miR-24-3p对宫颈癌细胞增殖与迁移的作用及机制研究

黄逸云[1] 户丽君[1] 林璐[1] 彭棋[1] 胡琴[1] 查何[2] 周兰[1]*

([1]重庆医科大学检验医学院 临床检验诊断学教育部重点实验室, 重庆 400016;
[2]遵义市第一人民医院/遵义医科大学第三附属医院, 遵义 563000)

摘要 该文探讨了miR-24-3p对宫颈癌细胞增殖和迁移的促进作用及其机制。采用miR-24-3p抑制剂下调宫颈癌细胞中miR-24-3p的表达后, 通过MTT、Transwell实验和Western blot检测细胞增殖、迁移和PCNA蛋白水平; 采用生物信息学方法预测miR-24-3p的靶基因并进行功能注释和筛选; 双荧光素酶报告实验和Western blot验证靶基因类血管动蛋白-2(angiomotin-like 2, *AMOTL2*), 并通过siRNA抑制*AMOTL2*检测其对宫颈癌细胞迁移的影响。结果显示, 下调miR-24-3p能抑制宫颈癌细胞的增殖和迁移能力并减少PCNA蛋白表达; 其靶基因主要存在细胞与细胞连接组分中, 显著富集于蛋白激酶活性分子功能、蛋白质自身磷酸化生物学过程和癌症中microRNA信号通路; miR-24-3p能靶向负调控最佳靶基因*AMOTL2*, 下调*AMOTL2*可促进宫颈癌细胞CaSki的迁移。总之, miR-24-3p可调控多靶基因参与多个生物学过程和多条信号通路, 在宫颈癌中可促进细胞增殖且通过靶向*AMOTL2*促进迁移。

关键词 宫颈癌; miR-24-3p; 增殖; 迁移; *AMOTL2*

The Effect and Mechanism of miR-24-3p on Proliferation and Migration in Human Cervical Cancer Cells

HUANG Yiyun[1], HU Lijun[1], LIN Lu[1], PENG Qi[1], HU Qin[1], ZHA He[2], ZHOU Lan[1]*

([1]*Key Laboratory of Diagnostic Medicine Designated by the Chinese Ministry of Education, Chongqing Medical University, Chongqing 400016, China*; [2]*The First People's Hospital of Zunyi/Third Affiliated Hospital of Zunyi Medical University, Zunyi 563000, China*)

Abstract This study aimed to investigate the role of miR-24-3p in the proliferation and migration of cervical cancer cells and its mechanism. miR-24-3p inhibitors were used to down-regulate the expression of miR-24-3p in cervical cancer cells, then MTT Transwell assays and Western blot were used to measure cell proliferation, migration and the protein level of the proliferating cell nuclear antigen. The target genes of miR-24-3p were predicted and then used for function annotation through bioinformatic methods. The relationship between miR-24-3p and target gene *AMOTL2* (angiomotin-like 2) was verified by dual-luciferase reporter system and Western blot, siRNA inhibited *AMOTL2* was used to detect its effect on cervical cancer cell migration. Data showed that down-regulating miR-24-3p could inhibit the proliferation and migration ability of cervical cancer cells and reduce PCNA protein expression. Its target genes were mainly present in the cell-cell junction component, and were sig-

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*通讯作者。Tel: 18523323947, E-mail: zhoulan@cqmu.edu.cn
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*Corresponding author. Tel: +86-18523323947, E-mail: zhoulan@cqmu.edu.cn
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nificantly enriched in biological processes including protein kinase activity molecular function, proteins autophosphorylation and microRNA in cancer signaling pathway. miR-24-3p was a negative regulator of the optimal target gene *AMOTL2*, and the decreased expression of *AMOTL2* promoted the migration of cervical cancer cell CaSki. In conclusion, miR-24-3p can regulate multiple target genes, which are involved in varied biological processes and signaling pathways, also promote cell proliferation and facilitate cell migration by targeting *AMOTL2* in cervical cancer.

Keywords cervical cancer; miR-24-3p; proliferation; migration; *AMOTL2*

宫颈癌(cervical cancer)是世界范围内最常见的恶性肿瘤之一, 其发病率居女性生殖器恶性肿瘤之首[1]。据统计, 2018年全球宫颈癌新发病例超过50万, 其中接近一半的患者死亡[2], 严重危及女性生命健康。目前宫颈癌的早期治疗效果较好, 但复发和转移性宫颈癌一般无法根治, 预后较差, 其生物标志物研究还处于临床试验阶段。因此, 进一步研究宫颈癌进展的机制, 对寻找新的治疗靶点具有重要意义。

