论　著　　基础研究

circAPLP2对结直肠癌细胞SW480侵袭与转移的影响及其机制

张鲁青，项利迪，缪淳迪，柯孔亮*

宁波市杭州湾医院普外科，浙江慈溪　315300

[中图分类号]　R73-37　　[文献标志码]　A　　[DOI]　10.11855/j.issn.0577-7402.2023.05.0577
[声明]　本文所有作者声明无利益冲突
[引用本文]　张鲁青, 项利迪, 缪淳迪, 等. circAPLP2对结直肠癌细胞SW480侵袭与转移的影响及其机制[J]. 解放军医学杂志, 2023, 48(5): 577-586.
[收稿日期]　2022-04-19　　[录用日期]　2022-06-28　　[上线日期]　2022-10-29

[摘要]　**目的**　探讨circAPLP2对结直肠癌细胞SW480侵袭与转移的调控作用及其分子机制。**方法**　采用在线数据库Starbase预测circAPLP2与miR-497-5p的结合位点，TargetScan预测miR-497-5p与FGFR1的结合位点。采用qRT-PCR检测LoVo、DLD1、SW480、SW620、Caco-2、HCoEpiC细胞中circAPLP2、miR-497-5p的相对表达水平，Western blotting检测FGFR1蛋白的相对表达水平。取SW480细胞，设置：(1)siNC组(转染siControl)与sicircAPLP2组(转染sicircAPLP2)，采用qRT-PCR检测circAPLP2、miR-497-5p、*FGFR1* mRNA的相对表达水平，Western blotting检测FGFR1蛋白的表达；(2)Vector组(转染对照空载质粒)与circAPLP2组(转染circAPLP2过表达质粒)，采用qRT-PCR检测miR-497-5p、*FGFR1* mRNA的相对表达水平，Western blotting检测FGFR1蛋白的相对表达水平；(3)siNC组(转染阴性对照)、sicircAPLP2组(转染sicircAPLP2)与sicircAPLP2+miR-497-5p inhibitor组(转染sicircAPLP2)，采用Transwell实验检测细胞侵袭能力，划痕实验检测细胞迁移能力，Western blotting检测上皮-间质转化(EMT)标志蛋白(E-cadherin、Twist1、N-cadherin、Vimentin)的表达；(4)NC-miRNA组(转染NC-miRNA)、miR-497-5p mimics组(转染miR-497-5p mimics)与NC-inhibitor组(转染NC-inhibitor)、miR-497-5p inhibitor组(转染miR-497-5p inhibitor)，采用qRT-PCR和Western blotting检测FGFR1的表达；(5)pcDNA-Control组(转染pcDNA-Control)与pcDNA-FGFR1组(转染pcDNA-FGFR1)，采用Western blotting检测FGFR1蛋白的相对表达水平；(6)NC-miRNA组(转染阴性对照)、miR-497-5p mimics组(转染miR-497-5p mimics)与miR-497-5p mimics+pcDNA-FGFR1组(共转染miR-497-5p mimics和pcDNA-FGFR1)，采用Transwell实验检测细胞侵袭能力，划痕实验检测细胞迁移能力，Western blotting检测FGFR1和EMT标志蛋白(E-cadherin、Twist1、N-cadherin、Vimentin)的表达。将circAPLP2-WT或circAPLP2-MT报告质粒、FGFR1-WT或FGFR1-MT报告质粒分别与miR-497-5p mimics和circAPLP2共转染SW480细胞48 h，采用荧光素酶报告基因检测系统检测荧光素酶的活性。**结果**　在线数据库Starbase、TargetScan分析结果显示，circAPLP2与miR-497-5p、miR-497-5p与FGFR1存在结合的位点。与人结肠上皮细胞HCoEpiC比较，结直肠癌细胞LoVo、DLD1、SW480、SW620、Caco-2中circAPLP2相对表达水平明显升高，miR-497-5p相对表达水平明显降低，FGFR1蛋白相对表达水平明显升高(P<0.05)。敲低circAPLP2的表达后，与siNC组比较，sicircAPLP2组侵袭细胞数减少(P<0.001)，划痕愈合率降低(P<0.001)，E-cadherin蛋白表达增加，Twist1、N-cadherin、Vimentin蛋白表达降低(P<0.05)。双荧光素酶报告实验结果显示，miR-497-5p mimics明显降低了circAPLP2-MT荧光素酶的活性(P<0.001)。miR-497-5p inhibitor可逆转sicircAPLP2对结直肠癌细胞EMT、迁移和侵袭的抑制作用，表现为侵袭细胞数增多(P<0.001)，划痕愈合率升高(P<0.01)，E-cadherin表达降低，Twist1、N-cadherin、Vimentin蛋白表达增加(P<0.05)。双荧光素酶报告实验结果显示，miR-497-5p mimics明显降低了FGFR1-MT荧光素酶的活性(P<0.001)。过表达FGFR1可逆转miR-497-5p过表达对结直肠癌细胞迁移和侵袭的抑制作用，表现为侵袭细胞数增多(P<0.001)，划痕愈合率升高(P<0.01)，E-cadherin表达降低，Twist1、N-cadherin、Vimentin蛋白表达增加(P<0.05)。**结论**　circAPLP2在结直肠癌细胞中表达水平升高，可能通过竞争性结合miR-497-5p促进FGFR1的表达，从而促进结直肠癌细胞的EMT、侵袭和迁移。

[关键词]　结直肠癌；circAPLP2；miR-497-5p；成纤维细胞生长因子受体1；侵袭转移

[基金项目]　宁波市自然科学基金(202003N4068)
[作者简介]　张鲁青，硕士研究生，主治医师，主要从事结直肠肿瘤及肛周疾病的诊断与治疗研究
[通信作者]　柯孔亮，E-mail：kekongliang1998@163.com

Effect and molecular mechanism of circAPLP2 on invasion and metastasis of colorectal cancer cell SW480

Zhang Lu-Qing, Xiang Li-Di, Miao Chun-Di, Ke Kong-Liang*

Department of General Surgery, Ningbo Hangzhou Bay Hospital, Cixi, Zhejiang 315300, China

**Corresponding author, E-mail: kekongliang1998@163.com*

This work was supported by the Natural Science Foundation of Ningbo City (202003N4068)

