEO chip data set GSE45267. The differentially expressed genes (DEGs) in HCC tissues as comparing to normal tissues in the two groups were screened by using GEO2R and Venn chart software. The Protein-Protein Interaction (PPI) network of the specific DEGs in young group was constructed by bioinformatics tools STRING and Cytoscape to screen the Hub genes and significant modules. The Hub genes were verified by GEPIA database, and the overall survival time was analyzed by Kaplan-Meier. Finally, Gene Ontology (GO) Enrichment analysis and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis were used to analyze the DEGs specific to young group and the common DEGs of the two groups by DAVID. **Results:** Finally, 117 up-regulated and 179 down-regulated DEGs specific to the young group were screened out, and PPI network screened 10 most connected genes as Hub genes, among which 7 Hub genes were concentrated in the

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first module. Six up-regulated Hub genes, including TYMS, CDC6, BUB1, TPX2, OIP5 and KIF23, were indicated to associate with the poor prognosis in young HCC patients by GEPIA and Kaplan-Meier analysis. GO function and KEGG pathway analyses showed that the DEGs specific to young HCC patients were mainly involved in biological processes such as ATP binding, and were mainly enriched in S phase of cell cycle; while the common DEGs of two groups were mainly involved in biological processes such as cyclooxygenase P450 and cell division, and were mainly enriched in the G2/M phase of the cell cycle. **Conclusion:** In this study, 6 up-regulated DEGs specific to young group that suggested poor prognosis were identified, which may be the potential therapeutic and prognostic targets for young patients with HCC.

**[Key words]** young; hepatocellular carcinoma (HCC); bioinformatics analysis; Hub gene; GSE45267

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资料[1]显示，肝癌是全球第六大常见癌症和第四大癌症死亡原因。在中国，肝癌在所有癌症中生存率最低，年龄标准化的5年相对生存率仅为12.1%[2]。一般来说，大多数肝癌患者为中老年。据估计，2015年被诊断的男性肝癌病例中约92.9%的患者年龄≥45岁[3]。然而，近年来发生肝癌的年轻人有增多趋势[4-5]。肝细胞癌（hepatocellular carcinoma, HCC）作为最常见的肝癌类型，约占肝癌的75%~85%[1]，与年老患者相比，40岁以下HCC患者可能具有肿瘤直径大、病理分期晚且预后差的特点[5-6]。

虽然对HCC形成和发展的分子机制已有广泛的研究，但少有研究关注年龄因素对HCC发病的影响。本研究运用生物信息学方法，筛选参与年轻人HCC癌变和进展的差异表达基因（differentially expressed gene，DEG），以期揭示年轻人HCC发生发展分子机制并寻找与预后相关的生物标志物。

## 1　资料与方法

### 1.1　HCC组织基因表达谱数据获取

HCC芯片数据GSE45267来源于美国国立生物技术信息中心（NCBI）的GEO数据库（Gene Express Ominibus，http://www.ncbi.nlm.nih.gov/geo），该数据采用的芯片平台是GPL570（Affymetrix Human Genome U133 Plus 2.0 Array）。该数据集共包含87个组织样品的基因表达谱，其中有48例HCC新鲜冰冻HCC组织和39例正常组织。芯片将确诊HCC时年龄≤40岁的样本列入年轻组，共16例HCC组织和15例正常肝组织；年龄>40岁的样本列入年老组，共32例HCC组织和24例正常肝组织。

### 1.2　筛选HCC差异表达基因

使用GEO2R在线分析工具（http://www.ncbi.nlm.nih.gov/geo/geo2r），以矫正后$P$<0.05和$\log_2$（fold change）>2作为截取标准，进行年轻组和年老组HCC组织相对正常肝组织的DEGs分析。并利用Venn软件（http://bioinformatics.psb.ugent.be/webtools/Venn/）在线工具统计各组特有及两组共有的上、下调基因。

