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下调 UCA1 通过靶向 miR-23b 及其下游靶基因抑制胃癌细胞的增殖和转移 *

张志勇[1#] 潘 妍[2#] 赵 艳[1] 任牡丹[1] 李雅睿[1] 卢桂芳[1] 和水祥[1△]

（1 西安交通大学第一附属医院消化内科 陕西 西安 710061；2 空军军医大学第一附属医院消化病医院 陕西 西安 710032）

摘要 目的：探讨长链非编码 RNA 尿路上皮癌相关基因 1（UCA1）调控胃癌细胞增殖和转移的分子机制。**方法：**将人胃癌细胞株 SGC7901 分为：对照组、siRNA-NC 组、siRNA-UCA1 组、inhibitor-NC 组和 miR-inhibitor 组、si-UCA1+inhibitor-NC 组和 si-UCA1+miR-inhibitor 组。对 SGC7901 细胞分别转染 siRNA-UCA1 及阴性对照（siRNA-NC）、miR-inhibitor 及阴性对照（inhibitor-NC），未转染的细胞作为对照组。通过 RT-qPCR 检测细胞中 UCA1 和 miR-23b-3p 的水平。通过 CCK-8 法、伤口愈合实验和 Transwell 实验评价细胞的增殖、迁移和侵袭能力。通过 Western blot 分析细胞中 IL6R、BCL2 和 HSP90B1 蛋白的表达。使用 pcDNA-UCA1/pcDNA-NC 与 pGL3-miR-23b-3p-WT/pGL3-miR-23b-3p-Mut 共转染细胞，通过双荧光素酶报告实验验证 UCA1 与 miR-23b-3p 的靶向关系。**结果：**细胞培养 48 h 和 72 h 后，与对照组比较，siRNA-UCA1 组的细胞活力分别降低了 31.58%和 31.40%（P<0.05）。与对照组比较，siRNA-UCA1 组的细胞迁移率[(61.46± 5.43)% vs (23.16± 3.17)%]、侵袭细胞数量（109.17± 9.66 vs 50.83± 6.96）、IL6R、BCL2 和 HSP90B1 的蛋白相对表达量均显著降低，而 miR-23b-3p 相对表达量升高（P<0.05）。与 pGL3-miR-23b-3p-WT 共转染后，与 pcDNA-NC 组比较，pcDNA-UCA1 组的相对荧光酶活性降低了 66.12%（P<0.05）。与 si-UCA1+inhibitor-NC 组比较，si-UCA1+miR-inhibitor 组的细胞活力、细胞迁移率、侵袭细胞数量、IL6R、BCL2 和 HSP90B1 的蛋白相对表达量均显著升高（P<0.05）。**结论：**下调 UCA1 通过靶向 miR-23b-3p 及其下游基因 IL6R、BCL2 和 HSP90B1 来抑制胃癌细胞的增殖和转移。

关键词：胃癌；尿路上皮癌相关基因 1；miR-23b-3p；增殖；转移

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Down-regulation of UCA1 Inhibits the Proliferation and Metastasis of Gastric Cancer Cells by Targeting miR-23b and Its Downstream Target Genes*

ZHANG Zhi-yong[1#], PAN Yan[2#], ZHAO Yan[1], REN Mu-dan[1], LI Ya-rui[1], LU Gui-fang[1], HE Shui-xiang[1△]

(1 Department of Gastroenterology, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, Shaanxi, 710061, China;

2 Xijing Hospital of Digestive Diseases, Air Force Military Medical University, Xi'an, Shaanxi, 710032, China)

ABSTRACT Objective: To explore the molecular mechanism of long non-coding RNA urothelial carcinoma-associated 1 (UCA1) regulating the proliferation and metastasis of gastric cancer cells. **Methods:** The human gastric cancer cell line SGC7901 was divided into the following groups: control group, siRNA-NC group, siRNA-UCA1 group, inhibitor-NC group, miR-inhibitor group, si-UCA1+inhibitor-NC group, si-UCA1+miR-inhibitor group. SGC7901 cell was transfected with siRNA-UCA1 or negative control (siRNA-NC), miR-inhibitor or negative control (inhibitor-NC) respectively, and the untransfected cells were used as control group. The levels of UCA1 and miR-23b-3p in the cells were detected by RT-qPCR. The CCK-8 method, wound healing experiment and Transwell experiment were used to evaluate cell proliferation, migration and invasion ability. The expression of IL6R, BCL2 and HSP90B1 protein in the cells was analyzed by Western blot. Cells were co-transfected with pcDNA-UCA1/pcDNA-NC and pGL3-miR-23b-3p-WT/pGL3-miR-23b-3p-Mut, and the target relationship between UCA1 and miR-23b-3p was verified by dual luciferase report experiment. **Results:** After culturing for 48 h and 72 h, compared with Control group, the cell viability of siRNA-UCA1 group was reduced by 31.58% and 31.40%, respectively (P<0.05). Compared with Control group, the cell migration rate [(61.46± 5.43)% vs (23.16± 3.17)%], the number of inva-

