WCJD 世界华人消化杂志®

在线投稿: http://www.baishideng.com/wcjd/ch/index.aspx
帮助平台: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.11569/wcjd.v23.i9.1379

世界华人消化杂志 2015年3月28日; 23(9): 1379-1386
ISSN 1009-3079 (print) ISSN 2219-2859 (online)

述评 EDITORIAL

癌胚型GPC-3特异诊断原发性肝细胞癌及其靶向治疗进展

潘刘翊, 姚 敏, 王 理, 姚登福

潘刘翊, 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
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
姚登福, 教授, 主要从事分子消化病学和临床分子生物学的研究.
国家国际科技合作专项基金资助项目, No. 2013DFA32150
江苏省“六大人才高峰”基金资助项目,
Nos. 2014-YY-028, 2013-WSW-011
江苏省高校优秀青年教师青蓝工程基金资助项目,
No. 2014-NTU-YM
作者贡献分布: 此述评由潘刘翊、姚敏及王理共同撰写; 由姚登福审校.
通讯作者: 姚登福, 教授, 226001, 江苏省南通市西寺路20号, 南通大学附属医院临床医学研究中心. yaodf@ahnmc.com
电话: 0513-85052523
收稿日期: 2015-01-24 修回日期: 2015-02-14
接受日期: 2015-03-06 在线出版日期: 2015-03-28

Oncofetal glypican-3: Specific diagnosis and targeted-therapy for primary liver cancer

Liu-Hong Pan, Min Yao, Li Wang, Deng-Fu Yao

Liu-Hong Pan, Deng-Fu Yao, Research Center of Clinical Medicine, 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
Supported by: the International S&T Cooperation Program of China, No. 2013DFA32150; the Projects of Jiangsu Six Talents Park, Nos. 2014-YY-028 and 2013-WSW-011; the Qinglan Project of Jiangsu Higher Education, No. 2014-NTU-YM
Correspondence to: Deng-Fu Yao, Professor, Research Center of Clinical Medicine, Affiliated Hospital of Nantong University, 20 West Temple Road, Nantong 226001, Jiangsu Province, China. yaodf@ahnmc.com
Received: 2015-01-24 Revised: 2015-02-14
Accepted: 2015-03-06 Published online: 2015-03-28

Abstract

The development and progression of primary hepatocellular cancer (PHC) are a multi-factorial, multi-step, and multi-center complex process. Its early diagnosis and effective treatments are of the most importance. Glypican-3 (GPC-3) plays a crucial role in PHC progression. Increased GPC-3 expression has been found during hepatocyte malignant transformation. GPC-3 levels in PHC patients are related to HBV infection, TNM stage, periportal cancerous embolus, and extra-hepatic metastasis. Circulating GPC-3 or GPC-3 mRNA with AFP enhances the positive rate up to 94.3% for PHC diagnosis. Down-regulating GPC-3 by specific siRNA could alter liver cancer cell biological behaviors such as migration, metastasis, and invasion; and inhibit nude mouse xenograft growth with decreased β-catenin, p-GSK3β, and cyclin D1 expression, suggesting that oncofetal GPC-3 is not only a specific diagnostic biomarker for PHC, but also a promising target for PHC therapy.

Key Words: Primary hepatocellular cancer; Glypican-3; Diagnosis; Targeted therapy

Pan LH, Yao M, Wang L, Yao DF. Oncofetal glypican-3: Specific diagnosis and targeted-therapy

背景资料
磷脂酰肌醇蛋白多糖(glypican, GPC)家族6个亚型, 其中*GPC-3*基因大于900 kb, 5'-端朝向端粒区, 3'-端朝向中心粒区, 8个外显子和7个内含子, 分子量为66 kDa. GPC-3是硫酸乙酰肝素蛋白家族中的一员, 参与调控在细胞的增殖, 分化和迁移等过程. 在肝癌特异表达, 可用于诊断与治疗.

同行评议者
王阁, 教授, 中国人民解放军第三军医大学第三附属医院

研发前沿
抗GPC-3人单抗HS20, 能有效识别并结合GPC-3硫酸乙酰肝素链, 破坏Wnt3a和GPC-3结合, 阻断Wnt3a/β-catenin信号; 能抑制Wnt3a依赖的肝癌细胞增殖, 抑制裸鼠体内移植瘤生长, 为PHC分子靶向治疗的潜在靶点.

for primary liver cancer. Shijie Huaren Xiaohua Zazhi 2015; 23(9): 1379-1386 URL: http://www.wjgnet.com/1009-3079/23/1379.asp DOI: http://dx.doi.org/10.11569/wcjd.v23.i9.1379

摘要

原发性肝细胞性肝癌(primary hepatocellular cancer, PHC)的发生、发展是一个多因素、多步骤、多中心的复杂过程, 他的早期诊断和有效治疗至关重要. 磷脂酰肌醇蛋白多糖-3(glypican-3, GPC-3)在PHC进展过程中起重要作用. 在肝细胞恶性转化过程中GPC-3呈进行性升高表达; 人肝癌GPC-3阳性率与乙型肝炎表面抗原(hepatitis B surface antigen, HbsAg)、TNM分期、门脉癌栓及肝外转移显著相关. GPC-3及mRNA与甲胎蛋白(alpha fetoprotein, AFP)联合检测, 对PHC诊断阳性率可提高至94.3%; 沉默GPC-3可改变肝癌细胞迁移、转移和侵袭的生物学行为, 伴随β-catenin、p-糖原合成激酶3β(p-glycogen synthase kinase 3β, p-GSK3β)和cyclin D1表达明显减少, 提示癌胚性GPC-3不仅是PHC特异诊断标志物, 而且有可能成为PHC治疗的潜在靶点.

