

下调GPC-3基因转录对肝癌细胞侵袭及血管新生的抑制作用

邵伯军, 姚敏, 王理, 钱琦, 时杨, 蔚丹丹, 陆少林, 姚登福

邵伯军, 南通大学附属医院感染性疾病科 江苏省南通市 226001

姚敏, 南通大学医学院免疫学教研室 江苏省南通市 226001

王理, 南通大学医学院医学信息学教研室 江苏省南通市 226001

钱琦, 蔚丹丹, 南通大学附属医院肿瘤科 江苏省南通市 226001

时杨, 陆少林, 姚登福, 南通大学附属医院临床医学研究中心 江苏省南通市 226001

邵伯军, 副主任医师, 主要从事肝癌的早期诊断与发病机制的研究。

国家国际科技合作专项基金资助项目, No. 2013DFA32150

中国博士后科学基金资助项目, No. 2013M531395

江苏省博士后基金资助项目, No. 苏人社发(2012)468号

作者贡献分布: 此课题由邵伯军与姚敏共同设计; 实验与数据整理由姚敏、蔚丹丹及王理完成; 样本收集及统计学处理由钱琦、时杨、蔚丹丹及陆少林完成; 论文写作由姚敏与姚登福共同完成。

通讯作者: 姚敏, 副教授, 226001, 江苏省南通市启秀路19号, 南通大学医学院免疫学教研室. yaodf@ahnmc.com

电话: 0513-85052297

收稿日期: 2014-02-24 修回日期: 2014-04-13

接受日期: 2014-04-17 在线出版日期: 2014-06-08

Down-regulation of glypican-3 gene transcription inhibits hepatoma cell invasion and angiogenesis

Bo-Jun Tai, Min Yao, Li Wang, Qi Qian, Yang Shi, Dan-Dan Yu, Shao-Lin Lu, Deng-Fu Yao

Bo-Jun Tai, Department of Infectious Diseases, Affiliated Hospital of Nantong University, Nantong 226001, Jiangsu Province, China.

Min Yao, Department of Immunology, Medical School, Nantong University, Nantong 226001, Jiangsu Province, China

Li Wang, Department of Medical Informatics, Medical School, Nantong University, Nantong 226001, Jiangsu Province, China

Qi Qian, Dan-Dan Yu, Department of Oncology, Affiliated Hospital of Nantong University, Nantong 226001, Jiangsu Province, China

Yang Shi, Shao-Lin Lu, Deng-Fu Yao, Research Center of Clinical Medicine, Affiliated Hospital of Nantong University, Nantong 226001, Jiangsu Province, China

Supported by: the International S&T Cooperation Program of China, No. 2013DFA 32150; the Chinese Postdoctoral Scientific Funds, No. 2013M531395; and the Jiangsu Postdoctoral Scientific Funds, No. JS2012-468

Correspondence to: Min Yao, Associate Professor, Department of Immunology, Medical School, Nantong University, 19 Qixiu Road, Nantong 226001, Jiangsu Province,

China. yaodf@ahnmc.com

Received: 2014-02-24 Revised: 2014-04-13

Accepted: 2014-04-17 Published online: 2014-06-08

Abstract

AIM: To investigate whether down-regulation of glypican-3 (GPC-3) gene transcription by short hairpin RNAs (shRNAs) inhibits hepatoma MHCC-97H cell invasion and angiogenesis.

METHODS: Specific GPC-3 shRNAs were transfected into MHCC-97H cells. GPC-3 mRNA and protein expression was analyzed by fluorescence quantitative PCR and Western blot, respectively. Hepatoma cell proliferation was detected by 5-ethynyl-2'-deoxyuridine and sulforhodamine B assay, and cell migration and invasion were assessed by wound healing and transwell assays.

RESULTS: After MHCC-97H cells were transfected with shRNA1, GPC-3 mRNA expression was down-regulated by 75.6% ($t = 15.473$, $P < 0.001$), cell proliferation was inhibited significantly, and cell migration and invasion were decreased. β -catenin expression was down-regulated by 67.7%, and glioma-associated oncogene 1 (Gli1) expression was up-regulated by 53.5% in MHCC-97H cells. The expression of vascular endothelial growth factor (VEGF) was significantly decreased (54.2%, $t = 46.746$, $P < 0.001$) in cells transfected with shRNA1 compared with control cells.

CONCLUSION: shRNA-mediated GPC-3 knock-down inhibits the migration, invasion and angiogenesis of hepatoma cells possibly through Wnt/ β -catenin and Hh signaling pathways.

© 2014 Baishideng Publishing Group Inc. All rights reserved.

Key Words: Hepatocellular carcinoma; GPC-3; shRNA; Angiogenesis

Tai BJ, Yao M, Wang L, Qian Q, Shi Y, Yu DD, Lu SL, Yao

■背景资料

肝癌是我国常见的恶性肿瘤, 他的早期诊断与转移监测是全球医学界的难题。胎肝和肝癌组织中磷脂酰肌醇蛋白多糖-3(glypican-3, GPC-3)过表达, GPC-3分析已是肝癌特异诊断的生物标志, 有望成为抑制肝癌生长与转移的靶目标。

■同行评议者

高润平, 教授, 吉林大学第一医院肝胆胰内科

■ 研发前沿

癌胚型GPC-3激活促进肝细胞恶性转化与肝癌的转移发生, 新近证实GPC-3表达与Wnt/ β -catenin信号通路与肝癌发展密切相关, 调控肝细胞生长、分化、增殖和凋亡, 可为肝癌基因治疗带来新契机。

DF. Down-regulation of glypican-3 gene transcription inhibits hepatoma cell invasion and angiogenesis. Shijie Huaren Xiaohua Zazhi 2014; 22(16): 2221-2228 URL: <http://www.wjgnet.com/1009-3079/22/2221.asp> DOI: <http://dx.doi.org/10.11569/wjcd.v22.i16.2221>

摘要

目的: 观察以短发夹RNA(short hairpin RNA, shRNA)下调磷脂酰肌醇蛋白多糖-3(glypican-3, GPC-3)基因转录, 对抑制肝癌MHCC-97H细胞侵袭和血管新生的作用与机制。

方法: 将对GPC-3特异性shRNA转染肝癌MHCC-97H细胞, 以荧光定量PCR和Western blot分析GPC-3 mRNA及蛋白表达; 以5-ethynyl-2'-deoxyuridine(EdU)和Transwell法评估细胞增殖、迁移和侵袭能力。