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为共同第一作者

作者简介：张志勇（1983-），男，博士研究生，主治医师，主要研究方向：消化系统肿瘤发生发展的分子机制，
E-mail: zhangzhy1983@163.com，电话：18792860648；
潘研（1984-），女，硕士研究生，主治医师，主要研究方向：消化系统肿瘤发生发展的分子机制

△ 通讯作者：和水祥（1964-），男，博士生导师，主要研究方向：消化系统肿瘤发生发展的分子机制，
E-mail: dyyyjxk@mail.xjtu.edu.cn，电话：13991380928

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sive cells (109.17± 9.66 vs 50.83± 6.96), and the relative expression levels of IL6R, BCL2 and HSP90B1 proteins in siRNA-UCA1 group were all significant reduced, but the relative expression of miR-23b-3p significant increased (P<0.05). After co-transfection with pGL3-miR-23b-3p-WT, compared with pcDNA-NC group, the relative luciferase activity of pcDNA-UCA1 group was reduced by 66.12% (P<0.05). Compared with si-UCA1+inhibitor-NC group, cell viability, cell migration rate, number of invaded cells, relative expression of IL6R, BCL2 and HSP90B1 protein in si-UCA1+miR-inhibitor group were significantly increased (P<0.05). **Conclusion:** Down-regulation of UCA1 inhibits the proliferation and metastasis of gastric cancer cells by targeting miR-23b-3p and its downstream genes IL6R, BCL2 and HSP90B1.

Key words: Gastric cancer; Urothelial carcinoma-associated 1; MiR-23b-3p; Proliferation; Metastasis

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前言

胃癌是发病率最高的癌症之一[1,2],放化疗耐药性的出现导致胃癌患者的预后仍然较差[3,4]。此外,胃癌的早期诊断比较困难，探索有效生物标志物具有重要意义。长链非编码 RNA（long non-coding RNA, lncRNA）在功能上被归类为非编码转录本,长度超过 200 个核苷酸,没有潜在的蛋白质编码能力。据报道,lncRNA 的异常表达、缺失或突变与包括癌症在内的许多复杂疾病有关[5,6]。lncRNA 参与多种生物学过程,包括染色质相互作用、转录调控、mRNA 转录后调控和表观遗传调控[7,8]。此外,越来越多的实验证据支持 lncRNA 作为竞争性内源性 RNA（competing endogenous RNAs,ceRNA），与 microRNA（miRNA)竞争,上调靶基因的表达。在胃癌中发现了许多 lncRNA 的异常表达,并与胃癌的发生、诊断和预后密切相关[9]。一项研究表明,lncRNA BC032469 可以作为 miR-1207-5p 的 ceRNA,上调 hTERT 的表达,促进胃癌细胞的增殖[10]。lncRNA 尿路上皮癌相关基因 1(urothelial carcinoma-associated 1,UCA1)在多种癌症中高度表达,包括胃癌、结直肠癌、肺癌和乳腺癌,并且与患者的预后有关[11]。Luan 等[12]研究表明,UCA1 在结直肠癌组织中上调并用通过 miR-143/MYO6 轴促进结直肠癌细胞增殖和转移。另外,也有研究表明 UCA1 调节化疗耐药性[13]。然而,UCA1 在胃癌中的具体作用机制仍有待阐明,因此,本研究旨在揭示 UCA1 调控胃癌细胞增殖和转移的具体机制。

1 材料与方法

1.1 实验材料

青霉素、链霉素、胎牛血清、RPMI-1640 培养基购自美国 Invitgen 公司;Lipofectamine 2000 购自美国 Thermo Fisher Science 公司;Trizol 试剂用、CCK-8 溶液、RIPA 裂解缓冲液购自碧云天生物技术研究所;M-MLV 逆转录酶购自美国 Invitgen 公司;Universal SYBR Green Master Mix 购自瑞士 Roche 公司;Transwell、Matrigel 购自美国 Corning 公司;白细胞介素 6 受体(IL6R)、BCL2 和热休克蛋白 90B1(HSP90B1)和 GAPDH 一抗以及 HRP 标记的二抗购自英国 Abcam 公司。

