circFNDC3B/miR-655-3p轴调控乳腺癌细胞增殖、细胞周期和凋亡

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摘要 为了探讨circFNDC3B对乳腺癌细胞增殖、细胞周期和凋亡的影响及可能机制, 该研究首先采用RT-qPCR法检测了83例乳腺癌组织及乳腺癌细胞系(MCF-7、T47D、Bcap-37)中circFNDC3B和miR-655-3p表达; 然后分别转染circFNDC3B小干扰RNA、miR-655-3p模拟物、miR-655-3p抑制剂或共转染circFNDC3B小干扰RNA与miR-655-3p抑制剂至MCF-7细胞中, 采用CCK-8法检测了细胞增殖, 流式细胞术检测了细胞凋亡和周期, Western blot法检测了细胞中CyclinD1与Cleaved-caspase-3的蛋白表达, 双荧光素酶报告基因实验验证了miR-655-3p与circFNDC3B的调控关系。结果显示, 乳腺癌组织和细胞系中circFNDC3B表达升高(P<0.05), 而miR-655-3p表达降低(P<0.05)。下调circFNDC3B或上调miR-655-3p后, MCF-7细胞增殖活性和CyclinD1蛋白表达量降低, 细胞周期进程受到阻滞, 细胞凋亡率与Cleaved-caspase-3蛋白表达量增加, 差异均具有统计学意义(P<0.05)。circFNDC3B靶向结合并负调控miR-655-3p。下调miR-655-3p对MCF-7细胞增殖、细胞周期和凋亡的影响与上调miR-655-3p相反。下调miR-655-3p逆转下调circFNDC3B对MCF-7细胞增殖、细胞周期和凋亡的影响。这说明, circFNDC3B可能通过抑制miR-655-3p的表达促进乳腺癌细胞增殖和细胞周期进程, 并阻碍细胞凋亡。

关键词 乳腺癌; circFNDC3B; miR-655-3p; 细胞增殖; 细胞周期; 凋亡

circFNDC3B/miR-655-3p Axis Regulates the Cell Proliferation, Cell Cycle and Apoptosis of Breast Cancer Cells

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Abstract In this study, in order to explore the effect of circFNDC3B on breast cancer cells proliferation, cell cycle and apoptosis and its possible mechanism, RT-qPCR was first used to detect the expression of circFNDC3B and miR-655-3p in 83 breast cancer tissues and breast cancer cell lines (MCF-7, T47D, Bcap-37). And then circFNDC3B small interfering RNA, miR-655-3p mimic or miR-655-3p inhibitor was transfected into MCF-7 cells, or circFNDC3B small interfering RNA and miR-655-3p inhibitor were co-transfected into MCF-7 cells. CCK-8 method was used to detect cell proliferation. Flow cytometry was used to detect cell apoptosis and cycle. Western blot was used to detect the protein expression of CyclinD1 and Cleaved-caspase-3. The dual luciferase reporter gene experiment verified the

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regulatory relationship between miR-655-3p and circFNDC3B. The results showed that the expression of circFNDC3B in breast cancer tissues and cell lines were increased (P<0.05), but the expression of miR-655-3p were decreased (P<0.05). After down-regulating circFNDC3B or up-regulating miR-655-3p, the proliferation activity of MCF-7 cells and the protein expression of CyclinD1 decreased, and cell cycle progression was blocked, but the apoptosis rate and the protein expression of Cleaved-caspase-3 increased, and the difference were statistically significant (P<0.05). circFNDC3B negatively regulated the expression of miR-655-3p. The effects of down-regulation of miR-655-3p on the proliferation, cell cycle and apoptosis of MCF-7 cells were opposite to those of up-regulation of miR-655-3p. Down-regulation of miR-655-3p reversed the effect of down-regulation of circFNDC3B on the proliferation, cell cycle and apoptosis of MCF-7 cells. This indicated that circFNDC3B might promote the proliferation and cell cycle progression of breast cancer cells, and hinder cell apoptosis by inhibiting the expression of miR-655-3p.

Keywords breast cancer; circFNDC3B; miR-655-3p; cell proliferation; cell cycle; apoptosis

目前, 乳腺癌发病机制尚未明确。原癌基因的激活与抑癌基因的失活在乳腺癌的发生发展中起重要作用[1], 探究乳腺癌中异常表达的基因分子可为其治疗提供分子靶点。环状RNA(circular RNA, circRNA)和微小RNA(microRNA, miRNA)是两类非编码RNA, 且circRNA可竞争性结合miRNA, 调控miRNA靶基因的表达, 进而发挥生物学功能, 参与肿瘤的发展进程[2]。含有纤连蛋白III型结构域的蛋白3B环状RNA(fibronectin type III domain-containing protein 3B circular RNA, circFNDC3B)是近年来新发现的一种circRNA, 其基因ID为circ_0006156。有报道称, circFNDC3B在乳腺癌细胞系中高表达, 可促进乳腺癌细胞迁移和侵袭[3]。Starbase靶基因预测软件显示, circFNDC3B可能靶向结合miR-655-3p。研究显示, 在口腔鳞癌[4]、神经胶质瘤[5]和肝细胞癌[6]等肿瘤中, miR-655-3p表达下调, 且通过上调miR-655-3p能够降低肿瘤细胞的增殖、迁移和侵袭能力, miR-655-3p可作为肿瘤治疗的分子靶点。有报道称, 乳腺癌患者血浆中miR-655的表达低于健康对照, 且对乳腺癌诊断具有良好的敏感度和特异度, 受试者工作特征曲线下面积为0.879, 另外该研究还指出, 血浆miR-655水平和乳腺癌组织学分级存在相关性, miR-655可作为乳腺癌早期诊断的生物标志物[7]。但目前, miR-655-3p对乳腺癌细胞恶性表型的影响及circFNDC3B能否靶向miR-655-3p影响乳腺癌细胞的恶性表型均未知。本研究主要探究了circFNDC3B和miR-655-3p对乳腺癌细胞增殖、细胞周期和凋亡的影响及circFNDC3B能否在靶向调控miR-655-3p中发挥作用, 以期了解circFNDC3B/miR-655-3p轴对乳腺癌发生发展的影响, 为乳腺癌的治疗提供分子靶点。