关键词: 原发性肝细胞性肝癌; 磷脂酰肌醇蛋白多糖-3; 诊断; 靶向治疗

核心提示: 磷脂酰肌醇蛋白多糖-3(glypican-3, *GPC-3*)基因位于人类X染色体(Xq26), 通过糖基磷脂酰肌醇锚定于细胞膜; 正常肝组织中无表达, 胎肝和肝癌组织呈高表达状态, 为原发性肝细胞性肝癌(primary hepatocellular cancer, PHC)特异诊断标志物. GPC-3羧基端近胞膜区域含两条具有功能的硫酸类肝素聚糖链, 可与成纤维生长因子、Wnts和Hedgehog等信号通路介导肝癌的发生发展; 干预GPC-3表达或转录, 可在体内、外明显抑制肝癌细胞增殖、诱导凋亡, 可望成为PHC基因治疗的潜在靶点.

潘刘翊, 姚敏, 王理, 姚登福. 癌胚型GPC-3特异诊断原发性肝细胞癌及其靶向治疗进展. 世界华人消化杂志 2015; 23(9): 1379-1386 URL: http://www.wjgnet.com/1009-3079/23/1379.asp DOI: http://dx.doi.org/10.11569/wcjd.v23.i9.1379

0 引言

原发性肝细胞性肝癌(primary hepatocellular cancer, PHC)为常见恶性肿瘤, 其防治仍是全世界的医学难题. PHC进展快、复发率高和预后差, 因患者早期通常无明显症状, 诊断困难, 确诊后仅10%-20%患者具有手术切除机会. 手术切除和肝移植仍是主要治疗方法, 术后易复发, 且对放、化疗不敏感, 寻找有效治疗方法尤为重要[1]. 磷脂酰肌醇蛋白多糖-3(glypican-3, *GPC-3*)基因位于人类X染色体(Xq26), 通过糖基磷脂酰肌醇(glycosyl-phosphatidyl inositol, GPI)锚定于细胞膜; 正常肝组织未见GPC-3表达, 胚胎肝和肝癌组织中GPC-3呈高表达状态, 被证实为PHC诊断的特异标志物[2]. 新近发现GPC-3分子羧基端近胞膜区域, 含两条具有功能的硫酸类肝素聚糖链, 可结合成纤维生长因子, 与Wnts、Hedgehog信号通路等协同作用, 介导PHC发生、发展. 在体内、外干扰或下调*GPC-3*基因转录, 可使肝癌细胞增殖周期发生阻滞、诱导细胞凋亡, 并抑制肝癌移植瘤生长, 提示该基因可望成为PHC基因治疗新的潜在靶点[3]. 本文述评了GPC-3作为诊断PHC生物标志及分子靶向治疗PHC的应用前景.

1 GPC-3与磷脂酰肌醇蛋白多糖家族

GPC家族主要有6个亚型, 基因各定位于不同区域, 在胚胎和成人组织中表达各异(表1). *GPC-3*基因全长大于900 kb, 5'-端朝向端粒区, 3'端朝向中心粒区, 由8个外显子和7个内含子组成. 分子量为66 kDa, 羧基端与糖基磷脂酰肌醇共价结合锚定于胞膜, 氨基端游离于胞外, 其内经多个二硫键连接, 使其具有球形立体结构. 蛋白聚糖由核心蛋白和糖胺聚糖(glycosaminoglycan, GAG)侧链构成, 核心蛋白富含14个半胱氨酸残基的独特保守序列, 位于中央区域; GAG侧链为肝素和硫酸乙酰肝素插入位点由羧基端50个氨基酸残基决定, 使该链靠近细胞膜. GPC-3是硫酸乙酰肝素蛋白家族中的一员, 他参与调控在细胞的增殖, 分化和迁移等过程[2,3,10-12].

GPC-3以GPI锚定在细胞膜上调控细胞生长过程, 在Arg358和Ser359位点处断裂后形成两个亚型: 可溶性N-端和与细胞膜结合C-端. Ser560是GPC-3核心蛋白和HS链结合Hh或FGF-2的一个切割位点. GPC-3与Hh信号分子结合或介导成纤维细胞生长因子2(fibroblast

表 1 GPC家族亚型、基因定位与组织表达

亚型	别名	基因定位	氨基酸	胚胎表达	成人表达	文献
GPC-1	GPC	2q35-37	558	骨、表皮、肾	多数组织	[4]
GPC-2	Cerebroglycan	7q22.1	579	神经系统	尚不清楚	[5]
GPC-3	OCI-5	Xq26	580	肝、胎盘等	卵巢、肺等	[6]
GPC-4	K-glypican	Xq26.1	556	脑、肾、肺	多数组织	[7]
GPC-5	-	13q21	572	脑、肺、肝等	脑	[8]
GPC-6	-	13q21	555	肝和肾等	卵巢、肾等	[9]

GPC: 磷脂酰肌醇蛋白多糖.