[**Abstract**] **Objective** To investigate the regulatory effect and molecular mechanism of circAPLP2 on invasion and metastasis of colorectal cancer cell SW480. **Methods** The online database Starbase was used to predict the binding sites of circAPLP2 and miR-497-5p, and TargetScan was used to predict the binding sites of miR-497-5p and FGFR1. The relative expression levels of circAPLP2 and miR-497-5p in LoVo, DLD1, SW480, SW620, Caco-2 and HCoEpiC cells were detected by qRT-PCR, and the relative expression level of FGFR1 protein was detected by Western blotting. SW480 cells were taken and set up as follows: (1) siNC group (transfected with siControl) and sicircAPLP2 group (transfected with sicircAPLP2), the expressions of circAPLP2, miR-497-5p, *FGFR1* mRNA were detected by qRT-PCR, and the expression of FGFR1 protein was detected by Western blotting. (2) Vector group (transfected with empty plasmid) and circAPLP2 group (transfected with circAPLP2 overexpression plasmid), the expressions of miR-497-5p and *FGFR1* mRNA were detected by qRT-PCR, and the expression of FGFR1 protein was detected by Western blotting. (3) siNC group (transfected with siControl), sicircAPLP2 group (transfected with sicircAPLP2) and sicircAPLP2+miR-497-5p inhibitor group (transfected with sicircAPLP2 and miR-497-5p inhibitor), the cell invasion was detected by Transwell, the cell migration was detected by scratch test, and the expressions of EMT marker proteins (E-cadherin, Twist1, N-cadherin and Vimentin) were detected by Western blotting. (4) NC miRNA group (transfected with NC-miRNA) and miR-497-5p mimics group (transfected with miR-497-5p mimics), or NC-inhibitor group (transfected with NC-inhibitor) and miR-497-5p inhibitor group (transfected with miR-497-5p inhibitor), the expression of FGFR1 was detected by qRT-PCR and Western blotting. (5) pcDNA-Control group (transfected with pcDNA-Control) and pcDNA-FGFR1 group (transfected with pcDNA-FGFR1), the expression of FGFR1 protein was detected by Western blotting. (6) NC-miRNA group (transfected with negative control), miR-497-5p mimics group (transfected with miR-497-5p mimics) and miR-497-5p mimics+pcDNA-FGFR1 group (co transfected with miR-497-5p mimics and pcDNA-FGFR1), the cell invasion, migration and the expression of FGFR1 and EMT marker proteins (E-cadherin, Twist1, N-cadherin, Vimentin) were detected by Transwell, scratch test or Western blotting. The circAPLP2-WT or circAPLP2-MT report plasmid was co-transfected with NC-miRNA or miR-497-5p mimics respectively for 48 h in SW480 cells, and the FGFR1-WT or FGFR1-MT report plasmid was co-transfected with miR-497-5p mimics and circAPLP2 respectively for 48 h in SW480 cells, and the luciferase activity was detected by the luciferase reporter gene detection system. **Results** The analysis results by Starbase and TargetScan showed that binding sites existed between circAPLP2 and miR-497-5p, and between miR-497-5p and FGFR1. Compared with human colon epithelial cell HCoEpiC, the relative expression level of circAPLP2 in colorectal cancer cells LoVo, DLD1, SW480, SW620 and Caco-2 increased significantly, while of miR-497-5p significantly decreased, and the relative expression level of FGFR1 protein significantly increased (P<0.05). After knocking down the expression of circAPLP2, compared with siCN group, the number of invasive cells in sicircAPLP2 group decreased (P<0.001), and the cell healing rate of scratches decreased (P<0.001), the expression of E-cadherin protein increased, and the protein expressions of Twist1, N-cadherin and Vimentin decreased (P<0.05). The results of dual luciferase reporter assay showed that miR-497-5p mimics decreased circAPLP2-MT luciferase activity significantly (P<0.001); MiR-497-5p inhibitor reverses the inhibitory effect of sicircAPLP2 on EMT, migration and invasion of colorectal cancer cells, as the number of invasive cells increased (P<0.001), the scratch healing rate increased (P<0.01), the expression of E-cadherin protein decreased, and the expression of Twist1, N-cadherin, and Vimentin protein increased (P<0.05). Dual luciferase reporter assay results showed that miR-497-5p mimics significantly reduced FGFR1-MT luciferase activity (P<0.001). Over-expression of FGFR1 reversed the inhibition of miR-497-5p overexpression on colorectal cancer cell migration and invasion, manifested as increased number of invasive cells (P<0.001), increased scratch healing rate (P<0.01), decreased expression of E-cadherin, increased expressions of N-cadherin, Twist1 and Vimentin (P<0.05). **Conclusion** The expression level of circAPLP2 increases in colorectal cancer cells, it may promote the expression of FGFR1 through competitive combination with miR-497-5p, thus promoting EMT, invasion and migration of colorectal cancer cells.

[**Key words**] colorectal cancer; circAPLP2; miR-497-5p; fibroblast growth factor receptor 1; invasion and metastasis

结直肠癌是全球范围内最常见的恶性肿瘤之一，其发病率在恶性肿瘤中居第4位，病死率居第2位，严重威胁人类的健康[1]。近年来，手术切除联合化疗、放疗的临床治疗方案在一定程度上改善了结直肠癌患者的生存质量，但病死率仍高达10.2%，而结直肠癌侵袭、转移是导致患者死亡的

主要原因[2-3]。侵袭、转移作为肿瘤发生发展中的恶性生物学行为，严重影响患者的预后，上皮-间质转化(epithelial-mesenchymal transition，EMT)是肿瘤发生侵袭、转移的前提[4]，深入阐明结直肠癌EMT、侵袭、转移的发生机制是延长患者生存期的关键。近年来，RNA组学成为生命科学关注的热点。在人类基因组中超过95%的基因不编码蛋白质，被称为非编码RNA[5]，其中环状RNA(circRNA)是一类特殊的非编码RNA，也是RNA领域最新的研究热点[6]。circRNA因其组织特异性、稳定性和高度保守性，已成为肿瘤诊断及预后预测的重要标志物[7]。既往研究发现，在结直肠癌中下调miR-497-5p表达，可导致成纤维细胞生长因子受体1(fibroblast growth factor receptor 1，FGFR1)表达增加，从而促进癌细胞增殖[8]。本研究探讨了circAPLP2竞争性结合miR-497-5p上调FGFR1在结直肠癌细胞中的作用，以期为结直肠癌靶向药物研发提供新的思路。

1 材料与方法

1.1 主要试剂及仪器 Trizol试剂、Lipofectamine 3000购自美国Invitrogen公司；Gibco胎牛血清、cDNA反转录试剂盒、SYBR Green PCR试剂盒和TaqMan miRNA测定试剂盒购自美国Thermo Fisher Scientific公司；circAPLP2小干扰RNA(sicircAPLP2)及其阴性对照(siControl)、miR-497-5p mimics及其阴性对照(NC-miRNA)、miR-497-5p inhibitor及其阴性对照(inhibitor-NC)、慢病毒质粒pLVX-circAPLP2(circAPLP2)及其阴性对照(pLVX)、pcDNA-FGFR1过表达质粒(pcDNA-FGFR1)及其阴性对照(pcDNA-Control)由上海锐博生物科技有限公司提供；E-cadherin(#14472)、Twist1(#69366)、N-cadherin(#13116)、Vimentin(#5741)、FGFR1(#9740)和GAPDH(#5174)抗体购自美国Cell Signaling Technology公司。ABI 7300系统购自美国Thermo Fisher Scientific公司。

1.2 miR-497-5p与FGFR1、circAPLP2的结合位点预测 采用在线数据库Starbase预测circAPLP2与miR-497-5p的结合位点，在线数据库TargetScan预测miR-497-5p与FGFR1的结合位点。

1.3 细胞培养及分组 结直肠癌细胞系LoVo、DLD1、SW480、SW620、Caco-2及人正常结肠上皮细胞HCoEpiC由中科院上海细胞研究所提供，置于含10%胎牛血清(FBS)的DMEM培养基中，在37 ℃、5% CO_2培养箱中培养。

