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·临床研究·

miR-133a-3p在胃癌组织和血浆中的表达及其对胃癌细胞增殖的影响

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[摘 要] **目的**:检测miR-133a-3p在胃癌组织及患者血浆中的表达水平及其对胃癌细胞增殖的影响,并探讨其与患者预后的关系。**方法**:收集河北医科大学第四医院普外科2012年5月至2013年5月胃癌手术切除的组织标本及术前外周静脉血标本52例,每例组织标本采集肿瘤组织(非坏死部分)和配对的癌旁组织。采用实时荧光定量RT-PCR(RT-qPCR)法检测miR-133a-3p在胃癌组织、癌旁组织、血浆及健康体检者血浆(35例)的表达情况,分析miR-133a-3p的表达水平与胃癌患者中位DFS及临床病理特征的关系。CCK-8法检测过表达或沉默miR-133a-3p对胃癌SGC7901细胞增殖的影响。**结果**:miR-133a-3p在胃癌组织中的表达明显降低(P<0.01),其表达水平与肿瘤TNM分期、肿瘤侵润深度(T)、淋巴结转移(N)及脉管瘤栓相关(P<0.01);在胃癌患者血浆中的表达明显升高(P<0.01),其表达水平与肿瘤TNM分期、淋巴结转移(N)及脉管瘤栓相关(P<0.05);与患者血清中CA199的表达水平正相关(P<0.01)。胃癌组织中miR-133a-3p高表达组患者中位DFS明显高于低表达组(20.8 *vs* 14.8个月,P<0.05)。血浆中miR-133a-3p的高表达组患者中位DFS明显低于低表达组(14.4 *vs* 20.3个月,P<0.05)。过表达miR-133a-3p可明显抑制SGC7901细胞的增殖能力,同样沉默其表达可明显促进SGC701细胞的增殖能力(均P<0.05)。**结论**:miR-133a-3p可明显抑制胃癌细胞SGC7901的增殖能力,在胃癌组织和血浆中存在明显异常表达,其表达与患者预后明显相关,可作为胃癌早诊早治及患者临床预后判定的潜在标志物。

[关键词] 胃癌;SGC7901细胞;miR-133a-3p;增殖;预后
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Expression of miR-133a-3p in gastric cancer tissues and plasma and its effect on proliferation of gastric cancer cells

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[Abstract] Objective: To detect the expression of miR-133a-3p in gastric cancer (GC) tissues and plasma of GC patients, and to investigate its effect on the proliferation of GC cells as well as its correlation toprognosis of GC patients. **Methods:** 52 cases of cancertissues (non-necrosis part) and corresponding adjacent tissues as well as the pre-operative peripheral blood samples from GC patients, who underwent surgery at Department of General Surgery, the Forth Hospital of Hebei Medical University(Shijiazhuang, China) between May 2012 and May 2013, were collected for this study. The plasma sample (n=35) from healthy donors were obtained during their physical examination. RT-qPCR was adopted to detect the expression of miR-133a-3p in gastric cancer tissues, adjacent tissuesand plasma samples of GC patients and healthy volunteers. The relationships between miR-133a-3p expression and the median DFS as well as clinicopathological parameters were also analyzed. CCK-8 assay was adopted to detect the effect of miR-133a-3p silence or over-expression on proliferation of gastric cancer SGC7901 cells. **Results:** miR-133a-3p was dramatically decreased in gastric cancer tissues (P<0.01), and its expression was associated with TNM stage, tumor infiltration (T), lynphonode metastasis (N), and vascular tumor thrombus (all P<0.01); miR-133a-3p was significantly increased in the plasma of GC patients (P<0.01), and its expression was associated with TNM stage, lynphonode metastasis (N), and vascular tumor thrombus (all P<0.05). miR-133a-3p expression was positively correlated with serum CA199 level of GC patients (P<0.01). The median DFS of patients with high miR-133a-3pexpression in cancer tissues was significantly longer than that of the patients with low expression(20.8 *vs* 14.8 months, P<0.05); The median DFS of patients with high plasma

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miR-133a-3p expression was significantly shorter than that of the patients with low expression (14.4 *vs* 20.3 months, $P<0.05$). Over-expression of miR-133a-3p could significantly inhibit the proliferation of gastric cancer SGC7901 cells, while miR-133a-3p silence could significantly promote the proliferation (all $P<0.05$). **Conclusion:** miR-133a-3p could significantlyinhibit the proliferation of SGC7901 cells; miR-133a-3p aberrantlyexpressed in gastric cancer tissues and plasma, and obviously correlated with prognosis of gastric cancer patients, which may be used as a potential clinical bio-maker for early diagnosis and treatment as well as the prognosis prediction of gastric cancer.