相关报道
Sawada等报道经Ⅰ期临床试验显示GPC-3衍生肽疫苗耐受性良好, 抗肿瘤效果显著. GPC-3肽特异性CTL数高者总生存率更高, 可作为肝癌患者接种肽疫苗后总生存率的预测指标.

growth factor 2, FGF-2)和人骨成型蛋白7 (human bone morphogenetic protein 7, BMP-7)信号分子, 参与调节细胞迁移、侵袭和凋亡[10,11].

2 GPC-3表达与肝细胞恶性转化

GPC-3是激活整合素信号通路的关键蛋白, 促进肝细胞恶性转化、增值及侵袭. GPC-3在胎肝组织中表达丰度最高, 正常肝组织未见表达; 肝细胞恶性转化是机体通过多种生物效应分子作用, 调节多信号传导的复杂过程, 该过程中*GPC-3*基因被激活. GPC-3表达经活化的经典Wnt信号通路, 促进PHC细胞增殖; 可导致Bax/Bcl-2/细胞色素c/Caspase3信号功能障碍, 抵抗细胞凋亡, 调节细胞增殖[13-16]; 可抑制BMP-7信号促使肝细胞恶性转化; 且可下调TGF-β2表达, 促进肝癌细胞增殖[17].

GPC-3可通过结合细胞外基质、生长因子和蛋白酶等参与调节肿瘤细胞的增殖、分化、黏附和转移等[3]. GPC-3异常表达与硫酸脂酶-2、锌指和同源框2和甲胎蛋白(alpha fetoprotein, AFP)等表达相关, 上调后激活整合素、IGF-Ⅱ和Wnt信号通路等促肝癌细胞生长, 影响多种信号传导途径, 导致肝细胞增殖、分化及癌变. 临床上, 肝癌在确诊时已多属中、晚期, 缺乏有效治疗, 预后较差[13]. GPC-3作为对肝癌的发生发展密切相关的分子, 已证实是PHC早期诊断的特异标志物.

3 人肝癌组织GPC-3异常表达

免疫组织化学显示70%-100%的PHC组织中, 可检测到GPC-3阳性表达(表2), 在病理组织学上呈现棕色巢状分布, 主要分布于细胞质和细胞膜上. GPC-3是调节发育的癌胚蛋白, 且作为早期PHC临床相关的分子标志物, 且在肝细胞恶性转化过程中为最先转录. 对PHC、癌旁和远癌组织的对照研究, 发现PHC组织中GPC-3阳性细胞数和染色深度, 均明显高于癌旁和远癌组织[18,24].

PHC组织GPC-3高表达, 纤维板层型肝癌组织GPC-3表达为64%[18], 肝母细胞瘤组织GPC-3全数表达, 另在高度不典型增生肝组织GPC-3阳性率为6%-22%, 胆管细胞型肝癌组织GPC-3表达在0%-10%[19,22](表2). 肝穿(hepatic fine needle aspirates, FNA)免疫组织化学显示HCC组织GPC-3免疫反应达83%-90%, 而良性病变组织和转移性肝癌除神经内分泌特征未分化癌外, 均无免疫反应[23].

4 肝病患者外周血GPC-3表达水平

AFP是现在临床常规使用以诊断PHC, 评估治疗疗效的一个相对特异指标. 然而单凭存在着假阴性或假阳性AFP指标, 有时无法鉴别PHC和良性肝病. 寻找一个合适的筛查指标, 将有助于早期诊断PHC并提高治疗效果. 目前, 虽有很多种作为诊断PHC的分子标记, 已在临床使用, 但只有为数不多的几个标志物, 具有较高敏感性和特异性如肝癌特异性γ-谷氨酰移换酶(HS-GGT), 肝癌特异性AFP(AFP-L3)和癌胚性GPC-3. 临床应用发现外周血中GPC-3, 在诊断和监测PHC转移方面的价值已被证实; 和血中AFP相比, GPC-3准确性更高, 是PHC患者的诊断、治疗疗效和预后监测的可靠指标[24].

对不同肝病组患者的外周血GPC-3分析发现, GPC-3阳性率在PHC组为52.8%, 良性肝病(急性肝炎、慢性肝炎、肝硬化)组为0.0%-1.4%, 非肝肿瘤组为2.0%, 组间差异显著; 良性肝病组除1例肝硬化外, 其余病例均阴性; 急、慢性肝炎组和健康对照组中, 未检出阳性病例; 和

创新盘点

人源化GC33缺乏糖基, 通过诱导ADCC和补体依赖性细胞毒性反应, 发挥抗肿瘤活性; 与索拉非尼联合治疗, 较单用索拉非尼对移植瘤抑制效率更高, 现已进入Ⅱ期临床试验.