非编码RNA, 如微小RNA(microRNAS, miRNAs)、长链非编码RNA(long non-coding RNAs, lncRNAs)等, 在肿瘤的发生发展过程中发挥着重要的调控作用。其中, miRNAs是一类约含22个核苷酸的内源性非编码单链小分子RNA, 它能在转录或转录后水平降解、抑制或活化靶基因mRNA[3], 其在肿瘤增殖、转移、侵袭和凋亡等过程中发挥重要作用[4]。在宫颈癌中, miRNAs可作为疾病进展的标志物, 其中miR-21促进宫颈癌细胞的增殖和迁移[5], miR-148a通过核糖体合成调控因子1(regulator of ribosome synthesis, *RRS1*)抑制宫颈癌细胞增殖促进细胞凋亡[6]。有报道称, miR-24在角质细胞分化过程中发挥重要作用, 其表达受到人乳头瘤病毒(human papilomavirus, HPV)致癌蛋白E6、E7调控[7], 提示miR-24家族可能参与宫颈癌变。miR-24-3p属于miR-24家族, 在肿瘤进展过程中起着重要的作用。研究表明, miR-24-3p能促进胃癌细胞生长, 抑制细胞凋亡[8], 也能通过靶向p27Kip1促进乳腺癌细胞增殖[9]。miR-24-3p被证实促进多种肿瘤进展, 如乳腺癌、膀胱癌、胶质瘤、头颈部鳞癌和霍奇金淋巴瘤[10-13]。然而, 在宫颈癌中miR-24-3p对细胞的增殖和迁移调控作用及机制尚未被阐明。因此, 本研究探究了miR-24-3p对宫颈癌细胞增殖和迁移的影响, 进一步预测和分析miR-24-3p的靶基因并加以验证, 旨在为宫颈癌分子诊断和预后提供理论依据。

1 材料与方法

1.1 材料

1.1.1 细胞 人宫颈癌细胞SiHa、CaSki由重庆医科大学检验医学院保存; CaSki细胞为宫颈腺癌细胞, SiHa细胞为宫颈鳞癌细胞, 均为HPV阳性并代表两种不同类型的宫颈癌细胞。

1.1.2 主要试剂 DMEM高糖培养基购自美国HyClone公司; 胎牛血清FBS购自以色列BI公司; Transwell小室购自Corning公司; Trizol试剂和Lipofectamine 2000转染试剂购自Invitrogen公司; qRT-PCR(quantitative Real-time PCR)相关试剂购自日本TaKaRa公司; MTT试剂和结晶紫染液购自北京索莱宝科技有限公司; 兔抗人PCNA(proliferation cell nuclear antigen)多克隆抗体购自沈阳万类生物科技有限公司; 鼠抗人*AMOTL2*单克隆抗体购自Santa Cruz Biotechnology公司; 小鼠抗人β-actin单克隆抗体、辣根过氧化物酶(HRP)标记的山羊抗兔IgG和山羊抗鼠IgG购自北京中杉金桥生物技术有限公司; pGL6-miR报告质粒、Western blot及蛋白提取相关试剂购自购自上海碧云天生物技术有限公司; 双荧光素酶报告检测试剂盒购自美国Progema公司; ECL发光液购自Millipore公司; miR-24-3p、*U6*、*AMOTL2*和*GAPDH*的引物由金斯瑞生物科技有限公司合成; miR-24-3p抑制剂和抑制剂阴性对照均购自上海吉玛制药技术有限公司; si*AMOTL2*和阴性对照NC购自百奥迈科生物技术有限公司。

1.2 方法

1.2.1 细胞培养 宫颈癌细胞SiHa和CaSki用含10% FBS、100 U/mL青霉素和100 μg/mL链霉素的DMEM高糖培养基培养, 当细胞密度约90%时, 用胰蛋白酶消化以1:2传代, 置于37 °C、5% CO_2的恒温培养箱中培养。

1.2.2 细胞转染 将宫颈癌细胞以每孔5.0×10^5个铺于6孔板内, 过夜培养, 待细胞密度达到70%时进

行转染。按照Lipofectamine 2000说明书步骤分别转染抑制剂阴性对照以及miR-24-3p抑制剂, 每孔转染终浓度为100 nmol/L, 转染6 h后将培养基换成含血清的培养基, 24 h或者48 h后收集细胞进行后续实验; 以相同的方法转染si*AMOTL2*和NC。miR-24-3p抑制剂和si*AMOTL2*以及阴性对照的序列见表1。

1.2.3 总RNA提取及qRT-PCR检测 Trizol法提取抑制剂阴性对照组、miR-24-3p抑制剂组的宫颈癌细胞SiHa和CaSki中的总RNA, 取1 μg用茎环法逆转录成cDNA, 用qRT-PCR检测miR-24-3p的mRNA水平, *U6*作为内参; Trizol法提取转染si*AMOTL2*或NC的CaSki细胞中总RNA, 一步法逆转录成cDNA后用qRT-PCR检测*AMOTL2*的mRNA水平, *GAPDH*作为内参。引物序列见表2。

1.2.4 MTT实验 将miR-24-3p抑制剂或抑制剂阴性对照转染24 h后的细胞用胰蛋白酶消化, 以每孔2 000个细胞接种于96孔板, 分别于0、24、48、72 h加入10 μL MTT溶液, 37 °C、5% CO_2恒温培养箱避光孵育4 h后, 在酶联免疫检测仪492 nm处读取各孔吸光度值。

1.2.5 Western blot检测PCNA和AMOTL2的蛋白水平 收集转染后48 h的宫颈癌细胞, 提取细胞总蛋白质, BCA法检测蛋白质的浓度, 取35 μg的蛋白质经10%的SDS-PAGE分离, 然后在210 mA恒流下进行湿转, 将蛋白质转移到PVDF膜上, 5% BSA室温封闭2 h后加入一抗, 在4 °C孵育过夜, 第2天用TBST洗去游离抗体, 加入HRP标记的山羊抗小鼠或兔IgG二抗, 室温孵育1 h; TBST洗膜3次, 每次10 min, ECL发光液显色。显色结果用Image Lab软件分析灰度值, 蛋白质相对表达水平=目的蛋白质灰度值/β-actin蛋白质灰度值。