[Key words] gastric cancer; SGC7901 cells; miR-133a-3p; proliferation; prognosis

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胃癌是全球发病率第4位、病死率第3位的恶性肿瘤，东亚地区是全球胃癌发病率最高的地区，患病人数约占全球全部胃癌患病人数的2/3[1]。我国胃癌致死病例占全世界胃癌致死总数的40%左右[2]。早期、局部晚期和存在远处转移胃癌患者的5年生存率存在显著差异，分别为65.4%、29.9%和4.5%[3]。由于早期胃癌无明显临床症状，加之我国胃癌的早诊早治工作开展时间短，辐射范围相对有限，其发现率仅为10%～20%，大部分胃癌患者确诊时已属晚期[4]。因此，寻找一种廉价、省时、省力，能够在早期诊断和术后预警胃癌患者生存的常规检测指标具有重要的临床意义。miRNA是一种长度19～24 nt的单链非编码RNA，通过与其靶基因3′非编码区（3′ untranslated region, 3′UTR）的相互结合抑制靶基因mRNA的表达或直接降解靶基因mRNA而发挥转录后调控的作用[5-6]。miR-133a在食管癌[7]、非小细胞肺癌[8]、膀胱癌[9]、结直肠癌[10]、口腔鳞状细胞癌[11]、胰腺癌[12]等多种肿瘤中存在异常表达，并在肿瘤的侵袭转移、增殖、凋亡及药物耐药方面发挥重要的调节作用。有研究[13-15]发现，miR-133a在胃癌中的表达也存在显著异常，并抑制胃癌发生发展。本研究探讨miR-133a-3p在胃癌患者癌组织及血浆中的表达情况，分析其与胃癌患者预后、临床病理特征及胃癌肿瘤标记物的关系；并观察miR-133a-3p对胃癌细胞增殖的影响，以期为胃癌的早诊早治和预后判定提供参考。

1　资料与方法

1.1　临床资料

收集河北医科大学第四医院普外科2012年5月至2013年5月胃癌手术切除的组织标本及术前外周静脉血标本52例。每例标本采集肿瘤组织(非坏死部分)和配对癌旁组织(距肿瘤组织>5 cm)，取出后立即放入冻存管液氮保存。纳入标准：病理学诊断为胃腺癌，患者术前未行任何放疗及辅助治疗；排除标准：病理学诊断为非腺癌。于病案室收集患者姓名、性别、年龄、手术记录、病理号码、病理报告等临床资料。52例胃癌患者中，男性46例、女性6例，年龄41～78岁、平均(59.2±8.72)岁。按临床病理资料进行分析，包括浸润深度(T1、T2、T3 和 T4)、淋巴结转移(N0、N1、N2、N3)、肿瘤TNM分期(I、II、III和IV)、病理组织学分级（高-中分化和低分化）和脉管瘤栓(阴性/阳性)，其中TNM分期依照第七版《AJCC癌症分期手册》进行。52例胃癌患者具体临床资料见表1。同时收集健康体检者外周静脉血标本35例，男性25例、女性10例，年龄24～77岁、平均(50.6±14.9)岁。研究方案已经通过医院伦理委员会批准。

1.2　主要试剂

TRIzol试剂、CCK-8细胞增殖检测试剂盒、实验相关引物均购自美国Invitrogen公司，逆转录试剂盒、GoTaq® qPCR Master Mix、GoTaq® Hot Start Green Master Mix均购自美国Fementas公司，miRNeasy Serum / Plasma Kit、miRNeasy Serum / Plasma Spike-In Control、miScript miR-133a-3p mimic、miScript miR-133a-3p inhibitor、AllStars Negative Control siRNA、HiperFect transfection Reagent均购自美国QIAGEN公司，所有无RNA酶相关耗材均购自美国AXYGEN公司。