(1)取LoVo、DLD1、SW480、SW620、Caco-2、HCoEpiC细胞，接种于96孔板中，采用实时荧光定量PCR(qRT-PCR)检测circAPLP2、miR-497-5p的相对表达水平，Western blotting检测FGFR1蛋白的相对表达水平。(2)取SW480细胞，设置siNC组(转染50 nmol/L siControl)、sicircAPLP2组(转染50 nmol/L sicircAPLP2)，转染48 h后，采用qRT-PCR检测circAPLP2、miR-497-5p及*FGFR1* mRNA的相对表达水平，Western blotting检测FGFR1蛋白的相对表达水平。(3)取SW480细胞，设置Vector组(转染2 μg对照空载质粒)、circAPLP2组(转染2 μg circAPLP2过表达质粒)，转染48 h后，采用qRT-PCR检测miR-497-5p及*FGFR1* mRNA的相对表达水平，Western blotting检测FGFR1蛋白的相对表达水平。(4)取SW480细胞，设置siNC组(转染50 nmol/L阴性对照)、sicircAPLP2组(转染50 nmol/L sicircAPLP2)与sicircAPLP2+miR-497-5p inhibitor组(转染50 nmol/L sicircAPLP2和50 nmol/L miR-497-5p inhibitor)，转染48 h后，采用Transwell实验检测细胞侵袭能力，划痕实验检测细胞迁移能力，Western blotting检测EMT标志蛋白(E-cadherin、Twist1、N-cadherin、Vimentin)的表达。(5)取SW480细胞，设置NC-miRNA组(转染50 nmol/L NC-miRNA)、miR-497-5p mimics组(转染50 nmol/L miR-497-5p mimics)与NC-inhibitor组(转染50 nmol/L NC-inhibitor)、miR-497-5p inhibitor组(转染50 nmol/L miR-497-5p inhibitor)，转染48 h后，采用qRT-PCR和Western blotting检测FGFR1的表达。(6)取SW480细胞，设置pcDNA-Control组(转染2 μg pcDNA-Control)、pcDNA-FGFR1组(转染2 μg pcDNA-FGFR1)，采用Western blotting检测FGFR1蛋白的相对表达水平。(7)取SW480细胞，设置NC-miRNA组(转染阴性对照)、miR-497-5p mimics组(转染50 nmol/L miR-497-5p mimics)与miR-497-5p mimics+pcDNA-FGFR1组(转染50 nmol/L miR-497-5p mimics和2 μg pcDNA-FGFR1)，采用Transwell实验检测细胞侵袭能力，划痕实验检测细胞迁移能力，Western blotting检测FGFR1和EMT标志蛋白(E-cadherin、Twist1、N-cadherin、Vimentin)的表达。

1.3.1 qRT-PCR检测circAPLP2、miR-497-5p及*FGFR1* mRNA的相对表达水平 采用Trizol试剂提取细胞总RNA，使用cDNA反转录试剂盒反转录成cDNA。采用ABI 7300系统通过SYBR Green PCR试剂盒测定circAPLP2及*FGFR1* mRNA的相对表达水平，TaqMan miRNA法测定miR-497-5p的相对表达水平。反应体系(25 μl)：12.5 μl SYBR Premix Ex Taq(2×)，0.5 μl Primer F，0.5 μl Primer R，2 μl RT产物，9.5 μl ddH_2O。反应条件：95 ℃预变性30 s；95 ℃变性5 s，70 ℃退火20 s，72 ℃延伸30 s，共40

个循环。以GAPDH或U6作为内参，采用$2^{-\Delta\Delta Ct}$法计算circAPLP2、miR-497-5p及*FGFR1* miRNA的相对表达水平。引物序列如表1所示。

表1 qRT-PCR引物序列

Tab.1 Primer sequences for qRT-PCR

基因	引物序列(5'-3')
circAPLP2	正义：TGCCGGAGGGACAAAAAGCA
	反义：TGAGGCTCAGCAACAGCAAAT
miR-497-5p	正义：AGCGAAGTTTTGAGCCGATCGGGC
	反义：GCCGTGAGTCAGAGGTGGT
FGFR1	正义：AACCTGACCACAGAATTGGAGGCT
	反义：ATGCTGCCGTACTCATTCTCCACA
U6	正义：CTCGCTTCGGCAGCACATATACT
	反义：ACGCTTCACGAATTTGCGTGTC
GAPDH	正义：CGCTCTCTGCTCCTCCTGTTC
	反义：ATCCGTTGACTCCGACCTTCAC

FGFR1. 成纤维细胞生长因子受体1

1.3.2 划痕实验检测细胞迁移能力 将结直肠癌细胞接种在含DMEM(加入10% FBS)培养基的6孔板中，当细胞生长至80%密度时，用无菌200 μl枪头尖端垂直划痕；PBS洗涤去除漂浮细胞，加入不含血清的培养基培养48 h。使用NIS-Elements AR 3.1软件(日本Nikon公司)检测细胞迁移能力。

1.3.3 Transwell实验检测细胞侵袭能力 使用24孔Matrigel涂层Millicell系统(BD Biosciences)检测细胞的侵袭能力。将2×10^4个SW480细胞接种到24孔Transwell室(Corning)中，上室加入200 μl无血清DMEM，下室加入含10% FBS的DMEM，孵育24 h；去除表面未侵入的细胞，并将底部的细胞固定在100%甲醇中，加入0.5%结晶紫溶液染色，于显微镜下观察拍照，每个腔室随机选取5个区域计算侵袭细胞数，取平均值。

1.3.4 Western blotting检测FGFR1和EMT标志蛋白的表达 用含蛋白酶抑制剂混合物的冷RIPA缓冲液裂解细胞。使用10% SDS-PAGE分离蛋白质样品，并转移到PVDF膜上，加入特异性一抗E-cadherin(1:1000)、Twist1(1:1000)、N-cadherin(1:1000)、Vimentin(1:1000)、FGFR1(1:1000)和GAPDH(1:1000)，4 ℃孵育12 h；加入辣根过氧化物酶，用二抗室温孵育2 h；加入SuperPico化学发光底物用于可视化信号。采用ImageJ软件分析目的蛋白的相对表达水平。

1.4 双荧光素酶报告实验测定miR-497-5p与FGFR1及circAPLP2的结合作用 通过PCR扩增circAPLP2或FGFR1中与miR-497-3p结合的位点序列片段，然后亚克隆到pmirGLO质粒中。将2 μg circAPLP2-WT或2 μg circAPLP2-MT报告质粒分别与50 nmol/L NC-miRNA或50 nmol/L miR-497-5p mimics利用Lipofectamine 3000共转染SW480细胞中；将2 μg FGFR1-WT或2 μg FGFR1-MT报告质粒分别与50 nmol/L NC-miRNA或50 nmol/L miR-497-5p mimics利用Lipofectamine 3000共转染SW480细胞中；将50 pmol/孔miR-497-5p mimics和800 ng/孔荧光素酶报告质粒利用Lipofectamine 3000共转染到SW480细胞中。转染48 h后，采用荧光素酶报告基因检测系统检测荧光素酶活性。

1.5 统计学处理 采用SPSS 19.0软件进行统计分析。计量资料以$\bar{x}\pm s$表示，多组间比较采用单因素方差分析，进一步两两比较采用LSD-*t*检验。$P<0.05$为差异有统计学意义。

2 结 果

2.1 circAPLP2及miR-497-5p在结直肠癌细胞中的表达情况 qRT-PCR检测结果显示，与结肠上皮细胞HCoEpiC比较，结直肠癌细胞LoVo、DLD1、SW480、SW620、Caco-2中circAPLP2相对表达水平升高($P<0.05$，图1A)，miR-497-5p相对表达水平下降($P<0.05$，图1B)，SW480细胞circAPLP2相对表达水平最高、miR-497-5p相对表达水平最低，选择该细胞进行后续实验。

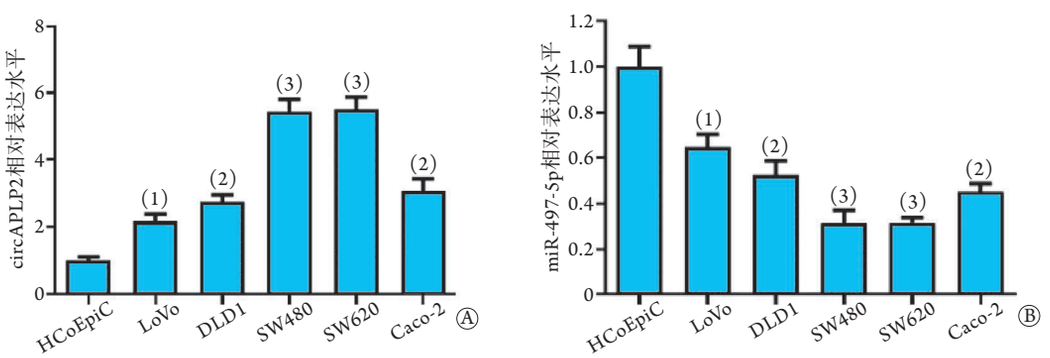

图1 circAPLP2 (A)及miR-497-5p (B)在结直肠癌细胞中的表达情况

Fig.1 Expressions of circAPLP2 (A) and miR-497-5p (B) in colorectal cancer cells

与结肠上皮细胞HCoEpiC比较，(1)$P<0.05$，(2)$P<0.01$，(3)$P<0.001$

2.2 上调或下调circAPLP2对结直肠癌细胞中miR-497-5p表达的影响 在线数据库Starbase预测结果显示，circAPLP2与miR-497-5p存在结合位点(图2A)。