### 1.3　年轻人HCC DEGs的PPI网络构建及Hub基因鉴定

STRING数据库（https://string-db.org/cgi/input.pl）用于预测蛋白质及研究蛋白质间的相互作用关系。本研究将年轻组特有的DEGs导入STRING数据库，构建蛋白质相互作用（protein protein interaction，PPI）网络图，筛选条件为combine score≥0.4，在移除了孤立的和部分连接节点后，使用Cytoscape软件中的CytoHubba插件计算每种蛋白质的连接度，再运用MCODE插件对PPI网络进行模块分析，基于重要性程度筛选关键Hub基因。

### 1.4　GEPIA验证年轻人HCC Hub基因表达水平及生存分析

运用基因表达谱动态数据分析库GEPIA（http://gepia.cancer-pku.cn/），以$\log_2$（fold change）>1、$P$<0.01为标准对筛选得到的Hub基因进行表达水平的验证。并应用在线工具Kaplan Meier plotter（http://kmplot.com/analysis）评估Hub基因在HCC患者中的预后价值。

### 1.5　GO功能富集和KEGG通路富集分析

GO功能富集分析内容包括生物过程（biological process，BP）、分子功能（molecular function, MF）和细胞成分（cellular component, CC）。本研究将DEGs导入在线富集分析工具DAVID数据库（https://david.ncifcrf.gov/），分别进行GO功能富集及KEGG通路富集分析。

## 2　结　果

### 2.1　HCC及正常肝组织中的DEGs

应用GEO2R和Venn对基因芯片GSE45267进行分析，在年老组中检测到267个差异倍数达4倍以上的显著DEGs，包括75个上调基因和192个下调基因（图1A）；在年轻组中检测到635个显著DEGs，包括186个上调基因和449个下调基因（图1B）。两组中有69个共同上调基因，年轻组特有117个上调基因（图2A）；两组中有179个共同下调基因，年轻组特有270个下调基因（图2B）。