1.3　RT-qPCR检测miR-133a-3p在胃癌组织、胃癌患者血浆及健康志愿者血浆中的表达

TRIzol法提取组织中总RNA，按miRNeasy Serum/Plasma Kit和miRNeasy Serum/Plasma Spike-In Control说明书提取血浆中的总RNA，并加入C. elegans miR-39 miRNA mimic作为对照。检测提取的RNA浓度、纯度和完整性，应用Fementas公司的反转录试剂盒将miRNA反转录为cDNA。参照GoTaq® qPCR Master Mix说明书推荐配制反应溶液，将PCR反应溶液置于ABI 7500仪器Real-time PCR仪上进行PCR反应。循环设置为：预变性（95 ℃ 2 min→95 ℃ 15 s）、热循环（60 ℃ 1 min→95 ℃ 15 s）、熔解曲线（60 ℃ 15 s→95 ℃ 15 s）。数据采用$RQ=2^{-\triangle\triangle Ct}$计算相对定量结果，组织标本以*U6*作为内参，血液标本以C. elegans miR-39作为内参，每个组织样本独立实验3次。所用特异引物如下：hsa-miR-133a-3p - RT: GTCGTATCCAGTGCGTGTCGTGGAGTCGGCAATTGCACTGGATACGACAGCTG；hsa-U6-RT:CGCTTCACGAATTTGCGTGTCAT；hsa-miR-133a-3p-Forward: GGCCTTTGGTCCCCTTCAAC；*U6*-

Forward:GCTTCGGCAGCACATATACTAAAAT；Universal Primer: CAGTGCGTGTCGTGGAGT。C. elegans miR-39所需引物为试剂盒提供。

1.4 CCK-8法检测过表达或沉默miR-133a-3p对胃癌细胞SGC7901细胞增殖的影响

按照HiperFect transfection Reagent试剂盒说明书对胃癌细胞SGC7901分别进行miR-133a-3p mimics、miR-133a-3p inhibitor和Negative Control siRNA转染。将转染后SGC-7901细胞按照3 000个/孔的浓度接种于96孔培养板，每组设5个复孔，37 ℃ 5% CO_2培养箱内培养。按CCK-8试剂盒说明书分别检测各转染组细胞在24、48和72 h的增殖情况。

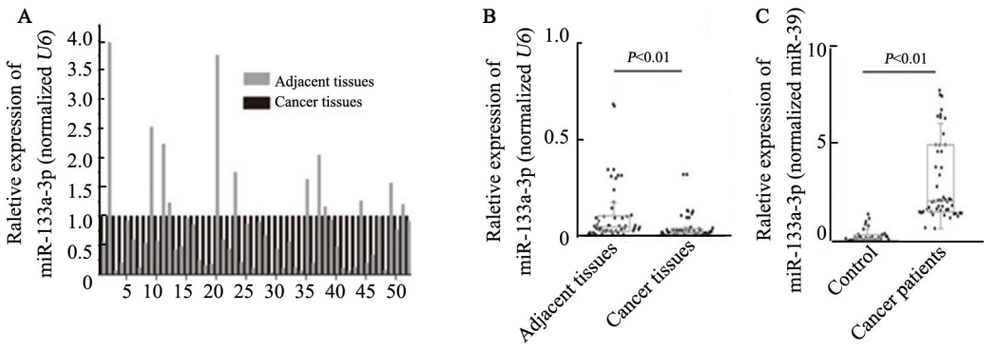
1.5 统计学处理

利用SPSS16.0统计学软件，组织及血浆中基因的表达用中位数和四分位间距表示，采用Wilcoxon和Mann-Whitney U符号秩检验分析其表达差异；分析miR-133a-3p的表达水平与临床病理参数的相关性时，两组比较用Mann-Whitney U秩和检验，3组及以上组间比较采用Kruskal-Wallis检验，采用Spearman检验miR-133a-3p与患者血浆肿瘤标记物的关系。以中位数为界点将46例根治术患者分组，采用所Kaplan-Meier生存曲线和Breslow-Wilcoxon检验miR-133a-3p表达水平与DFS的关系。以P<0.05或P<0.01表示差异有统计学意义。