表 2 PHC和其他恶性肝肿瘤GPC-3表达的免疫组织化学分析

组织	*n*	阳性数*n*(%)	抗体
肝细胞性肝癌	20	18(90.0)	单抗-IG12[18]
	56	47(84.0)	单抗, GPC-3-CO2[19]
	59	46(78.0)	单抗-IG12[20]
	58	46(79.0)	单抗-IG12[21]
	54	38(70.0)	单抗-IG12[22]
	42	40(95.2)	单抗-IG12[23]
	36	29(80.6)	单抗, 抗-GPC-3[24]
	58	55(94.8)	单抗-IG12[25]
	14	14(100.0)	PcAb, 抗-GPC-3[26]
纤维板层型肝癌	11	7(64.0)	单抗-IG12[21]
肝母细胞瘤	5	1(20.0)	单抗-IG12[22]
	6	6(100.0)	单抗, GPC-3-CO2[19]
	65	65(100.0)	单抗-IG12[27]
高度不典型增生	8	6(75.0)	单抗, GPC-CO2[19]
	33	2(6.0)	单抗-IG12[20]
	9	2(22.0)	单抗-IG12[22]
胆管细胞型肝癌	16	0(0.0)	单抗, GPC-3-CO2[19]
	10	1(10.0)	单抗-IG12[22]

PHC: 原发性肝细胞性肝癌; GPC-3: 磷脂酰肌醇蛋白多糖-3.

GPC-3相比, AFP阳性率在PHC组虽高达70.73%, 但良性肝病组, AFP假阳性率达14.3%-20.0%; PHC患者血GPC-3表达和瘤体大小相关(P<0.01)[24]. 外周血GPC-3诊断PHC敏感度53%, 特异性为99%[28]. PHC组GPC-3和AFP两者间并未见明显相关性, 联合检测可互补诊断PHC, 对小肝癌的诊断敏感性可达75%[28,29], 且有助于PHC的鉴别诊断.

5 GPC-3与PHC的预后

肝癌患者的GPC-3表达与其预后相关. 免疫组织化学分析107例肝癌患者术后标本GPC-3表达, 随访5年, 发现GPC-3阳性组患者死亡率明显高于阴性组(87.7% *vs* 54.5%); 手术切除的80例患者中, 16例GPC-3阴性患者中, 5年内未见死亡(0/16), 多因素分析显示GPC-3表达是肝癌预后的独立相关因素[30]. 另一研究[28]提示, 肝癌复发患者血GPC-3阳性率高达61.1%, 提示GPC-3可能与肝癌复发相关, 定期监测治疗后肝癌患者血GPC-3, 可早期诊断肝癌复发. PHC患者经导管肝动脉栓塞术(transcatheter arterial embolization, TAE)治疗前血GPC-3及AFP表达水平, 均显著高于正常对照组, 经1-3次TAE治疗后, 有效组患者血GPC-3水平, 较治疗前显著降低(P<0.05), 而无效组GPC-3变化不明显, 提示GPC-3可作为PHC患者预后判断的有用指标.

6 GPC-3作为新PHC靶向治疗

6.1 抗GPC-3抗体使用 GPC-3作为PHC的一个新靶点, 对GPC-3抗体的研究文献较多, 前期研究中, 将M18D04、M19B11、A1836A和GPC-3-CO2等单抗, 用于肝癌组织GPC-3免疫组织化学分析[31]. 首个治疗单抗GC33通过与GPC-3羧基末端的表位作用, 诱导抗体依赖性的细胞毒性作用, 显著抑制肝癌HepG2和Huh7细胞的移植瘤及原位癌生长, 还可明显降低血AFP水平, 对不表达GPC-3的肝癌SK-HEP-1细胞无明显作用; 人源化GC33(humanized GC33, hGC33)具有和上述GC33相似的抑制移植瘤增殖效应, hGC33缺乏糖基, 主要通过诱导ADCC和补体依赖性细胞毒性反应, 发挥抗肿瘤活性; 肿瘤相关的巨噬细胞与GC33非ADCC机制所致的抗肿瘤效应密切相关, 可通过增加HCC细胞对化疗药物敏感性, 抑制GPC-3阳性细胞增殖. hGC33与索拉非尼联合治疗, 较单用索拉非尼对移植瘤抑制效率更高, 现已进入Ⅱ期临床试验[32]. 抗GPC-3人单抗HS20, 能有效识别