与siNC组比较，sicircAPLP2组结直肠癌细胞SW480中circAPLP2相对表达水平明显降低(*P*<0.001，图2B)，表明转染成功。

与siNC组比较，敲低circAPLP2后，结直肠癌细胞SW480中miR-497-5p相对表达水平明显升高(*P*<0.001，图2C)；过表达circAPLP2后，结直肠癌细胞SW480中miR-497-5p相对表达水平明显降低(*P*<0.001，图2D)。双荧光素酶报告实验结果显示，与NC-miRNA组比较，miR-497-5p mimics组circAPLP2-WT荧光素酶活性明显降低(*P*<0.001)，但circAPLP2-MT荧光素酶活性差异无统计学意义(*P*>0.05，图2E)。

2.3 circAPLP2调控miR-497-5p对结直肠癌侵袭、迁移及EMT的影响 Transwell实验、划痕实验和Western blotting检测结果显示，与siNC组比较，sicircAPLP2组侵袭细胞数减少(*P*<0.001)，划痕愈合率降低(*P*<0.001)，E-cadherin蛋白相对表达水平增高(*P*<0.001)，Twist1、N-cadherin、Vimentin蛋白相对表达水平降低(*P*<0.001)；与sicircAPLP2组比较，sicircAPLP2+miR-497-5p inhibitor组侵袭细胞数增多(*P*<0.001)，划痕愈合率升高(*P*<0.01)，E-cadherin蛋白相对表达水平降低(*P*<0.01)，Twist1、N-cadherin、Vimentin蛋白相对表达水平增高(*P*<0.05) (图3)。

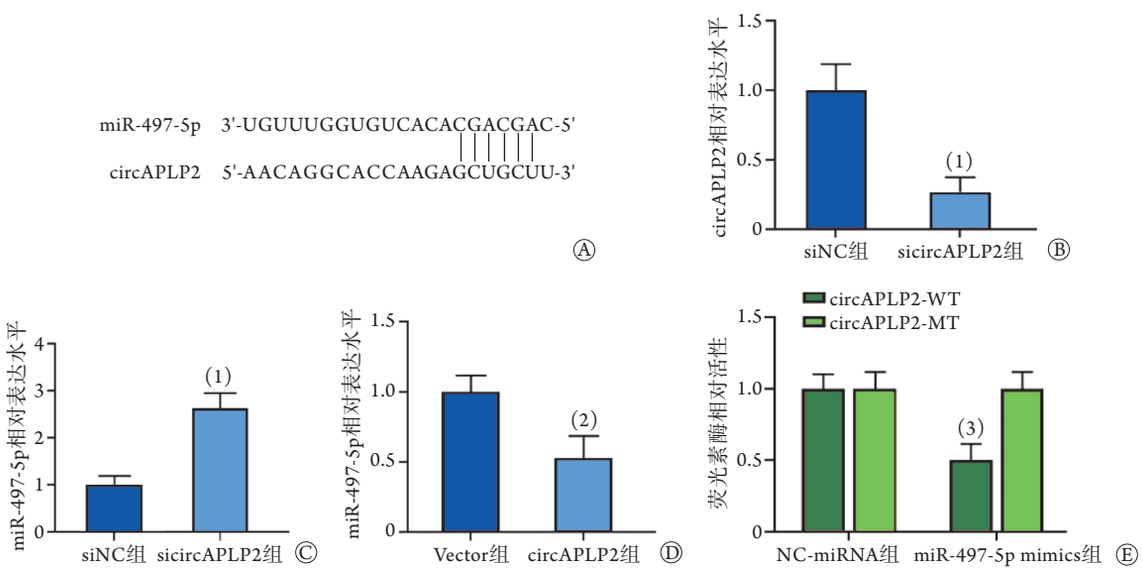

图2 上调或下调circAPLP2对结直肠癌细胞中miR-497-5p表达的影响

Fig.2 Effects of circAPLP2 up-regulation or down-regulation on miR-497-5p expression in colorectal cancer cells

A. Starbase预测circAPLP2与miR-497-5p的结合序列；B—C. SW480细胞转染sicircAPLP2或siControl 48 h后，qRT-PCR检测circAPLP2 (B)、miR-497-5p (C)相对表达水平；D. 过表达circAPLP2质粒或对照空载转染SW480细胞48 h后，qRT-PCR检测miR-497-5p相对表达水平；E. 将circAPLP2-WT或circAPLP2-MT报告质粒分别与NC-miRNA或miR-497-5p mimics共转染SW480细胞48 h后，荧光素酶报告基因检测系统检测荧光素酶活性。与siNC组比较，(1)*P*<0.001；与Vector组比较，(2)*P*<0.001；与NC-miRNA组比较，(3)*P*<0.001

2.4 上调或下调miR-497-5p对结直肠癌细胞中FGFR1表达的影响 TargetScan在线数据库分析结果显示，*FGFR1*是miR-497-5p的潜在靶基因(图4A)。

Western blotting检测结果显示，与结肠上皮细胞HCoEpiC比较，结直肠癌细胞LoVo、DLD1、SW480、SW620、Caco-2中FGFR1蛋白相对表达水平增高(*P*<0.01，图4B)。转染miR-497-5p mimics后，结直肠癌细胞SW480中*FGFR1* mRNA和蛋白相对表达水平降低(*P*<0.001，图4C)；转染miR-497-5p inhibitor后，结直肠癌细胞SW480中*FGFR1* mRNA和蛋白相对表达水平增高(*P*<0.001，图4D)。

双荧光素酶报告实验结果显示，与NC-miRNA组比较，miR-497-5p mimics组FGFR1-WT荧光素酶活性明显降低(*P*<0.001)，但FGFR1-MT荧光素酶活性差异无统计学意义(*P*>0.05，图4E)。

2.5 miR-497-5p靶向抑制FGFR1对结直肠癌侵袭、迁移及EMT的影响 转染FGFR1过表达质粒后，结直肠癌细胞中FGFR1蛋白相对表达水平明显增高(*P*<0.001，图5A)，表明转染成功。

Transwell实验、划痕实验和Western blotting检测结果显示，与NC-miRNA组比较，miR-497-5p mimics组侵袭细胞数减少(*P*<0.001)，划痕愈合率降低(*P*<0.001)，E-cadherin蛋白相对表达水平增高(*P*<0.001)，Twist1、N-cadherin、Vimentin蛋白相对表达水平降低(*P*<0.001)；与miR-497-5p mimics