1.2 实验方法

1.2.1 **细胞培养** 人胃癌细胞株 SGC7901 细胞购自美国 ATCC。SGC7901 细胞以 1.0× 10^3 个 / 孔的密度接种于 96 孔培养板中，每孔加入含有青霉素（100 U/mL）、链霉素(100 mg/mL)和 10%胎牛血清(FBS)的 RPMI-1640 培养基,在 37℃、5% CO_2 条件下培养。

1.2.2 **细胞分组及转染** 将细胞分为以下几组，对照组、siRNA-NC 组、siRNA-UCA1 组、inhibitor-NC 组、miR-inhibitor 组、si-UCA1+inhibitor-NC 组、si-UCA1+miR-inhibitor 组。siRNA-UCA1 及阴性对照（siRNA-NC)、pcDNA-UCA1 及其阴性对照（pcDNA-NC)、miR-inhibitor 及其阴性对照(inhibitor-NC)均购自上海吉玛制药技术有限公司。根据说明书，用 Lipofectamine 2000 转染上述产物 48 h。未转染的细胞作为对照组。

1.2.3 RT-qPCR **检测** mRNA **表达水平** Trizol 试剂用于提取总 RNA。应用 M-MLV 逆转录酶合成 cDNA。使用 Universal SYBR Green Master Mix 在 ABI 7900HT 荧光定量 PCR 仪上进行 qRT-PCR 扩增，扩增条件为:95℃预变性 5 min,95℃变性 10 s,60℃退火 20 s,70℃延伸 10 s,35 个循环。引物序列如下:UCA1，正向:5'-CCGCTCGAGAGCGCGTGTGGCGGCCGAGCAC-3'；反向:5'-CGCGGATCCAGACACGAGGCCGGCCACGCCACG-3';miR-23b-3p，正向:5'-CTCCCCAGCATCTTCGATCC-3'；反向:5'-GAGGTCATCGCTGGGCATAA-3';GAPDH，正向:5'- ACAGTCAGCCGCATCTTCTT-3'；反向:5'-GACAAGCTTCCCTTCTCAG -3';U6，正向:5'-GCTTCGGCAGCACATATACT-3';反向:5'-ACGCTTCACGAATTTGCGTG-3'。以 GAPDH 作为 UCA1 的内部对照。以 U6 作为 miR-23b-3p 的内部对照。

1.2.4 CCK-8 **检测细胞增殖能力** 转染后的细胞以 5.0× 10^3 个 / 孔的密度接种在 96 孔细胞培养板中，在 37℃、5% CO_2 环境中培养 24、48 和 72 h,然后在每孔中加入 10 μL CCK-8 溶液培养 2 h,用 Thermo Fisher Science Varioskan Flash 多功能酶标仪检测 450 nm 处的吸光度。实验重复 3 次。

1.2.5 **伤口愈合实验检测细胞迁移能力** 在伤口愈合实验中,将密度为 1× 10^5 个 /mL 的细胞在含 10%胎牛血清的 RPMI1640 培养基中培养,生长至 90%融合。然后,用移液枪尖端在细胞上划出划痕。随后将培养液换成无血清 RPMI1640 并培养 24 h,然后计算细胞迁移率 =(0 h 伤口面积 -24 h 伤口面积)/0 h 伤口面积。实验重复 3 次。

1.2.6 Transwell **实验检测细胞侵袭能力** Transwell 实验中(孔径为 8 μm),将密度为 1× 10^5 个 /mL 的细胞在含有无血清培养基的上室中培养，上室底部涂有 Matrigel。然后将含有 20%胎牛血清的培养基加入下室。孵育 36 h 后,用 0.05%结晶紫(美国 Sigma 公司)染色。在显微镜下随机选择 6 个视野拍摄并计数侵袭的细胞。

1.2.7 Western blot **检测蛋白表达水平** 用含有蛋白酶抑制剂的 RIPA 裂解缓冲液提取总蛋白。总蛋白经 10% SDS-PAGE 分离后转移到 PVDF 膜上。用 5%的脱脂牛奶来封闭膜 1 h 后，将抗 IL6R（1:1000）、BCL2（1:1000）、HSP90B1（1:2000）和 GAPDH（1:1000）的一抗分别与膜在 4℃下孵育过夜。然后，将膜与 HRP 标记的二抗（1:1000 稀释）在室温下孵育 1 h。采用 ECL 检测液进行显影，并使用 Image Lab 软件对蛋白表达水平进行定量并归一化为 GAPDH。实验重复 3 次。

