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MiR–466 对胃癌细胞增殖和迁移的影响及机制

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[摘　要]　**目的：**研究miR-466对胃癌细胞系增殖和迁移的影响，并阐明其可能的机制。**方法：**实时聚合酶链反应 (real-time polymerase chain reaction，real-time PCR)测定胃癌细胞系AGS，BSG823，MGC-803，Hs746T及正常胃黏膜上皮细胞系GES-1中miR-466的表达。将胃癌细胞系Hs746T分成3组：miR-466过表达组(miR-466组)、阴性对照组(miR-NC组)及空白对照组(Mock组)。用Lipofectamine™ 3000分别转染miR-466 mimic，miR-NC序列，Mock组为空白对照。细胞计数试剂盒-8(cell counting kit-8，CCK-8)法和细胞划痕实验分别测定3组细胞增殖和迁移能力，蛋白质印迹测定3组Runt相关转录因子2(Runt-related transcription factor 2，RUNX2)蛋白表达。**结果：**miR-466在胃癌细胞系AGS，BSG823，MGC-803和Hs746T中的表达量低于正常胃黏膜上皮细胞系GES-1 (P<0.05)。转染0，24，48 h，$OD_{450\ nm}$值3组差异无统计学意义(P>0.05)；转染后72及96 h，miR-466组$OD_{450\ nm}$低于miR-NC组和Mock组(P<0.05)。miR-466组划痕愈合率低于miR-NC组[(19.7±6.8)% vs (69.3±7.8)%，P<0.01]，miR-NC组与Mock组划痕愈合率差异无统计学意义(P>0.05)。miR-466组RUNX2蛋白相对表达量低于miR-NC组(0.38±0.04 vs 1.00±0.03，P<0.05)；miR-NC组与Mock组RUNX2蛋白表达量差异无统计学意义(P>0.05)。**结论：**在胃癌细胞系中miR-466低表达，上调miR-466表达可抑制胃癌细胞增殖和迁移，其机制可能与RUNX2蛋白下调表达有关。

[关键词]　miR-466；胃癌；增殖；迁移；Runt相关转录因子2

Effect of miR-466 on the proliferation and migration of gastric carcinoma cell line and its mechanism

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Abstract　**Objective:** To investigate the effect of miR-466 on the proliferation and migration of gastric carcinoma cell lines, and explain its mechanism. **Methods:** Real-time polymerase chain reaction (real-time PCR) was used to detect the expression of miR-466 in normal gastric cell line, GES-1 and gastric cancer cell lines, AGS, BSG823, MGC-803 and Hs746T. The Hs746T cell line was assigned into three groups: miR-466 over-expression group (miR-466 group), negative control group (miR-NC group) and blank control group (Mock group), which was transfect with miR-466 mimic, miR-NC sequence and phosphate buffered saline with Tween-20 (PBST) by Lipofectamine™

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3000. The proliferation and migration ability were analyzed using Cell Counting Kit-8 (CCK-8) and wound scratch assay between three groups. The expression of Runt-domain containing protein (RUNX2) protein was analyzed by Western blot between three groups. **Results:** The expression of miR-466 in four gastric carcinoma cell lines, AGS, BSG823, MGC-803 and Hs746T, was significantly lower than that in normal gastric cell line, GES-1 (P<0.05). There was no significant difference in $OD_{450\ nm}$ value between the three groups at 0, 24 and 48 h after transfection (P>0.05). The $OD_{450\ nm}$ value of miR-466 group was significantly lower than that in miR-NC group and Mock group after transfecting for 72 and 96 h, respectively (P<0.05). Scar healing rate of miR-466 group was significantly lower than that in miR-NC group [(19.7±6.8)% vs (69.3±7.8)%, P<0.01]. There was no significant difference in scratch healing rate between miR-NC group and Mock group (P>0.05). The expression level of RUNX2 in miR-466 group was significantly lower than in miR-NC group (0.38±0.04 vs 1.00±0.03, P<0.05). There was no significant difference in the expression of RUNX2 protein between miR-NC group and Mock group (P>0.05). **Conclusion:** The expression of miR-466 in gastric cancer cell lines is significantly decreased. Over-expression of miR-466 could inhibit the proliferation and migration of gastric cancer cells, probably by targeting RUNX2.