结果: shRNA1转染MHCC-97H细胞, GPC-3在mRNA水平下降75.6%($t = 15.473$, $P < 0.001$), 蛋白表达同步下调; 干预GPC-3基因转录, 可抑制肝癌细胞增殖, 细胞迁移与侵袭能力减低, 与抗癌药物具协同作用; Wnt/ β -catenin中 β -catenin表达下调67.7%; Hedgehogs通路中脑胶质瘤相关癌基因1(glioma-associated oncogene 1, *Gli1*)表达上调53.3%; shRNA1转染72 h细胞血管内皮生长因子(vascular endothelial growth factor, VEGF)表达下调54.2%($t = 46.746$, $P < 0.001$)。

结论: shRNA下调GPC-3转录, 经Wnt/ β -catenin和Hh信号通路抑制癌细胞迁移、侵袭和血管新生, 为肝癌基因治疗潜在的分子靶目标。

© 2014年版权归百世登出版集团有限公司所有。

关键词: 肝细胞性癌; 磷脂酰肌醇蛋白多糖-3; shRNA; 血管新生

核心提示: 以特异性短发夹RNA(short hairpin RNA, shRNA)沉默磷脂酰肌醇蛋白多糖-3(glypican-3, GPC-3)基因转录, 分析Wnt/ β -catenin通路 β -catenin和Hedgehogs通路中脑胶质瘤相关癌基因1(glioma-associated oncogene 1, *Gli1*)表达的作用, 干预GPC-3转录抑制肝癌细胞侵袭和血管新生。

邵伯军, 姚敏, 王理, 钱琦, 时杨, 蔚丹丹, 陆少林, 姚登福. 下调GPC-3基因转录对肝癌细胞侵袭及血管新生的抑制作用. 世界华人消化杂志 2014; 22(16): 2221-2228 URL: <http://www.wjgnet.com/1009-3079/22/2221.asp> DOI: <http://dx.doi.org/10.11569/wjcd.v22.i16.2221>

0 引言

磷脂酰肌醇蛋白聚糖-3(glypican-3, GPC-3)在正常肝组织中未见表达, 胎肝和肝癌(hepatocellular carcinoma, HCC)组织呈过表达状态^[1,2], 是HCC肝癌特异诊断最有应用前景的生物标志^[3,4]. HCC进展快、复发率高和预后差, 与肝癌的血管新生、癌细胞的高侵袭性等诸多因素相关^[5,6]. GPC-3蛋白锚定于细胞膜, 与Wnt/ β -catenin和Hedgehogs(Hh)等信号通路密切相关^[7,8]; 肝癌发生发展过程中, Wnt/ β -catenin通路异常激活, β -连环蛋白积聚, 对肝癌细胞增殖和生长起促进作用; Hh通路信号增强, 激活锌指转录因子脑胶质瘤相关癌基因1(glioma-associated oncogene 1, *Gli1*), 在组织架构、癌细胞增殖和分化方面发挥作用^[9-11]; 然而, 肝组织GPC-3过表达与肝癌细胞侵袭和血管新生的关系以及作用机制尚待阐明^[12,13]. 本文以特异性shRNA沉默GPC-3基因转录, 观察对Wnt/ β -catenin通路中 β -catenin和Hh通路中*Gli1*表达的作用, 分析干预GPC-3转录对肝癌细胞迁移侵袭和血管新生的影响。

1 材料和方法

1.1 材料 人HepG2及MHCC-97H细胞株购自中科院上海细胞库; DMEM完全培养基购自Gibco公司; GPC-3和GAPDH引物由TaKaRa公司设计合成; β -catenin和*Gli1*引物由Invitrogen公司合成; TRIzol试剂、LipoD293TM和GenJetTM转染试剂均购自Invitrogen公司; GPC-3购自美国Abcam公司; 基质胶购自BD公司; 血管内皮生长因子(vascular endothelial growth factor, VEGF)ELISA检测试剂盒购自上海蓝基公司。

1.2 方法

1.2.1 质粒构建和转染: 按文献[14]合成并构建4种干扰序列和阴性对照序列. 细胞以含10%FBS DMEM完全培养基于37 °C含5%CO₂培养箱培养. 转染前24 h将融合度为70%左右细胞接种6孔板中, 于转染前1 h更换新培养基. 最有效的shRNA1(正向: 5'-CACC GCG GTTACTGCAATGTGGTCATTCAAGAGATGACCA CATTG-CAGTAACCGCTTTTTTG-3', 反向: 5'-GATC-CAAAAAAGCGGT TACTGCAATGTGGT-CATCTCTTGAATGACCACATTGCAGTAACCGC-3')作为干扰组及阴性对照(正向: 5'-CACC-GTTCTCCGAACGTGT CACGTCAAGAGAT-

TACGTGACACGTTCCGAGAATTTTTTG-3', 反向5'-GATCCAAAAAATTCTCCGAACGTGTCACGTAATCTCTTGACGTGACACGTTCCGAGAAC-3')质粒作为阴性对照组分别用LipoD293TM转染试剂转染至MHCC-97H细胞, 并培养未处理细胞作为空白对照. 将shRNA(沉默GPC-3有效序列)作为干扰组及阴性对照质粒作为阴性对照组分别用GenJetTM(Ver II for HepG2细胞)转染试剂转染至HepG2细胞, 培养未处理HepG2细胞作为空白对照. 12-18 h后换液, 继续培养.

1.2.2 总RNA提取、引物及荧光定量PCR: MHCC-97H细胞分别设转染组4组, 阴性对照及空白对照($n = 3$). 转染48 h后, 以TRIzol试剂提取细胞总RNA. 检测总RNA及 $A_{260/280}$. 引物序列(5'-3'): GPC-3(NM_004484), F-CGAGATAAGCACCTTTCACAACC和R-AGAAGAAGCACACCACGAGA; β -catenin(NM-001904), F-AAAGCGGCTGTTAGTCACTGG和R-AAAGCGGCTGTTAGTCACTGG; Gli1(NM-005269), F-GCACCACATCAACAGTGAGC和R-GCGTCTTGAGGTTTTCAAGG; GAPDH(NM_017008), F-CAAGGTCATCCATGACAACCTTTG和R-GTCCACCACCCTGTTGCTGTAG. RT-PCR扩增参数如下: 95 °C 30 s, 94 °C 5 s, 60 °C 45 s, 40个循环, 4 °C 保存. 以Ct比较法对GPC-3基因相对定量: 即 $\Delta Ct = Ct_{\text{目的基因}} - Ct_{\text{内参基因}}$, 相对表达量 = $2^{-\Delta Ct}$.