1.2.6 Transwell实验 在24孔板中加入600 μL含15% FBS的DMED培养基, 平稳地将小室放入, 取200 μL细胞悬液(悬液密度为2×10^5个/mL)滴加到小室里面, 在37 °C、5% CO_2恒温培养箱孵育24 h后将小室取出, 轻柔地用PBS清洗, 接着用4%多聚甲醛固定20 min后, 用棉签擦去上层未穿过细胞, PBS清洗后用结晶紫染色15 min, 自来水冲洗染料后将小室放入37 °C烘箱干燥, 最后在倒置显微镜下拍照计数。

1.2.7 miR-24-3p靶基因的预测和功能注释 靶

表1 miRNA抑制剂和小干扰RNA序列

Table 1 Sequences of miRNA inhibitor and siRNA

miRNA和小干扰RNA miRNA and siRNA	序列(5′→3′) Sequence (5′→3′)
miR-24-3p inhibitor	CUG UUC CUG CUG AAC UGA GCC A
NC inhibitor	CAG UAC UUU UGU GUA GUA CAA
si*AMOTL2*	Forward: GUC AGA ACA ACU GCG AGA GdT dT Reverse: CUC UCG CAG UUG UUC UGA CdT dT
NC	Forward: UUC UCC GAA CGU GUC ACG UdT dT Reverse: ACG UGA CAC GUU CGG AGA AdT dT

表2 引物序列

Table 2 Primer sequences

基因名称 Gene name	序列(5′→3′) Sequence (5′→3′)
miR-24-3p	RT: GTC GTA TCC AGT GCA GGG TCC GAG GTA TTC GCA CTG GAT ACG ACC TG TTC
U6	RT: CGC TTC ACG AAT TTG CGT GTC AT
miR-24-3p	Forward: GCG TGG CTC AGT TCA GCA G Reverse: AGT GCA GGG TCC GAG GTA TT
U6	Forward: GCT TCG GCA GCA CAT ATA CTA AAT Reverse: CGC TTC ACG AAT TTG CGT GTC AT
AMOTL2	Forward: TAA AGG TGC TCC ATG CCC AG Reverse: ATC TCT GCT CCC GTG TTT GG
GAPDH	Forward: CAG CGA CAC CCA CTC CTC Reverse: TGA GGT CCA CCA CCC TGT

基因预测采用miRDB(http://www.mirdb.org/)、TargetScan(http://www.targetscan.org/)、mirtarbase(http://mirtarbase.mbc.nctu.edu.tw/index.php)和starBase(http://starbase.sysu.edu.cn/index.php)这4个在线数据库进行预测, 并将4个数据库的共同预测结果上传至Metascape(http://metascape.org/)数据库, 然后选择H.sapiens进行Custom Analysis, 选取P<0.01的结果进行生物学过程、分子功能、细胞组分和信号通路的功能注释。

1.2.8 双荧光素酶报告实验 TargetScan 7.2数据库中显示了miR-24-3p与靶基因的结合位点, 将包含了结合位点的*AMOTL2* 3′UTR的野生型和突变型片段分别插入到pGL6-miR报告质粒的多克隆酶切位点区间, 利用Lipofectamine 2000转染试剂将*AMOTL2* 3′UTR的野生型(wt)和突变型重组质粒(mut)分别与miR-24-3p mimic或NC mimic共转染进HEK 293T细胞, 海肾荧光素酶作为底物, 48 h后检测萤火虫荧光素酶和海肾荧光素酶的活性。

1.2.9 统计学分析 各实验均独立重复3次, 使用GraphPad Prism 5.0软件进行统计学分析。定量资料结果数据以均数±标准差($\bar{x}\pm s$)表示, 两组间数据比较采用t检验, 多组间数据采用方差分析, P<0.05表示差异有统计学意义。