Keywords miR-466; gastric carcinoma; proliferation; migration; Runt-domain containing protein 2

在中国，胃癌的发病率和病死率仅次于肺癌，位于第二位[1]。由于胃癌早期临床症状不易发现，多数患者在确诊时已进入进展期，导致患者预后不佳，晚期患者5年生存率仅20%~30%[2-3]。微小RNA (miRNA)长度19~22 nt，可通过与靶基因的mRNA 3'-非翻译区(3'-untranslated region，3'-UTR)区结合，调控靶标蛋白质的表达[4]。miRNA在肿瘤发生发展进程中发挥类似抑癌基因或原癌基因的功能，参与肿瘤细胞的增殖、转移及侵袭等生物学过程[5]。miR-466位于染色体3p23，在前列腺癌[6]和结肠癌[7]中低表达，与癌细胞增殖、侵袭有关。本研究旨在检测胃癌细胞系及正常人胃黏膜上皮细胞系中miR-466的表达，并探讨其对胃癌细胞迁移及增殖的影响。

1 材料与方法

1.1 材料

胃癌细胞系BSG823，AGS，Hs746T，MGC-803及GES-1正常胃黏膜上皮细胞系均购自美国典型培养物保藏中心(American Type Culture Collection，ATCC)细胞库；Runt相关转录因子2(Runt-related transcription factor 2，RUNX2)及GAPDH一抗购自美国Cell Signaling公司；RPMI-1640细胞培养基购自美国Sigma公司；细胞计数试剂盒-8(cell counting kit-8，CCK-8)购自美国GlpBio公司；二抗购自美国Invitrogen公司；转染所需的miR-466 scramble，mimics序列及转染试剂Lipofectamine™ 3000购自上海吉玛生物科技有限公司。

1.2 细胞培养及分组

实验所需的对数生长期Hs746T细胞系，按实验设计分阴性对照组(miR-NC组)，miR-466过表达组(miR-466组)及空白对照组(Mock组)，采用Lipofectamine™ 3000分别转染miR-466 scramble，mimics及含吐温-20的磷酸盐缓冲液(phosphate buffered saline with Tween-20，PBST)处理。Scramble序列：正向引物5'-UUCUCCGAACGUGUCACGUTT-3'，反向引物5'-ACGUGACACGUUCGGAGAATT-3'。miR-466 mimics序列：正向引物5'-CAAAGCGCUCCCCUUUAGAGGU-3'；反向引物5'-CUCUAAAGGGGAGCGCUUUGUU-3'，Mock组以PBST为空白对照。转染浓度为300 nmol/孔。

1.3 RNA提取及实时聚合酶链反应

对总RNA采用提取纯化一体试剂盒(美国Invitrogen公司)提取后，采用反转录法合成cDNA，U6小核为内参，采用$2^{-\Delta\Delta Ct}$法定量，反应条件为：95 ℃预变性30 s，95 ℃ 5 s，60 ℃ 20 s，共40个循环，采用$2^{-\Delta\Delta Ct}$法测定miR-466相对表达量。

1.4 细胞增殖能力测定

CCK-8法用于评估Hs746T细胞系增殖，将每组细胞以2×10^3个/孔种植，经0，24，48，72，96 h培

养后，以20 μL/孔加入MTT，使用酶标仪在450 nm波长下测定吸光度值，绘制细胞增殖曲线。

1.5 细胞迁移能力测定

采用细胞划痕实验检测Hs746T细胞系迁移能力：使用无菌孔板培养3组细胞，待细胞融合后，用无菌枪头画直线，于0及24 h观察融合情况，计算划痕面积，并根据划痕面积计算划痕愈合率。划痕愈合率越高表明迁移能力越强，重复3次实验，取均值。