1.2.3 Western blot检测蛋白浓度: 转染细胞及空白对照细胞, 于转染48 h后收集细胞, 提取蛋白后以BCA法定量. 制备10%SDS聚丙烯酰胺凝胶, 每泳道蛋白量50 μ g, 加5 \times SDS上样缓冲液, 封口膜封口后于沸水煮8 min上样, 电泳恒压60 V \times 40 min, 后110 V \times 60 min, 转移至PVDF膜(膜在甲醇浸泡于转膜缓冲液中平衡10 min), 配制5%脱脂牛奶封闭液(脱脂奶粉2.5 g+TBS-T 50 mL)于玻璃平皿中, 室温下脱色摇床封闭1 h. 取膜用TBS-T漂洗5 min \times 4次. 加兔抗人GPC-3(1:250)或 β -catenin(美国CST公司, 1:1000)或Gli1(美国CST公司, 1:1000), β -actin抗体(1:1000)作为内参, 4 °C过夜, 洗涤, 辣根过氧化物酶标记的羊抗兔及羊抗鼠IgG二抗(1:1000)孵育1 h, 洗涤后ECL显色并拍照.

1.2.4 细胞增殖分析: 细胞转染48 h后, 胰蛋白酶消化细胞, 调整细胞浓度为 1×10^4 /孔, 细胞贴壁后每孔加入100 μ L 50 μ mol/L EdU培养基孵育

2 h, PBS洗涤2遍, 加4%多聚甲醛固定液, 室温孵育30 min, 每孔加入100 μ L 1 \times Apollo[®]染色反应液, 室温、避光、脱色摇床孵育30 min后加入100 μ L含0.5%TritonX-100的PBS脱色摇床清洗3次, 每孔加入100 μ L 1 \times Hoechst33342反应液, 避光、室温、脱色摇床孵育30 min, PBS漂洗3遍, 立即于倒置荧光显微镜下观察并拍照.

1.2.5 划痕试验分析细胞愈合能力: MHCC-97H细胞于转染48 h后, 当融合度为90%左右时, 用10 μ L无菌移液器枪头在培养细胞的6孔板平皿中央划出一条直径为1 mm左右的直线划痕, PBS轻洗3遍, 洗去漂浮的脱落细胞, 每孔加入2 mL无血清培养基, 在倒置相差显微镜下取划痕处几个不同视野拍照(0 h), 继续于37 °C含5%CO₂培养箱中培养24 h并拍照, 观察各组细胞由划痕边缘向划痕中央迁移后剩下距离(mm, $\times 200$), 根据划痕部位细胞愈合的相对距离判断细胞运动能力. 划痕愈合率(%) = (0 h划痕宽度-24 h划痕宽度)/0 h划痕宽度 $\times 100\%$.

1.2.6 Transwell法分析迁移能力: 先将Matrigel胶用无血清DMEM-H培养基1:3稀释, 每个侵袭小室加60 μ L, 迁移实验不加Matrigel. 各细胞转染48 h后, 胰蛋白酶消化, PBS洗涤2遍, 用无血清培养基调整细胞浓度为 1.0×10^6 /mL, 每孔取100 μ L接种于Transwell小室上室, 下室加入600 μ L完全培养基, 37 °C, 5%CO₂培养箱培养24 h后检测, 迁移20 h后检测. 棉签擦去滤膜上层贴壁生长但未能穿过膜细胞, 将上室置于950 mL/L乙醇室温固定30 min, 0.1%结晶紫染色15 min后, 置显微镜下观察并拍照, 任取中央及四周5个低倍视野(200 \times)细胞计数($n = 3$), 计算平均值; 33%醋酸脱色, 酶标仪测 A_{570} , 侵袭抑制率(%) = [(A对照-A转染)/A对照] $\times 100\%$.

1.2.7 β -catenin及胰岛素样生长因子II(insulin-like growth factor II, IGF-II)荧光分析: 转染48 h后, 胰酶消化细胞, 调整细胞为 2×10^5 /mL, 接种于铺有无菌盖玻片6孔板内, 培养24 h后细胞贴壁爬片, 取出盖玻片, PBS洗3遍, 用4%多聚甲醛固定30 min, 0.5%Triton-100透膜处理15 min, PBS洗3遍后用含5%BSA封闭1 h, PBS漂洗, 兔抗人 β -catenin抗体(1:100)、鼠抗人IGF-II抗体(1:100)分别于4 °C过夜, PBS漂洗3遍后用Cy3标记抗兔或抗小鼠荧光二抗(1:1000)室温避光孵育1 h, PBS漂洗3遍, 添加DAPI避光染色15 min, 洗涤后滴加抗荧光淬灭封闭液封片, 于倒置荧光显微镜下观察并拍照.

■相关报道

Yao等报道人肝癌组织GPC-3阳性率为80.6%, 远癌组织未见阳性; 肝癌患者外周血GPC-3 mRNA阳性率为70.7%, 肝癌伴远处转移患者阳性为100%, 提示GPC-3及基因转录与肝癌密切相关, 是否具有基因治疗价值有待证实.