1.2.8 **双荧光素酶报告实验** 通过生物信息学预测 UCA1 在 miR-23b-3p 上的结合位点。含有野生型（pGL3-miR-23b-3p-WT）和突变型（pGL3-miR-23b-3p-Mut）lncRNA UCA1 和 miR-23b-3p 结合位点序列的 pGL3 荧光素酶报告载体由美国 Promega 公司合成。用 Lipofectamine 2000 将 pcDNA-UCA1/pcDNA-NC 与 pGL3-miR-23b-3p-WT/pGL3-miR-23b-3p-Mut 报告载体共转染细胞。转染 48 h 后，用美国 Promega 公司的双荧光素酶报告分析系统测定荧光素酶活性，并归一化为海肾荧光素酶活性。实验重复 3 次。

1.3 **统计学分析**

两组比较采用 t 检验，多组比较采用单因素方差分析，然后进行 Tukey 事后检验，使用 SPSS21.0 软件进行数据分析并以均值± 标准差表示。P<0.05 说明差异具有统计学意义。

2 结果

2.1 **下调 UCA1 抑制 SGC7901 细胞的增殖**

与对照组比较，siRNA-UCA1 组 SGC7901 细胞的 UCA1 相对表达量降低了 76.16%（P<0.05）。CCK-8 检测结果显示，细胞培养 48 h 时，与对照组比较，siRNA-UCA1 组的细胞活力降低了 31.58%（P<0.05）；细胞培养 72 h 时，与对照组比较，siRNA-UCA1 组的细胞活力降低了 31.40%（P<0.05）。见图 1。

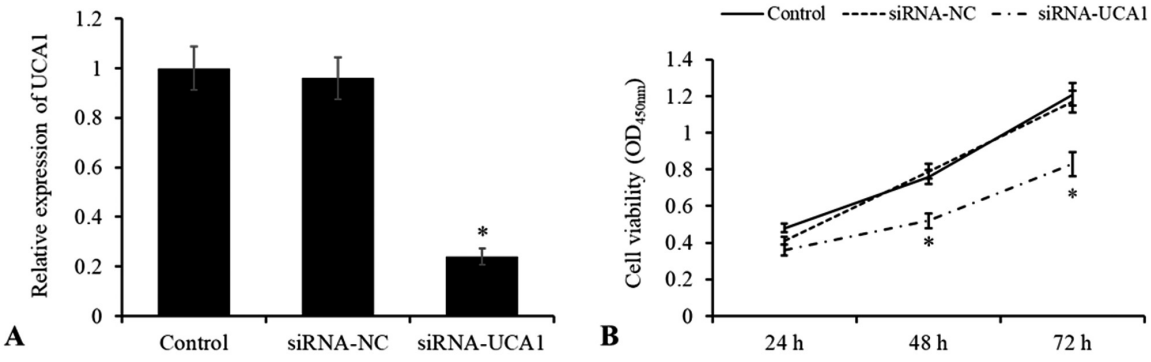

图 1 转染 siRNA-UCA1 对 SGC7901 细胞增殖的影响

Fig.1 The effect of transfection of siRNA-UCA1 on the proliferation of SGC7901 cells

Note: A: Transfection of siRNA-UCA1 down-regulated UCA1 expression level; B: CCK-8 to detect cell viability after transfection of siRNA-UCA1; Compared with control group, *P<0.05.

2.2 **下调 UCA1 抑制 SGC7901 细胞的迁移和侵袭**

伤口愈合实验结果显示，细胞培养 24 h 时，与对照组比较，siRNA-UCA1 组的细胞迁移率显著降低[(61.46± 5.43)% vs (23.16± 3.17)%，P<0.05]。Transwell 实验结果显示，与对照组比较，siRNA-UCA1 组的侵袭细胞数量显著降低（109.17± 9.66 vs 50.83± 6.96，P<0.05）。见图 2。

2.3 UCA1 **靶向抑制 SGC7901 细胞中的** miR-23b-3p

生物信息学网站（mircode 数据库）预测 UCA1 靶向调控 miR-23b-3p，UCA1 与 miR-23b-3p 的潜在结合位点如图 3 所示。双荧光素酶报告实验结果显示，与 pGL3-miR-23b-3p-WT 共转染后，与 pcDNA-NC 组比较，pcDNA-UCA1 组的相对荧光酶活性降低了 66.12%（P<0.05）。此外，与对照组比较，siRNA-UCA1 组的 miR-23b-3p 相对表达量升高了 1.65 倍（P<0.05）。见图 3。