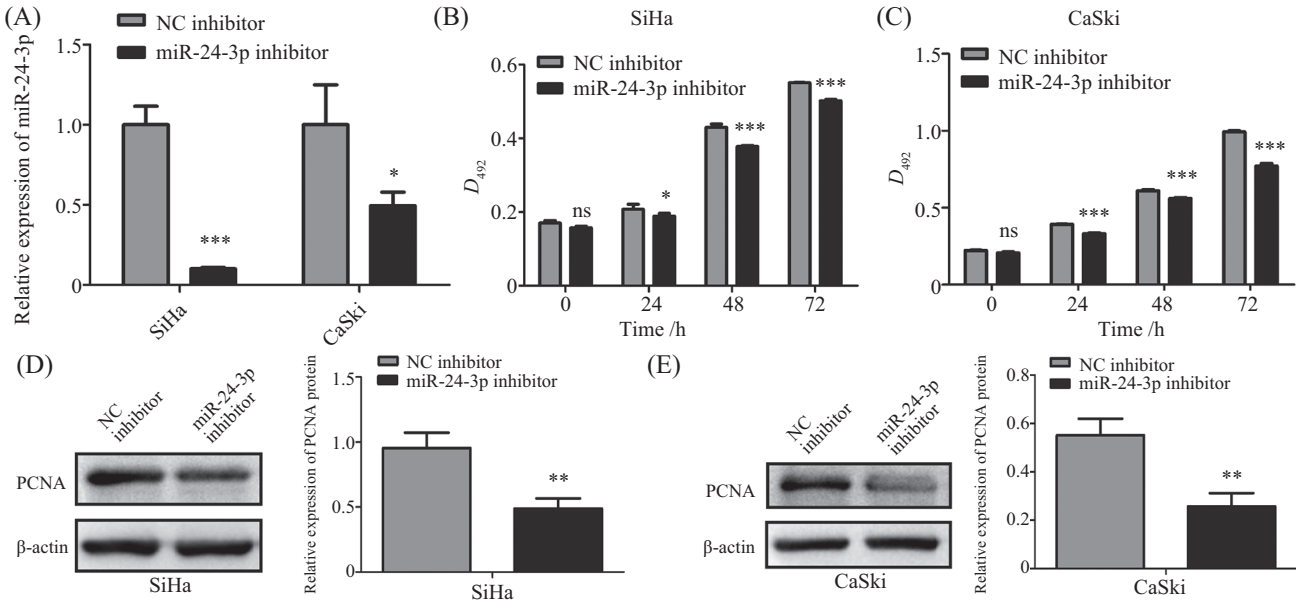
2 结果

2.1 下调miR-24-3p可抑制宫颈癌细胞的增殖

首先qRT-PCR结果(图1A)表明, 转染miR-24-3p抑制剂后, SiHa和CaSki细胞中miR-24-3p的mRNA水平显著低于对照组(P<0.001, P<0.05), 说明转染成功。如图1B和图1C MTT结果所示, 下调miR-24-3p后SiHa和CaSki细胞的增殖能力在48 h和72 h均明显低于对照组(P<0.001)。增殖细胞核抗原PCNA与细胞DNA合成关系密切, 在细胞增殖的启动上起重要作用, 是反映细胞增殖状态的良好指标, 因此进一步用Western blot检测细胞PCNA蛋白的表达(图1D和图1E), 与对照组相比, miR-24-3p抑制剂组中SiHa和CaSki细胞的PCNA蛋白水均下调明显, 细胞增殖减弱(P<0.01)。

2.2 下调miR-24-3p可抑制宫颈癌细胞的迁移

Transwell实验结果见图2A和图2B, miR-24-3p



A: 用miR-24-3p抑制剂或阴性对照处理SiHa和CaSki细胞24 h, qRT-PCR检测细胞miR-24-3p的表达水平; B、C: MTT检测细胞的增殖情况, miR-24-3p的下调抑制了细胞增殖; D、E: Western blot检测细胞中PCNA蛋白水平, 下调miR-24-3p减少了PCNA蛋白的表达。*P<0.05, **P<0.01, ***P<0.001, 与抑制剂阴性对照组相比。

A: SiHa and CaSki cells were transfected with miR-24-3p inhibitor or NC inhibitor, then the mRNA level of miR-24-3p was detected by qRT-PCR; B,C: MTT assay was used to evaluate cell proliferation, downregulation of miR-24-3p suppressed proliferation of two types of cells; D,E: the expression of PCNA protein was measured by Western blot, down-regulating miR-24-3p reduced PCNA protein expression. *P<0.05, **P<0.01, ***P<0.001 *vs* NC inhibitor group.

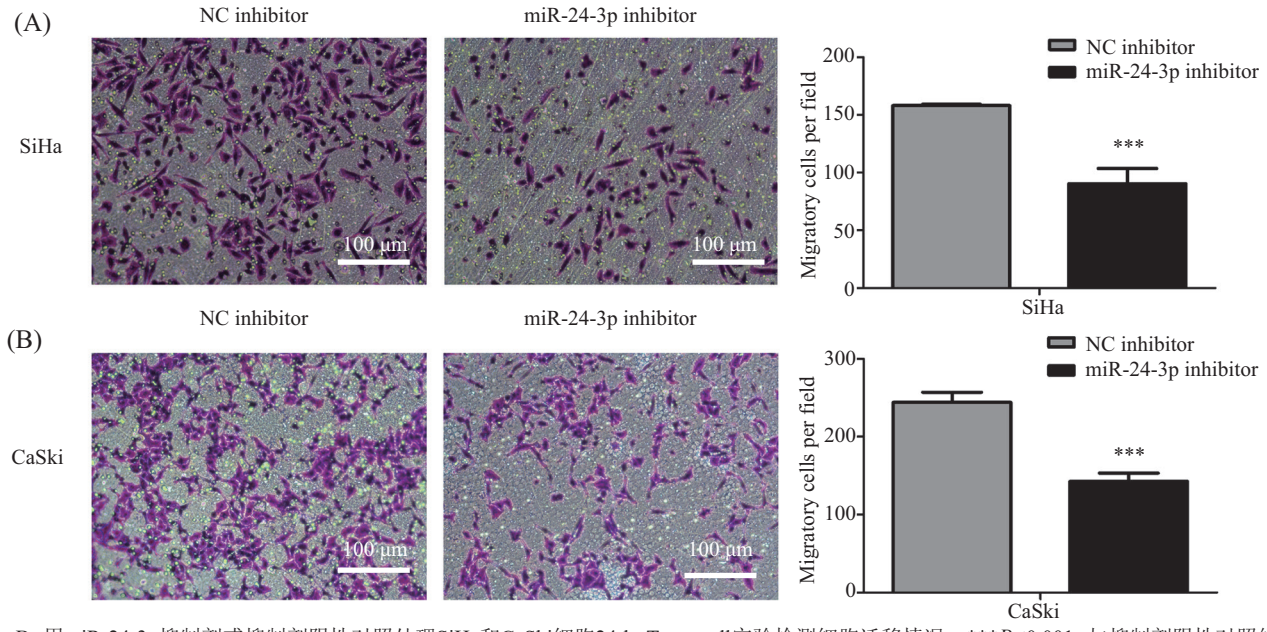
图1 下调miR-24-3p可抑制宫颈癌细胞的增殖

Fig.1 Downregulation of miR-24-3p inhibits the proliferation of cervical cancer cells