表 3 GPC-3作为肝癌治疗新靶目标的研究进展

组别	名称	种属	抗原或基因	进展
抗体	M18D04, M19B11	鼠	GPC-3 N末端(残基: 25-358)	前期基础研究[31]
	A1836A	鼠	GPC-3 N末端	前期基础研究[31]
	GPC3-C02	鼠	GPC-3 C末端	前期基础研究[31]
	GC33	鼠	GPC-3 C末端(残基: 52-563)	临床前期研究[31]
	hGC33	人GC33	GPC-3 C末端(残基: 524-563)	Ⅱ期临床试验[32]
	HS20	人	GPC-3硫酸乙酰肝素链	临床前试验[4]
	MDX-1414	人	-	临床前评估阶段[33]
	YP7	人	-	临床前评估阶段[34]
	HN3	人	-	临床前评估阶段[35]
	sGPC-3	人	-	临床前试验[36]
疫苗	GPC-3 298-306	鼠	多肽298-306	Ⅱ期临床试验[37,38]
	GPC-3 144-152	鼠	多肽144-152	Ⅱ期临床试验[40]
miRNA	miR-219-5p	人	GPC-3 mRNA	体外及体内研究[15]
	miR-520c-3p	人	GPC-3 mRNA	体外研究[45]
	miR-1271	人	GPC-3 mRNA	体外及体内研究[46]
shRNA	GPC-3 shRNA	人	GPC-3 mRNA	体外及体内研究[48]
siRNA	GPC-3 siRNA	人	GPC-3 mRNA	体外及体内研究[49]

GPC-3: 磷脂酰肌醇蛋白多糖-3.

应用要点
GPC-3及其mRNA已用于肝癌诊断. 针对GPC-3的PHC治疗研究, 已进入Ⅱ期临床试验. 特异靶向于肝癌细胞, 降低GPC-3表达, 抑制移植瘤生长, 具有明显的应用前景, 期待深入研究.

并结合GPC-3硫酸乙酰肝素链, 破坏Wnt3a和GPC-3结合, 阻断Wnt3a/β-catenin信号. 此外, HS20能抑制体外Wnt3a依赖的肝癌细胞增殖, 也能抑制裸鼠体内移植瘤生长, 为PHC分子靶向治疗的潜在靶点[4].

人源化MDX-1414、YP7及HN3抗体, 现处于临床前评估阶段. 抗重链可变区抗体HN3, 与细胞表面GPC-3有高亲和力, 可调节YAP通路使细胞周期阻滞在G_1期, 抑制GPC-3阳性肝癌细胞增殖, 显著抑制裸鼠移植瘤生长[33-35]. 因GPC-3可通过促进或稳定与Frizzled间作用, 激活Wnt通路, 缺乏HS链的sGPC-3可阻断硫酸肝素类生长因子激活Wnt信号通路, 抑制肝癌细胞增殖和血管生长; 另还可抑制Erk-1/2和Akt相关通路, 阻断肝癌Huh7和HepG2细胞的Erk1/2和Akt磷酸化作用[36].

6.2 GPC-3肿瘤疫苗 针对GPC-3肿瘤疫苗已进入临床试验阶段. 以鼠GPC-3转染Colon26细胞株后接种GPC-3衍生和Kd-限制性细胞毒性T淋巴细胞(cytotoxic T lymphocyte, CTL)表位肽抑制肿瘤生长, 证实GPC-3具较高免疫原性, 有效诱导抗肿瘤免疫反应且无自身免疫性. HLA-A24(A*2402)和H-2Kd-限制性GPC-3298-306(EYILSLEEL)或HLA-A2(A*0201)-限制性GPC-3144-152(FVGEFFTDV)与弗氏不完全佐剂混合能诱导产生GPC-3 CTLs, GPC-3衍生肽疫苗通过诱导CTL反应发挥抗肿瘤效应, 疫苗免疫效应呈剂量依赖性, 该疫苗处于中晚期肝癌治疗Ⅱ期临床试验阶段[37,38]. Ⅰ期临床试验显示GPC-3衍生肽疫苗耐受性良好, 抗肿瘤效果显著. GPC-3肽特异性CTL数高者总生存率更高, 可作为肝癌患者接种肽疫苗后总生存率的预测指标[39]. 系列研究[40]表明CTLs具高度亲和力, 对接种GPC-3-144-152肽疫苗有临床疗效的肝癌患者, CTLs可诱导产生肿瘤细胞天然抗原特异性杀伤活性.

6.3 *GPC-3*基因治疗 已有GPC-3 miRNA、shRNA和siRNA抑制PHC增殖的报道[41-44]. miR-219-5p在肝癌细胞株中表达明显降低, 与瘤体大小、分化及总存活时间密切相关. 体外可抑制细胞增殖并使细胞周期G_1期阻滞, 降低GPC-3 mRNA及蛋白水平, 抑制癌细胞增殖[15]. miR-520c-3p及miR-1271能特异性靶向作用于肝癌细胞株, 降低GPC-3表达, 诱导肝癌细胞凋亡并抑制增殖与侵袭[45,46]. 另特异性shRNA沉默*GPC-3*基因转录, 能明显抑制肝癌细胞增殖, 细胞周期阻滞在G_1期, 且能诱导肝癌细胞凋亡. 移植瘤模型转染shRNA能有效抑制瘤体生长[47,48]. GPC-3 siRNA可明显抑制HepG2及Huh7细胞增殖与侵袭, 均已得到体内、外研究结果的证实[49,50](表3).

名词解释
蛋白聚糖: 由核心蛋白和糖胺聚糖(GAG)侧链构成, 核心蛋白富含14个半胱氨酸残基的独特保守序列, 位于中央区域; GAG侧链为肝素和硫酸乙酰肝素插入位点由羧基端50个氨基酸残基决定, 使该链靠近细胞膜.

