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·临床研究·

胃癌患者血清中miR-133a水平及其与病理特征的相关性

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[摘要] **目的**:探讨血清中miR-133a水平对胃癌的诊断价值及其与病理特征的相关性。**方法**:取2010年3月2011年4月于南京医科大学附属常州市第二附属医院胃肠外科住院的胃癌手术患者94例(胃癌组),男64例、女30例,年龄32~82岁,平均(61.72±8.59)岁;取30例健康对照者作为(对照组)。实时荧光定量PCR法检测两组血清中miR-133a水平,应用SPSS22.0和Graphpad5.0统计软件进行统计学分析。**结果**:胃癌患者血清中miR-133a水平明显低于与正常对照组(1.95±1.51 *vs* 4.47±0.56,*P*<0.01)。患者miR-133a水平与其年龄、性别、肿瘤大小无明显相关性(*P*>0.05),与肿瘤分化程度、浸润深度、淋巴结转移及TNM分期有关(*P*<0.05)。受试者工作特征曲线(ROC)分析结果显示,miR-133a在预测胃癌发生和淋巴结转移时具有较好的敏感性和特异性[ROC曲线下面积(AUC)分别为0.883(95%CI:0.8257-0.9402)和0.984(95%CI:0.9633-1.005),均*P*<0.01]。miR-133a高表达组患者5年生存率显著高于miR-133a低表达组(40.49% vs 19.37%,*P*<0.05)。**结论**:胃癌患者血清miR-133a表达水平改变与胃癌的发生发展、临床病理特征及临床预后密切相关,miR-133a可能成为胃癌诊断及预后判断的液体活检标志物。

[关键词] miR-133a;血清;胃癌;TNM分期;预后
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Correlation between serum miR-133a level and pathologic features in gastric cancer patients

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[Abstract] Objective: To investigate the correlation between serum miR-133a level and pathological features of gastric cancer, and to explore the diagnostic and prognostic value of miR-133a. **Method:** Ninety-four gastric cancer patients treated in the Department of Gastroenterology Surgery of Affiliated Changzhou N0.2 People's Hospital of Nanjing Medical University from March 2010 to April 2011 were included in this study; there were 64 male cases and 30 female cases, aged from 32-82 years old with an mean age of (61.72±8.59) years old; Another 30 healthy subjects were enrolled as control. RT-PCR was used to detect the serum miR-133a expression of two groups. SPSS22.0 and Graphpad5.0 were used for statistical analysis. **Result:** The levels of serum miR-133a in gastric cancer patients was obviously lower than those in healthy controls (1.95±1.51 *vs* 4.47±0.56, *P*=0.000). miR-133a expression was significantly associated with the degree of differentiation、depth of invasion, lymph node metastasis and TNM stage (*P*<0.05), but not obviously associated with age、sex and tumor size (*P*>0.05). ROC curve analysis showed high sensitivity and specificity of miR-133a in the prediction of gastric cancer (the AUC value of miR-133a was 0.883, 95% CI 0.8257-0.9402) and the prediction of lymph node metastasis (the AUC value of miR-133a was 0.984, 95%CI 0.9633-1.005). The 5-year survival rate of patients with high miR-133a expression was significantly higher than that of low expression group (40.49% *vs* 19.37%, *P*<0.05). Conclusion: The expression of miRNA-133a was closely associated with gastric cancer development, its clinicopathological features and patient's clinical prognosis. It may be used as a potential diagnostic bio-marker and a prognostic predictor for gastric cancer.

[Key words] miR-133a; serum; gastric cancer; TNM stage; prognosis
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胃癌是常见的消化道恶性肿瘤，由于其早期临床表现不明显，故绝大多数患者被确诊时已经为进展期。miRNA是一类小分子非编码RNA，包含约22个核苷酸，在肿瘤的发生发展中发挥了重要作用[1][2]。多种miRNA具有促癌或抑癌作用。血液中miRNA能够游离于细胞之外，稳定存在于循环血液中[3-5]，其异常表达与多种人类疾病密切相关，并在多种疾病诊断和预后判断中展现了独特的价值。血清miRNA检测，不仅创伤小、方法简便、结果准确可靠，而且可以提高疾病诊断、癌症分类、预后评估、疗效及复发预测的精度。前期研究[6]发现，miR-133a的在胃癌组织中表达水平显著下调，但是外周血中表达水平如何尚不清楚，也未见其与胃癌的诊断、肿瘤分型及预后的相关性报道。因此本实验拟通过实时荧光定量PCR（qPCR）检测胃癌患者及健康正常对照者外周血中miR-133a的表达水平，探讨miR-133a与胃癌的诊断、临床病理特征和预后分析的相关性。