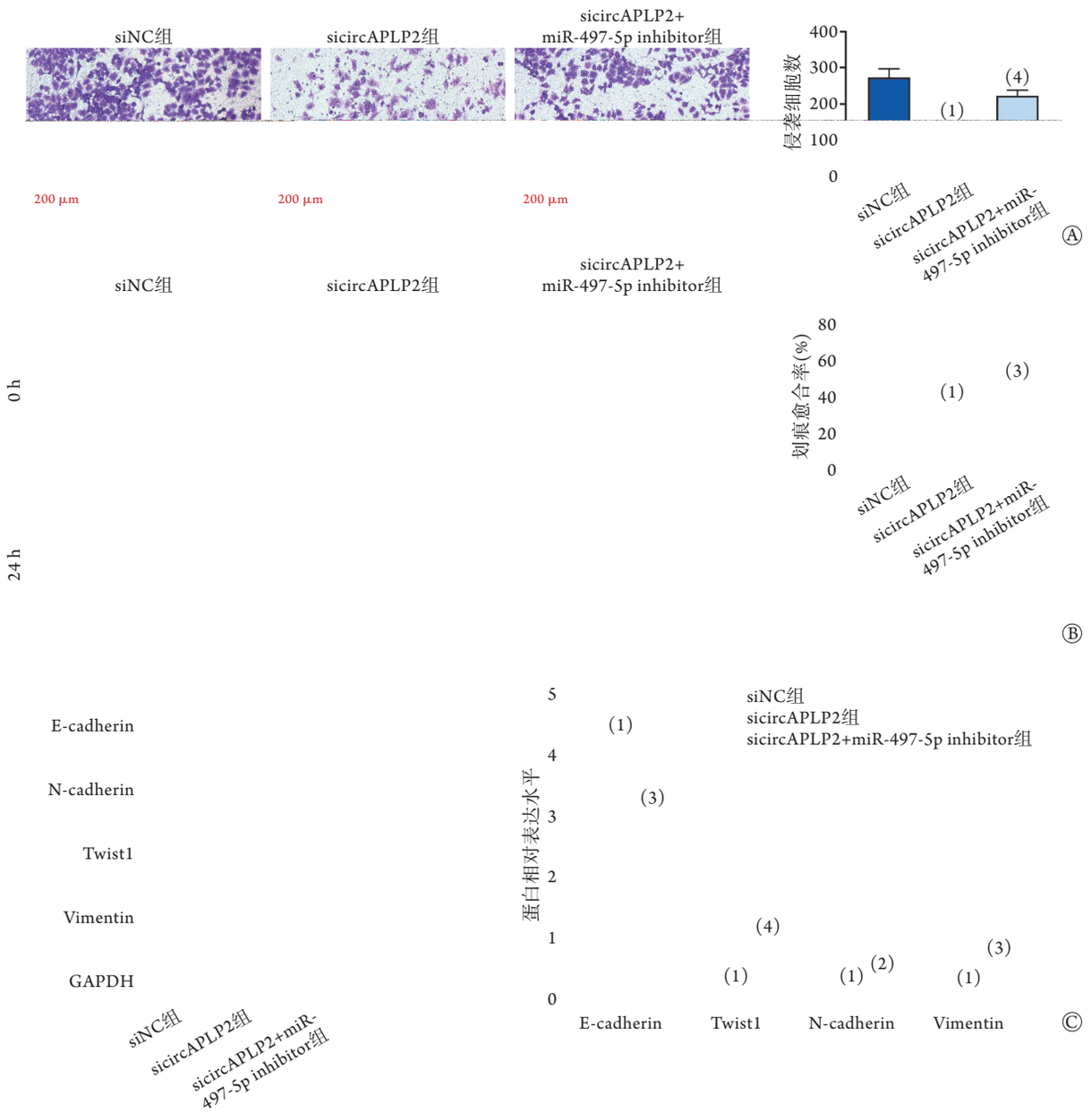

图3 circAPLP2调控miR-497-5p对结直肠癌侵袭、迁移及EMT的影响

Fig.3 Effects of miR-497-5p regulated by circAPLP2 on invasion, migration and EMT of colorectal cancer

EMT. 上皮-间质转化；SW480细胞共转染sicircAPLP2或siControl、NC-miRNA或miR-497-5p inhibitor 48 h后，Transwell实验检测细胞侵袭能力(A)，划痕实验检测细胞迁移能力(B)，Western blotting检测EMT标志蛋白的表达(C)；与siNC组比较，(1)P<0.001；与sicircAPLP2组比较，(2)P<0.05，(3)P<0.01，(4)P<0.001

组比较，miR-497-5p mimics+pcDNA-FGFR1组侵袭细胞数增多(P<0.001)，划痕愈合率升高(P<0.01)，E-cadherin蛋白相对表达水平降低(P<0.01)，Twist1、N-cadherin、Vimentin蛋白相对表达水平增高(P<0.05，图5B—D)。

2.6 上调或下调circAPLP2对结直肠癌细胞中FGFR1表达的影响 过表达circAPLP2后，结直肠癌细胞中*FGFR1* mRNA和蛋白相对表达水平明显上调(P<0.001，图6A)；敲低circAPLP2的表达后，结直肠癌细胞中*FGFR1* mRNA和蛋白相对表达水平明显下调(P<0.001，图6B)。

双荧光素酶报告实验结果显示，与NC-miRNA组比较，miR-497-5p mimics组FGFR1-WT荧光素酶活性明显降低(P<0.001)；与miR-497-5p mimics组比较，miR-497-5p mimics+circAPLP2组FGFR1-WT荧光素酶活性明显升高(P<0.001，图6C)。

Western blotting检测结果显示，转染miR-497-5p mimics后，结直肠癌细胞中FGFR1蛋白相对表达水平降低(P<0.001)；在此基础上过表达circAPLP2后，FGFR1蛋白相对表达水平增高(P<0.05，图6D)。