2.4 **下调** miR-23b-3p **逆转了** UCA1 **对 SGC7901 细胞增殖、迁移和侵袭的影响**

与对照组比较，miR-inhibitor 组的 miR-23b-3p 相对表达量降低了 73.92%（P<0.05）。细胞培养 72 h 后，与 si-UCA1+inhibitor-NC 组比较，si-UCA1+miR-inhibitor 组的细胞活力升高了 29.55%（P<0.05）。细胞培养 24 h 后，与 si-UCA1+inhibitor-NC 组比较，si-UCA1+miR-inhibitor 组的细胞迁移率显著升高[(23.24± 2.05)% vs (53.12± 4.70)%，P<0.05]。与 si-UCA1+inhibitor-NC 组比较，si-UCA1+miR-inhibitor 组的侵袭细胞数量显著升高[(50.50± 4.46) vs (85.33± 7.55)，P<0.05]。见图4。

2.5 UCA1 **通过** miR-23b-3p **调控 SGC7901 细胞中 IL6R、BCL2 和 HSP90B1 的蛋白表达**

生物信息学网站（Targetscan 数据库）预测 miR-23b-3p 靶向调控 IL6R、BCL2 和 HSP90B1。Western blot 检测结果显示，与对照组比较，siRNA-UCA1 组的 IL6R、BCL2 和 HSP90B1 的蛋白相对表达量均显著降低（P<0.05）。与 si-UCA1+inhibitor-NC 组比较，si-UCA1+miR-inhibitor 组的 IL6R、BCL2 和 HSP90B1 的蛋白相对表达量均显著升高（P<0.05）。见图 5。

3 讨论

非编码 RNA 是一种不能编码蛋白质的 RNA 分子。根据长度不同，非编码 RNA 可分为两大类。大 RNA（大于 50 nt）：包括 lncRNA、核仁小 RNA、环状 RNA、tRNA 和 rRNA [14]。小 RNA（小于 50 nt）：包括 miRNA、siRNA 和 piRNA[15]。大量研究表明，非编码 RNA 在胃癌进展中起着重要作用。lncRNA 是一种长度大于 200 nt 的 RNA 转录本，不具备编码蛋白质的能力[16]。

在功能上，lncRNA 在任何水平上调节基因的表达，包括染色质修饰、转录和转录后处理。lncRNA 对胃癌细胞的增殖、细胞周期、凋亡、侵袭、迁移、转移和致瘤性具有明显的调控作用[17-19]。其他研究报道了 lncRNA UCA1 在胃癌中的过度表达与患者的预后有关，并且通过海绵吸附抗肿瘤 miRNAs 调节癌细胞的生长、转移、化疗耐药性[20,21]。本研究结果表明，下调 UCA1 可抑制胃癌细胞的增殖、迁移和侵袭，与前人研究结果一致。

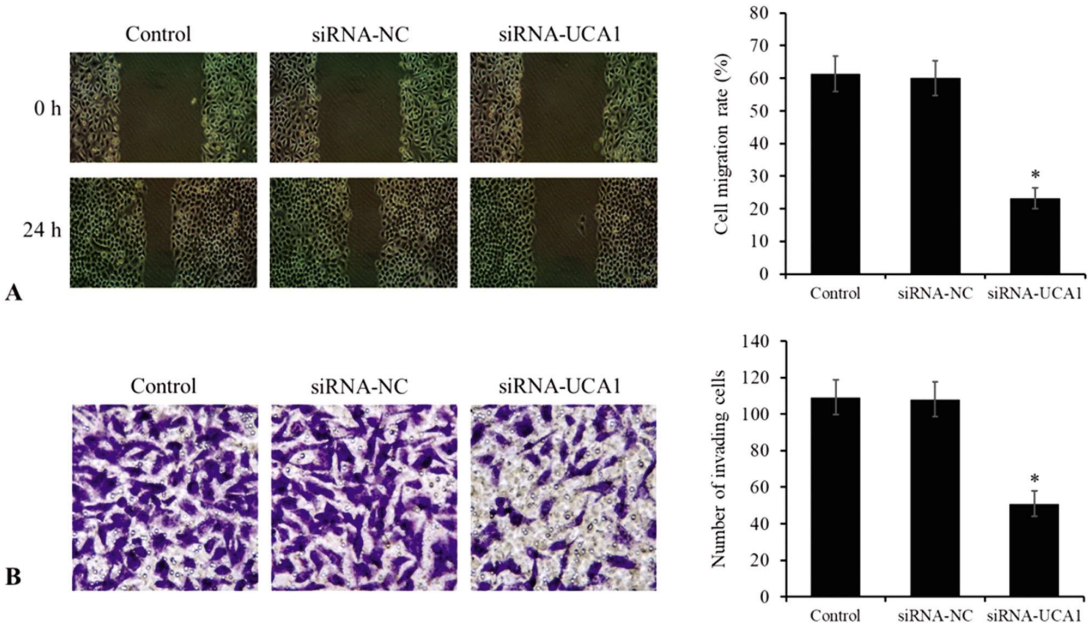

图 2 转染 siRNA-UCA1 对 SGC7901 细胞迁移和侵袭的影响

Fig.2 The effect of transfection of siRNA-UCA1 on the migration and invasion of SGC7901 cells

