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肝癌组织中 miR-338-3p 的表达及与临床病理参数的关系

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摘要 目的：探讨肝癌组织中微小 RNA-338-3p（miR-338-3p）的表达及与临床病理参数的关系。**方法**：选取 2015 年 1 月至 2016 年 6 月我院手术获得的 67 例肝癌组织标本，同时每例标本均取癌旁正常组织标本作为配对对照，采用实时定量逆转录聚合酶链反应（RT-qPCR）对两组组织标本中的 miR-338-3p 进行检测，并分析其与肝癌临床病理特征的关系。**结果**：45 例（67.16%）miR-338-3p 表达下调，22 例（32.85%）表达上调；RT-qPCR 结果显示，肝癌组织中 miR-338-3p 的相对含量为（0.76± 0.38），低于癌旁正常组织中的（1.23± 0.45），差异有统计学意义（t=-6.259，P=0.000）。miR-338-3p 在低分化、TNM 分期 III+IV 期、肿瘤浸润深度 T3+T4 期、有淋巴结转移肝癌患者肝癌组织中的表达下调率高于中高分化、I+II 期、T1+T2 期、无淋巴结转移肝癌患者，差异有统计学意义（P<0.05）。不同性别、年龄、病理类型、肿瘤大小肝癌患者肝癌组织中 miR-338-3p 表达下调率差异无统计学意义（P>0.05）。**结论**：miR-338-3P 在肝癌组织中呈低表达水平，与分化程度、TNM 分期、肿瘤浸润深度、淋巴结转移有关，可能参与了肝癌的发生发展过程，早期检测可作为评估肝癌病情的指标。

关键词：肝癌；组织；miR-338-3p；病理参数；临床意义

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Expression of miR-338-3p and its Relationship with Clinicopathological Parameters in Hepatocellular Carcinoma

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ABSTRACT Objective: To explore the expression of microRNA-338-3p (miR-338-3p) in hepatocellular carcinoma and its relationship with clinicopathological parameters. **Methods:** A total of 67 hepatocellular carcinoma tissue samples obtained by surgery from January 2015 to June 2016 in our hospital were selected, and adjacent normal tissue samples matched to each hepatocellular carcinoma tissue sample were selected. The miR-338-3p in tissue samples of the two groups were detected by the real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR), and the relationship between miR-338-3p and clinicopathologic features of hepatocellular carcinoma was analyzed. **Results:** There were 45 cases (67.16%) of miR-338-3p down expression, 22 cases (32.85%) of up expression; RT-qPCR results showed that the relative content of miR-338-3p in hepatocellular carcinoma tissues was (0.76± 0.38), lower than (1.23 ± 0.45) in the adjacent normal tissues, the difference was statistically significant (t=-6.259, P=0.000). The expression down-regulation rate of miR-338-3p in hepatocellular carcinoma tissue samples of patients with poorly differentiated, III&IV stages of TNM, T3&T4 stages of tumor infiltrating depth, lymph node metastasis were higher than those with well differentiated, I&II stages, T1&T2 stages, without lymph node metastasis, the differences were statistically significant (P<0.05). The differences of the expression down-regulation rate of miR-338-3p in different gender, age, pathological type and tumor size were not statistically significant (P>0.05). **Conclusion:** MiR-338-3p expression was low in hepatocellular carcinoma tissues, which may be related to the degree of differentiation, TNM stage, depth of tumor invasion and lymph node metastasis. MiR-338-3p may be involved in the development and progression of hepatocellular carcinoma, and early detection of miR-338-3p can be used as an indicator to evaluate hepatocellular carcinoma condition.

Key words: Hepatocellular carcinoma; Tissue; miR-338-3p; Pathological parameters; Clinical significance

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前言

肝癌是临床常见的恶性肿瘤，具有发病率高、预后差等特点，报道显示[1,2]，肝癌的发病率呈逐渐上升趋势，我国每年的肝癌死亡人数占全球 45%，高达 11 万人。肝癌发病隐匿，早期无特异性的临床症状和体征，很多患者确诊时已处于中晚期。根治性切除术联合术后放、化疗是目前临床治疗肝癌的常见方式，但是临床报道显示，患者术后易发生淋巴结转移，预后差，5 年生存率低[3]。早发现、早诊断及早治疗是有效改善肝癌患者预后的重要措施[4]。近年来，在分子水平上研究恶性肿瘤的发生发展过程成为研究热点。微小 RNA（microRNA，miRNA）长约 22 个核苷酸，是真核生物中的非编码小分子 RNA。miR-338-3p 是新近发现的 miRNA，报道表明[5,6]，它通过介导肿瘤细胞的侵

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袭、转移而参与结直肠癌、肝癌的发生发展过程。关于miR-338-3p在肝癌中的表达,目前相关的研究相对较少,本研究通过检测肝癌组织中miR-338-3p的表达水平,并分析其与临床病理特征的关系,旨在探讨miR-338-3p在肝癌发生发展过程中的作用。