1 资料与方法

1.1 临床资料

83例乳腺癌组织及对应的癌旁组织来自2016年4月至2019年12月于四川省人民医院确诊并行手术治疗的乳腺癌患者, 患者平均年龄为(52.36±7.19)岁。纳入标准: 均经病理确诊; 首次确诊。排除标准: 合并其他恶性肿瘤; 合并高血压、糖尿病等慢性病患者。参与研究的所有患者均了解本研究具体内容并签订知情同意书。本研究经四川省人民医院伦理委员会批准。

1.2 细胞和试剂

正常乳腺上皮细胞MCF-10A和乳腺癌细胞系(MCF-7、T47D、Bcap-37)购自中国科学院上海细胞库; RPMI 1640培养基、细胞计数试剂盒-8(cell counting kit-8, CCK-8)、膜联蛋白V(Annexin V)-异硫氰酸荧光素(fluorescein isothiocyanate, FITC)/碘化丙啶(propidium iodide, PI)凋亡试剂盒和二喹啉甲酸(bicinchoninicacid, BCA)蛋白检测试剂盒购自北京索莱宝科技有限公司; 胎牛血清(fetal bovine serum, FBS)购自浙江天杭生物科技有限公司; PCR引物、miR-655-3p模拟物(mimcs)及模拟对照序列(miR-con)、miR-655-3p抑制剂(anti-miR-655-3p)及抑制剂阴性序列(anti-miR-con)、circFNDC3B小干扰RNA(si-circFNDC3B)及乱序无意义阴性序列(si-con)、circFNDC3B野生型质粒(WT-circFNDC3B)和突变型质粒(MUT-circFNDC3B)购自上海生工生物工程股份有限公司; RNA抽提试剂盒、逆转录试剂盒和PCR试剂盒购自大连宝生物工程有限公司; Li-

pofectamine™ 2000试剂盒购自美国Invitrogen公司; 兔抗人细胞周期蛋白D1(CyclinD1)与活化的半胱天冬酶-3(Cleaved-caspases-3)抗体购自美国Santa Cruz公司; 双荧光素酶活性检测试剂盒购自上海碧云天生物技术有限公司。

1.3 方法

1.3.1 RT-qPCR检测组织中circFNDC3B和miR-655-3p的表达 RNA抽提试剂盒提取组织中的总RNA, 经逆转录后, 行PCR扩增。扩增操作流程为: 95 ℃ 5 min; 95 ℃ 10 s—60 ℃ 30 s—72 ℃ 30 s, 持续35个循环。引物序列: circFNDC3B上游5′-CTG ACG ACT AGC AAT ACG ATA G-3′, 下游5′-CGA TAC GAC GAA CTA AG-3′; *β-actin*上游5′-CGA TAC GAT ACG GGC TAA CG-3′, 下游5′-CGA TAC ACG AAG AGC GCG-3′; miR-655-3p上游5′-CGA CGA CTA CGA TAA CAG GAG-3′, 下游5′-CGA TAG TCA GGC GGT GC-3′; *U6*上游5′-CGA TAC CTG CTC AGT CGA CG-3′, 下游5′-CGA TAC GCG GCG CGA CGA-3′。$2^{-\Delta\Delta Ct}$法计算circFNDC3B相对于*β-actin*、miR-655-3p相对于*U6*的表达水平。

1.3.2 细胞培养 复苏MCF-10A、MCF-7、T47D、Bcap-37细胞, 均用含10% FBS的RPMI 1640培养基培养。将对数期的MCF-10A、MCF-7、T47D、Bcap-37细胞均以5.0×10^{5}个/孔的密度接种于6孔板中, 每个细胞设置3个复孔。培养24 h后, 收集细胞, RT-qPCR法检测细胞中circFNDC3B和miR-655-3p表达, 方法同1.3.1。实验重复3次, 选择较MCF-10A细胞表达差异最显著的乳腺癌细胞MCF-7进行后续实验。

1.3.3 MCF-7细胞转染 将MCF-7细胞以5.0×10^{5}个/孔的密度接种于6孔板中, 用Lipofectamine™ 2000脂质体转染法, 分别转染si-circFNDC3B(si-circFNDC3B组)、si-con(si-con组)、miR-655-3p mimcs(miR-655-3p组)、miR-con(miR-con组)、anti-miR-655-3p(anti-miR-655-3p组)、anti-miR-con(anti-miR-con组)、共转染si-circFNDC3B与anti-miR-655-3p(si-circFNDC3B+anti-miR-655-3p组)、si-circFNDC3B与anti-miR-con(si-circFNDC3B+anti-miR-con组), 共转染6 h。转染完成后转移到新鲜培养基上继续培养24 h, 培养完成后收集细胞。

1.3.4 CCK-8法检测细胞增殖 MCF-7细胞转染完成后接种在96孔板中, 密度为2.5×10^{4}个/孔。同时设置对照组(NC组), 接种未转染的MCF-7细胞。每组设置3个复孔。培养24 h后, 加10 μL CCK-8。37 ℃孵育2 h后, 于酶标仪450 nm处测吸光度(*D*)值。实验重复3次。*D*值越大, 说明细胞增殖活性越强。

1.3.5 流式细胞术检测细胞周期和细胞凋亡 将各组转染后的MCF-7细胞和NC组细胞均以5.0×10^{5}个/孔的密度接种于6孔板中, 每组设置3个复孔。培养24 h后, 收集细胞, 并用PBS清洗2次, 1 500 r/min离心5 min, 弃上清。①细胞周期检测。加预冷的75%乙醇, 4 ℃固定过夜。1 500 r/min离心5 min, 弃上清。加适量PBS清洗细胞, 除去乙醇。加入400 μL PI和100 μL RNase A(100 μg/mL), 4 ℃避光孵育30 min, 上流式细胞仪检测细胞周期。②细胞凋亡检测。利用Annexin V-FITC/PI试剂盒, 将500 μL结合缓冲液和细胞充分混合, 重悬细胞。重悬完成后置入Annexin V-FITC和PI, 用量分别为10 μL与5 μL, 反应时间均为15 min, 然后使用流式细胞仪察看细胞凋亡情况。实验重复3次。