7 结论

癌胚型GPC-3和PHC发生, 发展及预后相关. 癌组织GPC-3 mRNA和蛋白表达明显升高, 是肝癌诊断和预后的相关指标. 针对GPC-3的PHC靶向治疗研究正在深入, 已进入Ⅱ期临床试验阶段. 小干扰RNA的干预作用, 在体内、外研究模型中已得到证实, miR-219-5p、miR-520c-3p及miR-1271能特异靶向于肝癌细胞, 降低GPC-3表达, 抑制移植瘤生长, 具有应用前景, 期待着更深入的临床研究.

8 参考文献

1 El-Serag HB. Epidemiology of viral hepatitis and hepatocellular carcinoma. *Gastroenterology* 2012; 142: 1264-1273.e1 [PMID: 22537432 DOI: 10.1053/j.gastro.2011.12.061]
2 Filmus J, Capurro M. The role of glypican-3 in the regulation of body size and cancer. *Cell Cycle* 2008; 7: 2787-2790 [PMID: 18787398]
3 Kwack MH, Choi BY, Sung YK. Cellular changes resulting from forced expression of glypican-3 in hepatocellular carcinoma cells. *Mol Cells* 2006; 21: 224-228 [PMID: 16682817]
4 David G, Lories V, Decock B, Marynen P, Cassiman JJ, Van den Berghe H. Molecular cloning of a phosphatidylinositol-anchored membrane heparan sulfate proteoglycan from human lung fibroblasts. *J Cell Biol* 1990; 111: 3165-3176 [PMID: 2148568]
5 Stipp CS, Litwack ED, Lander AD. Cerebroglycan: an integral membrane heparan sulfate proteoglycan that is unique to the developing nervous system and expressed specifically during neuronal differentiation. *J Cell Biol* 1994; 124: 149-160 [PMID: 8294498]
6 Filmus J, Church JG, Buick RN. Isolation of a cDNA corresponding to a developmentally regulated transcript in rat intestine. *Mol Cell Biol* 1988; 8: 4243-4249 [PMID: 3185547]
7 Watanabe K, Yamada H, Yamaguchi Y. K-glypican: a novel GPI-anchored heparan sulfate proteoglycan that is highly expressed in developing brain and kidney. *J Cell Biol* 1995; 130: 1207-1218 [PMID: 7657705]
8 Veugelers M, Vermeesch J, Reekmans G, Steinfeld R, Marynen P, David G. Characterization of glypican-5 and chromosomal localization of human GPC5, a new member of the glypican gene family. *Genomics* 1997; 40: 24-30 [PMID: 9070915]
9 Paine-Saunders S, Viviano BL, Saunders S. GPC6, a novel member of the glypican gene family, encodes a product structurally related to GPC4 and is colocalized with GPC5 on human chromosome 13. *Genomics* 1999; 57: 455-458 [PMID: 10329016]
10 Gao W, Kim H, Feng M, Phung Y, Xavier CP, Rubin JS, Ho M. Inactivation of Wnt signaling by a human antibody that recognizes the heparan sulfate chains of glypican-3 for liver cancer therapy. *Hepatology* 2014; 60: 576-587 [PMID: 24492943 DOI: 10.1002/hep.26996]
11 Gao W, Ho M. The role of glypican-3 in regulating Wnt in hepatocellular carcinomas. *Cancer Rep* 2011; 1: 14-19 [PMID: 22563565]
12 Wang Y, Yang H, Xu H, Lu X, Sang X, Zhong S, Huang J, Mao Y. Golgi protein 73, not Glypican-3, may be a tumor marker complementary to α-Fetoprotein for hepatocellular carcinoma diagnosis. *J Gastroenterol Hepatol* 2014; 29: 597-602 [PMID: 24236824 DOI: 10.1111/jgh.12461]
13 Fico A, Maina F, Dono R. Fine-tuning of cell signaling by glypicans. *Cell Mol Life Sci* 2011; 68: 923-929 [PMID: 18087675]
14 Lee YL, Ahn BC, Lee Y, Lee SW, Cho JY, Lee J. Targeting of hepatocellular carcinoma with glypican-3-targeting peptide ligand. *J Pept Sci* 2011; 17: 763-769 [PMID: 21976137 DOI: 10.1002/psc.1400]
15 Huang N, Lin J, Ruan J, Su N, Qing R, Liu F, He B, Lv C, Zheng D, Luo R. MiR-219-5p inhibits hepatocellular carcinoma cell proliferation by targeting glypican-3. *FEBS Lett* 2012; 586: 884-891 [PMID: 22449976 DOI: 10.1016/j.febslet.2012.02.017]
16 Filmus J, Capurro M. Glypican-3: a marker and a therapeutic target in hepatocellular carcinoma. *FEBS J* 2013; 280: 2471-2476 [PMID: 23305321 DOI: 10.1111/febs.12126]
17 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 DOI: 10.3892/mmr.2013.1279]
18 Kandil D, Leiman G, Allegretta M, Trotman W, Pantanowitz L, Goulart R, Evans M. Glypican-3 immunocytochemistry in liver fine-needle aspirates : a novel stain to assist in the differentiation of benign and malignant liver lesions. *Cancer* 2007; 111: 316-322 [PMID: 17763368]
19 Yamauchi N, Watanabe A, Hishinuma M, Ohashi K, Midorikawa Y, Morishita Y, Niki T, Shibahara J, Mori M, Makuuchi M, Hippo Y, Kodama T, Iwanari H, Aburatani H, Fukayama M. The glypican 3 oncofetal protein is a promising diagnostic marker for hepatocellular carcinoma. *Mod Pathol* 2005; 18: 1591-1598 [PMID: 15920546]
20 Libbrecht L, Severi T, Cassiman D, Vander Borght S, Pirenne J, Nevens F, Verslype C, van Pelt J, Roskams T. Glypican-3 expression distinguishes small hepatocellular carcinomas from cirrhosis, dysplastic nodules, and focal nodular hyperplasia-like nodules. *Am J Surg Pathol* 2006; 30: 1405-1411 [PMID: 17063081]
21 Shafizadeh N, Ferrell LD, Kakar S. Utility and limitations of glypican-3 expression for the diagnosis of hepatocellular carcinoma at both ends of the differentiation spectrum. *Mod Pathol* 2008; 21: 1011-1018 [PMID: 18536657 DOI: 10.1038/modpathol.2008.85]
22 Wang XY, Degos F, Dubois S, Tessiore S, Allegretta M, Guttmann RD, Jothy S, Belghiti J, Bedossa P, Paradis V. Glypican-3 expression