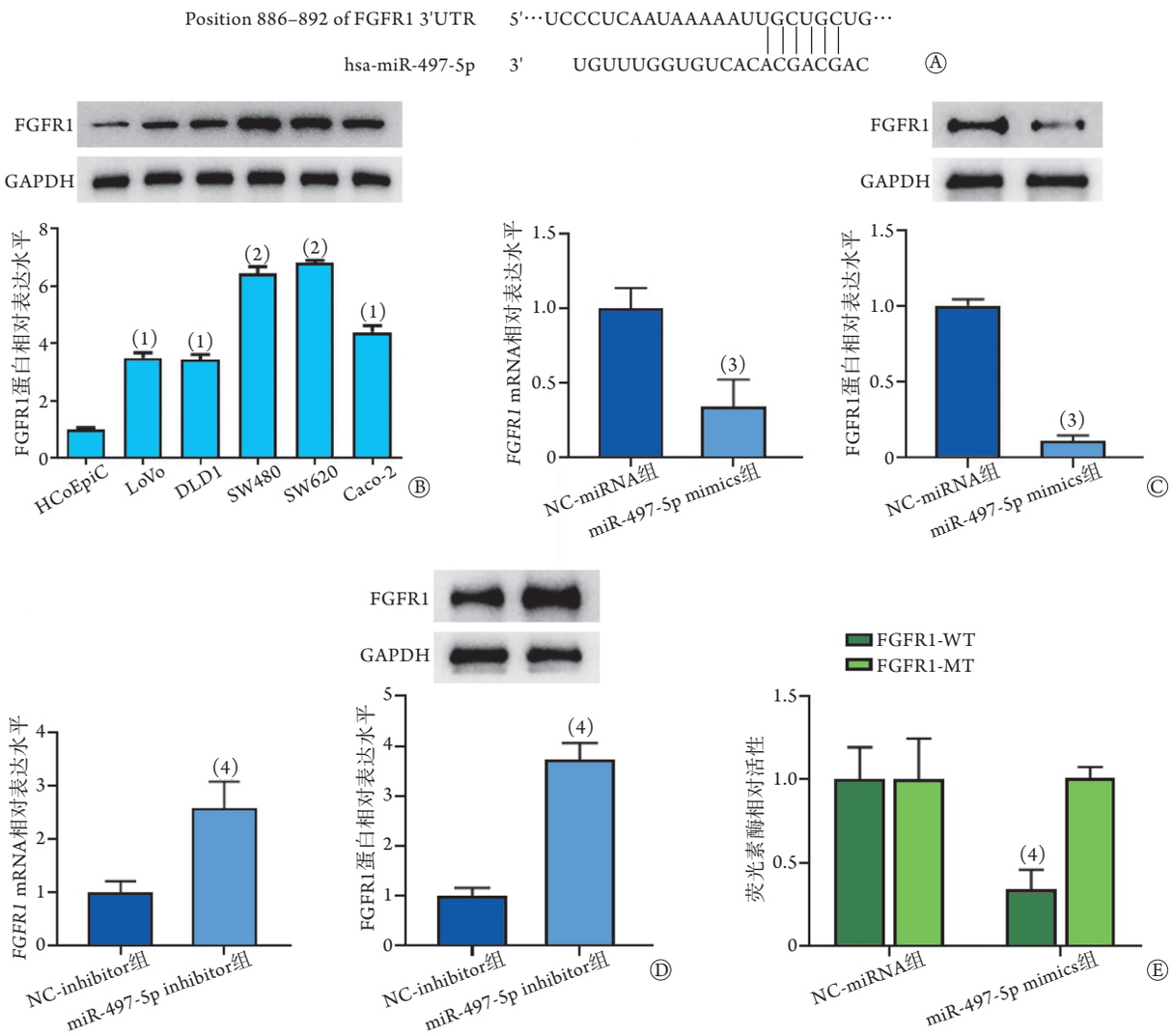

图4 上调或下调miR-497-5p对结直肠癌细胞中FGFR1表达的影响

Fig.4 Effects of miR-497-5p upregulation or downregulation on FGFR1 expression in colorectal cancer cells

FGFR1. 成纤维细胞生长因子受体1；A. 在线数据库TargetScan预测FGFR1与miR-497-5p的结合序列；B. Western blotting检测结直肠癌细胞中FGFR1的表达；C. NC-miRNA或miR-497-5p mimics转染SW480细胞48 h后，qRT-PCR及Western blotting检测FGFR1的表达；D. NC-inhibitor或miR-497-5p inhibitor转染SW480细胞48 h后，qRT-PCR及Western blotting检测FGFR1的表达；E. FGFR1-WT或FGFR1-MT报告质粒分别与NC-miRNA或miR-497-5p mimics共转染SW480细胞48 h后，荧光素酶报告基因检测系统检测荧光素酶活性；与结肠上皮细胞HCoEpiC比较，(1)P<0.01，(2)P<0.001；与NC-miRNA组比较，(3)P<0.001；与NC-inhibitor组比较，(4)P<0.001

3 讨　论

结直肠癌是我国癌症相关死亡的第五大原因，尽管随着治疗方法的不断进步，结直肠癌患者5年生存率有所提高，但术后复发和远处转移仍是患者死亡的主要原因[9]。EMT是肿瘤增殖和转移的重要组成部分，在其发生过程中，上皮细胞由上皮类型转变为间质类型，失去细胞间连接及极性，获得间质细胞形态，增强了细胞的迁移和侵袭能力[10]。研究发现，结直肠癌肝转移患者癌组织中上皮细胞标志物E-cadherin表达降低[11]；结直肠癌细胞发生EMT后，间质细胞标志物Twist1表达增加，细胞侵袭与迁移能力增强[12]。因此探究结直肠癌细胞EMT、增殖和转移能力的调控机制，对制订结直肠癌的治疗策略尤为重要。

circRNAs作为机体非编码RNA的重要组成部分，在肿瘤的发展中发挥了关键作用。已有研究证实，在结直肠癌中circPTK2、circCAMSAP1、circHIPK3等多种circRNA表达水平异常，是患者预后不良的重要预测指标[13-14]。circRNA参与调控肿瘤EMT、侵袭、转移等过程，如circLMNB1可抑制E-cadherin的表达，上调N-cadherin的表达，促进结直肠癌细胞发生EMT和体内转移[15]。敲低hsa_circ_0005273可抑制结直肠癌细胞的EMT进程，从而抑制结直肠癌的侵袭、转移[16]。circAPLP2(hsa_circ_0000372)位于11号染色体q24.3，在结直肠癌肝

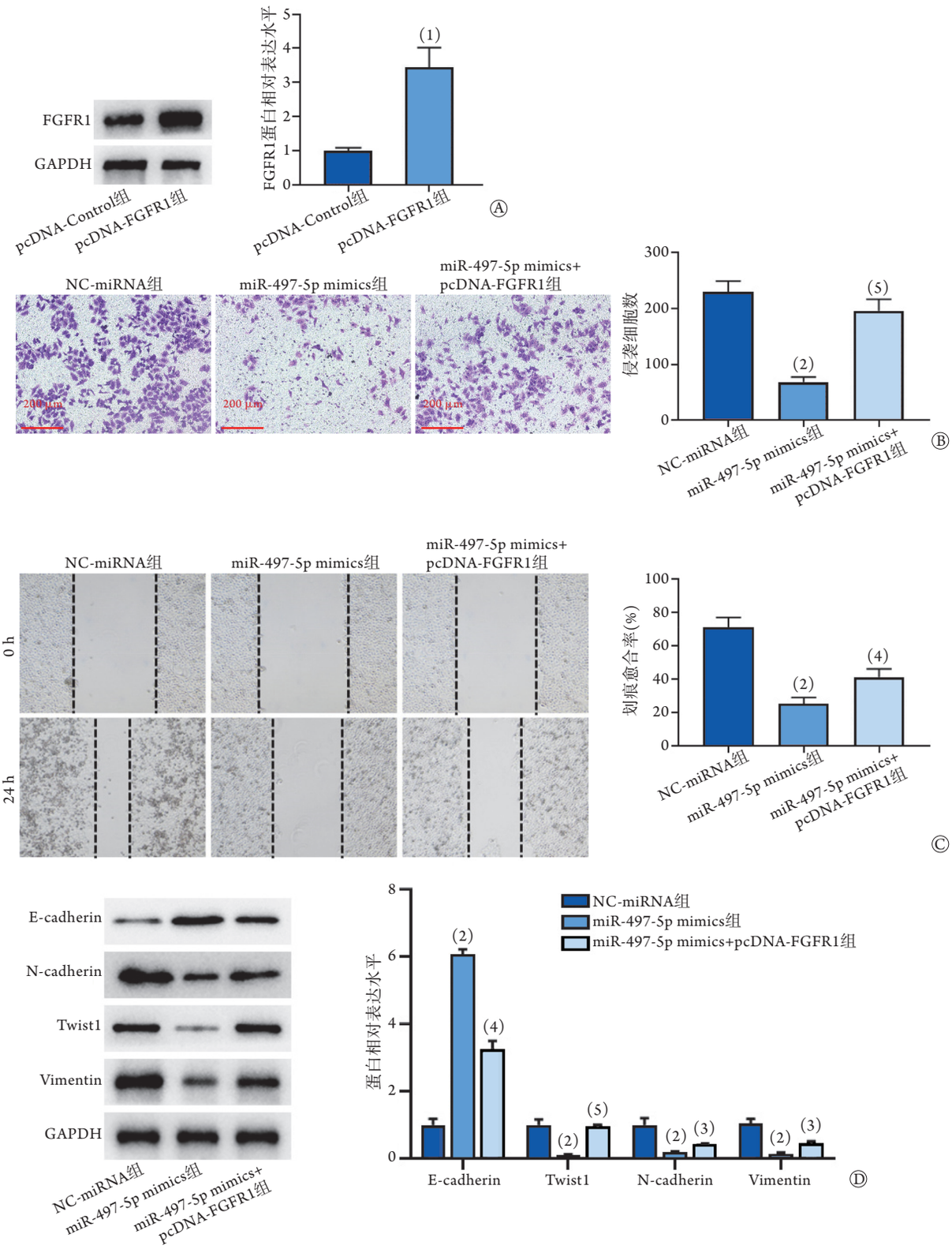

图5 miR-497-5p抑制FGFR1表达对结直肠癌侵袭、迁移及EMT的影响

Fig.5 Effects of miR-497-5p inhibits FGFR1 expression on invasion, migration and EMT of colorectal cancer