1.3.6 Western blot法检测CyclinD1、Cleaved-caspase-3蛋白表达水平 将各组转染后的MCF-7细胞和NC组细胞接种在6孔板中, 接种密度为5.0×10^{5}个/孔, 每组设置3个复孔。24 h后收集细胞备用。总蛋白使用RIPA试剂提取, 使用BCA法定量、SDS-PAGE电泳、转膜及5%脱脂奶粉封闭后, 分别置于CyclinD1(1:500)、Cleaved-caspase-3(1:500)和β-actin(1:500)一抗孵育液中, 在4 ℃的环境下静置过夜。再于山羊抗兔二抗(1:2 000)孵育液中, 37 ℃孵育1 h。加显影液避光显影, 曝光拍照, ImageJ软件分析CyclinD1、Cleaved-caspase-3相对于β-actin的表达水平。实验重复3次。

1.3.7 双荧光素酶报告基因实验 将MCF-7细胞以5.0×10^{5}个/孔的密度接种于6孔板中, 用Lipofectamine™ 2000脂质体转染法, 分别共转染WT-circFNDC3B与miR-655-3p mimcs、WT-circFNDC3B与miR-con、MUT-circFNDC3B与miR-655-3p mimcs、MUT-circFNDC3B与miR-con, 每组设置3个复孔。转染6 h后, 换新鲜培养基。培养24 h, 收集细胞并裂解, 1 500 r/min离心5 min, 取上清, 检测荧光素酶活性。实验重复3次。

1.4 统计学处理方法

研究数据均使用SPSS 22.0统计学软件处理。组间计量数据使用*t*检验, 使用均数±标准差($\bar{x}\pm s$)表示。使用单因素方差分析多组间的数据, 进一步两组间比较使用*SNK-q*检验。数据是否存在统计学差异使用*P*表示, *P*<0.05证明数据间的差异有统计学意义。

2 结果

2.1 乳腺癌组织及细胞系中circFNDC3B、miR-655-3p的表达情况

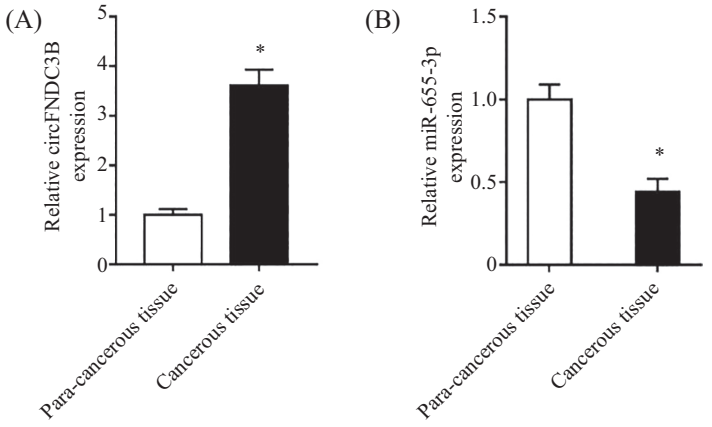
与癌旁组织比较，乳腺癌组织中circFNDC3B表达量升高，而miR-655-3p表达量降低，且差异均显著(P<0.05)；与正常乳腺上皮细胞MCF-10A比较，乳腺癌细胞系(MCF-7、T47D、Bcap-37)中circFNDC3B表达量升高，而miR-655-3p表达量降低，差异均显著(P<0.05)，且MCF-7细胞中circFNDC3B和miR-655-3p表达差异最显著，因此，后续实验以MCF-7细胞为研究对象(图1和图2)。

2.2 下调circFNDC3B对乳腺癌细胞MCF-7增殖、细胞周期及凋亡的影响

转染si-circFNDC3B的MCF-7细胞中circFNDC3B表达明显低于转染si-con的MCF-7细胞(P<0.05)，说明下调circFNDC3B的MCF-7细胞构建成功。与si-con组比较，si-circFNDC3B组MCF-7细胞增殖活性降低，细胞周期G_0/G_1期延长，而S期缩短，细胞凋亡率、Cleaved-caspase-3蛋白均呈上升趋势，CyclinD1蛋白表达量呈下降趋势，数据间的差异均有统计学意义(P<0.05)；而NC组与si-con组各检测指标比较均无显著差异(P>0.05)，说明下调circFNDC3B阻碍MCF-7细胞的增殖及细胞周期进程，而加剧其凋亡(图3)。

2.3 上调miR-655-3p对乳腺癌细胞MCF-7增殖、细胞周期及凋亡的影响

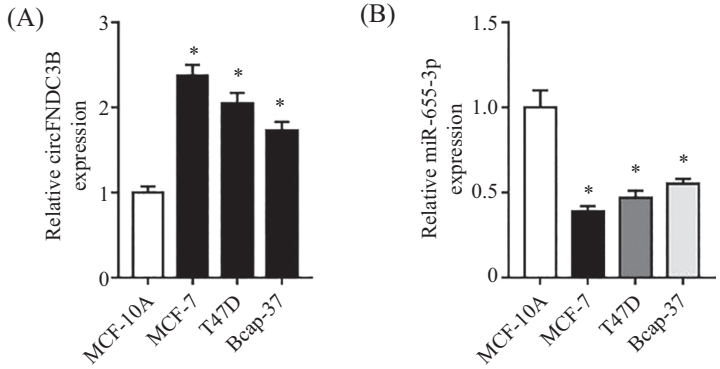
转染miR-655-3p mimics的MCF-7细胞中miR-655-3p表达明显高于转染miR-con的MCF-7细胞(P<0.05)，说明上调miR-655-3p的MCF-7细胞构建成功。与miR-con组比较，miR-655-3p组MCF-7细胞增殖活性降低，细胞周期G_0/G_1期延长，而S期缩短，细胞凋亡率及Cleaved-caspase-3蛋白均明显上



*P<0.05.