in hepatocellular tumors: diagnostic value for preneoplastic lesions and hepatocellular carcinomas. *Hum Pathol* 2006; 37: 1435-1441 [PMID: 16949914]
23 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]
24 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]
25 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]
26 Nakatsura T, Kageshita T, Ito S, Wakamatsu K, Monji M, Ikuta Y, Senju S, Ono T, Nishimura Y. Identification of glypican-3 as a novel tumor marker for melanoma. *Clin Cancer Res* 2004; 10: 6612-6621 [PMID: 15475451]
27 Zynger DL, Gupta A, Luan C, Chou PM, Yang GY, Yang XJ. Expression of glypican 3 in hepatoblastoma: an immunohistochemical study of 65 cases. *Hum Pathol* 2008; 39: 224-230 [PMID: 17949790]
28 Tangkijvanich P, Chanmee T, Komtong S, Mahachai V, Wisedopas N, Pothacharoen P, Kongtawelert P. Diagnostic role of serum glypican-3 in differentiating hepatocellular carcinoma from non-malignant chronic liver disease and other liver cancers. *J Gastroenterol Hepatol* 2010; 25: 129-137 [PMID: 19793164 DOI: 10.1111/j.1440-1746.2009.05988.x]
29 Liu H, Li P, Zhai Y, Qu CF, Zhang LJ, Tan YF, Li N, Ding HG. Diagnostic value of glypican-3 in serum and liver for primary hepatocellular carcinoma. *World J Gastroenterol* 2010; 16: 4410-4415 [PMID: 20845507]
30 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 in hepatocellular carcinoma. *Cancer Sci* 2009; 100: 1403-1407 [PMID: 19496787 DOI: 10.1111/j.1349-7006]
31 Ho M, Kim H. Glypican-3: a new target for cancer immunotherapy. *Eur J Cancer* 2011; 47: 333-338 [PMID: 21112773 DOI: 10.1016/j.ejca.2010.10.024]
32 Allegretta M, Filmus J. Therapeutic potential of targeting glypican-3 in hepatocellular carcinoma. *Anticancer Agents Med Chem* 2011; 11: 543-548 [PMID: 21554204]
33 Feng M, Ho M. Glypican-3 antibodies: a new therapeutic target for liver cancer. *FEBS Lett* 2014; 588: 377-382 [PMID: 24140348 DOI: 10.1016/j.febslet.2013.10.002]
34 Phung Y, Gao W, Man YG, Nagata S, Ho M. High-affinity monoclonal antibodies to cell surface tumor antigen glypican-3 generated through a combination of peptide immunization and flow cytometry screening. *MAbs* 2012; 4: 592-599 [PMID: 22820551 DOI: 10.4161/mabs.20933]
35 Feng M, Gao W, Wang R, Chen W, Man YG, Figg WD, Wang XW, Dimitrov DS, Ho M. Therapeutically targeting glypican-3 via a conformation-specific single-domain antibody in hepatocellular carcinoma. *Proc Natl Acad Sci U S A* 2013; 110: E1083-E1091 [PMID: 23471984 DOI: 10.1073/pnas.1217868110]
36 Takahashi-Yanaga F, Kahn M. Targeting Wnt signaling: can we safely eradicate cancer stem cells? *Clin Cancer Res* 2010; 16: 3153-3162 [PMID: 20530697 DOI: 10.1158/1078-0432.CCR-09-2943]
37 Sawada Y, Yoshikawa T, Fujii S, Mitsunaga S, Nobuoka D, Mizuno S, Takahashi M, Yamauchi C, Endo I, Nakatsura T. Remarkable tumor lysis in a hepatocellular carcinoma patient immediately following glypican-3-derived peptide vaccination: an autopsy case. *Hum Vaccin Immunother* 2013; 9: 1228-1233 [PMID: 23466818 DOI: 10.4161/hv.24179]
38 Nobuoka D, Yoshikawa T, Sawada Y, Fujiwara T, Nakatsura T. Peptide vaccines for hepatocellular carcinoma. *Hum Vaccin Immunother* 2013; 9: 210-212 [PMID: 23442593 DOI: 10.4161/hv.22473]
39 Sawada Y, Yoshikawa T, Nobuoka D, Shirakawa H, Kuronuma T, Motomura Y, Mizuno S, Ishii H, Nakachi K, Konishi M, Nakagohri T, Takahashi S, Gotohda N, Takayama T, Yamao K, Uesaka K, Furuse J, Kinoshita T, Nakatsura T. Phase I trial of a glypican-3-derived peptide vaccine for advanced hepatocellular carcinoma: immunologic evidence and potential for improving overall survival. *Clin Cancer Res* 2012; 18: 3686-3696 [PMID: 22577059 DOI: 10.1158/1078-0432.CCR-11-3044]
40 Tada Y, Yoshikawa T, Shimomura M, Sawada Y, Sakai M, Shirakawa H, Nobuoka D, Nakatsura T. Analysis of cytotoxic T lymphocytes from a patient with hepatocellular carcinoma who showed a clinical response to vaccination with a glypican 3 derived peptide. *Int J Oncol* 2013; 43: 1019-1026 [PMID: 23903757 DOI: 10.3892/ijo.2013.2044]
41 Sun CK, Chua MS, He J, So SK. Suppression of glypican 3 inhibits growth of hepatocellular carcinoma cells through up-regulation of TGF-β2. *Neoplasia* 2011; 13: 735-747 [PMID: 21847365]
42 Liu S, Li Y, Chen W, Zheng P, Liu T, He W, Zhang J, Zeng X. Silencing glypican-3 expression induces apoptosis in human hepatocellular carcinoma cells. *Biochem Biophys Res Commun* 2012; 419: 656-661 [PMID: 22382024 DOI: 10.1016/j.bbrc.2012.02.069]
43 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]
44 Dong Z, Yao M, Wang L, Yang J, Yao D. Down-regulating Glypican-3 Expression: Molecular-