FGFR1. 成纤维细胞生长因子受体1；EMT. 上皮-间质转化；A. 过表达FGFR1质粒或空载转染SW480细胞48 h后，Western blotting检测FGFR1蛋白的表达；B—D. 过表达FGFR1质粒或空载分别与NC-miRNA或miR-497-5p mimics共转染SW480细胞48 h后，Transwell实验检测细胞侵袭能力(B)，划痕实验检测细胞迁移能力(C)，Western blotting检测FGFR1和EMT标志蛋白的表达(D)；与pcDNA-Control组比较，(1)P<0.001；与NC-miRNA组比较，(2)P<0.001；与miR-497-5p mimics组比较，(3)P<0.05，(4)P<0.01，(5)P<0.001

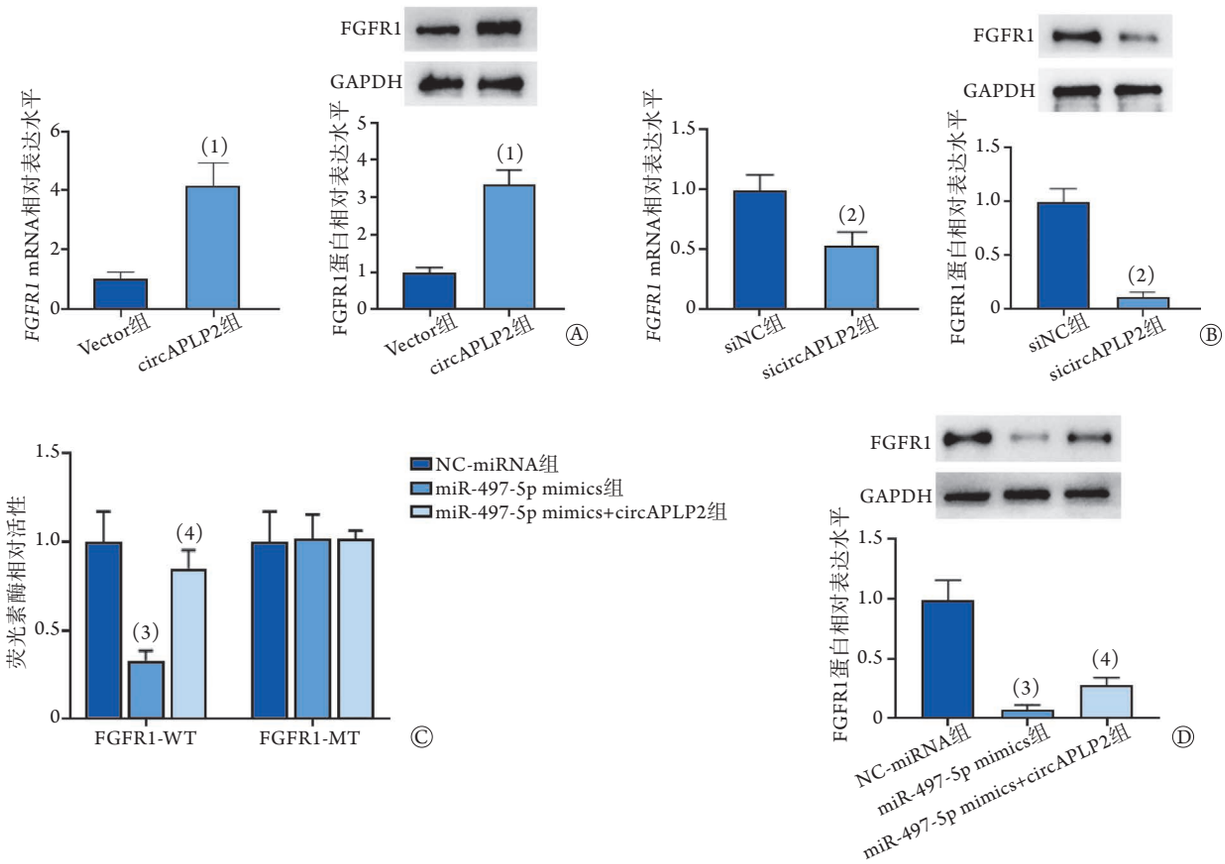

图6 上调或下调circAPLP2对结直肠癌细胞中FGFR1表达的影响

Fig.6 Effects of circAPLP2 up-regulation or down-regulation on FGFR1 expression in colorectal cancer cells

FGFR1. 成纤维细胞生长因子受体1；A. circAPLP2或空载转染SW480细胞48 h后，qRT-PCR及Western blotting检测FGFR1的表达；B. sicircAPLP2或siCtrl转染SW480细胞48 h后，qRT-PCR及Western blotting检测FGFR1的表达；C. 将FGFR1野生型或突变型报告质粒分别与miR-497-5p mimics和circAPLP2共转染SW480细胞48 h后，荧光素酶报告基因检测系统检测荧光素酶活性；D. circAPLP2或空载分别与NC-miRNA或miR-497-5p mimics共转染SW480细胞48 h后，Western blotting检测FGFR1蛋白的表达；与Vector组比较，(1)P<0.001；与siNC组比较，(2)P<0.001；与NC-miRNA组比较，(3)P<0.001；与miR-497-5p mimics组比较，(4)P<0.01

转移患者中表达水平明显升高[17]。Liu等[18]研究发现，敲低结直肠癌细胞SW480中circAPLP2的表达，可上调E-cadherin的表达，下调Vimentin的表达，本研究结果与之一致。Wu等[19]研究发现，circAPLP2竞争性结合miR-101可促进结直肠癌细胞的侵袭和迁移。本研究发现，在结直肠癌细胞中circAPLP2表达水平升高，敲低circAPLP2可抑制结直肠癌细胞的EMT、侵袭和迁移，提示circAPLP2在结直肠癌中发挥了促癌作用。

circRNA通过相同的miRNA识别元件(MRE)竞争性结合miRNA，参与miRNA调控，在肿瘤中发挥作用[20-21]。例如，circ_0001666竞争性结合miR-576-5p可抑制结直肠癌细胞的增殖和侵袭[22]，circ_100876竞争性结合miR-516b可促进结直肠癌细胞的增殖、侵袭和EMT过程[23]。本研究发现，circAPLP2竞争性结合miR-497-5p可促进结直肠癌细胞的EMT、侵袭和迁移。miRNA可通过与靶基因3'-UTR MRE配对，调控靶基因的表达水平。FGFR1是一种跨膜蛋白，属于受体酪氨酸激酶，与配体成纤维细胞生长因子(FGF)结合后，可激活下游MAPK/PI3K/Akt、RAS/ERK、STAT3等多条信号通路，调控血管生成、细胞增殖和分化等过程[24]。有研究发现，肿瘤中FGFR1信号通路被异常激活，非小细胞肺癌、乳腺癌、卵巢癌等多种癌症中FGFR1表达水平均升高[25]；FGFR1信号通路持续激活导致前列腺癌发生侵袭、转移，同时体内、体外实验均观察到明显的EMT改变[26]。FGFR1可激活ERK1/2-SOX2通路，促进肺癌细胞的EMT、侵袭和迁移[27]。在肺癌中miR-214-3p可通过与FGFR1 3'-UTR MRE配对，抑制FGFR1的表达[28]。在结直肠癌中miR-214水平与FGFR1水平呈负相关，可抑制*FGFR1* mRNA和蛋白的表达，促进结直肠癌肝转移[29]。本研究发现，在结直肠癌细胞中FGFR1表达增加，miR-497-5p靶向抑制FGFR1的表达可促进结直肠癌的EMT、侵袭和迁移，此外circAPLP2竞争性结合miR-497-5p可促进FGFR1的表达。