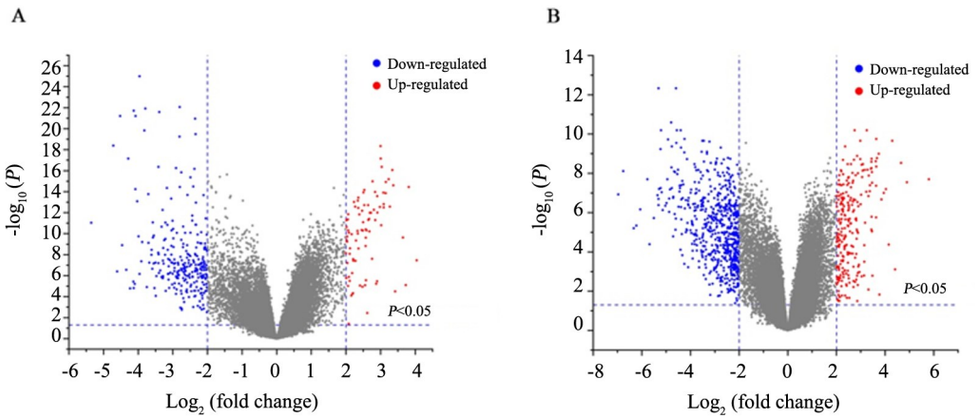



图1 年老组(A)及年轻组(B)HCC中显著DEGs的火山图

**Fig.1 Volcano plots reflecting significant DEGs of HCC in young (A) and old (B) groups**

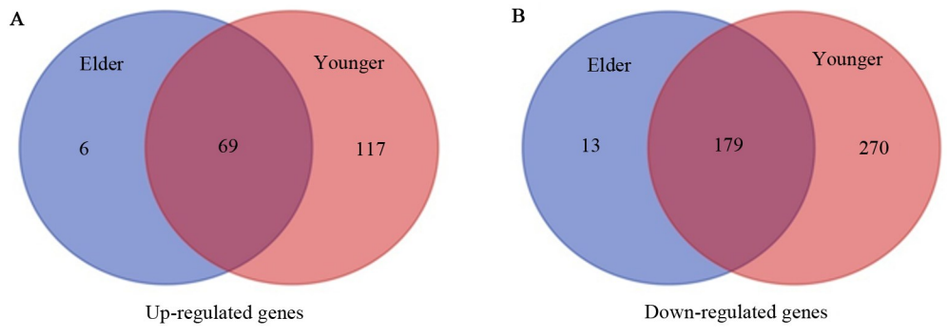
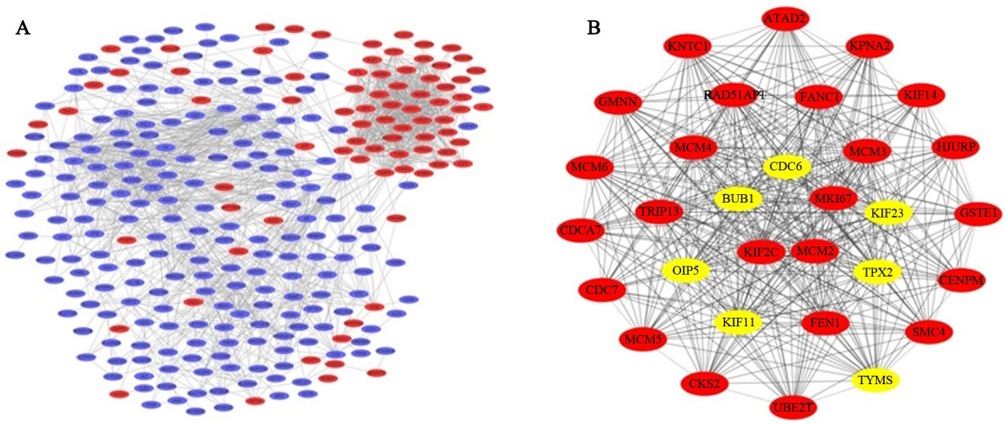



图2 两组HCC中上调(A)及下调(B)DEGs的韦恩图

**Fig.2 Up-regulated (A) and down-regulated (B) DEGs in HCC of two groups by Venn diagram**

2.2 年轻组特有DEGs的PPI网络筛选获取的Hub基因

将筛选得到年轻组特有的387个DGEs输入到STRING网站，导入Cytoscape软件得到可视化PPI网络图，显示有285个节点和1 568个边，包括204个下调基因和81个上调基因（图3A）。运用CytoHubba计算每个蛋白质的连接度，选取得分最高的前10个基因为Hub基因（表1）。基于重要性程度，运用Cytoscape软件中MCODE插件筛选出一个由31个节点、407条边组成的显著模块，并发现10个Hub基因中有7个集中在第一显著模块，且均为上调基因（图3B）。




Red nodes denote up-regulated genes; Blue nodes denote down-regulated genes;Yellow nodes denote Hub genes

图3 年轻组特有DEGs构建的PPI网络(A)和显著模块分析(B)

**Fig.3 The PPI network (A) and significant module analysis (B) of specific DEGs in the young group**

表1 蛋白质互作网络中连接度排名前10的中心节点相关基因

**Tab. 1 The central node related genes in the PPI network (Top 10 in connectivity)**

| Gene | Degree | Gene | Degree |
|---|---|---|---|
| TYMS | 44 | BUB1 | 37 |
| FGA | 41 | TPX2 | 37 |
| SERPINC1 | 41 | OIP5 | 37 |
| KIF11 | 40 | FGB | 36 |
| CDC6 | 39 | KIF23 | 36 |

TYMS：Thyidylate synthase，胸苷酸合成酶；FGA：Fibrinogen alpha chain，纤维蛋白原α链多肽；SERPINC1：Serpin peptidase inhibitor clade C (antithrombin) member 1，丝氨酸蛋白酶抑制剂肽酶抑制蛋白进化枝C（抗凝血酶）成员1；KIF11：Kinesin family member 11，驱动蛋白超家族11；CDC6：Cell division cycle 6，细胞分裂周期蛋白6；BUB1：Budding uninhibited by benzimidazoles-1，苯并咪唑出芽抑制物解除同源物蛋白-1；TPX2：Targeting protein for Xenopus kinesin-like protein 2，爪蟾驱动蛋白样蛋白2靶蛋白；OIP5：Opa interacting protein 5，Opa相互作用蛋白5；FGB：Fibrinogen beta chain，纤维蛋白原β链多肽；KIF23：Kinesin family member 23，驱动蛋白超家族23