1.6 蛋白质印迹法

3组细胞裂解、蛋白变性及定量后，行蛋白质印迹法，电泳条件为常规条件：即浓缩胶80 V 80 min，分离胶100 V 100 min，一抗浓度为1:200，二抗浓度为1:500，显影液显影后测定蛋白灰度值，重复实验3次，取3次平均值行数据分析。

1.7 统计学处理

采用SPSS 20.0统计软件进行数据分析。计量资料用均数±标准差($\bar{x}\pm s$)表示，两组数据比较采用t检验，P<0.05为差异有统计学意义。

2 结果

2.1 胃癌细胞系中 miR-466 呈低表达

MiR-466在正常胃黏膜上皮细胞系GES-1中的表达量为1.00±0.05，在AGS，BSG823，MGC-803及Hs746T胃癌细胞系中miR-466相对表达量分别为0.51±0.04，0.42±0.04，0.27±0.03及0.21±0.02，胃癌细胞系AGS，BSG823，MGC-803及Hs746T中miR-466表达量低于正常胃黏膜上皮细胞系GES-1(P<0.05，图1)。

2.2 MiR-466 对 Hs746T 细胞系增殖的影响

取相对表达量最低的Hs746T细胞系分成3组，转染24 h后，miR-466组中miR-466相对表达量显著高于miR-NC组和Mock组(P<0.05)；转染0，24，48，72及96 h后，miR-466组和miR-NC组的$OD_{450\ nm}$值分别为0.36±0.04 vs 0.35±0.03 (P>0.05)，0.45±0.04 vs 0.50±0.05 (P>0.05)，0.58±0.05 vs 0.71±0.07 (P>0.05)，0.76±0.06 vs 1.04±0.09 (P<0.05)及0.95±0.08 vs 1.57±0.10 (P<0.01)。Mock组为0.33±0.03，0.51±0.04，0.72±0.07，1.14±0.09及1.65±0.11，与miR-NC组比较差异无统计学意义(P>0.05，图2)。

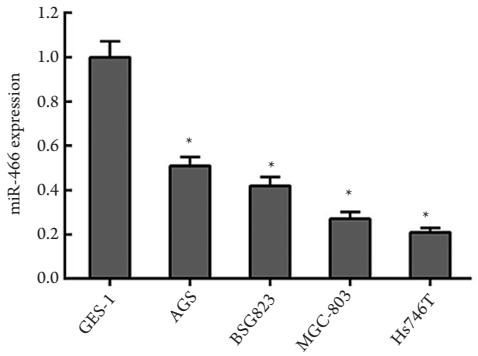

图1 MiR-466在胃癌及正常胃黏膜上皮细胞系中的表达

Figure 1 Expression of miR-466 in gastric cancer and normal gastric cell lines

与正常胃黏膜上皮细胞系相比，*P<0.05。

Compared with normal gastric cell lines, *P<0.05.

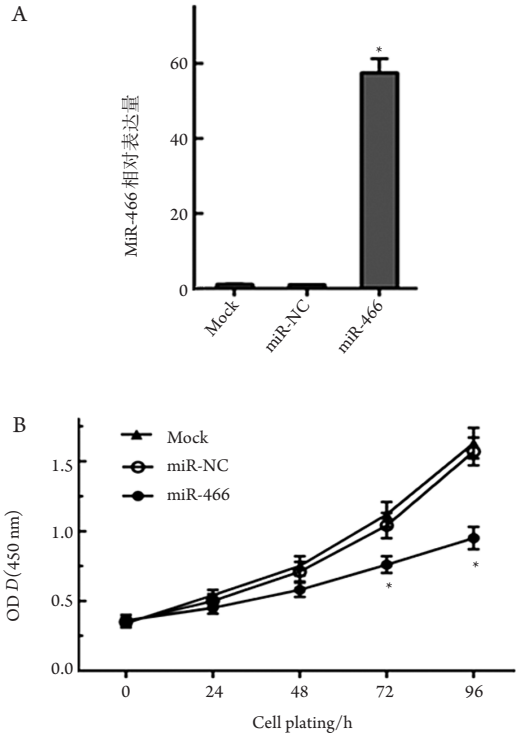

图2 MiR-466抑制胃癌细胞增殖

Figure 2 MiR-466 inhibited the proliferation of gastric cancer cell

(A)转染后，3组miR-466相对表达量的比较；(B)3组细胞增殖曲线比较。与miR-NC组比较，*P<0.05。

(A) Comparison of miR-466 expression between the three groups after transfect; (B) Comparison of proliferation curve between the three groups. Compared with the miR-NC group, *P<0.05.