2 结 果

2.1 miR-133a-3p在胃癌组织和血浆中的表达呈显著负相关

miR-133a-3p在组织及血浆中的表达水平均采用中位数（四分位间距）表示，在癌组织和癌旁组织中的表达分别为0.02（0.02）和0.05（0.08），在胃癌患者和健康志愿者血浆中的表达分别为2.07（3.45）和0.15（0.25）。胃癌组织中miR-133a-3p的表达显著低于癌旁组织（P<0.01，图1A、B）。与健康志愿者相比，miR-133a-3p在胃癌患者血浆中的表达显著升高（P<0.01，图1C）。miR-133a-3p在胃癌患者组织和血浆中的表达呈显著负相关（r=−0.85，P<0.01）。



A, B: RT-qPCR analysis of miR-133a-3p in gastric cancer tissues and paired adjacent tissues. C: RT-qPCR analysis of miR-133a-3p in plasma of healthy controls and gastric cancer patients. Data of miR-133a-3p expression ware the average of 3 separate experiments.

图1 miR-133a-3p在胃癌组织、癌旁组织及外周静脉血中的表达

Fig.1 Expression of miR-133a-3p in gastric cancer tissues, adjacent tissues and plasma

2.2 胃癌组织和血浆中miR-133a-3p表达水平与患者临床病理特征的关系

miR-133a-3p在胃癌组织中的表达水平与患者肿瘤脉管瘤栓、TNM分期及T、N、M相关（P<0.01）。患者血浆中表达水平与脉管瘤栓、肿瘤TNM分期及N、M相关（P<0.01），且与患者外周血CA199的水平正相关（r=0.454，P<0.01）。

2.3 胃癌组织和外周血中miR-133a-3p表达水平与患者中位DFS的关系

胃癌组织中miR-133a-3p高表达组与低表达组的中位DFS为20.8个月和14.8个月，miR-133a-3p的表达水平与患者中位DFS呈正相关（P=0.013，图2A）。胃癌患者血浆中miR-133a-3p高表达组与低表达组的中位DFS为14.4个月和20.3个月，miR-141-3p的表达水平与患者中位DFS呈负相关（r=−1.01，P=0.028，图2B）。

2.4 miR-133a-3p对胃癌细胞株SGC-7901细胞增殖的影响

与阴性对照组比较，SGC-7901细胞转染48 h后，miR-133a-3p在miR-133a-3p mimics组细胞中的表达显著升高（P<0.01），在miR-133a-3p inhibitor组细胞中的表达显著降低（P<0.01，图3A）。与阴性对照组比较，转染72 h后miR-133a-3p mimics组细胞增殖能力出现明显抑制，miR-133a-3p inhibitor组细胞增殖

能力明显增强（P<0.01，图3B）。

表1 胃癌组织和患者血浆中miR-133a-3p的表达与胃癌患者临床病理特征的关系

Tab.1 Correlations of miR-133a-3p expression in gastric cancer specimens with the clinicopathological features