Note: A: Wound healing experiment to evaluate cell migration after transfection of siRNA-UCA1, magnification: × 100; B: cell migration rate; C: Transwell experiment to evaluate cell invasion after transfection of siRNA-UCA1, magnification: × 400; D: The number of invading cells in each field of view; Compared with control group, **P*<0.05.

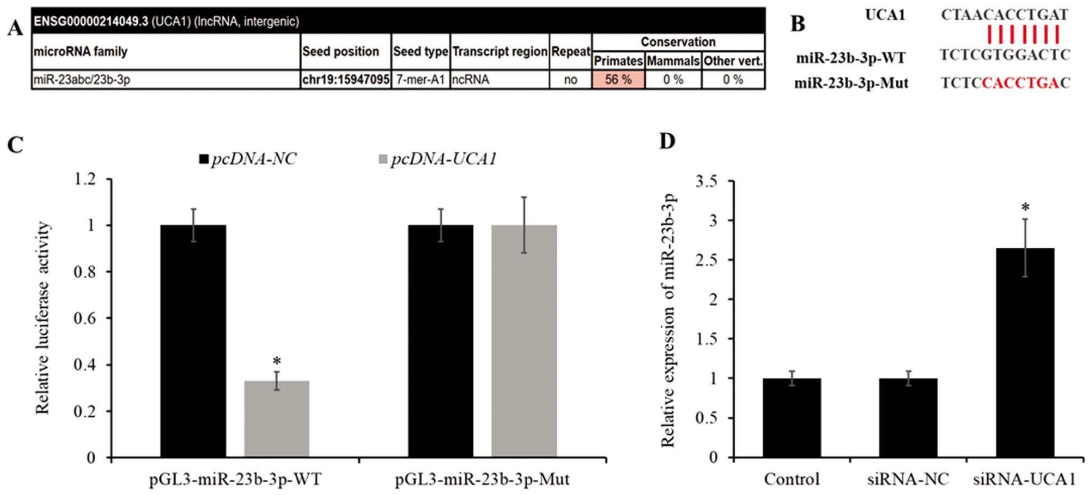

图 3 UCA1 靶向抑制 SGC7901 细胞中的 miR-23b-3p

Fig.3 UCA1 targeted miR-23b-3p in SGC7901 cells

Note: A: Bioinformatics website (mircode database) predicted that UCA1 targeted miR-23b-3p; B: UCA1 and miR-23b-3p binding sites; C: Relative luciferase activity after co-transfection of pcDNA-UCA1 and pGL3-miR-23b-3p-WT; Compared with pcDNA-NC group, **P*<0.05; D: The effect of transfection of siRNA-UCA1 on the expression of miR-23b-3p; Compared with control group, **P*<0.05.

基于 miRNA 的癌症治疗可能是一条充满希望的途径。一方面，基于 miRNA 的药物可以通过抑制相关信号通路来抑制肿瘤进展[22]。另一方面，miRNA 在耐药中也起着重要作用。miRNA 可以显著影响药物转运体、药物代谢酶、转录因子和核受体[23]。例如，HOTAIR 与 EZH2 和 SUZ12 结合形成复合物，通过 H3K27me3 修饰直接与 miR-34a 启动子结合，抑制其表达。miR-34a 的低表达显著促进 HGF/c-met 的激活，从而诱导 Snail、PI3K/Akt 和 NF-kB 信号促进肿瘤的进展[24]。已知 lncRNA 在转录水平上通过表观遗传沉默 miRNA 的表达，从而促进胃癌的进展。本研究结果显示胃癌细胞中 UCA1 与

miR-23b-3p 存在靶向调控关系，下调 UCA1 可引起 miR-23b-3p 水平的升高。进一步的实验结果表明，下调 miR-23b-3p 逆转了 UCA1 对 SGC7901 细胞增殖、迁移和侵袭的影响。本课题组在前期实验中发现，miR-23b-3p 可作为胃癌总生存期的预测因子。miR-23b-3p 的过表达在体外通过抑制 ATG12 和 HMGB2 介导的自噬逆转了胃癌细胞对多种化疗药物的耐药性，并在体内增强了肿瘤对化疗的敏感性[25]。基于前人研究结果，本研究推测，在胃癌细胞中，UCA1 的过表达通过海绵吸附 miR-23b-3p 导致其活性抑制，进而促进了胃癌的进展。