图1 乳腺癌患者癌组织中circFNDC3B和miR-655-3p的表达水平

Fig.1 The expression levels of circFNDC3B and miR-655-3p in cancerous tissues of breast cancer patients

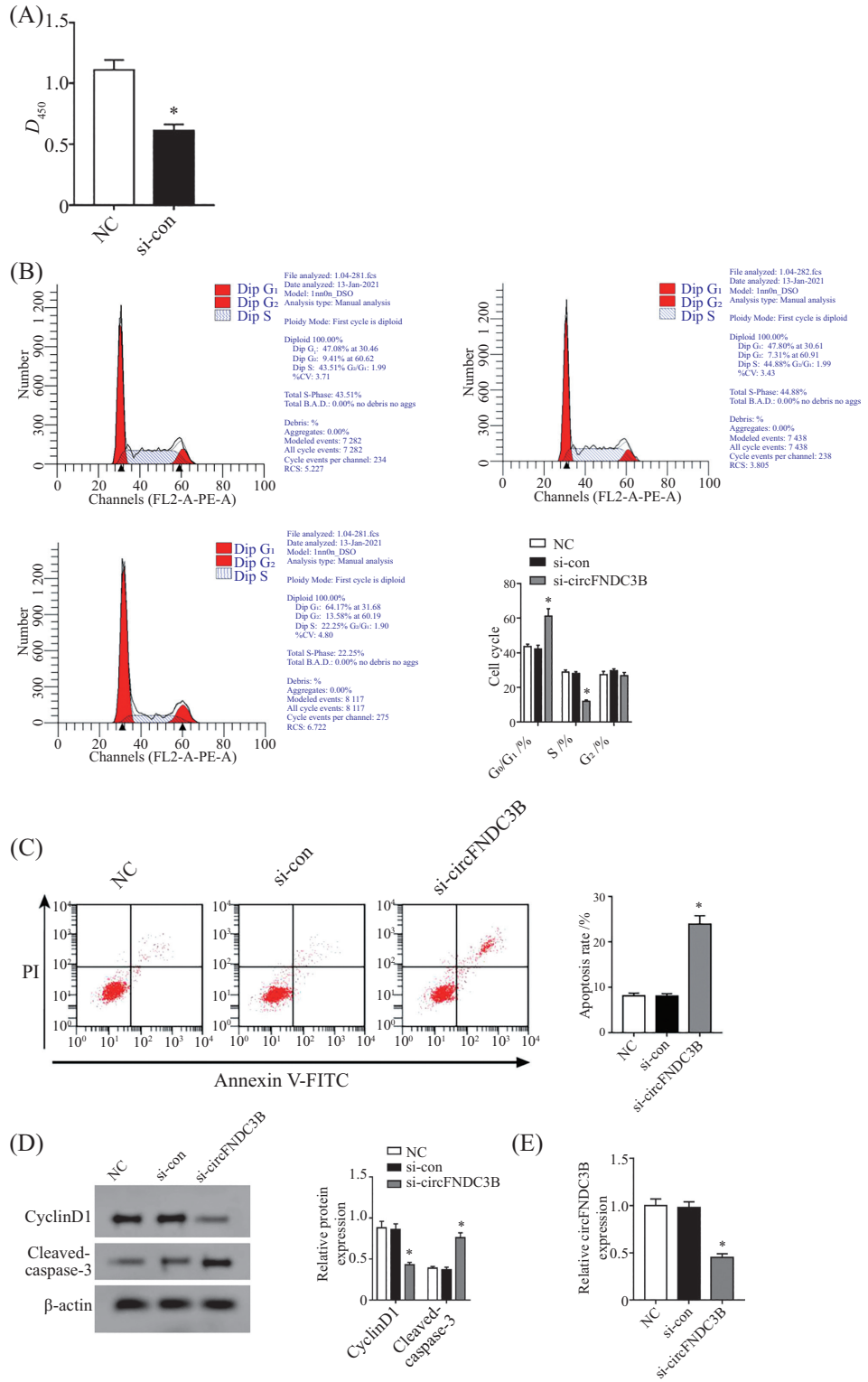


*P<0.05, 与MCF-10A细胞比较。

*P<0.05 compared with the MCF-10A cells.

图2 乳腺癌细胞系中circFNDC3B和miR-655-3p的表达水平

Fig.2 The expression levels of circFNDC3B and miR-655-3p in breast cancer cell lines



A: 下调circFNDC3B降低MCF-7细胞增殖活性; B: 下调circFNDC3B阻滞MCF-7细胞周期进程; C: 下调circFNDC3B促进MCF-7细胞凋亡; D: 下调circFNDC3B抑制MCF-7细胞中CyclinD1蛋白表达而促进Cleaved-capspse-3蛋白表达; E: MCF-7细胞中circFNDC3B表达降低, 说明转染成功。*P<0.05, 与si-con组比较。

A: down-regulation of circFNDC3B reduces MCF-7 cell proliferation activity; B: down-regulation of circFNDC3B blocks MCF-7 cell cycle progression; C: down-regulation of circFNDC3B promotes MCF-7 cell apoptosis; D: down-regulation of circFNDC3B inhibits the protein expression of CyclinD1 in MCF-7 cells, but promotes the protein expression of Cleaved-capspse-3; E: the expression of circFNDC3B in MCF-7 cells decreased, indicating that the transfection was successful. *P<0.05 compared with the si-con group.