综上所述，本研究结果表明，circAPLP2在结直肠癌细胞中呈高表达，可通过竞争性结合miR-497-5p促进FGFR1的表达，从而促进结直肠癌细胞的侵袭和迁移，这为结直肠癌靶向药物的研发提供了理论依据。但由于缺乏相关动物实验支持，circAPLP2竞争性结合miR-497-5p促进FGFR1表达在结直肠癌荷瘤小鼠中的作用尚不明确，因此，circAPLP2是否可作为结直肠癌的诊断标志物仍需进一步研究。

【参考文献】

[1] Siegel RL, Miller KD, Fuchs HE, *et al*. Cancer statistics, 2022[J]. CA Cancer J Clin, 2022, 72(1): 7-33.

[2] Bray F, Ferlay J, Soerjomataram I, *et al*. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries[J]. CA Cancer J Clin, 2018, 68(6): 394-424.

[3] Hu Z, Ding J, Ma Z, *et al*. Quantitative evidence for early metastatic seeding in colorectal cancer[J]. Nat Genet, 2019, 51(7): 1113-1122.

[4] Lüönd F, Sugiyama N, Bill R, *et al*. Distinct contributions of partial and full EMT to breast cancer malignancy[J]. Dev Cell, 2021, 56(23): 3203-3221.

[5] Winkle M, El-Daly SM, Fabbri M. Noncoding RNA therapeutics - challenges and potential solutions[J]. Nat Rev Drug Discov, 2021, 20(8): 629-651.

[6] Xue C, Li G, Zheng Q, *et al*. The functional roles of the circRNA/Wnt axis in cancer[J]. Mol Cancer, 2022, 21(1): 108.

[7] Kristensen LS, Jakobsen T, Hager H, *et al*. The emerging roles of circRNAs in cancer and oncology[J]. Nat Rev Clin Oncol, 2022, 19(3): 188-206.

[8] Hong S, Yan Z, Song Y, *et al*. LncRNA AGAP2-AS1 augments cell viability and mobility, and confers gemcitabine resistance by inhibiting miR-497 in colorectal cancer[J]. Aging (Albany NY), 2020, 12(6): 5183-5194.

[9] Vatandoust S, Price TJ, Karapetis CS. Colorectal cancer: Metastases to a single organ[J]. World J Gastroenterol, 2015, 21(41): 11767-11776.

[10] Lee CC, Lin JC, Hwang WL, *et al*. Macrophage-secreted interleukin-35 regulates cancer cell plasticity to facilitate metastatic colonization[J]. Nat Commun, 2018, 9(1): 3763.

[11] Wang J, Chen D, Song W, *et al*. ATP6L promotes metastasis of colorectal cancer *via* inducing epithelial-mesenchymal transition[J]. Cancer Sci, 2020, 111(2): 477-488.

[12] Shen X, Hu X, Mao J, *et al*. The long noncoding RNA TUG1 is required for TGF-beta/TWIST1/EMT-mediated metastasis in colorectal cancer cells[J]. Cell Death Dis, 2020, 11(1): 65.

[13] Yan Y, Su M, Qin B. CircHIPK3 promotes colorectal cancer cells proliferation and metastasis *via* modulating of miR-1207-5p/FMNL2 signal[J]. Biochem Biophys Res Commun, 2020, 524(4): 839-846.

[14] Song W, Fu T. Circular RNA-associated competing endogenous RNA network and prognostic nomogram for patients with colorectal cancer[J]. Front Oncol, 2019, 9: 1181.

[15] He C, Huang C, Zhou R, *et al*. CircLMNB1 promotes colorectal cancer by regulating cell proliferation, apoptosis and epithelial-mesenchymal transition[J]. Onco Targets Ther, 2019, 12: 6349-6359.

[16] Yang H, Li X, Meng Q, *et al*. CircPTK2 (hsa_circ_0005273) as a novel therapeutic target for metastatic colorectal cancer[J]. Mol Cancer, 2020, 19(1): 13.

[17] Xu H, Wang C, Song H, *et al*. RNA-Seq profiling of circular RNAs in human colorectal cancer liver metastasis and the potential biomarkers[J]. Mol Cancer, 2019, 18(1): 8.

[18] Liu J, Zhang J, Wang Z, *et al*. Knockdown of circAPLP2 inhibits progression of colorectal cancer by regulating miR-485-5p/FOXK1 axis[J]. Cancer Biother Radiopharm, 2021, 36(9): 737-752.

[19] Wu HB, Huang SS, Lu CG, *et al*. CircAPLP2 regulates the proliferation and metastasis of colorectal cancer by targeting miR-101-3p to activate the Notch signalling pathway[J]. Am J Transl Res, 2020, 12(6): 2554-2569.

[20] Xu D, Wu Y, Wang X, *et al*. Identification of functional circRNA/miRNA/mRNA regulatory network for exploring prospective therapy strategy of colorectal cancer[J]. J Cell Biochem, 2020. doi: 10.1002/jcb.29703.

[21] Repetto M, Crimini E, Giugliano F, *et al*. Selective FGFR/FGF pathway inhibitors: Inhibition strategies, clinical activities, resistance mutations, and future directions[J]. Expert Rev Clin Pharmacol, 2021, 14(10): 1233-1252.

[22] Zhou J, Wang L, Sun Q, *et al*. Hsa_circ_0001666 suppresses the progression of colorectal cancer through the miR-576-5p/PCDH10 axis[J]. Clin Transl Med, 2021, 11(11): e565.

[23] Zhang J, Wang H, Wu K, *et al*. Dysregulated circRNA_100876 contributes to proliferation and metastasis of colorectal cancer by targeting microRNA-516b (miR-516b)[J]. Cancer Biol Ther, 2020, 21(8): 733-740.

[24] Lian Z, Du W, Zhu Y, *et al*. Anlotinib can overcome acquired resistance to EGFR-TKIs *via* FGFR1 signaling in non-small cell lung cancer without harboring EGFR T790M mutation[J]. Thorac Cancer, 2020, 11(7): 1934-1943.

[25] Ko J, Meyer AN, Haas M, *et al*. Characterization of FGFR signaling in prostate cancer stem cells and inhibition *via* TKI treatment[J]. Oncotarget, 2021, 12(1): 22-36.

[26] Wang K, Ji W, Yu Y, *et al*. FGFR1-ERK1/2-SOX2 axis promotes cell proliferation, epithelial-mesenchymal transition, and metastasis in FGFR1-amplified lung cancer[J]. Oncogene, 2018, 37(39): 5340-5354.

[27] Beermann J, Piccoli MT, Viereck J, *et al*. Non-coding RNAs in development and disease: Background, mechanisms, and therapeutic approaches[J]. Physiol Rev, 2016, 96(4): 1297-1325.

[28] Yang Y, Li Z, Yuan H, *et al*. Reciprocal regulatory mechanism between miR-214-3p and FGFR1 in FGFR1-amplified lung cancer[J]. Oncogenesis, 2019, 8(9): 50.

[29] Chen DL, Wang ZQ, Zeng ZL, *et al*. Identification of microRNA-214 as a negative regulator of colorectal cancer liver metastasis by way of regulation of fibroblast growth factor receptor 1 expression[J]. Hepatology, 2014, 60(2): 598-609.

(责任编辑：纪方方)