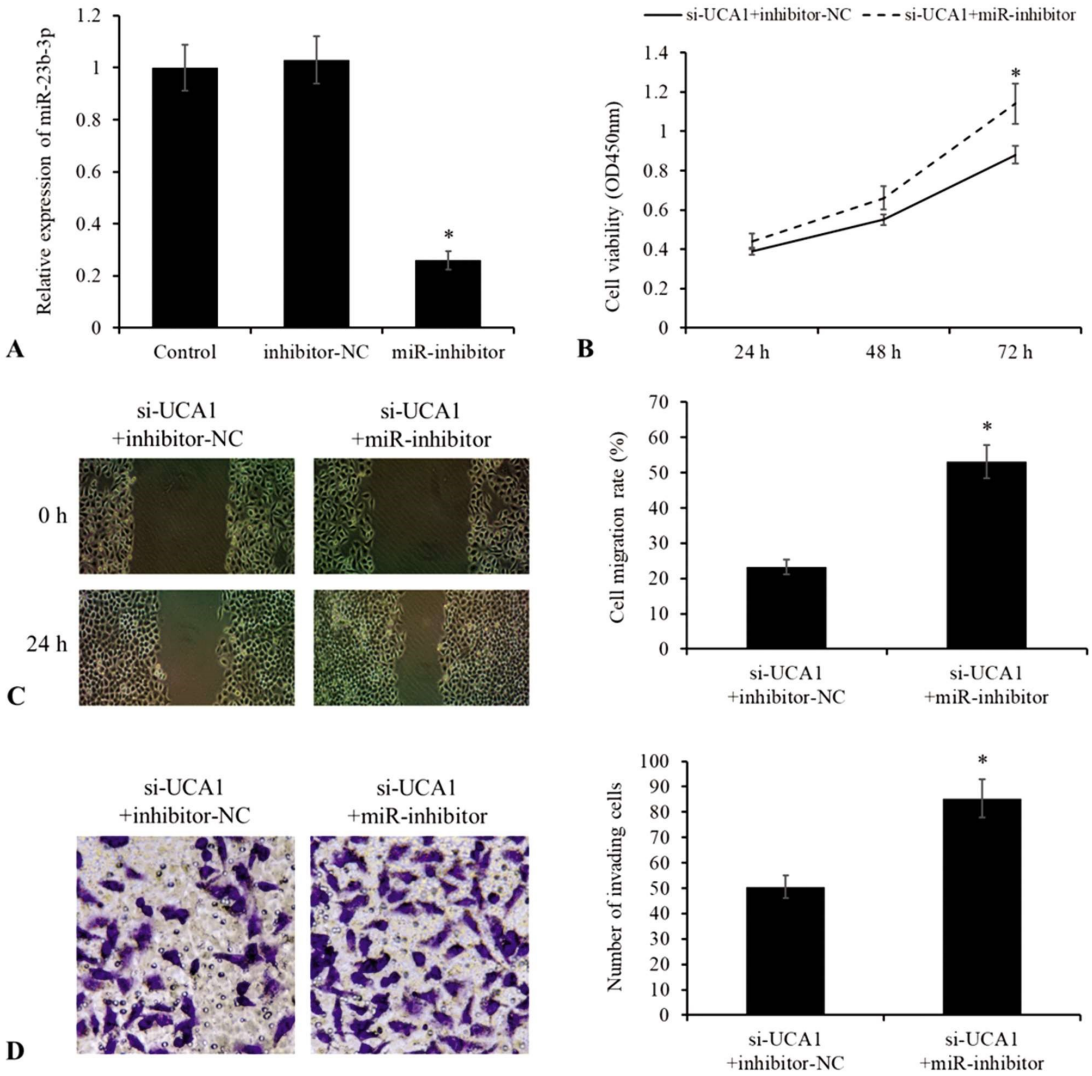

图 4 下调 miR-23b-3p 逆转了 UCA1 对 SGC7901 细胞增殖、迁移和侵袭的影响

Fig.4 Down-regulation of miR-23b-3p reversed the effects of UCA1 on the proliferation, migration and invasion of SGC7901 cells

Note: A: Transfection of miR-inhibitor down-regulated miR-23b-3p; B: Transfection of miR-inhibitor increased cell viability; C: Transfection of miR-inhibitor promoted cell migration, magnification: × 100; D: miR-inhibitor transfection promoted cell invasion, magnification: × 400; Compared with si-UCA1+inhibitor-NC group, *P<0.05.