1 材料与方法

1.1 研究对象

选取2010年3月2011年4月于南京医科大学附属常州二院胃肠外科住院的胃癌手术患者94例（胃癌组），男64例、女30例，年龄32~82岁，平均（61.72±8.59）岁。所有病例术前均未进行过放疗和化疗，术后均经病理证实为胃癌。另选取同期健康体检者30例（对照组），均排除消化系统良性病变及肿瘤相关疾患，男19例、女11例，年龄49~71岁，平均（61.88±6.42）岁。本研究均征得患者及家属知情同意并签署知情同意意书，并获得医院伦理委员会的批准。

1.2 主要试剂

RNA提取试剂盒TRIzol购自Invitrogen公司，逆转录试剂盒购自TaKaRa公司，qPCR试剂购自Vazyme公司，miR-133a、内参U6引物均购自广州锐博生物公司。

1.3 方法

术前采集患者空腹静脉血5ml，3 000×*g*离心10 min分离血清，置于−80℃保存。按照产品说明书提取RNA，以U6作为内参，qPCR检测血清中miR-133a的表达水平，结果采用$2^{-\Delta\Delta Ct}$法进行相对定量分析。实验独立重复3次。

1.4 统计学方法

采用SPSS22.0和Graphad Prism 5.0统计软件分析，组间比较采用独立样本*t*检验，受试者工作特征曲线（ROC）用于分析miR-133a在预测胃癌发生和淋巴转移时的敏感度及特异度，Kaplan-Mieimer法及Log-Rank法用于生存分析和生存率比较，$P<0.05$或$P<0.01$表示差异有统计学意义。

2 结　果

2.1 miR-133a在胃癌患者血清中表达水平下降

miR-133a在胃癌患者血清中的水平明显低于对照组血清（1.95±1.51 *vs* 4.47±0.56，$P<0.01$），有淋巴结转移的患者血清中miR-133a水平显著低于无淋巴结转移患者（1.02±0.30 *vs* 3.92±1.11，$P<0.05$；图1）。

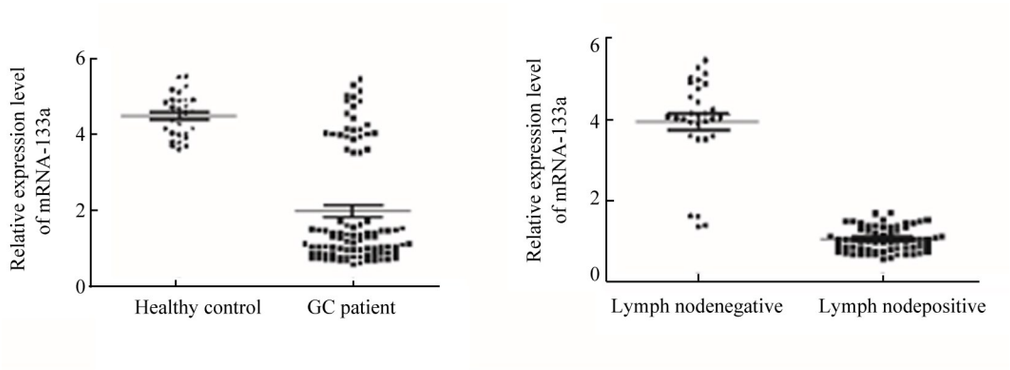

图1 miR-133a在胃癌患者血清中表达水平下降

Fig.1 Expression of miR-133a decreased in serum of GC patients

2.2 血清miR-133a的表达水平与胃癌临床病理特征间的关系

在胃癌患者外周血清中，miR-133a水平与患者年龄、性别、肿瘤大小无明显相关性（$P>0.05$）；但与肿瘤分化程度、浸润深度、淋巴结转移及TNM分期有关（均$P<0.05$）。肿瘤分化程度差、浸润深度深、淋巴结转移、TNM分期晚的胃癌患者外周血miR-133a表达水平较低（表1）。

2.3 血清miR-133a预测胃癌发生有较高敏感性和特异性

ROC分析结果（图2）显示，miR-133a预测胃癌发生时，其ROC曲线下面积（AUC）为0.883(95%CI为

0.8257~0.9402,P<0.0001)。诊断界值为3.62时,敏感度为76.60%(95%CI为0.6674~0.8471),特异度为96.67%(95%CI为0.8278~0.9992)。miR-133a预测胃癌发生有较高敏感性和特异性。

表1 血清中miR-133a表达与胃癌临床病理特征相关性($\bar{x}\pm s$)

Tab.1 Correlation between serum miR-133a expression and clinicopathological parameters in patients with gastric cancer