### 2.3 Hub基因在HCC标本中高表达验证及其与患者预后生存的关系

GEPIA验证了7个上调Hub基因中有6个在HCC标本中高表达，与正常肝组织标本形成鲜明对比。为了进一步探究Hub基因对HCC预后的影响，用Kaplan Meier plotter确定Hub基因的生存数据，发现年轻组特有的6个Hub基因高表达均与HCC患者预后不良相关。

### 2.4 DEGs涉及的生物过程、分子功能、细胞成分和信号通路

2.4.1 年轻组特有DEGs的GO和KEGG富集分析　为了解显著模块中蛋白质相互作用连接度最高的31个基因的富集情况，将其导入David在线网站进行GO及KEGG富集分析。GO富集结果表明，显著模块中的基因主要与微小染色体维持蛋白（minichromosome maintenance proteins，MCM）复合体及细胞核质组分有关，参与ATP结合过程（图5，表2）。KEGG通路分析显示，显著模块中基因主要与细胞周期及DNA复制有关（图6，表3）。

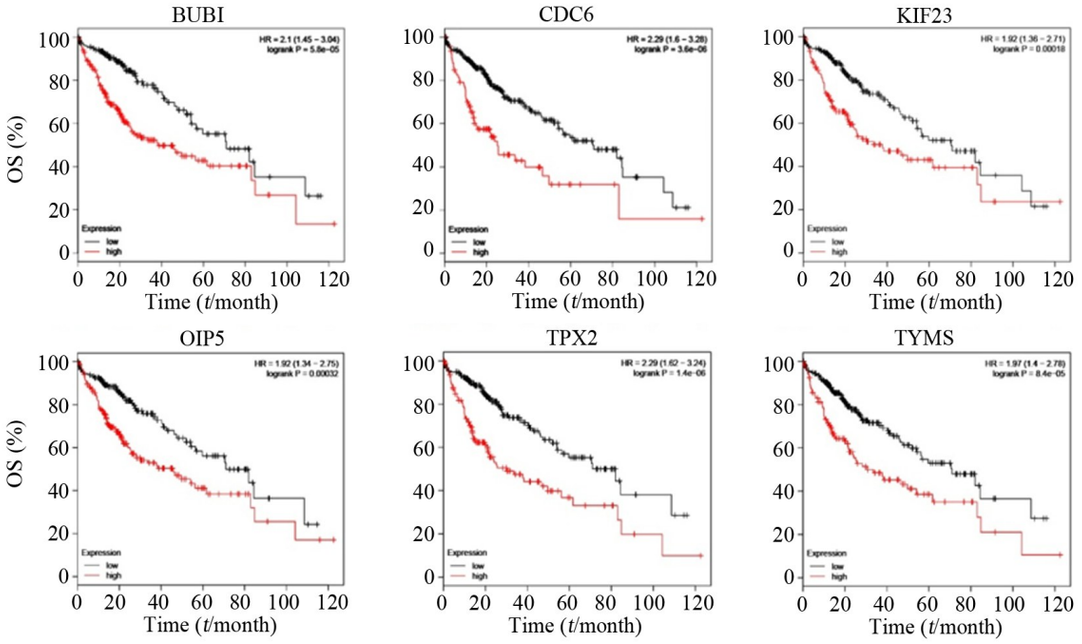



**图4 6个Hub基因的高表达均与患者5年OS呈明显负相关（Kaplan-Meier曲线）**

**Fig.4 High expression of 6 Hub genes was significantly negatively correlated with the 5-year OS of patients (Kaplan-Meier curves)**