■创新盘点

本研究构建靶向肝癌GPC-3的高效、特异的shRNA干扰质粒,在体外,成功转染具高转移潜能MHCC-97H细胞,下调GPC-3基因转录明显抑制肝癌细胞增殖,证实其机制为抑制Wnt/ β -catenin通路关键信号分子发挥作用。

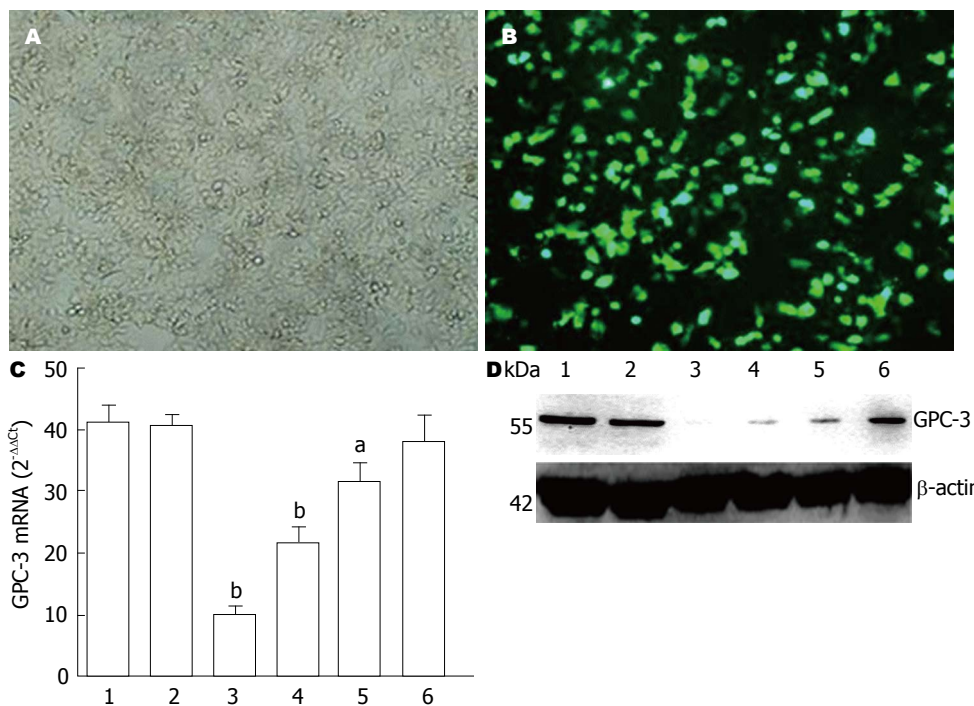


图1 shRNA1转染影响肝癌细胞GPC-3转录与蛋白表达。A: MHCC97H细胞转染明场图; B: 荧光图($\times 100$); C: 采用实时定量PCR检测GPC-3 mRNA表达。* $P < 0.05$, ^b $P < 0.01$ vs 空白组; D: Western blot分析MHCC-97H细胞GPC-3表达。1: 空白组; 2: 阴性组; 3: GPC-3 shRNA1; 4: GPC-3 shRNA2; 5: GPC-3 shRNA3; 6: GPC-3 shRNA4。GPC-3: 磷脂酰肌醇蛋白多糖-3。

1.2.8 VEGF定量: 将shRNA1质粒及阴性对照转染至细胞, 设置未处理细胞为空白组, 分别于24、48和72 h收集细胞上清液($n = 3$), 按ELISA试剂盒说明操作检测VEGF含量。

统计学处理 所有数据采用SPSS17.0和GraphPad Prism 5.0统计分析软件进行分析处理及作图, 所有计量资料以mean \pm SD表示, 样本均数两两比较采用独立样本 t 检验, 多样本均数比较采用单因素方差分析(One-way ANOVA), $P < 0.05$ 为差异有统计学意义。

2 结果

2.1 shRNA转染下调肝癌细胞GPC-3转录与表达 将GPC-3特异的shRNA转染人肝癌MHCC-97H细胞, 培养、荧光显微镜观察($n = 5$)、拍照, 计算转染效率(图1A, B)。结果显示转染效率在80%以上, 4种shRNA转染对MHCC-97H细胞GPC-3 mRNA均有干扰, shRNA1、shRNA2、shRNA3和shRNA4分别为75.6%、47.5%、22.8%和6.7%, 其中shRNA1对MHCC-97H细胞GPC-3 mRNA转录沉默效果最佳(图1C), ($t = 15.473$, $P < 0.001$); 在MHCC-97H细胞GPC-3蛋白水平上, Western blot分析显示以shRNA1明显抑制GPC-3蛋白表达, 干扰效果最好(图1D)。

2.2 shRNA抑制肝癌细胞迁移及侵袭能力 干预

MHCC-97H细胞GPC-3转录后, 空白组、对照组和干扰组划痕实际平均宽度($n = 3$), 0 h分别为210.7、201.3和228.3 mm, 24 h分别为142.7、141.6和213.6 mm, 划痕愈合率(图2A)分别为32.2%、27.4%和6.4%, 干扰组MHCC-97H细胞的划痕愈合速度明显慢于空白组($t = 32.697$, $P < 0.001$), (图2B); 以Transwell法分析MHCC-97H细胞侵袭能力, 沉默GPC-3表达后显示肝癌细胞侵袭力明显受抑。与空白组相比, MHCC-97H细胞侵袭抑制率为69.1%($t = 39.647$, $P < 0.001$)。

2.3 shRNA转染影响 β -catenin和Gli1表达 转染GPC-3-shRNA1及阴性对照质粒于HCC细胞, 检测 β -catenin及Gli1 mRNA水平, 显示下调 β -catenin mRNA(图3A)并上调Gli1 mRNA表达(图3B); 与空白组相比, MHCC97-H细胞中 β -catenin mRNA抑制67.7%($t = 18.4$, $P < 0.001$); Gli1 mRNA上调53.3%($t = -4.824$, $P = 0.008$); Western blot分析显示 β -catenin明显下调, Gli1明显上调(图3C)。

2.4 shRNA转染下调IGF-II表达 免疫荧光显示IGF-II广泛存在于MHCC-97H细胞膜、细胞浆和细胞核; shRNA1转染沉默MHCC-97H细胞中GPC-3表达后, 细胞中IGF-II表达明显下调, MHCC-97H细胞凋亡明显增加(图4)。