2.3 MiR-466 过表达抑制 Hs746T 细胞系迁移

100倍视野下，miR-466组细胞划痕愈合率为(19.7±6.8)%，miR-NC组为(69.3±7.8)%，miR-466组细胞划痕愈合率低于miR-NC组(P<0.05)，Mock组为(70.2±5.4)%，Mock组与miR-NC组细胞划痕愈合率差异无统计学意义(P>0.05，图3)。

2.4 MiR-466 过表达对 RUNX2 蛋白表达的影响

蛋白质印迹法示：miR-466组RUNX2蛋白相对表达量为0.38±0.04，miR-NC组为1.04±0.03。miR-466组RUNX2蛋白表达量显著低于miR-NC组(P<0.05，图4)。

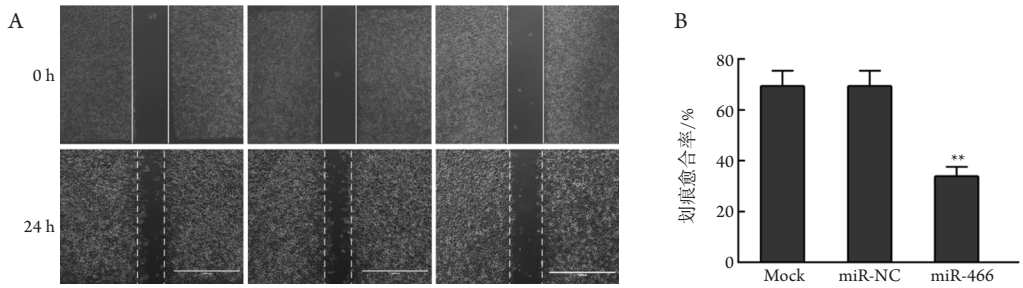

图3 MiR-466过表达抑制胃癌细胞迁移

Figure 3 Over-expression of miR-466 inhibited the migration of gastric cancer cell

(A)3组细胞划痕实验比较(×100)；(B)3组划痕愈合率比较。与miR-NC组比较，**P<0.01。

(A) Comparison of wound scratch assay results between the three groups (×100); (B) Comparison of scar healing rate between the three groups. Compared with the miR-NC group, **P<0.01.

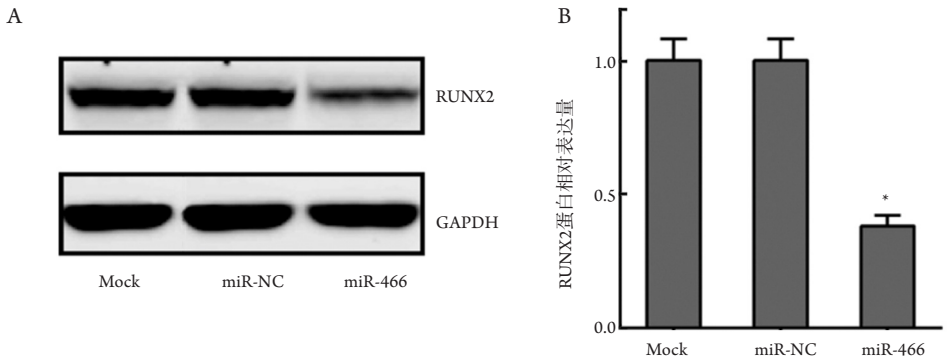

图4 MiR-466过表达对RUNX 2蛋白表达的影响

Figure 4 Effect of miR-466 over-expression on RUNX 2 protein expression

(A)蛋白质印迹示3组RUNX2表达；(B)3组RUNX2蛋白表达量的比较。与miR-NC组比较，*P<0.05。

(A) Western blot shows expression of RUNX2 protein in the three groups; (B) Comparison of RUNX2 protein expression between the three groups. Compared with the miR-NC group, *P<0.05.