2.4.2 年轻组和年老组共同涉及的生物学过程、细胞组分、功能和通路　年轻组和年老组共有的248个DEGs提示可能与年龄因素无关，使用David网站研究其功能注释和信号通路。GO功能富集分析表明，与年龄因素无关的DEGs主要参与环氧酶P450、氧化还原、有丝分裂核分裂、细胞分裂及药物代谢等生物学过程（图2A）；主要与细胞器膜、细胞质、血液微粒、外泌体及着丝粒等细胞组分有关（图2B）；其主要分子功能为氧结合、氧化还原酶活性、血红素结合及铁离子结合等（图2C）。KEGG分析显示，年龄因素无关的DEGs主要富集在视黄醇代谢、化学致癌、药物代谢、细胞周期及补体等信号通路（图2D）。

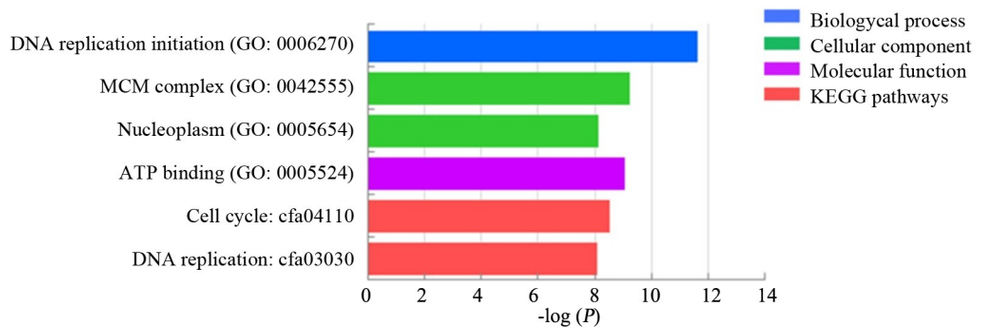



**图5 显著模块中基因显著关联的分子功能和信号通路**

**Fig.5 Molecular functions and signaling pathways significantly associated with the genes in significant module**

**表2 显著模块中31个基因涉及的主要分子功能**

**Tab.2 Major molecular functions related to 31 genes in significant module**

| ID & Term function | Count | *P* | Gene |
|---|---|---|---|
| GO_BP_ID:0006270 DNA replication initiation | 7 | 2.52E-12 | CDC7, CDC6, MCM2, MCM3, MCM4, MCM5, MCM6 |
| GO_CC_ID: 0042555 MCM complex | 5 | 6.66E-10 | MCM2, MCM3, MCM4, MCM5, MCM6 |
| GO_CC_ID: 0000796 Nucleoplasm | 15 | 8.13E-09 | KIF23, CDC7, CDC6, TPX2, ATAD2, MCM2, MCM3, MCM4, MCM5, SMC4, MCM6, FANCI, BUB1, KPNA2, FEN1 |
| GO_MF_ID: 0005524 membrane | 16 | 9.41E-10 | KIF14, KIF23, CDC7, CDC6, KIF11, ATAD2, MCM2, MCM3, MCM4, MCM5, SMC4, MCM6, KIF2C, BUB1, UBE2T, TRIP13 |

**表3 显著模块中31个基因明显涉及的通路**

**Tab.3 Pathways clearly associated with 31 genes in significant module**

| Pathway ID | Term | Count | *P* | Gene |
|---|---|---|---|---|
| cfa04110 | Cell cycle | 8 | 3.21E-09 | CDC7, CDC6, BUB1, MCM2, MCM3, MCM4, MCM5, MCM6 |
| cfa03030 | DNA replication | 6 | 9.34E-09 | MCM2, MCM3, MCM4, MCM5, FEN1, MCM6 |

## 3 讨 论

年龄已被广泛认可为各种实体肿瘤的预后因素之一。如在胃癌[7]和乳腺癌[8]中，年轻患者比年老患者具有更不利的病理特征及更差的临床预后。相反，在甲状腺癌[9]和前列腺癌[10]中，年轻患者的临床预后较好。而年龄是否影响HCC患者的预后仍存在争议。有些学者认为，与年轻患者相比，年老HCC患者的存活率更低[11-12]，有些研究[13-14]则显示相反的结果。