抑制剂组中穿膜的SiHa细胞数量为90.3±13.3, CaSki细胞数量为142.7±10.6, 对照组中穿膜的SiHa细胞数量为158.0±1.0, CaSki细胞数量为244.0±13.0。可见相比于对照组, 下调miR-24-3p后, SiHa细胞和CaSki细胞的迁移能力显著减弱(P<0.001)。

2.3 miR-24-3p靶基因的预测及功能注释

用miRDB、mirtarbase、TargetScan和starBase在线预测miR-24-3p的靶基因, 靶基因数目分别为959、761、862和4 249个, 取其交集得到87个基因(表3), 并将其作为功能富集分析的基因集合。GO功能注释结果见表4, 集合中的靶基因主要参与蛋白质自身磷酸化、钙调磷酸酶-NFAT级联信号的调控、激酶活性的正调控、细胞压力反应调节、细胞蛋白质定位调控、生殖发育过程、生长调节、血管形成、细胞增殖负向调节和昼夜节律调控基因的表达等20个生物学过程。分子功能显著富集于蛋白激酶活性、丝裂原激活蛋白激酶结合、蛋白酪氨酸激酶活性、泛素蛋白连接酶结合、腺苷三磷酸酶结合、磷酸酶绑定和蛋白羧基端结合(表5)。细胞组分主要富集于细胞间连接、PcG蛋白复合物、黏着斑、核斑点和内体再循环(表6)。KEGG信号通路分析显示miR-24-3p靶基因在癌症中miRNAs、干细胞多能性信号通路、Rap1信号通路、幽门螺杆菌感染的上皮细胞信号转导、p53信号通路、MAPK信号通路、细胞



A、B: 用miR-24-3p抑制剂或抑制剂阴性对照处理SiHa和CaSki细胞24 h, Transwell实验检测细胞迁移情况。***P<0.001, 与抑制剂阴性对照组相比。

A,B: SiHa and CaSki cells were transfected with miR-24-3p inhibitor or NC inhibitor. Transwell assay was taken to evaluate cell migration. ***P<0.001 *vs* NC inhibitor group.

图2 下调miR-24-3p可抑制宫颈癌细胞的迁移

Fig.2 Downregulation of miR-24-3p inhibits the migration of cervical cancer cells

表3 miR-24-3p预测靶基因

Table 3 Predicated target genes of miR-24-3p

miRNA	靶基因 Target genes
miR-24-3p	*TMEM216*, *HIC2*, *BBC3LMNB2*, *VGLL3*, *ABHD2*, *LIMD1*, *FGFR3*, *SH3PXD2A*, *NEK6*, *PDGFRB*, *BVES*, *ATP6V0A2*, *REPS2*, *NET1*, *MAPK7*, *NFAT5*, *MAGI1*, *AMOTL2*, *TSPAN14*, *AVL9*, *ATG4A*, *GBA2*, *MMP14*, *PTPRF*, *SESN1*, *FSCN1*, *FZD5*, *ABCB9*, *TSC22D2*, *CMTM4*, *SSR1*, *RAB11FIP1*, *PTGFRN*, *GUCD1*, *MARCKSL1*, *PAK4*, *RAP2C*, *SNTB1*, *RAD54L2*, *AAK1*, *PIM2*, *INSIG1*, *PPM1D*, *UGCG*, *ADD3*, *MATR3*, *VCPIP1*, *NDST1*, *C8orf58*, *STRADB*, *CRY2*, *YOD1*, *CDKN1B*, *SCML2*, *AGPAT3*, *DYRK2*, *RNF2*, *MAPK14*, *CSK*, *ZXDA*, *FURIN*, *RBBP4*, *ZNF217*, *MIDN*, *PER2*, *H2AFX*, *ZCCHC14*, *KCNK2*, *DEDD*, *DVL3*, *BCL2L11*, *MBD6*, *SP1*, *MXI1*, *ERBB3*, *RALA*, *SCML1*, *ACVR1B*, *MLEC*, *DNAJB12*, *PRSS8*, *ZXDB*, *TAOK1*, *POLR3D*, *CCDC58*, *TOP1*