2.5 shRNA转染下调VEGF表达 ELISA法检测

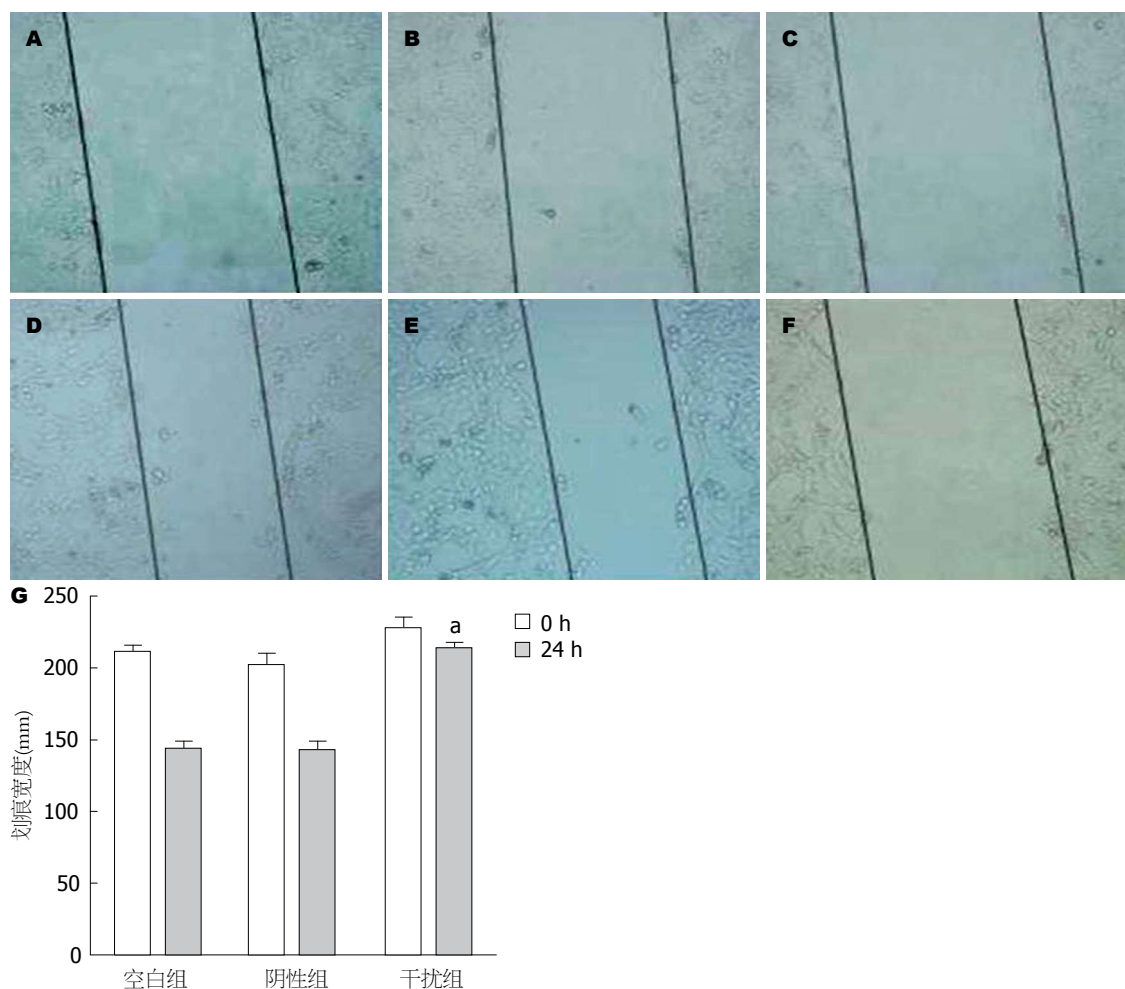


图2 沉默GPC-3后MHCC-97H细胞划痕结果. A: 0 h空白组; B: 0 h阴性组; C: 0 h干扰组; D: 24 h空白组; E: 24 h阴性组; F: 24 h干扰组; G: MHCC-97H细胞划痕后0和24 h的实际宽度(mm). ^aP<0.001 vs 空白组. GPC-3: 磷脂酰肌醇蛋白多糖-3.

■应用要点

在体外以高效、特异的shRNA干扰肝癌GPC-3转录, 明显抑制肝癌细胞增殖, 显示在肝癌过表达的GPC-3为新发现治疗靶点, 需更多的肝癌发生或动态体内模型深入研究, 论证干预GPC-3基因转录有助于肝癌的靶向治疗.

MHCC-97H细胞中VEGF表达, 显示下调MHCC-97H细胞中GPC-3的表达后VEGF明显下调且呈时间依赖性. MHCC-97H细胞干扰组24、48和72 h与MHCC-97H细胞空白组相比, VEGF表达分别下降36.8%($t_{24} = 14.543, P < 0.001$)、46.2%($t_{48} = 55.212, P < 0.001$)和54.2%($t_{72} = 46.746, P < 0.001$).

3 讨论

位于X染色体(Xq26)的GPC-3, 其核心蛋白由40 kDa的氨基末端亚基和30 kDa的羧基末端亚基组成, 通过糖基磷脂酰肌醇锚定于细胞膜表面^[15,16]. 羧基末端接近细胞膜的区域含有两条硫酸类肝素聚糖链. 葡糖氨基聚糖参与调节多条信号通路, 与多种生长因子如Wnts、Hh、胰岛素样生长因子II (insulin-like growth factor II, IGF-II)、转化生长因子-β、成纤维生长因子及骨形态生成蛋白等结合并介导HCC发生发展^[17,18]; 通过调节基质金属蛋白酶和尿激酶型纤溶酶原激活物水解细胞外基质、促进肿瘤侵袭与转移^[19,20]. 本

文构建高效特异的GPC-3-shRNA干扰质粒, 转染具有高转移潜能肝癌MHCC-97H细胞观察下调GPC-3基因转录对肝癌细胞增殖和血管形成的影响、分析对肝癌相关Wnt/β-catenin通路中关键信号分子的表达与改变特征.

GPC-3以调节自分泌/旁分泌的经典Wnt信号通路刺激HCC细胞生长, 其过表达可使细胞结合Wnt能力增强. Wnts与HS链结合形成磷脂酰蛋白聚糖-Wnt/生长因子复合物, 在硫酸酯酶-2(sulfatase 2, SULF-2)作用下Wnts从HS链上释放, 与Frizzled受体结合进而激活Wnt/Frizzled下游区域的信号转导, 促进HCC细胞增殖^[21,22]. shRNA有效干预GPC-3基因转录, 显示shRNA1可显著抑制细胞增殖、迁移和侵袭, 可影响肝癌相关Wnt/β-catenin和Hh通路. β-catenin是Wnt/β-catenin信号通路的关键分子, Wnt信号激活将致β-catenin在胞内大量积聚, 进入胞核触发肝癌发生等. shRNA1转染MHCC-97H细胞后, 在mRNA水平上β-catenin下调67.7%; 但Gli1分