1 资料与方法

1.1 一般资料

选取2015年1月至2016年6月我院手术获得的67例经病理组织学确诊为肝癌的组织标本,同时对于每1例肝癌患者,均取距离癌组织边缘>5 cm的癌旁正常组织标本为配对对照,其中肝癌患者男39例,女28例;年龄41~76岁,平均(59.5± 8.2)岁;分化程度:低分化23例,中、高分化44例;病理类型:腺癌35例,鳞癌24例,腺鳞癌8例;肿瘤大小:<5 cm 24例,≥ 5c m 43例;根据UICC第7版TNM分期[7]:I+II期38例,III+IV期29例;浸润深度:T1+T2 33例,T3+T4 34例;有淋巴结转移36例,无淋巴结转移31例。手术切除的两种组织在30 min内制成标本以提取RNA,将采集到的标本迅速置于液氮中进行冷冻,并放在-80℃的环境下保存。研究中纳入标本的患者在手术前及手术后均未接受放、化疗及其它抗肿瘤治疗,同时排除其它类型的恶性肿瘤,标本的使用获得患者或家属知情同意。本研究获得医院伦理委员会的批准。

1.2 方法

1.2.1 主要仪器及试剂 总TRIzol试剂盒由美国Invitrogen公司提供,Toyobo公司提供RNA逆转录试剂盒,ABI公司提供miR-338-3p试剂盒,美国Thermo Scientific公司提供的Revert Aid First Strand cDNA Synthesis试剂盒。

1.2.2 提取及鉴定RNA 将保存于液氮中的肝癌组织标本及癌旁正常组织标本碾碎为粉末,严格按照TRIzol试剂盒上的说明提取总RNA,并通过紫外分光光度法提取RNA的纯度和浓度,在RNA的OD260/OD280吸光度值为1.8~2.1时,提取得到的总RNA的纯度较好。采用琼脂糖电泳实验对RNA的完整性进行检测。

1.2.3 逆转录聚合酶链反应(RT-qPCR) miR-338-3p的基因序列为,根据该基因序列设计反转录引物以及上下游引物(由华大基因科技有限公司操作),反向通用引物为:,上游引物为:,下游引物为:,内参为RNU6B。

参照Revert Aid First Strand cDNA Synthesis试剂盒上的说明书逆转录合成cDNA第1链子,RT-qPCR总反应体系为2020 μL,分别为2 μL稀释比例为1:10的cDNA,10 μL的miRcute miRNA Premix(with SYBR&ROX)(2×),7.2 μL的灭菌用水,0.4 μL的前向引物,0.4 μL的反向引物。PCR反应条件:94℃环境下变性2 min,94℃12 s,60℃34s,如此反复40个循环,采用比较Ct法($2^{-\Delta\Delta\alpha}$法)对实验数据进行相对定量分析,Δ Ct=Ct标本-CtRNU6B,Δ Δ CT=Δ ct癌组织-Δ Ct癌旁正常组织,其中RNU6B为内参照,组织标本中miR-338-3p相对含量采用$2^{-\Delta\Delta\alpha}$表示,即肝癌组织中miR-338-3p相对癌旁正常组织的倍数。miR呈高水平表达则Δ Δ CT为负值,反之,miR表达下调则Δ Δ CT为正值。

1.3 统计学处理

实验中所有数据采用SPSS19.0软件录入及统计学分析,采用($\bar{x}\pm s$)对定量资料进行描述,配对样本的比较采用配对t检验,定性资料采用率(%)表示,比较采用χ^2检验,P<0.05提示差异有统计学意义。

2 结果

2.1 miR-338-3p在肝癌组织及癌旁正常组织中的表达比较

本研究中,45例(67.16%)miR-338-3p表达下调,22例(32.85%)表达上调;RT-qPCR结果显示,肝癌组织中miR-338-3p的相对含量为(0.76± 0.38),低于癌旁正常组织中的(1.23± 0.45),差异有统计学意义(t=-6.259,P=0.000),说明miR-338-3p在肝癌组织中呈低表达水平。

2.2 肝癌组织中miR-338-3p与临床病理特征的关系

miR-338-3p在低分化、TNM分期III+IV期、肿瘤浸润深度T3+T4期、有淋巴结转移肝癌患者肝癌组织中表达下调率高于中高分化、I+II期、T1+T2期、无淋巴结转移肝癌患者,差异有统计学意义(P<0.05),不同性别、年龄、病理类型、肿瘤大小肝癌患者肝癌组织中miR-338-3p表达下调率差异无统计学意义(P>0.05)。见表1。