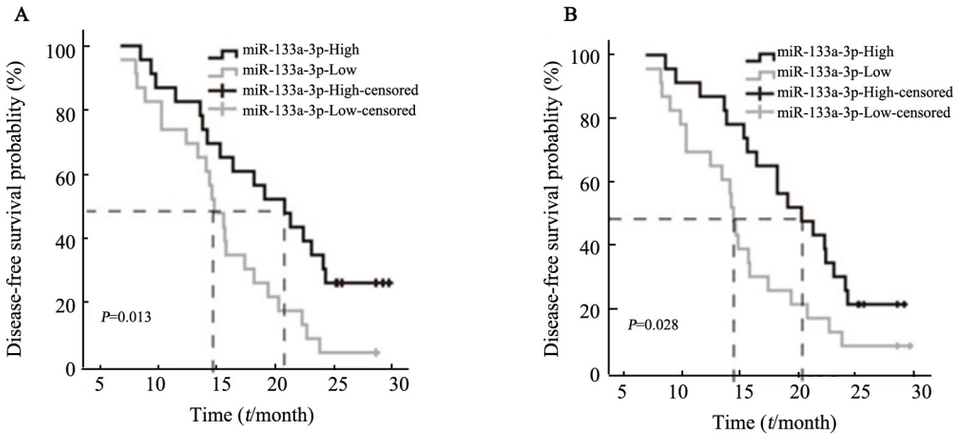
Clinicopathological features	N	Tissue interquartile			Plasma interquartile		
		Median	Range	P	Median	Range	P
Gender				0.856[a]			0.922[a]
Male	46	0.55	0.77		2.07	3.44	
Female	6	0.55	1.43		3.20	13.2	
Ages（t/a）				0.763[a]			0.564[a]
<60	25	0.59	0.86		2.10	3.10	
≥60	27	0.53	0.87		1.84	6.77	
Pathological differentiation*				0.688[a]			0.963[a]
Well-moderate	21	0.55	1.26		2.03	5.43	
Poorly	31	0.56	0.76		2.08	3.44	
TNM stage				0.001[b]			0.017[b]
I	4	1.44	0.51		1.57	0.13	
II	12	1.26	1.98		1.70	1.15	
III	30	0.50	0.61		2.08	4.06	
IV	6	0.13	0.09		6.92	7.86	
T stage				0.001[b]			0.280[b]
T2	7	1.62	2.50		1.63	0.52	
T3	2	1.43			2.30	1.07	
T4	43	0.47	0.75		2.13	6.71	
N stage				0.006[b]			0.037[b]
N0	14	1.22	1.12		1.59	1.09	
N1	13	0.43	0.60		2.17	2.73	
N2	11	0.59	0.45		1.66	0.98	
N3	14	0.19	0.36		4.67	6.41	
M stage				0.002[a]			0.003[a]
M0	46	0.59	0.94		1.83	2.51	
M1	6	0.13	0.09		6.92	7.86	
Venous invasion				0.001[a]			0.001[a]
Negative	33	0.91	0.94		1.64	0.71	
Positive	19	0.19	0.33		4.96	6.90	

*: Well-differentiated adenocarcinoma and moderately differentiated adenocarcinoma (well-moderate), poorly differentiated adenocarcinoma (poorly); a: Mann-Whitney U Test; b: Kruskal-Wallis Test

表2 外周血中miR-133a-3p的表达水平与CA50/CEA/CA199/CA724的关系

Tab. 2 Correlations of miR-133a-3p expression in blood samples of GC patients with the CA50/CEA/CA199/CA724

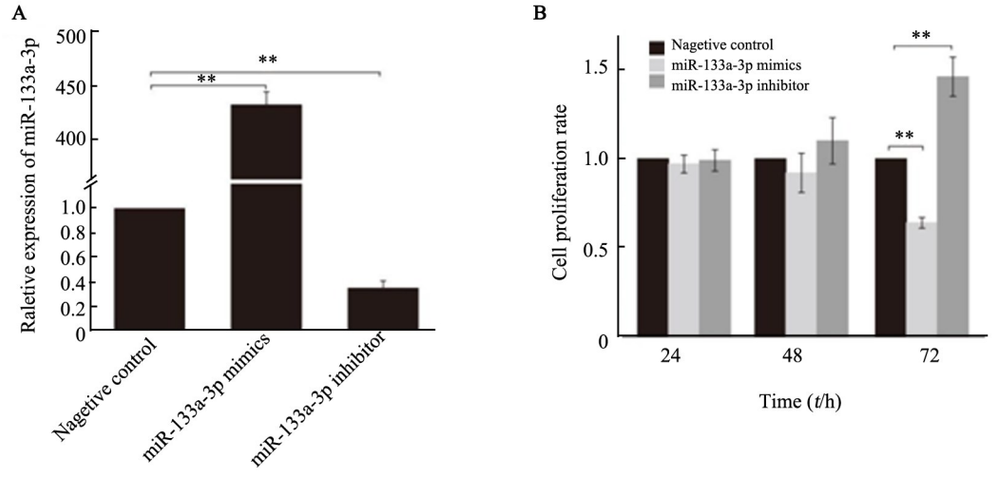
Type [Z/(U·ml^{-1})]	n	Median	Interquartile range	Spearman test	
				r	P
CA50	52	8.12	6.09	-0.069	0.636
CEA[ρ_B/(ng·ml^{-1})]	52	2.42	2.00	-0.021	0.886
CA199	52	15.6	19.9	0.454	0.001
CA724	52	1.93	4.62	0.115	0.426