表4 miR-24-3p靶基因生物学过程富集分析

Table 4 BP (biological processes) enrichment analysis of miR-24-3p target genes

ID	项目 Term	*P*-value	数量 Count
GO:0046777	Protein autophosphorylation	1.78E−07	11
GO:0070884	Regulation of calcineurin-NFAT signaling pathway	6.70E−06	13
GO:0033674	Positive regulation of kinase activity	6.90E−06	12
GO:0080135	Regulation of cellular response to stress	7.78E−05	11
GO:1903827	Regulation of cellular protein localization	6.05E−04	11
GO:0003006	Developmental process involved in reproduction	7.41E−04	15
GO:0040008	Regulation of growth	7.82E−04	16
GO:0022411	Cellular component disassembly	8.39E−04	10
GO:0001525	Angiogenesis	1.27E−03	10
GO:0000768	Syncytium formation by plasma membrane fusion	1.40E−03	6
GO:0032922	Circadian regulation of gene expression	1.47E−03	3
GO:0008285	Negative regulation of cell proliferation	1.69E−03	11
GO:0000077	DNA damage checkpoint	1.88E−03	8
GO:0071479	Cellular response to ionizing radiation	2.16E−03	7
GO:0033692	Cellular polysaccharide biosynthetic process	2.25E−03	5
GO:0006914	Autophagy	2.27E−03	7
GO:0035967	Cellular response to topologically incorrect protein	2.75E−03	5
GO:0007219	Notch signaling pathway	5.25E−03	4
GO:0043254	Regulation of protein complex assembly	6.98E−03	8
GO:0060349	Bone morphogenesis	8.15E−03	3

表5 miR-24-3p靶基因分子功能富集分析

Table 5 MF (molecular functions) enrichment analysis of miR-24-3p target genes

ID	项目 Term	*P*-value	数量 Count
GO:0004672	Protein kinase activity	4.30E−10	16
GO:0051019	Mitogen-activated protein kinase binding	1.24E−04	10
GO:0004713	Protein tyrosine kinase activity	1.39E−04	7
GO:0031625	Ubiqutin protein ligase binding	6.37E−04	6
GO:0051117	ATPase binding	3.03E−03	3
GO:0019902	Phosphatase binding	4.52E−03	4
GO:0008022	Protein C-terminus binding	4.70E−03	4

表6 miR-24-3p靶基因细胞组分富集分析

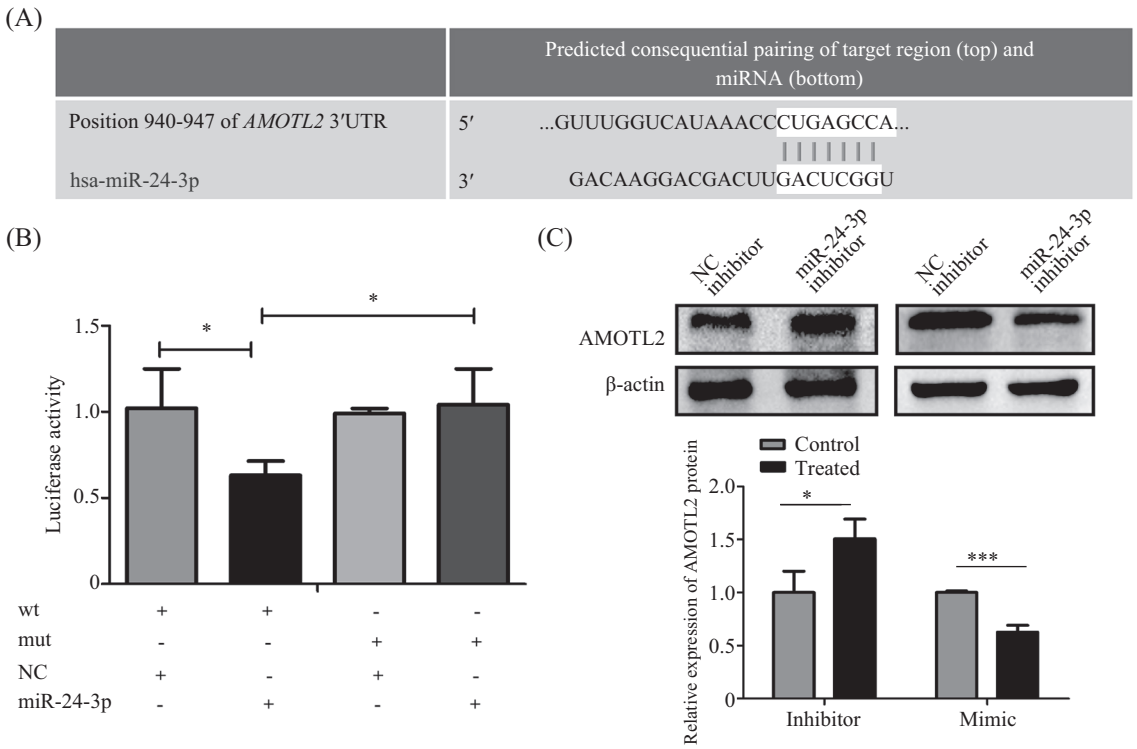
Table 6 CC (cellular components) enrichment analysis of miR-24-3p target genes

ID	项目 Term	*P*-value	数量 Count
GO:0005911	Cell-cell junction	6.03E−06	10
GO:0031519	PcG protein complex	6.53E−04	9
GO:0005925	Focal adhesion	6.82E−04	7
GO:0016607	Nuclear speck	3.13E−03	8
GO:0055037	Recycling endosome	3.86E−03	4

表7 miR-24-3p靶基因KEGG信号通路富集分析

Table 7 KEGG pathway enrichment analysis of miR-24-3p target genes

ID	项目 Term	*P*-value	数量 Count
hsa05206	MicroRNAs in cancer	1.26E−05	8
hsa04550	Signaling pathway regulating pluripotency of stem cells	1.49E−04	7
hsa04015	Rap1 signaling pathway	9.82E−04	9
hsa05120	Epithelial cell signaling in Helicobacter pylori infection	1.91E−03	3
hsa04115	p53 signaling pathway	1.99E−03	3
hsa04010	MAPK signaling pathway	2.31E−03	6
hsa04530	Tight junction	3.35E−03	4
hsa01522	Endocrine resistance	5.07E−03	3



A: TargetScan 7.2预测的miR-24-3p与*AMOTL2*的结合位点; B、C: 双荧光素酶报告实验和Western blot验证miR-24-3p靶向*AMOTL2*。*P<0.05, ***P<0.001。

A: the binding sites of miR-24-3p with AMOTL2 were observed in TargetScan 7.2; B,C: miR-24-3p targeting *AMOTL2* were validated by dual-luciferase reporter assay and Western blot. *P<0.05, ***P<0.001.