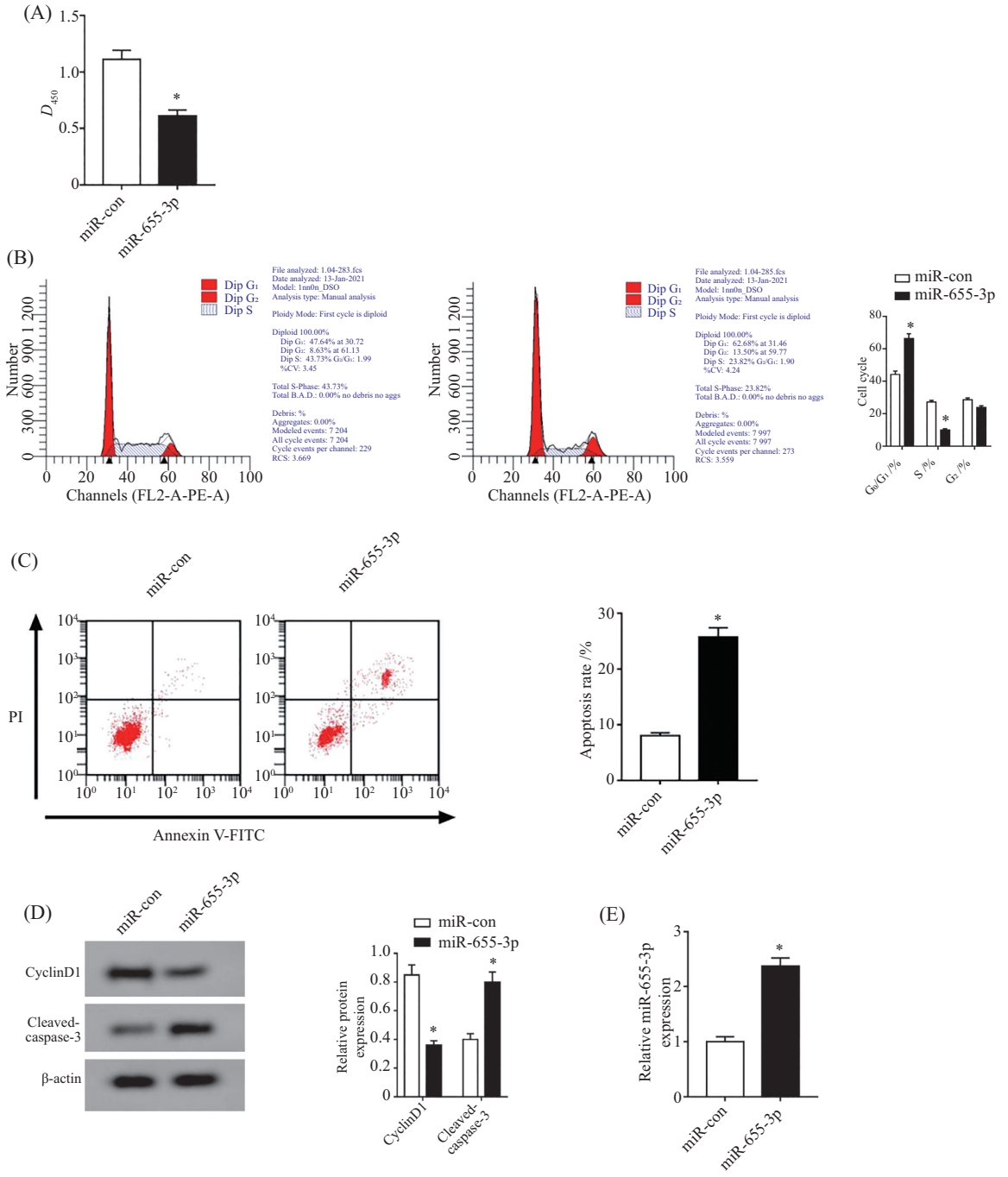
图3 下调circFNDC3B对MCF-7细胞的细胞增殖、细胞周期和凋亡的影响

Fig.3 The effects of down-regulation of circFNDC3B on cell proliferation, cell cycle and apoptosis of MCF-7 cells

升, CyclinD1蛋白表达量明显下降, 且差异均显著(P<0.05), 说明上调miR-655-3p阻碍MCF-7细胞的增殖及细胞周期进程, 而加剧其凋亡(图4)。

2.4 circFNDC3B靶向结合并负调控miR-655-3p

Starbase靶基因预测软件显示, circFNDC3B和miR-655-3p存在连续结合位点(图5)。双荧光素酶



A: 上调miR-655-3p降低MCF-7细胞增殖活性; B: 上调miR-655-3p阻滞MCF-7细胞周期进程; C: 上调miR-655-3p促进MCF-7细胞凋亡; D: 上调miR-655-3p抑制MCF-7细胞中CyclinD1蛋白表达而促进Cleaved-capspse-3蛋白表达; E: MCF-7细胞中miR-655-3p表达降低, 说明转染成功。*P<0.05, 与miR-con组比较。

A: up-regulation of miR-655-3p reduces MCF-7 cell proliferation activity; B: up-regulation of miR-655-3p blocks MCF-7 cell cycle progression; C: up-regulation of miR-655-3p promotes MCF-7 cell apoptosis; D: up-regulation of miR-655-3p inhibits the protein expression of CyclinD1 in MCF-7 cells, but promote the protein expression of Cleaved-capspse-3; E: the expression of miR-655-3p in MCF-7 cells decreased, indicating that the transfection was successful. *P<0.05 compared with the miR-con group.

图4 上调miR-655-3p对MCF-7细胞增殖、细胞周期和凋亡的影响

Fig.4 The effects of up-regulation of miR-655-3p on the proliferation, cell cycle and apoptosis of MCF-7 cells

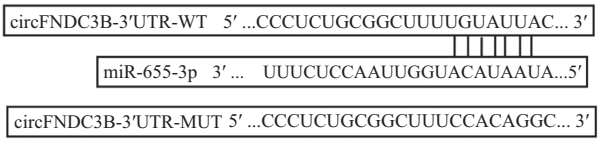


图5 circFNDC3B和miR-655-3p互补的结合位点

Fig.5 The complementary binding sites between circFNDC3B and miR-655-3p

表1 双荧光素酶报告基因实验结果

Table 1 Experimental results of dual luciferase reporter gene assay

组别 Groups	相对荧光素酶活性 Relative luciferase activity	
	WT	MUT
miR-con	1.00±0.10	1.00±0.07
miR-655-3p	0.41±0.04*	0.99±0.09
t	16.434	0.263
P	0.000	0.796

$\bar{x}\pm s$; n=9; *P<0.05, 与miR-con组比较。

$\bar{x}\pm s$; n=9; *P<0.05 compared with the miR-con group.