■同行评价

作者构建与筛选出对肝癌GPC-3干预具有的特异性shRNA, 转染肝癌MHCC-97H细胞, 显著抑制肝癌细胞迁移和侵袭作用。

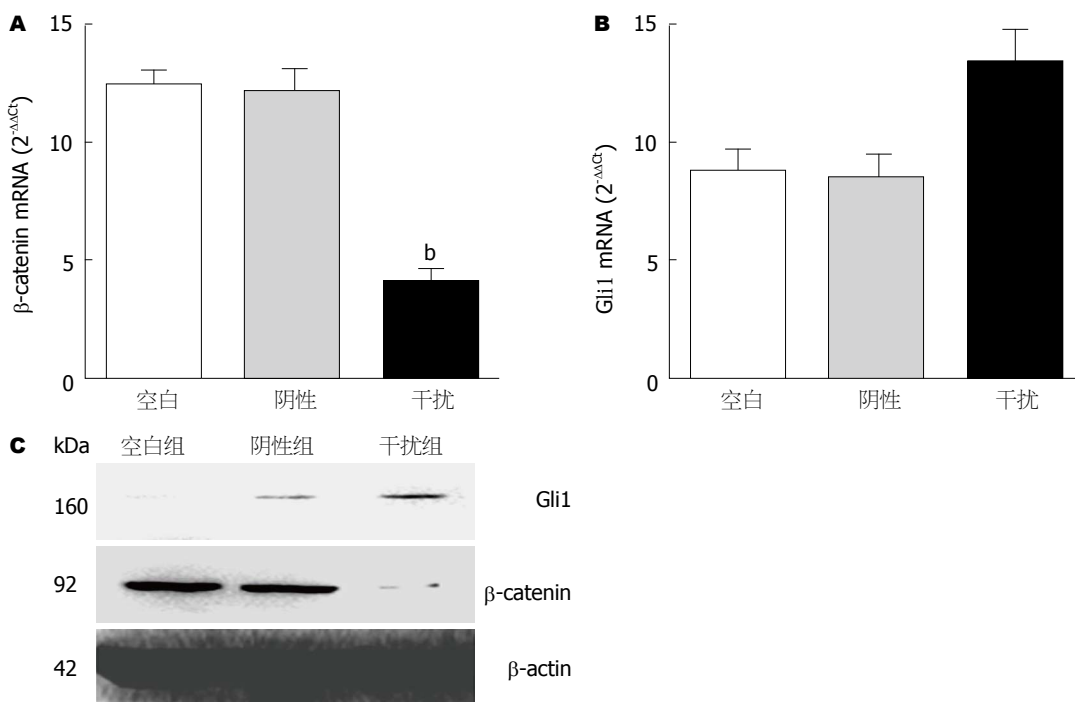


图3 沉默GPC-3后MHCC-97H细胞β-catenin下调和Gli1上调。A: β-catenin mRNA相对表达; B: Gli1 mRNA; C: β-catenin及Gli1蛋白表达。GPC-3: 磷脂酰肌醇蛋白多糖-3; Gli1: 脑胶质瘤相关癌基因1。^b $P < 0.001$ 。

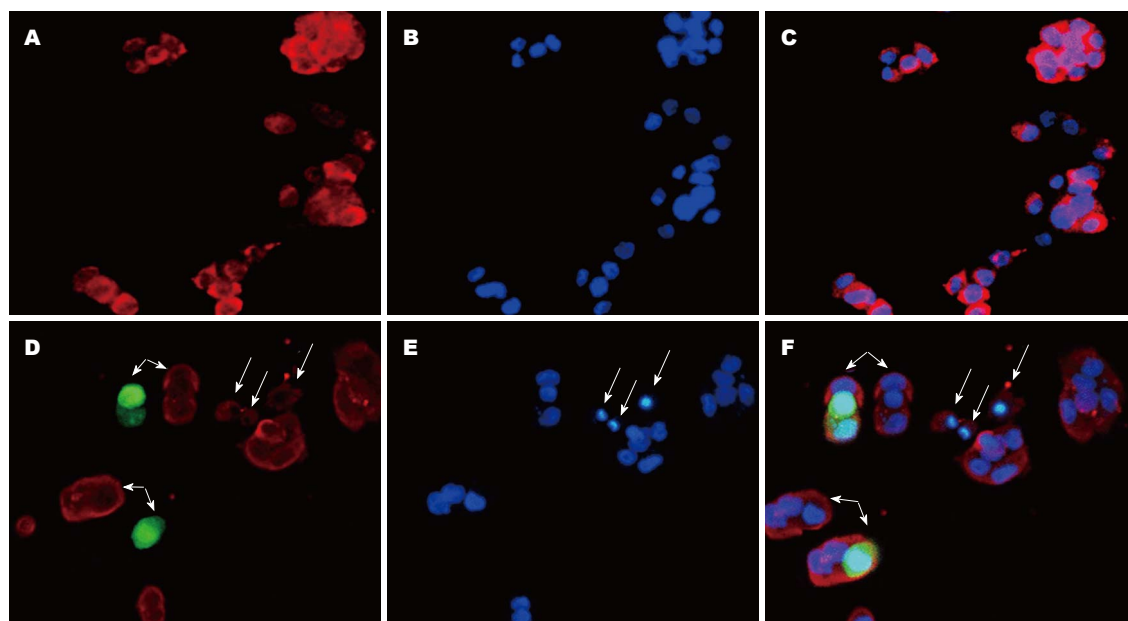


图4 沉默GPC-3下调MHCC-97H细胞IGF-II表达并诱导凋亡($\times 200$)。A: 对照组IGF-II图; B: 对照组DAPI图; C: 对照组Merge图; D: GPC-3 shRNA1干扰组IGF-II图; E: GPC-3 shRNA1 DAPI图; F: GPC-3 shRNA1 Merge图。白色箭头为β-catenin下调最显著, 单个箭头所示为细胞凋亡。GPC-3: 磷脂酰肌醇蛋白多糖-3。

别上调53.3%。沉默GPC-3可下调β-catenin并上调Gli1表达; 推测GPC-3可经上调β-catenin激活Wnt/β-catenin通路^[23,24], 与Hh竞争结合Ptch受体下调Gli1表达而抑制Hh通路^[9,25], 促进肝癌发展。

下调GPC-3基因转录影响肝癌相关信号通路, 是否影响新血管生成, 本文观察了对IGF-II

和VEGF的表达。肝脏是合成和分泌IGF-II主要器官, 癌变过程中激活启动子P3产生胚胎表型逆转, 使IGF-II过表达。IGF-II调节胚胎生长发育、刺激DNA合成、促进细胞有丝分裂, 在肝癌转移中起重要作用^[26,27]。下调MHCC-97H细胞GPC-3表达, IGF-II表达显著下调, 并诱导细胞