3 讨论

肝癌是临床常见的恶性肿瘤,严重威胁人类健康和生命质量,随着医学技术的不断进步,肝移植、外科切除等为肝癌的治愈提供了途径,但是,报道显示,肝癌术后复发和转移率较高,预后较差,5年生存率低[8,9]。miRNAs在胚胎发育、细胞分裂、分化、凋亡过程,以及恶性肿瘤的发生等方面有重要作用,Zhang Y[10]的研究显示,超过50%的miRNAs基因在肿瘤相关的基因组,如容易发生移位、断裂、重复或者缺失区域等脆弱位点,通过调控致癌基因或者抑癌基因而参与恶性肿瘤的侵袭、转移等过程。有研究显示,miR-193b、miR-125a-5p、miR-133b等在胃癌的形成过程中呈低水平表达,miR-421、miR-106a、miR-19a等在胃癌发生过程中表达上调,而miR-192与胃癌细胞的增殖及浸润密切关系,miR-451的表达下调则与胃癌的预后有关,可见miRNA参与了胃癌发生发展的整个过程[11-13]。此外,miRNAs中的miR-122a在肝癌组织中表达下调,宫颈癌中miR-143、miR-126表达下调,而在甲状腺瘤中miR-222、miR-221表达上调[14,15]。可见,miRNA表达谱在不同类型肿瘤发生过程中发生不同程度的改变。

miR-338-3p是由22个核苷酸所组成的位于第17号染色体上的单链非编码RNA小分子[16],miR-338-3p通过特异性的识别并且指导沉默复合体降解靶mRNA,以及结合靶mRNA3'端非翻译区,或者抑制靶mRNA的翻译而介导基因的表达过程[17]。有研究表明[11],在胃癌的发生及发展过程中,miRNA-338-3p通过调控P-Rex2a/PTEN/AKT通路而参与恶性肿瘤细胞的增殖、侵袭。Sun K[18]的研究显示,miR-338-3p在结直肠癌组织中低表达,作为抑癌基因,与结肠癌的侵袭、转移高度相关,可将其作为判断结直肠癌预后的重要生物标志物。关于miR-338-3p在肝癌组织中的表达情况,目前相关的研究相对较少。

本研究结果显示,肝癌组织中miR-338-3p的相对含量低

表 1 肝癌组织中 miR-338-3p 与临床病理特征的关系

Table 1 Relationship between clinicopathologic features and miR-338-3p in hepatocellular carcinoma tissues

Pathologic features		Cases	MiR-338-3p expression		χ^2	P
			Down-regulation	Up-regulation		
Gender	male	39	24(61.54)	15(38.46)	1.339	0.247
	female	28	21(75.00)	7(25.00)		
Age(year)	＜50	21	13(61.90)	8(38.10)	0.384	0.536
	≥ 50	46	32(69.57)	14(30.43)		
Histological grade	low differentiation	23	20(86.96)	3(13.04)	6.221	0.013
	high/medium differentiation	44	25(56.82)	19(43.18)		
Pathological type	adenocarcinoma	35	27(77.14)	8(22.86)	3.498	0.174
	squamous cell carcinoma	24	13(54.17)	11(45.83)		
	adenosquamous carcinoma	8	5(62.50)	3(37.50)		
Tumor size(cm)	＜5	24	13(54.17)	11(45.83)	2.865	0.091
	≥ 5	43	32(74.42)	11(25.58)		
TNM staging	I+II	38	21(55.26)	17(44.74)	5.638	0.018
	III+IV	29	24(82.76)	5(17.24)		
Depth of infiltration	T1+T2	33	18(54.55)	15(45.45)	4.695	0.030
	T3+T4	34	27(79.41)	7(20.59)		
Lymph node metastasis	yes	36	33(91.67)	3(8.33)	21.181	0.000
	no	31	12(38.71)	19(61.29)		

于癌旁正常组织，说明 miR-338-3p 在肝癌组织中呈低表达水平，其中 67.16%的肝癌组织 miR-338-3p 表达水平低于癌旁正常组织，提示 miR-338-3p 可能通过介导细胞周期蛋白 D1 而对 HBx 缺失突变体产生影响，进而参与肝癌的发生过程，可作为早期辅助诊断肝癌的重要分子标志物，与有关研究结果一致[19]，即 miR-338-3p 能够抑制肝癌细胞的侵袭和转移。肿瘤的 TNM 分期、分化程度、淋巴结转移等临床病理特征与肿瘤的发展过程密切相关，TNM 分期越晚，分化程度越低，淋巴结转移的患者肿瘤恶性程度越高，预后则越差。有研究显示[20]，miR-338-3p 与胃癌的 TNM 分期、病灶浸润深度以及淋巴结转移呈显著的相关性，TNM 分期越晚，病灶浸润越深、有淋巴结转移时，miR-338-3p 表达越下调，可作为评估胃癌预后的指标。本研究结果显示，miR-338-3p 在低分化、III+IV 期、T3+T4 期以及淋巴结转移肝癌患者肝癌组织中的表达下调率较高，而与性别、年龄、病理类型、肿瘤大小等特征无明显关系，miR-338-3p 与肝癌分化程度、TNM 分期、肿瘤浸润深度以及淋巴结转移明显相关，提示 miR-338-3p 可能参与了肝癌的发展过程。

综上所述，miR-338-3p 在肝癌组织中呈低表达水平，且和分化程度、TNM 分期、肿瘤浸润深度、淋巴结转移有关，可能参与了肝癌的发生及发展进程，早期检测有助于辅助诊断，以及评估肝癌病情严重程度，从而为临床制定干预措施改善预后提供指导。

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