为了找出可能与年轻HCC患者预后有关的关键基因，本研究在GSE45267数据集的基础上，运用生物信息学方法筛选出年轻及年老组HCC内的DGEs，随后将筛选得到的年轻组特有的387个DGEs构建PPI网络，选取连接度最高的前10个Hub基因，同时筛选出了由31个上调基因组成的显著模块，发现连接度最高的10个Hub基因中有7个集中于这一模块中。并且与正常样本相比，有6个基因（TYMS、CDC6、BUB1、TPX2、OIP5、KIF23）在HCC样本中高表达，其表达量越高，患者总生存期越低。最后通过David网站对31个基因组成的显著模块进行富集分析，其中8个基因参与细胞周期通路，集中富集在细胞周期中的S期。与年龄因素无关的DEGs中同样有基因参与细胞周期的过程，但大多在G2和M期（图7）。

TYMS是本研究中发现的连接度最高的Hub基因，提示其可能在年轻患者HCC的发生发展中起重要作用。TYMS是细胞增殖和DNA合成的重要酶，其在增殖细胞中活性很高，已被公认为癌症化疗药物的理想靶点。迄今为止，调控TYMS表达的机制尚不明确，但TYMS基因的多态性似乎是其表达

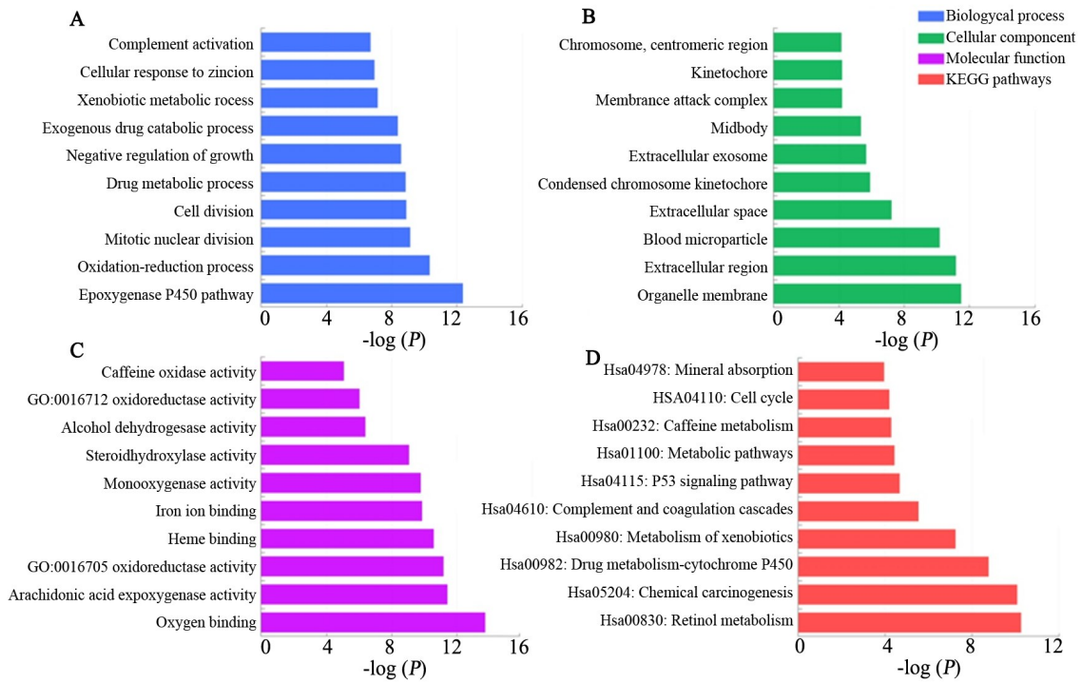
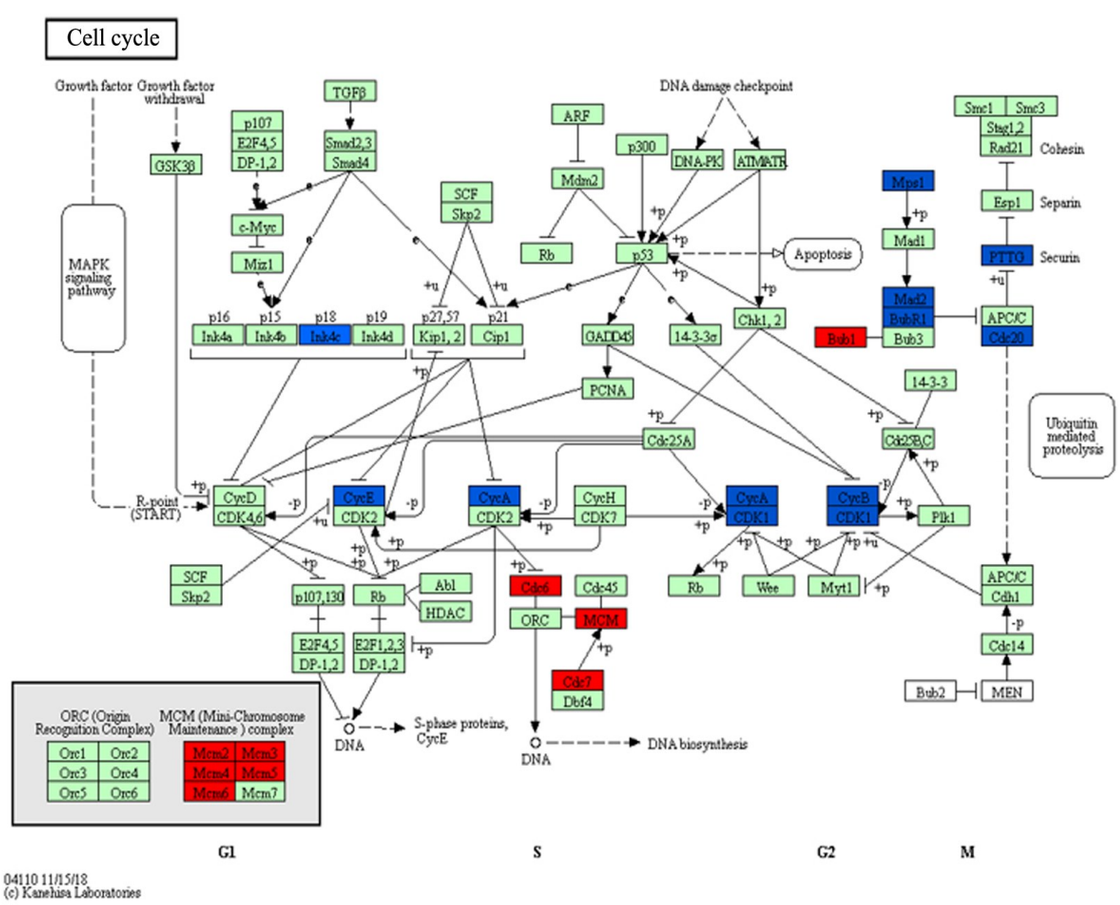



图6 与年龄因素无关DEGs涉及的生物学过程(A)、细胞组分(B)、分子功能(C)、和通路(D)

**Fig.6 Biological processes (A), cellular components (B), molecular functions (C), and pathways (D) involved in age-independent DEGs**




The red represents specific DEGs in younger group, while the blue represents age-independent DEGs