凋亡; VEGF为多功能细胞因子, 具有促内皮细胞分裂、增殖和血管构建, 细胞迁移, 诱导蛋白水解酶、间质胶原酶和组织因子表达。癌组织VEGF过表达, 启动新生血管形成是肝癌生长、侵袭和转移基础, 为癌细胞播散途径^[28,29]。下调GPC-3明显抑制VEGF表达, 且呈时间依赖性, 显示靶向GPC-3可潜在抑制肝癌细胞转移发生。

肝癌是我国常见的恶性肿瘤, 为典型多血管肿瘤, 肝细胞癌变机制复杂, 多种信号通路参与。新生血管形成同样是肝癌细胞增殖和转移的重要条件, 血管生长因子经与特异性受体结合, 直接刺激内皮细胞增殖, 并增加血管通透性, 不仅促进新生血管形成, 且有利于脱落的肝癌细胞进入血管或向肝外转移。肝癌GPC-3高表达, 为有助于早期诊断特异标志^[30]; 癌胚型GPC-3激活促进肝细胞恶性转化与肝癌的转移发生^[31], 新近证实GPC-3基因有可能成为HCC靶向治疗的潜在分子靶目标, Wnt/ β -catenin信号通路与HCC发展密切相关, 调控肝细胞生长、分化、增殖和凋亡, 也为癌症的诊断和治疗带来契机。

4 参考文献

- 1 Qiao SS, Cui ZQ, Gong L, Han H, Chen PC, Guo LM, Yu X, Wei YH, Ha SA, Kim JW, Jin ZT, Li S, Peng JR, Leng XS. Simultaneous measurements of serum AFP, GPC-3 and HCCR for diagnosing hepatocellular carcinoma. *Hepatogastroenterology* 2011; 58: 1718-1724 [PMID: 21940340]
- 2 Yao M, Yao DF, Bian YZ, Zhang CG, Qiu LW, Wu W, Sai WL, Yang JL, Zhang HJ. Oncofetal antigen glypican-3 as a promising early diagnostic marker for hepatocellular carcinoma. *Hepatobiliary Pancreat Dis Int* 2011; 10: 289-294 [PMID: 21669573 DOI: 10.1016/S1499-3872(11)60048-9]
- 3 Liu XF, Hu ZD, Liu XC, Cao Y, Ding CM, Hu CJ. Diagnostic accuracy of serum glypican-3 for hepatocellular carcinoma: a systematic review and meta-analysis. *Clin Biochem* 2014; 47: 196-200 [PMID: 24362268 DOI: 10.1016/j.clinbiochem.2013.12.007]
- 4 Zaakook M, Ayoub M, Sinna EA, El-Sheikh S. Role of glypican-3 immunocytochemistry in differentiating hepatocellular carcinoma from metastatic carcinoma of the liver utilizing fine needle aspiration cytology. *J Egypt Natl Canc Inst* 2013; 25: 173-180 [PMID: 24207089 DOI: 10.1016/j.jnci.2013.07.004]
- 5 Cheng J, Wang W, Zhang Y, Liu X, Li M, Wu Z, Liu Z, Lv Y, Wang B. Prognostic role of pre-treatment serum AFP-L3% in hepatocellular carcinoma: systematic review and meta-analysis. *PLoS One* 2014; 9: e87011 [PMID: 24498011 DOI: 10.1371/journal.pone.0087011]
- 6 Kennedy AS, Sangro B. Nonsurgical treatment for localized hepatocellular carcinoma. *Curr Oncol Rep* 2014; 16: 373 [PMID: 24488546]
- 7 Gao W, Ho M. The role of glypican-3 in regulating Wnt in hepatocellular carcinomas. *Cancer Rep* 2011; 1: 14-19 [PMID: 22563565]
- 8 Zheng X, Zeng W, Gai X, Xu Q, Li C, Liang Z, Tuo H, Liu Q. Role of the Hedgehog pathway in hepatocellular carcinoma (review). *Oncol Rep* 2013; 30: 2020-2026 [PMID: 23970376]
- 9 Lu JT, Zhao WD, He W, Wei W. Hedgehog signaling pathway mediates invasion and metastasis of hepatocellular carcinoma via ERK pathway. *Acta Pharmacol Sin* 2012; 33: 691-700 [PMID: 22543708 DOI: 10.1038/aps.2012.24]
- 10 Philips GM, Chan IS, Swiderska M, Schroder VT, Guy C, Karaca GF, Moylan C, Venkatraman T, Feuerlein S, Syn WK, Jung Y, Witek RP, Choi S, Michelotti GA, Rangwala F, Merkle E, Lascola C, Diehl AM. Hedgehog signaling antagonist promotes regression of both liver fibrosis and hepatocellular carcinoma in a murine model of primary liver cancer. *PLoS One* 2011; 6: e23943 [PMID: 21912653 DOI: 10.1371/journal.pone.0023943]
- 11 Allan BJ, Wang B, Davis JS, Parikh PP, Perez EA, Neville HL, Sola JE. A review of 218 pediatric cases of hepatocellular carcinoma. *J Pediatr Surg* 2014; 49: 166-171; discussion 171 [PMID: 24439603 DOI: 10.1016/j.jpedsurg.2013.09.050]
- 12 Huang X, Hollinger FB. Occult hepatitis B virus infection and hepatocellular carcinoma: a systematic review. *J Viral Hepat* 2014; 21: 153-162 [PMID: 24438677]
- 13 Higgins MC, Soulen MC. Combining locoregional therapies in the treatment of hepatocellular carcinoma. *Semin Intervent Radiol* 2013; 30: 74-81 [PMID: 24436520]
- 14 Yu D, Dong Z, Yao M, Wu W, Yan M, Yan X, Qiu L, Chen J, Sai W, Yao D. Targeted glypican-3 gene transcription inhibited the proliferation of human hepatoma cells by specific short hairpin RNA. *Tumour Biol* 2013; 34: 661-668 [PMID: 23192642 DOI: 10.1007/s13277-012-0593-y]
- 15 Enan ET, El-Hawary AK, El-Tantawy DA, Abu-Hashim MM, Helal NM. Diagnostic role of glypican 3 and CD34 for differentiating hepatocellular carcinoma from nonmalignant hepatocellular lesions. *Ann Diagn Pathol* 2013; 17: 490-493 [PMID: 24012547 DOI: 10.1016/j.anndiagpath.2013.08.001]
- 16 Witjes CD, ten Kate FJ, Verhoef C, de Man RA, IJzermans JN. Immunohistochemical characteristics of hepatocellular carcinoma in non-cirrhotic livers. *J Clin Pathol* 2013; 66: 687-691 [PMID: 23585667 DOI: 10.1136/jclinpath-2012-201156]
- 17 Yao M, Yao DF, Bian YZ, Wu W, Yan XD, Yu DD, Qiu LW, Yang JL, Zhang HJ, Sai WL, Chen J. Values of circulating GPC-3 mRNA and alpha-fetoprotein in detecting patients with hepatocellular carcinoma. *Hepatobiliary Pancreat Dis Int* 2013; 12: 171-179 [PMID: 23558072 DOI: 10.1016/S1499-3872(13)60028-4]
- 18 Lagana SM, Salomao M, Bao F, Moreira RK, Lefkowitz JH, Remotti HE. Utility of an immunohistochemical panel consisting of glypican-3, heat-shock protein-70, and glutamine synthetase in the distinction of low-grade hepatocellular carcinoma from hepatocellular adenoma. *Appl Immunohistochem Mol Morphol* 2013; 21: 170-176 [PMID: 22914605]
- 19 Shirakawa H, Suzuki H, Shimomura M, Kojima M, Gotohda N, Takahashi S, Nakagohri T, Konishi M, Kobayashi N, Kinoshita T, Nakatsura T. Glypican-3 expression is correlated with poor prognosis