报告基因实验结果显示, 共转染miR-655-3p与WT-circFNDC3B的细胞荧光素酶活性显著低于共转染miR-con与WT-circFNDC3B的细胞(P<0.05), 而共转染miR-655-3p与MUT-circFNDC3B的细胞荧光素酶活性与共转染miR-con与MUT-circFNDC3B的细胞比较无显著差异(P>0.05), 说明circFNDC3B可靶向结合miR-655-3p(表1)。此外, si-circFNDC3B组MCF-7细胞中circFNDC3B表达量低于si-con组(0.47±0.03比1.03±0.10), 而miR-655-3p表达量高于si-con组(2.04±0.16比0.99±0.10), 且差异均显著(P<0.05), 进一步说明circFNDC3B靶向结合并负调控miR-655-3p。

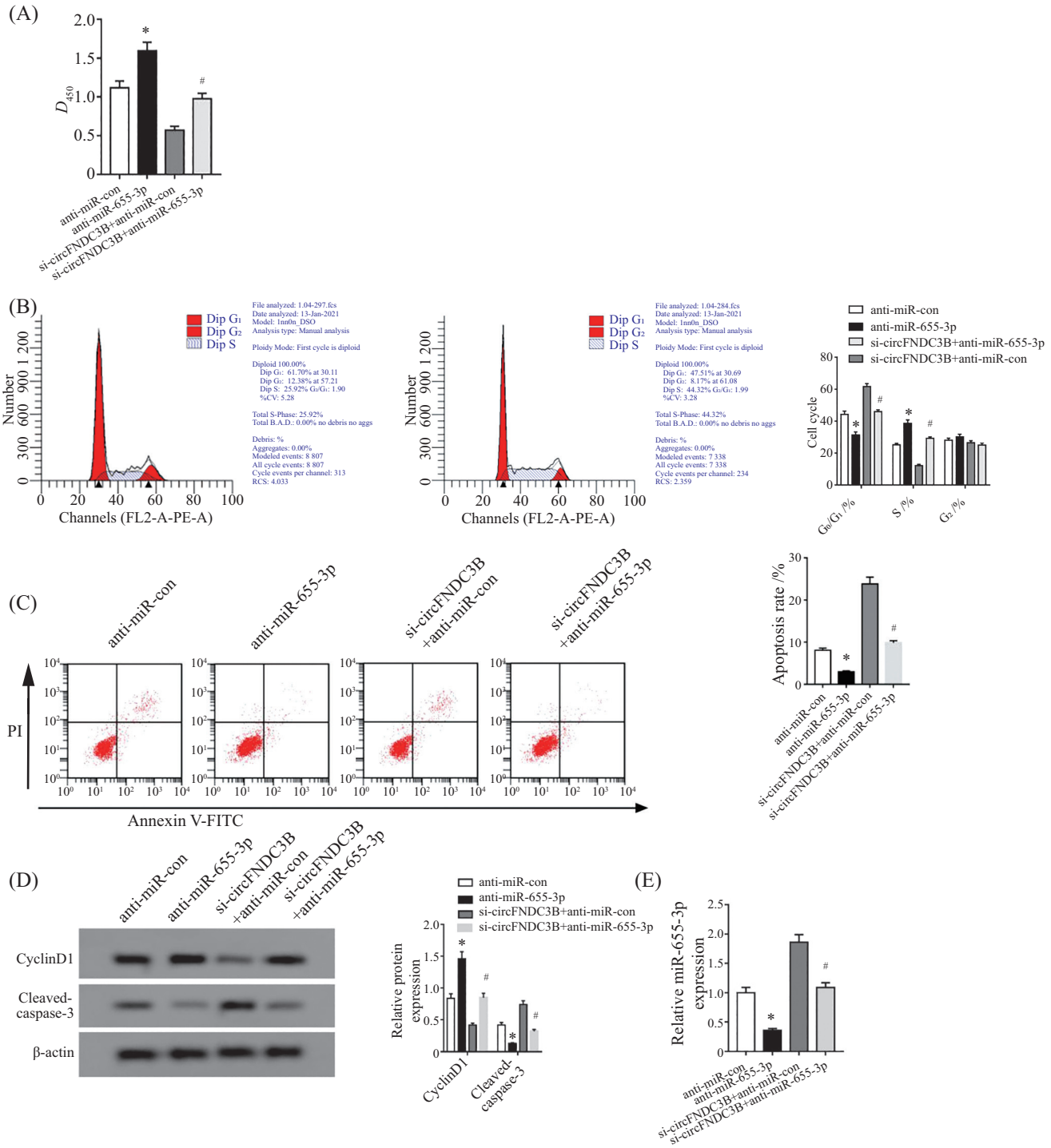
2.5 下调miR-655-3p逆转下调circFNDC3B对MCF-7细胞增殖、细胞周期及凋亡的影响

与anti-miR-con组比较, anti-miR-655-3p组MCF-7细胞中miR-655-3p表达量降低, 细胞增殖活性升高, 细胞周期G_0/G_1期缩短, 而S期延长, 细胞凋亡率、Cleaved-caspase-3蛋白表达量均明显下降, 而CyclinD1蛋白表达量明显升高, 且差异均显著(P<0.05), 说明下调miR-655-3p促进MCF-7细胞增殖, 并阻碍细胞的细胞周期进程及细胞凋亡; 与si-circFNDC3B+anti-miR-con组比较, si-circFNDC3B+anti-miR-655-3p组MCF-7细胞中miR-655-3p表达量降低, 细胞增殖活性升高, 细胞周期G_0/G_1期缩短, 而S期延长, 细胞凋亡率、Cleaved-caspase-3蛋白表达量均明显下降, 而CyclinD1蛋白表达量明显升高, 且差异均显著(P<0.05), 说明下调miR-655-3p逆转下调circFNDC3B对MCF-7细胞增殖、细胞周期及凋亡的影响(图6)。

3 讨论

近年来, 乳腺癌的发病呈年轻化趋势[8], 严重危害女性生命健康。乳腺癌的发生发展过程复杂, 与原癌基因的激活及抑癌基因的失活密切相关。探究乳腺癌中异常表达的基因分子及其对乳腺癌发生发展的影响, 对乳腺癌的早期诊断和治疗尤为重要。circRNA是真核生物中广泛存在的一类环状非编码RNA, 其结构稳定, 且具有高度保守性。有研究表明, circRNA和肿瘤的发病具有紧密关联, 其能够有效调控肿瘤细胞[9]。研究已表明, circTP63[10]、circABCC4[11]和circRNA_0000518[11]等多种circRNA促进乳腺癌的发展进程, 而CircNOL10[13]、circ_SETD2[14]和circRNA_000554[15]等circRNA对乳腺癌发生发展起抑制作用, 这些异常表达的circRNA为乳腺癌的治疗提供了分子靶点。