Item	n	Serum miR-133a	P
Sex			0.593
Male	64	2.01±1.61	
Female	30	1.82±1.31	
Age (t/a)			0.395
≤60	44	1.81±1.50	
＞60	50	2.08±1.52	0.059
Size(d/cm)			
≤5	70	1.77±1.41	
＞5	24	2.45±1.72	0.000
Degree of differentiation			
Well and moderately	26	4.30±0.56	
Poorly	68	1.05±0.31	0.000
Lymph node metastasis			
Yes	64	1.02±0.30	
No	30	3.92±1.11	0.000
Depth of invasion			
T1+T2	28	4.11±0.90	
T3+T4	66	1.04±0.30	0.000
TNM stage			
Ⅰ+Ⅱ	32	3.78±1.21	
Ⅲ+Ⅳ	62	1.01±0.28	

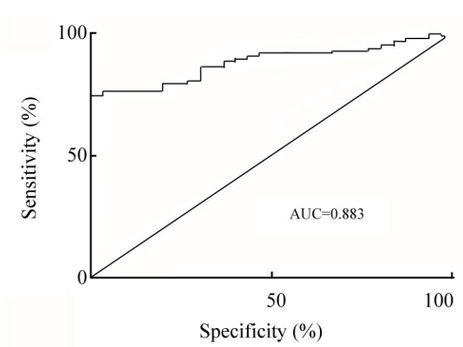

图2 ROC曲线显示miR-133a预测胃癌发生有较高敏感性和特异性

Fig.2 ROC curve shows that miR-133a has high sensitivity and specificity for predicting gastric cancer

2.4 血清miR-133a预测胃癌肿瘤淋巴转移有较高敏感性和特异性

进一步评价miR-133a预测肿瘤淋巴转移的价值,ROC曲线分析结果(图3)显示,血清miR-133a在预测淋巴结转移时,其AUC值为0.984(95%CI为0.9633~1.005,P<0.0001)。诊断界值为1.56时,敏感度为96.88%,95%CI为0.8916~0.9962,特异度为93.33%(95%CI为0.7793~0.9918)。miR-133a预测肿瘤淋巴转移发生有较高敏感性和特异性。

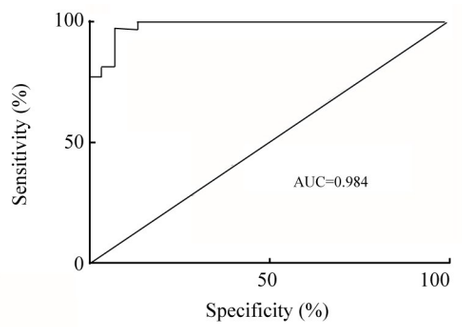

图3 血清miR-133a预测胃癌肿瘤淋巴转移有较高的敏感性和特异性

Fig.3 ROC curve shows that miR-133a has high sensitivity and specificity for predicting gastric cancer metastasis

2.5 血清miR-133a高表达组生存率显著高于miR-133a低表达组

根据胃癌患者血清中miR-133a表达水平差异(平均差异大于2倍)将患者分为低表达组(n=68)和高表达组(n=26)两组。分析结果(图4)显示,miR-133a高表达组5年生存率显著高于miR-133a低表达组(40.49% *vs* 19.37%,P<0.05)。

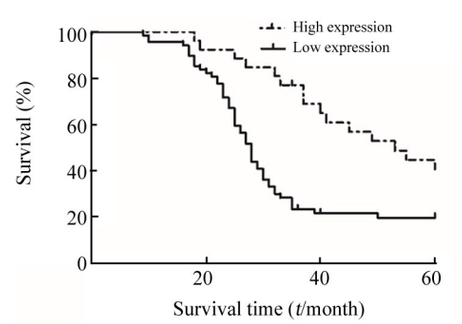

图4 胃癌患者的血清miRNA-133a水平与生存曲线

Fig.4 Kaplan-Meier survival curve of gastric cancer patients based on serum miR-133a expression level

3 讨 论

miRNA为一类长度约为18-25核苷酸的小型非编码RNA,在进化过程中高度保守。miRNA能够通过种子序列识别特定的目标mRNA,并在转录后水平通过促进目标mRNA的降解(或)抑制翻译过程而