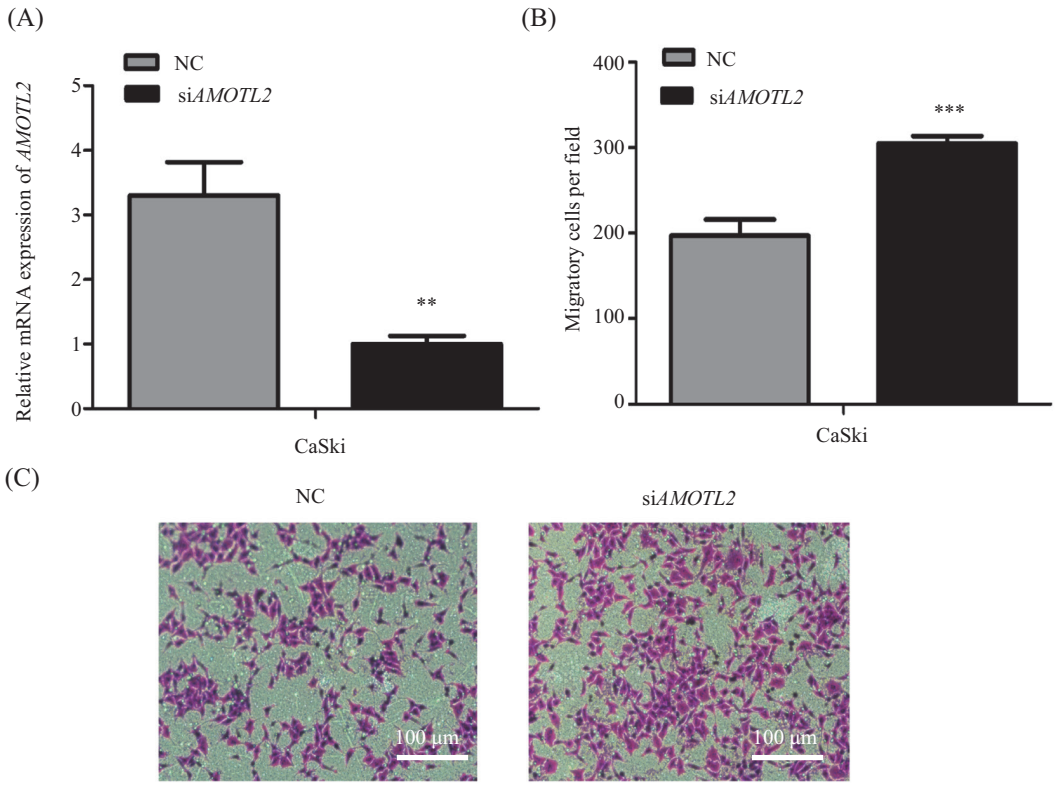
图3 *AMOTL2*是miR-24-3p的靶基因

Fig.3 *AMOTL2* is the target gene of miR-24-3p

紧密连接和内分泌耐药中显著富集(表7), 每个条目的*P*值和涉及的靶基因数目均展示在表中。

2.4 *AMOTL2*是miR-24-3p的靶基因

根据miR-24-3p对宫颈癌细胞增殖和迁移的作用, 将研究定位于参与血管形成和细胞增殖负调节生物学过程的靶基因, 筛选潜在的新型宫颈癌致病基因, 并结合RNA22靶基因预测数据库, 显示*AMOTL2*为miR-24-3p的最优预测靶基因。*AMOTL2* 3′UTR与miR-24-3p的结合位点见图3A, 其结合位点是*AMOTL2* 3′UTR的940到947位碱基。双荧光素酶报告实验结果见图3B, 将*AMOTL2* 3′UTR野生型报告质粒分别与NC mimic和miR-24-3p mimic共转染进HEK 293T细胞后, miR-24-3p mimic共转染组的荧光素酶活性明显低于NC mimic共转染组(P<0.05), 而将*AMOTL2* 3′UTR突变后, 荧光素酶活性又得以恢复。而且Western blot结果(图3C)显示, 在CaSki细胞中下调miR-24-3p后,



A: qRT-PCR检测*ANOTL2*的干扰效果; B、C: 敲低*AMOTL2*后, Transwell检测CaSki细胞的迁移。**P<0.01, ***P<0.001, 与NC组相比。

A: qRT-PCR was used to detect interference effect of *AMOTL2*; B,C: Transwell assay was taken to evaluate the migration of CaSki cells after knocking down *AMOTL2*. **P<0.01, ***P<0.001 *vs* NC group.