- sis in hepatocellular carcinoma. *Cancer Sci* 2009; 100: 1403-1407 [PMID: 19496787 DOI: 10.1111/j.1349-7006.2009.01206.x]
- 20 Daniele A, Divella R, Quaranta M, Mattioli V, Casamassima P, Paradiso A, Garrisi VM, Gadaleta CD, Gadaleta-Caldarola G, Savino E, Maci R, Bellizzi A, Fazio V. Clinical and prognostic role of circulating MMP-2 and its inhibitor TIMP-2 in HCC patients prior to and after trans-hepatic arterial chemoembolization. *Clin Biochem* 2014; 47: 184-190 [PMID: 24355694 DOI: 10.1016/j.clinbiochem.2013.11.022]
 - 21 Marquardt JU, Quasdorff M, Varnholt H, Curth HM, Mesghenna S, Protzer U, Goeser T, Nierhoff D. Neighbor of Punc E11, a novel oncofetal marker for hepatocellular carcinoma. *Int J Cancer* 2011; 128: 2353-2363 [PMID: 20658536 DOI: 10.1002/ijc.25567]
 - 22 O'Beirne J, Farzaneh F, Harrison PM. Generation of functional CD8+ T cells by human dendritic cells expressing glypican-3 epitopes. *J Exp Clin Cancer Res* 2010; 29: 48 [PMID: 20465843 DOI: 10.1186/1756-9966-29-48]
 - 23 Gu DL, Chen YH, Shih JH, Lin CH, Jou YS, Chen CF. Target genes discovery through copy number alteration analysis in human hepatocellular carcinoma. *World J Gastroenterol* 2013; 19: 8873-8879 [PMID: 24379610 DOI: 10.3748/wjg.v19.i47.8873]
 - 24 Liu J, Ruan B, You N, Huang Q, Liu W, Dang Z, Xu W, Zhou T, Ji R, Cao Y, Li X, Wang D, Tao K, Dou K. Downregulation of miR-200a induces EMT phenotypes and CSC-like signatures through targeting the β -catenin pathway in hepatic oval cells. *PLoS One* 2013; 8: e79409 [PMID: 24260215 DOI: 10.1371/journal.pone.0079409]
 - 25 Arzumanyan A, Sambandam V, Clayton MM, Choi SS, Xie G, Diehl AM, Yu DY, Feitelson MA. Hedgehog signaling blockade delays hepatocarcinogenesis induced by hepatitis B virus X protein. *Cancer Res* 2012; 72: 5912-5920 [PMID: 22986746 DOI: 10.1158/0008-5472.CAN-12-2329]
 - 26 Pan Z, Chen C, Long H, Lei C, Tang G, Li L, Feng J, Chen F. Overexpression of GPC3 inhibits hepatocellular carcinoma cell proliferation and invasion through induction of apoptosis. *Mol Med Rep* 2013; 7: 969-974 [PMID: 23338845]
 - 27 Kessler SM, Pokorný J, Zimmer V, Laggai S, Lammert F, Bohle RM, Kierner AK. IGF2 mRNA binding protein p62/IMP2-2 in hepatocellular carcinoma: antiapoptotic action is independent of IGF2/PI3K signaling. *Am J Physiol Gastrointest Liver Physiol* 2013; 304: G328-G336 [PMID: 23257922 DOI: 10.1152/ajpgi.00005.2012]
 - 28 Wang D, Luo L, Chen W, Chen LZ, Zeng WT, Li W, Huang XH. Significance of the vascular endothelial growth factor and the macrophage migration inhibitory factor in the progression of hepatocellular carcinoma. *Oncol Rep* 2014; 31: 1199-1204 [PMID: 24366206]
 - 29 Moeini A, Cornella H, Villanueva A. Emerging signaling pathways in hepatocellular carcinoma. *Liver Cancer* 2012; 1: 83-93 [PMID: 24159576]
 - 30 Bertino G, Ardiri A, Malaguarnera M, Malaguarnera G, Bertino N, Calvagno GS. Hepatocellular carcinoma serum markers. *Semin Oncol* 2012; 39: 410-433 [PMID: 22846859 DOI: 10.1053/j.seminoncol.2012.05.001]
 - 31 Nassar A, Cohen C, Siddiqui MT. Utility of glypican-3 and survivin in differentiating hepatocellular carcinoma from benign and preneoplastic hepatic lesions and metastatic carcinomas in liver fine-needle aspiration biopsies. *Diagn Cytopathol* 2009; 37: 629-635 [PMID: 19405109 DOI: 10.1002/dc.21075]

编辑 郭鹏 电编 鲁亚静