A: Gastric cancer tissue; B: Plasma

图2 胃癌组织和血浆miR-133a-3p的表达水平与胃癌患者中位DFS的关系

Fig. 2 Correlation analysis of miR-133a-3p expression and median DFS in gastric cancer tissue and plasma



$^{**}P<0.01$ *vs* Negative control group

A: Expression of miR-133a-3p in SGC-7901 cells; B: Proliferation of SGC-7901 cells in various groups

图3 miR-133a-3p对胃癌细胞SGC7901增殖的影响

Fig. 3 The influence of miR-133a-3p on proliferation of SGC7901 cells

3 讨 论

miRNA-133a是一种肌肉特异性miRNA,在果蝇、小鼠和人类肌肉组织中高度保守,位于3个不同的染色体上,分别是6p12.2、18q11.2和20q13.33[17]。miR-133a能够靶向调节运动相关基因,调节肌源性干细胞分化成为成肌细胞,有利于肌肉的生长和骨骼肌受损后的再生[18]。近年来,越来越多的研究[9-11,15,19-21]发现,miR-133a在多种肿瘤中的存在异常表达,并可通过靶向抑制*EGFR*、*IGF1R*、*DR5*、*SENP1*、*USP39*和*COL1A1*等抑制肿瘤细胞增殖、侵袭转移及耐药的产生,抑制肿瘤的发展。本研究通过RT-qPCR技术检测胃癌组织及配对癌旁组织miR-133a-3p的表达情况,结果显示,miR-133a-3p在胃癌组织中表达较癌旁组织显著降低。与既往研究[22-23]结果一致。miR-133a-3p在胃癌组织的表达水平与患者脉管瘤栓、肿瘤TNM分期及T、N、M相关,与患者中位DFS明显正相关,提示miR-133a在胃癌的发展和患者预后方面发挥着重要的作用。miRNA的补充替代疗法能够发挥显著抗肿瘤作用,故提高miR-133a-3p内源性的表达,可能是胃癌的治疗和预防潜在手段之一[24]。

体液分子标志物相比于其他来源的标志物具有易获得性的优点,在早期诊断及预后判定方面有更广阔的前景。有研究[25-27]报道,miRNA在外周血中以蛋白结合体或外泌体的形式存在,能够在外周血中

长时间稳定存在，且其特质不会因性别、年龄存在差异。外周血中miRNA主要来源于侵入循环中的肿瘤细胞及肿瘤细胞分泌的外泌体，由于肿瘤患者外周血中外泌体含量和循环肿瘤细胞数量的升高及外泌体包裹miRNA的选择性[28-31]，使外周血miRNA作为肿瘤早诊标志物成为可能。有研究[32]发现，相较于健康体检者，早期胃癌患者血浆中miR-106b、miR-20a明显升高，用于诊断胃癌的ROC曲线下面积分别为77.3%、85.9%和79.6%。miR-133a-3p在胃癌患者外周血中的表达情况至今未有深入研究，其是否能够起到胃癌早诊及预后判定作用尚不清楚。本研究发现，miR-133a-3p在胃癌患者外周血的表达显著升高，其表达水平与患者肿瘤TNM分期、N、M和脉管瘤栓相关，与患者中位DFS明显负相关，且与患者外周血CA199的水平正相关。miR-133a-3p在胃癌患者组织和血浆中的表达呈显著负相关，此结果可能是由于胃癌组织细胞通过外泌体途径对miR-133a-3p进行选择性外排造成的。结合外周血miRNA高度稳定性、易获得等优势，miR-133a-3p可成为胃癌早诊及判定患者预后的潜在优势生物标志物。有研究[33]发现，miR-133a在肿瘤的发生发展过程中发挥着抑癌基因的作用。本研究在胃癌细胞SGC7901中过表达或沉默miR-133a-3p，结果显示miR-133a-3p过表达能够显著抑制胃癌细胞的增殖能力，提示miR-133a-3p可通过调节胃癌细胞的增殖影响患者预后。

综上所述，miR-133a-3p在胃癌组织及外周血中存在异常表达，其表达与患者预后密切相关。另外，miR-133a-3p可抑制胃癌细胞的增殖，miR-133a-3p可作为胃癌早诊早治及患者临床预后判定的潜在标志物。

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