本课题组前期研究结果证实 miR-23b-3p 是一个抗癌 miRNA[25]，为了进一步考察 miR-23b-3p 影响胃癌的途径，本研究通过生物信息学网站预测了 IL6R、BCL2 和 HSP90B1 miR-23b-3p 的潜在靶基因。IL6R 的高表达可促进多种癌症进展并增加耐药性[26,27]。BCL2 是一种广泛研究的抗凋亡基因[28,29]。HSP90B1 的高表达与多种癌症患者的预后和进展有关[30,31]。本研究显示，下调 UCA1 抑制了这三种癌基因的表达，然而下调 miR-23b-3p 则逆转了这种作用。由于 miRNA 在转录后水平调节靶基因的表达。因此，高表达的 lncRNA 可以竞争性地与 miRNA 结合，起到海绵的作用，诱导 miRNA 功能丧失，从而促进肿瘤进展。因此，上述结果说明，UCA1 对胃癌细胞增殖和转移的促进作用部分是通过调控 miR-23b-3p 及其下游基因来实现的。

综上所述，本研究表明下调 UCA1 通过靶向 miR-23b-3p 及其下游基因 IL6R、BCL2 和 HSP90B1 来抑制胃癌细胞的增殖和转移，因此，该信号途径可能是胃癌治疗的一个潜在靶点。

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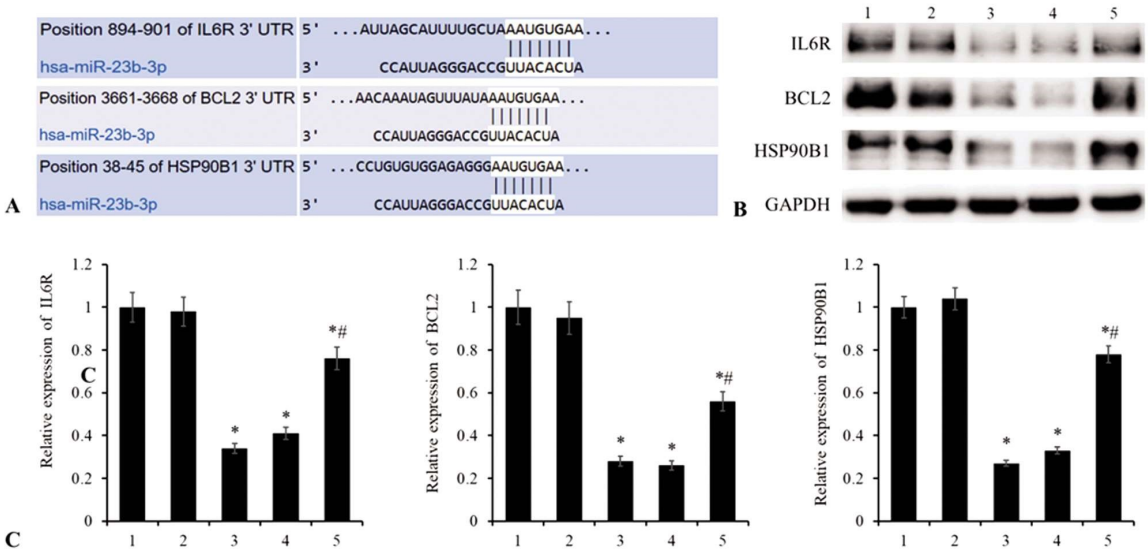

图 5 UCA1 通过 miR-23b-3p 调控 SGC7901 细胞中 IL6R、BCL2 和 HSP90B1 的蛋白表达

Fig.5 UCA1 regulated the protein expression of IL6R, BCL2 and HSP90B1 in SGC7901 cells through miR-23b-3p

Note: A: The bioinformatics website (Targetscan database) predicted that miR-23b-3p targeted and regulated IL6R, BCL2 and HSP90B1; B: Western blot analysis of the protein expression of IL6R, BCL2 and HSP90B1; C: The relative protein expression level of IL6R, BCL2 and HSP90B1; 1: Control group, 2: siRNA-NC group, 3: siRNA-UCA1 group, 4: si-UCA1+inhibitor-NC group, 5: si-UCA1+miR-inhibitor group; Compared with Control group, **P*<0.05; Compared with si-UCA1+inhibitor-NC group, #*P*<0.05.

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