发挥负调控基因表达的生物学作用，从而在细胞增殖、分化、凋亡以及应激反应中起到至关重要的调控作用[7]。另外，miRNA可以作为癌基因或抑癌基因参与癌症的发生与发展[8]。miRNA的异常表达在肿瘤的发生、进展、诊断、治疗和预后评估等方面极具研究价值[9]。

miR-133a属于miR-133家族,其编码基因包括miR-133a-1和miR-133a-2，分别位于第18和20号染色体，两者拥有共同的成熟序列：uuggucccuucaaccagcug。miR-133a是一类研究十分广泛且在肌肉组织中特异性表达的miRNA。研究[10]发现miR-133a、miR-1和miR-206在人和小鼠的心肌、骨骼肌中富集表达，这也首次将它们定义为肌肉特异性miRNAs。从此之后，越来越多的研究者对miR-133a的功能展开研究。Chen等[11]首次发现miR-133a能够调控肌肉细胞的增殖以及骨骼肌和心肌的分化。随后，大量研究[12]表明miR-133a在恶性肿瘤发生发展中发挥至关重要的调控作用。He等[13]报道miR-133a-3p可直接作用于COL1A1，减少其表达并抑制口腔鳞状细胞癌的增殖和迁移。Li等[14]通过一系列实验发现miR-133a在结肠癌中作用于eIF4A1，扮演肿瘤抑制者的角色。Cai等[15]研究发现miR-133a可通过AKT信号通路促进胰腺癌的进展，并且与胰腺癌较差的预后密切相关。Gao等[16]检测了126例食管癌组织，发现miR-133a明显降低，与食管癌肿瘤大小及TNM分期关系密切，并且可作为较差预后的独立预后因素。Wang等[17]报道在非小细胞肺癌中，miR-133a表达降低，与肿瘤的TNM分期、临床分期及生存期呈负相关。Huang等[18]发现miR-133a在胆囊癌中表达降低，过表达miR-133a抑制胆囊癌细胞的增殖、迁移和侵袭。此外该研究发现miR-133a作用于直接RBPJ，过表达RBPJ可逆转miR-133a对胆囊癌细胞的增殖、迁移和侵袭作用。Chen等[19]检测了64例结直癌患者癌组织及相应癌旁组织中miR-1-133a的表达量，发现miR-1-133a在癌组织中表达明显下调；进一步研究发现在miR-1-133a基因启动子区域富含CpG岛，且在结直肠癌组织中该CpG岛高度甲基化，这表明miR-1-133a的表达下调是由启动子区DNA高度甲基化介导的；同时还发现癌基因TAGLN2在结直肠癌表达增强。该研究表明DNA甲基化介导miR-1-133a沉默，从而使TAGLN2表达失控是结直肠癌发生的机制之一。以上研究表明miR-133a在多种肿瘤中通过调控不同的靶基因发挥抑癌作用，是潜在的肿瘤生物标志物。

课题组前期研究[6]结果显示，miR-133a在胃癌组织和胃癌细胞中低表达，而在胃癌细胞中过表达miR-133a能够抑制肿瘤细胞的增值、迁移和侵袭，并诱导肿瘤细胞凋亡，而这种抑癌作用是通过抑制下游靶基因IGF1R的表达实现的。IGF1R是酪氨酸激酶胰岛素受体家族成员之一，其在许多癌症中过度表达，是一个促癌基因[20]。更有研究[21-22]表明，IGF1R的异常表达与胃癌淋巴转移密切相关，而IGF1R阻滞剂能够降低胃肠肿瘤的浸润和增强对抗肿瘤药物的耐受性。在前期研究基础上，本课题进一步探讨了miR-133a作为血清生物标志物对胃癌患者诊断及预后的可能性，发现胃癌患者中血清miR-133a的表达显著低于正常对照组，且表达水平与患者的年龄、性别、肿瘤大小无明显相关性，但与肿瘤分化程度、浸润深度、淋巴结转移及TNM分期有关。肿瘤分化程度差、浸润深度深、淋巴结有转移的胃癌患者血清miR-133a的表达水平显著降低；同时Kaplan-Mieimer生存曲线分析显示，高表达miR-133a胃癌患者的5年生存率显著高于低表达组。为进一步评价miR-133a预测肿瘤发生及转移的价值，进行ROC曲线分析，结果显示在预测胃癌发生时，AUC值为0.883，诊断界值为3.62时，敏感度为76.60%，特异度为96.67%；在预测淋巴结转移中，其AUC值为0.984，诊断界值为1.56时，敏感度为96.88%，特异度为93.33%。以上结果说明胃癌患者血清中miR-133a的表达在胃癌的诊断和预后判断中有一定的价值，是一种潜在的新型肿瘤标志物。

综上所述，miR-133a在胃癌患者外周血中水平显著下调，与胃癌的肿瘤分化程度、浸润深度、TNM分期和淋巴结转移相关，提示血清miR-133a可以作为临床评估胃癌发生、发展、转移的潜在新型肿瘤标志物，并可通过液体活检作为判断预后的可靠指标，有可能进一步为胃癌的治疗提供新的思路和方向。

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