同行评价
本文重点阐述GPC-3与肝癌发生发展的关系以及其成为肝癌靶向治疗的潜在分子, 整篇文章结构合理, 数据详实可靠, 是一篇比较优秀的述评文章.

targeted Therapy for Hepatocellular Carcinoma. *Mini Rev Med Chem* 2014; 14: 1183-1193 [PMID: 25553423]

45 Miao HL, Lei CJ, Qiu ZD, Liu ZK, Li R, Bao ST, Li MY. MicroRNA-520c-3p inhibits hepatocellular carcinoma cell proliferation and invasion through induction of cell apoptosis by targeting glypican-3. *Hepatol Res* 2014; 44: 338-348 [PMID: 23607462 DOI: 10.1111/hepr.12121]

46 Maurel M, Jalvy S, Ladeiro Y, Combe C, Vachet L, Sagliocco F, Bioulac-Sage P, Pitard V, Jacquemin-Sablon H, Zucman-Rossi J, Laloo B, Grosset CF. A functional screening identifies five microRNAs controlling glypican-3: role of miR-1271 down-regulation in hepatocellular carcinoma. *Hepatology* 2013; 57: 195-204 [PMID: 22865282 DOI: 10.1002/hep.25994]

47 Yao M, Wang L, Dong Z, Qian Q, Shi Y, Yu D, Wang S, Zheng W, Yao D. Glypican-3 as an emerging molecular target for hepatocellular carcinoma gene therapy. *Tumour Biol* 2014; 35: 5857-5868 [PMID: 24633918 DOI: 10.1007/s13277-014-1776-5]

48 Ruan J, Liu F, Chen X, Zhao P, Su N, Xie G, Chen J, Zheng D, Luo R. Inhibition of glypican-3 expression via RNA interference influences the growth and invasive ability of the MHCC97-H human hepatocellular carcinoma cell line. *Int J Mol Med* 2011; 28: 497-503 [PMID: 21617840 DOI: 10.3892/ijmm.2011.704]

49 Miao HL, Pan ZJ, Lei CJ, Wen JY, Li MY, Liu ZK, Qiu ZD, Lin MZ, Chen NP, Chen M. Knockdown of GPC3 inhibits the proliferation of Huh7 hepatocellular carcinoma cells through down-regulation of YAP. *J Cell Biochem* 2013; 114: 625-631 [PMID: 23060277 DOI: 10.1002/jcb.24404]

50 Akutsu N, Yamamoto H, Sasaki S, Taniguchi H, Arimura Y, Imai K, Shinomura Y. Association of glypican-3 expression with growth signaling molecules in hepatocellular carcinoma. *World J Gastroenterol* 2010; 16: 3521-3528 [PMID: 20653060]

编辑: 郭鹏 电编: 都珍珍