作为一种circRNA, circFNDC3B参与多种肿瘤的发展进程。研究显示, circFNDC3B在甲状腺乳头状癌[16]、肾癌[17]和食管癌[18]等肿瘤中表达上调, 促进肿瘤发展进程; 而circFNDC3B在膀胱癌[19]、结肠癌[20]和胃癌[21]等肿瘤中表达下调, 发挥抑癌抑癌基因作用。本研究显示, circFNDC3B在乳腺癌组织及细胞系中的表达明显升高, 与牛恒等[3]报道结果一



A: 下调miR-655-3p促进MCF-7细胞增殖; B: 下调miR-655-3p促进MCF-7细胞的细胞周期进程; C: 下调miR-655-3p抑制MCF-7细胞凋亡; D: 下调miR-655-3p促进MCF-7细胞中CyclinD1蛋白表达而抑制Cleaved-capspse-3蛋白表达; E: MCF-7细胞中miR-655-3p表达降低, 表明低表达miR-655-3p的MCF-7细胞构建成功。*P<0.05, 与anti-miR-con组比较; #P<0.05, 与si-circFNDC3B+anti-miR-con组比较。

A: down-regulating miR-655-3p promotes the proliferation of MCF-7 cells; B: down-regulating miR-655-3p promotes the cell cycle progression of MCF-7 cells; C: down-regulating miR-655-3p inhibits MCF-7 cell apoptosis; D: down-regulation of miR-655-3p promotes the protein expression of CyclinD1 in MCF-7 cells, but inhibits the protein expression of Cleaved-capspse-3; E: the expression of miR-655-3p decreased in MCF-7 cells, indicating that MCF-7 cells that down-regulated miR-655-3p were successfully constructed. *P<0.05 compared with anti-miR-con group; #P<0.05 compared with si-circFNDC3B+anti-miR-con group.

图6 同时下调circFNDC3B和miR-655-3p对MCF-7细胞增殖、细胞周期和凋亡的影响

Fig.6 The effects of simultaneous down-regulation of circFNDC3B and miR-655-3p on the proliferation, cell cycle and apoptosis of MCF-7 cells

致, 提示circFNDC3B可能促进乳腺癌的发展进程; 通过转染circFNDC3B小干扰RNA下调其在乳腺癌细胞中的表达后, 细胞增殖能力及细胞周期进程受阻, 而凋亡加剧, 提示circFNDC3B可作为乳腺癌治疗的分子靶点。细胞增殖和凋亡受多种基因分子的调控, CyclinD1表达量增加能够加速细胞周期的转变, 从而加快细胞周期进程, 达到增加细胞数量的目的[22]; caspase-3是caspase级联反应的关键调控分子, 受到上游信号刺激后被活化生成Cleaved-caspase-3, 进而诱导细胞凋亡[23]。本研究显示, 下调circFNDC3B抑制了乳腺癌细胞中CyclinD1蛋白表达, 而促进了Cleaved-caspase-3蛋白表达, 提示其可能通过直接或间接的方式调控CyclinD1和Cleaved-caspase-3的表达来影响乳腺癌细胞增殖、细胞周期及凋亡。

为了进一步探究circFNDC3B调控乳腺癌细胞增殖、细胞周期及凋亡的分子机制, 本研究证实了circFNDC3B可靶向结合miR-655-3p。同时, 下调circFNDC3B促进乳腺癌细胞中miR-655-3p的表达, 进一步说明circFNDC3B可靶向结合并负调控miR-655-3p, 这也与本文乳腺癌组织及细胞系中circFNDC3B表达升高而miR-655-3p表达降低的结果一致。研究显示, miR-655-3p在卵巢癌中低表达, 上调其表达可阻碍卵巢癌细胞增殖、迁移及上皮间质转化过程[24]; miR-655-3p在非小细胞肺癌(non-small cell lung cancer, NSCLC)细胞系中的表达明显降低, miR-655-3p的过表达可靶向抑制PTTG1降低NSCLC细胞的迁移和侵袭性, 提示miR-655-3p可作为NSCLC治疗的分子靶点[25]。本研究显示, 上调miR-655-3p能够有效抑制乳腺癌细胞的增殖和细胞周期进程, 从而加速乳腺癌细胞凋亡, 而下调miR-655-3p对乳腺癌细胞增殖、凋亡和细胞周期进程的影响与上调miR-655-3p相反, 提示miR-655-3p可作为抑制乳腺癌发展的分子靶点。本研究还显示, 下调miR-655-3p逆转下调circFNDC3B对乳腺癌细胞增殖和细胞周期进程的抑制作用和凋亡促进作用, 进一步提示circFNDC3B可能通过靶向负调控miR-655-3p来影响乳腺癌细胞的恶性表型。

综上, circFNDC3B在乳腺癌组织及细胞系中高表达, 下调circFNDC3B可阻碍乳腺癌细胞增殖和细胞周期进程, 并加剧细胞凋亡, 其作用机制可能与靶向负调控miR-655-3p有关, 其可能成为乳腺癌治疗的分子靶点。但本研究尚未对miR-655-3p下游靶基因进行探究, 且仅通过体外细胞实验进行了初步探究, 接下来将进一步探究miR-655-3p下游靶基因对乳腺癌发生发展的影响, 并通过裸鼠移植瘤实验验证circFNDC3B/miR-655-3p轴对乳腺癌发生发展的影响。

参考文献 (References)

[1] GAO D F, QI X W, ZHANG X F, et al. hsa_circRNA_0006528 as a competing endogenous RNA promotes human breast cancer progression by sponging miR-7-5p and activating the MAPK/ERK signaling pathway [J]. Mol Carcinog, 2019, 58(4): 554-64.

[2] LI Z Y, ZHENG J H, LIN W X, et al. Circular RNA hsa_circ_0001785 inhibits the proliferation, migration and invasion of breast cancer cells *in vitro* and *in vivo* by sponging miR-942 to upregulate SOCS3 [J]. Cell Cycle, 2020, 19(21): 2811-25.