图4 下调*AMOTL2*促进CaSki细胞的迁移

Fig.4 Knocking down *AMOTL2* promotes the migration of CaSki cells

AMOTL2蛋白水平上调(P<0.05), 而上调miR-24-3p会使AMOTL2蛋白水平下调(P<0.001), 但干扰*AMOTL2*对宫颈鳞癌细胞SiHa的迁移无明显作用, 并未进行靶基因验证, 以上结果表明miR-24-3p在CaSki细胞中直接靶向*AMOTL2*。

2.5 下调*AMOTL2*可促进CaSki细胞的迁移

用siRNA干扰CaSki细胞中*AMOTL2*的表达, qRT-PCR检测干扰效果, 结果显示, 相比于NC组, si*AMOTL2*组中*AMOTL2*的mRNA水平显著下降(P<0.01)(图4A), 说明干扰效果显著, Transwell迁移结果见图4B和图4C, 下调*AMOTL2*后, CaSki细胞的迁移能力明显增强(P<0.001)。以上结果提示, *AMOTL2*在宫颈癌中可能发挥抑癌作用。

3 讨论

宫颈癌是最常见的女性生殖道肿瘤, 居发展中国家女性肿瘤发病率首位, 高危型HPV持续感染是主要的危险因素。miRNA在多种生物学过程中发挥重要作用, 并通过调控靶基因参与肿瘤的进程。有报道称miR-24参与角质细胞分化, 其表达受到HPV E6、HPV E7调控[7], 提示miR-24家族可能参与宫颈癌变。其中, miR-24-3p属于miR-24家族, 它能通过p53/p21信号通路抑制泪腺腺样囊性癌恶性进展, 但在乳腺癌、肺癌、肝癌、膀胱癌等大多数肿瘤中发挥促癌作用[14-16]。如miR-24-3p能和miR-27a-3p协同调控*MXI1*(max interactor 1)促进胶质瘤细胞增殖[11], 在恶性间皮瘤中miR-24-3p作为致癌miRNA参与细胞迁移过程[17]。然而, miR-24-3p对宫颈癌的具体作用及其确切的分子机制仍知之甚少。为了研究miR-24-3p在宫颈癌中的生物学功能, 在宫颈癌细胞CaSki和SiHa中转染miR-24-3p抑制剂成功干扰其表达。我们发现下调miR-24-3p后, 增殖细胞核抗原PCNA蛋白表达水平下调, 细胞增殖能力减弱, Transwell迁移实验表明, 下调miR-24-3p能抑制宫颈癌细胞的迁移。因此, 我们推测, miR-24-3p可能通过促进肿瘤细胞的增殖和迁移来调控宫颈癌的进程。

miRNA可作为肿瘤早期诊断和治疗的分子标

志物[18], 难点在于最佳候选体或靶点的选择。生物信息学可挖掘miRNA与疾病的关系并预测靶基因[19], 指导其在癌症进展中的研究。为探究miR-24-3p在宫颈癌中作用的分子途径, 本研究采用多个软件预测得到87个miR-24-3p的靶基因, 靶基因主要存在细胞与细胞连接的组分中, 具有蛋白激酶活性、丝裂原激活蛋白激酶结合和其他分子功能, 并涉及蛋白质自身磷酸化、激酶活性的正调控、血管形成、细胞增殖负向调节等多种生物学过程; 显著富集于癌症中miRNAs、干细胞多能性信号通路和Rap1等信号通路。本研究定位于有关细胞增殖和迁移的血管形成和细胞增殖负调节生物学过程的靶基因, 根据Genecards中宫颈癌致病基因, 筛选出*AMOTL2*、*PDGFRB*(platelet-derived growth factor receptor beta)和*FZD5*(frizzled-5)这三个潜在的新型靶基因, 但结合RNA22靶基因预测数据库, 显示*AMOTL2*为miR-24-3p的最优预测靶基因。

*AMOTL2*是血管动蛋白Amot家族成员之一, Amot蛋白属于血管抑素结合蛋白, 参与血管形成过程[20]。*AMOTL2*被证实是胚胎细胞正常运动的关键因子[21], 同时参与了内皮细胞的迁移和增殖[22]。本研究中双荧光素酶报告实验和Western blot证实了miR-24-3p能直接靶向*AMOTL2*并负向调控其蛋白表达。*AMOTL2*在癌症中的调控作用研究甚少, 其在乳腺癌和结直肠癌中作为促癌基因发挥作用[23-24], 但在胶质瘤中通过抑制*YAP*靶基因的活化作为抑癌基因参与肿瘤的生长和侵袭[25]。研究表明, *AMOTL2*抑制上皮细胞–间充质转化进程[26], 提示其对肿瘤的抑制作用, 为进一步研究*AMOTL2*对细胞迁移能力的影响, 我们采用siRNA干扰宫颈癌细胞CaSki中*AMOTL2*的表达。Transwell结果显示, 细胞的迁移能力明显增强, 因此我们认为, *AMOTL2*在miR-24-3p促进宫颈癌细胞迁移的过程中发挥重要作用。但本研究存在一定局限性, 在探究*AMOTL2*对细胞迁移作用的过程中, 发现其对SiHa细胞的作用并不显著, 其原因可能是在SiHa细胞中miR-24-3p通过其他靶基因发挥作用, 有待后续实验证实。

综上所述, miR-24-3p可通过多靶基因参与多个生物学过程和多条信号通路转导。在宫颈癌中, miR-24-3p促进细胞的增殖和迁移, 其对细胞迁移的影响是通过靶向*AMOTL2*来实现的, 这为miR-24-3p参与宫颈癌变的调控机制提供了新的证据和思路。

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