3 讨论

世界范围内，每年胃癌发病近百万例，其中发展中国家占比近60%[1]。胃癌的发生是个多步骤调控的过程，由于病情复杂，早期病程隐匿，导致多数患者确诊时已是晚期，5年生存期仅20%~30%[2-3,8]。

miR-466位于3p23的染色体位点，首次在黑色素瘤中发现[9]。miR-466在前列腺癌[6]和结肠癌[7]中低表达，同时与吸烟导致的肺癌[10]及人乳头瘤病毒(human papillomavirus，HPV)导致的宫颈癌[11]有密切关联。本研究发现：miR-466在胃癌细胞系中表达下调，这和以往其他肿瘤中的研究结果一致[6-7]。为进一步探究miR-466对胃癌细胞生物学功能的影响，本研究在Hs746T细胞中过表达miR-466，检测其对癌细胞增殖和迁移能力的影响。结

果发现：过表达miR-466可显著抑制胃癌细胞的增殖和迁移。一项在结肠癌中的研究[7]显示：miR-466在结肠癌中低表达，miR-466低表达与肿瘤大小、淋巴结转移、肿瘤分期、远处转移及患者的不良预后相关。在结肠癌细胞系中过表达miR-466抑制结肠癌细胞增殖、迁移和侵袭，并诱导癌细胞阻滞于G_0/G_1期，更高比例的细胞被诱导进入凋亡[7]。在前列腺癌中的研究[6]发现：miR-466低表达可用来区分前列腺癌细胞系和正常细胞系，过表达miR-466可抑制前列腺癌细胞增殖、浸润、迁移及抗凋亡能力。这项研究经荧光素酶实验发现：miR-466直接靶向作用于RUNX2，从而抑制前列腺癌细胞增殖和转移[6]。在胃癌中，miR-466的作用机制尚不清楚，本研究对RUNX2进行蛋白水平检测发现：过表达miR-466可显著抑制RUNX2的表达，提示在胃癌中miR-466可能也是通过作用于RUNX2发挥作用的。

RUNX2属于RUNX家族，该家族是一种谱系特异性转录因子，在成骨和肿瘤发生中起关键作用[12]。RUNX2的致癌潜能最初在T细胞淋巴瘤中被发现，后续发现其与多种肿瘤的进展有关[13-14]。研究[15]还发现：RUNX2通过促进钙黏蛋白转换、侵袭I型胶原和蛋白激酶B活化来加速前列腺癌的侵袭性。各种证据显示：在胫骨髓腔内注射肿瘤细胞后导致的前列腺癌[13]和乳腺癌[16]中，骨溶解的严重程度与RUNX2的水平相关。这些研究[16-17]表明：RUNX2可使肿瘤细胞具有潜在地破坏周围组织结构的能力，并帮助癌细胞适应和在这种外来环境中生长。RUNX2还可直接诱导与血管生成、侵袭、转移和刺激原发肿瘤上皮间质转化相关基因的表达[17-18]，提示RUNX2是一个前列腺癌发生和转移潜在的重要因素。在胃癌的研究结果中发现：RUNX2受到miR-466的直接靶标作用，表达被异常调控，这提示miR-466在胃癌中可能是通过调控RUNX2来发挥抑癌基因作用的。

本研究也存在不足之处，比如miR-466如何影响下游信号通路最终导致胃癌的发生，miR-466在体内组织中的表达及动物实验中的结果如何，都值得进一步研究。

综上所述，本研究发现miR-466在胃癌细胞系中表达下调，miR-466过表达可对胃癌细胞增殖和迁移产生抑制作用，其机制可能与miR-466过表达下调RUNX2蛋白表达有关，这为深入理解胃癌的发病机制提供了一个新的思路。

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