图7 细胞周期通路中的富集分析比较

**Fig.7 Comparison of enrichment analysis in cell cycle pathways**

水平的重要决定因素。有研究[15-16]报道了TYMS的表达及其基因多态性对亚洲HCC患者治疗和预后的重要性。BUB1是纺锤体组装检查点的一个组成部分，在有丝分裂期间控制染色体分离[17]。XU等[18]发现，miR-490-5p通过调节TGFβ/Smad信号通路，使BUB1低表达，从而抑制肝癌细胞的增殖、侵袭和迁移。TPX2是一种微管相关蛋白，已被证明为有丝分裂和纺锤体组装的关键因素。AGUIRRE-PORTOLES等[19]发现，TPX2的过表达与人类不同肿瘤的发展有关，并与染色体不稳定性密切相关。有研究[20]表明，在肝癌中TPX2与肝癌细胞的增殖、凋亡和上皮-间质转化有关，其高表达促进HCC的发生和转移。有学者认为TPX2可作为HCC治疗的潜在靶点[21]。OIP5属于癌症-睾丸抗原（cancer testis antigen，CTA），对癌症具有高度特异性，在正常组织中很少表达。最初WILLIAMS等[22]通过酵母双杂交分析发现OIP5与Opa相互作用编码具有卷曲螺旋结构域的25 kDa蛋白质。先前的研究[23-26]证明，OIP5在乳腺癌、大肠癌及胃癌中高度表达，且其高表达与肺癌以及食管癌患者不良预后密切相关。最近有研究[27]证实，OIP5基因敲除可抑制肝癌细胞增殖、减少集落形成，并将细胞周期阻滞在G0/G1或G2/M期，同时OIP5通过BMPR2/JUN/CHEK1/RAC1失调参与肝癌进程，以上均表现出该基因在HCC靶向治疗中的应用前景。CDC6对真核细胞中DNA复制的启动至关重要[28]。研究[29-30]发现CDC6高表达可加速卵巢癌细胞及肺癌细胞的DNA复制和增殖迁移。XIONG等[31]证明，CDC6过表达可能增加HCC的易感性，并且Cdc6-515A>G多态性可减弱CDC6的表达从而降低癌症风险。这表明CDC6或许可能作为识别肝癌易感亚群的生物学标志物，并实现个体化治疗。KIF23是驱动素样运动蛋白家族的成员，在胞质分裂中起重要作用。已有报道，KIF23的高表达与胶质瘤[32]、胃癌[33]、肺癌[34]等肿瘤的预后不良有关。XIONG等[35]在筛选HCC的差异基因时发现，KIF23在肝癌中高表达，在敲除KIF23 V1蛋白后HCC患者的总生存期延长。以上均显示出这6个基因与HCC的发生发展及预后相关，但从未有人探究过年轻HCC患者较于年老患者该6个基因表达及影响预后的差异情况。因此，本研究致力于通过生物信息学分析为年轻HCC患者个体化早期诊疗及预后评估提供新的方向。本研究在对年轻组特有DEGs进行富集分析时发现，MCM复合体不仅在细胞周期中有举足轻重的作用，同时也参与许多生物学过程。早前有研究[36]发现，6个MCM亚基组装的MCM2-7复合物参与DNA复制起始的所有事件。在HCC中已研究了一些亚基，例如，有研究[37]发现MCM3是HCC的独立预后因子，它通过激活NF-κB途径促进HCC的抗放射性。MCM6被报道可作为HCC早期复发的新型血清生物标志物，并通过MEK/ERK途径促进HCC的转移[38]。MCM7作为HCC的不良预后因子通过激活MAPK信号促进HCC进展[39]。那么MCM复合体在年轻HCC患者是否有不同于年老患者的作用机制，有待继续探索。

综上所述，本研究鉴定出6个仅与年轻HCC预后相关基因，并系统地介绍了与年轻HCC发生和发展密切相关的生物学过程和信号通路。本研究发现，相较于年老组，年轻组DEGs在细胞周期通路中靠近细胞周期前期，提示年轻组HCC患者具有更旺盛的细胞分裂能力。推测年轻HCC患者可能是通过6个Hub基因（TYMS、CDC6、BUB1、TPX2、OIP5、KIF23）调控细胞周期S期，进而参与HCC的发生发展过程，但还需在临床实验中进行深入探究，以期为年轻HCC患者提供更优选择。

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