[3] 牛恒, 邱爽. CircFndc3b对乳腺癌细胞侵袭和迁移的影响及机制预测[J]. 甘肃科学学报(NIU H, QIU S. Effect and mechanism prediction of CircFndc3b on invasion and migration of breast cancer cell [J]. J Gansu Sci), 2020, 32(3): 50-5.

[4] YU L J, HUO L L, SHAO X L, et al. LncRNA SNHG5 promotes cell proliferation, migration and invasion in oral squamous cell carcinoma by sponging miR-655-3p/FZD4 axis [J]. Oncol Lett, 2020, 20(6): 310-21.

[5] XIN J, ZHAO Y H, ZHANG X Y, et al. LncRNA NFIA-AS2 promotes glioma progression through modulating the miR-655-3p/ZFX axis [J]. Hum Cell, 2020, 33(4): 1273-80.

[6] ZHAO X Q, LIANG B, JIANG K, et al. Down-regulation of miR-655-3p predicts worse clinical outcome in patients suffering from hepatocellular carcinoma [J]. Eur Rev Med Pharmacol Sci, 2017, 21(4): 748-52.

[7] 禚守荣. 血浆miR-655作为乳腺癌早期诊断标志物的临床价值[J]. 中国医师杂志(ZHUO S R. The clinical value of plasma miR-655 as a marker for early diagnosis of breast cancer [J]. J Chin Physician), 2016, 18(9): 1396-8.

[8] 郭敏, 张亚男, 吕建鑫, 等. 乳腺病理Ki67指数和磁共振ADC值在乳腺癌诊疗中的应用研究进展[J]. 东南大学学报(医学版)(GUO M, ZHANG Y N, LÜ J X, et al. Progress in the application of breast pathological Ki67 index and magnetic resonance ADC value in the diagnosis and treatment of breast cancer [J]. J Southeast Univ, Med Sci Edi), 2019, 38(6): 1099-103.

[9] MIN J, PAN X, LÜ G. The circRNA circ_0000291 acts as a sponge of microRNA 326 to regulate E26 transformation-specific sequence-1 expression and promote breast cancer progression [J]. Pathol Int, 2020, 70(12): 953-64.

[10] DENG Y B, XIA J, XU Y E. Circular RNA circTP63 enhances estrogen receptor-positive breast cancer progression and malignant behaviors through the miR-873-3p/FOXM1 axis [J]. Anticancer Drugs, 2021, 32(1): 44-52.

[11] JIANG J C, CHENG X Q. Circular RNA circABCC4 acts as a ceRNA of miR-154-5p to improve cell viability, migration and invasion of breast cancer cells *in vitro* [J]. Cell Cycle, 2020, 19(20): 2653-61.

[12] JIANG J, LIN H, SHI S H, et al. Hsa_circRNA_0000518 facilitates breast cancer development via regulation of the miR-326/FGFR1 axis [J]. Thorac Cancer, 2020, 11(11): 3181-92.

[13] WANG F, WANG X C, LI J R, et al. CircNOL10 suppresses breast cancer progression by sponging miR-767-5p to regulate SOCS2/JAK/STAT signaling [J]. J Biomed Sci, 2021, 28(1): 4.

[14] SHEN Y Y, ZHANG M M, DA L S, et al. Circular RNA circ_SETD2 represses breast cancer progression via modulating the miR-155-5p/SCUBE2 axis [J]. Open Med (Wars), 2020, 15(1): 940-53.

[15] MAO Y, LÜ M, CAO W H, et al. Circular RNA 000554 represses epithelial-mesenchymal transition in breast cancer by regulating microRNA-182/ZFP36 axis [J]. FASEB J, 2020, 34(9): 11405-20.

[16] WU G J, ZHOU W H, PAN X H, et al. Circular RNA profiling reveals exosomal circ_0006156 as a novel biomarker in papillary thyroid cancer [J]. Mol Ther Nucleic Acids, 2020, 19: 1134-44.

[17] CHEN T, YU Q C, SHAO S X, et al. Circular RNA circFNDC3B protects renal carcinoma by miR-99a downregulation [J]. J Cell Physiol, 2020, 235(5): 4399-406.

[18] LUO G, LI R F, LI Z J. CircRNA circFNDC3B promotes esophageal cancer progression via cell proliferation, apoptosis, and migration regulation [J]. Int J Clin Exp Pathol, 2018, 11(8): 4188-96.

[19] WANG S Y, FU S Q, CHEN Q, et al. Prognostic role of circular RNAs expression in bladder carcinoma: a meta-analysis [J]. Genet Test Mol Biomarkers, 2020, 24(11): 692-700.

[20] ZENG W, LIU Y, LI W T, et al. CircFNDC3B sequestrates miR-937-5p to derepress TIMP3 and inhibit colorectal cancer progression [J]. Mol Oncol, 2020, 14(11): 2960-84.

[21] HE Y X, JU H, LI N, et al. Association between hsa_circ_0006156 expression and incidence of gastric cancer [J]. Eur Rev Med Pharmacol Sci, 2020, 24(6): 3030-6.

[22] YANG B R, DU K, YANG C H, et al. CircPRMT5 circular RNA promotes proliferation of colorectal cancer through sponging miR-377 to induce E2F3 expression [J]. J Cell Mol Med, 2020, 24(6): 3431-7.

[23] GENG Y, BAO Y X, ZHANG W, et al. Circular RNA hsa_circ_0014130 inhibits apoptosis in non-small cell lung cancer by sponging miR-136-5p and upregulating BCL2 [J]. Mol Cancer Res, 2020, 18(5): 748-56.

[24] ZHA J F, CHEN D X. MiR-655-3p inhibited proliferation and migration of ovarian cancer cells by targeting RAB1A [J]. Eur Rev Med Pharmacol Sci, 2019, 23(9): 3627-34.

[25] WANG W, CAO R H, SU W Y, et al. miR-655-3p inhibits cell migration and invasion by targeting pituitary tumor-transforming 1 in non-small cell lung cancer [J]. Biosci Biotechnol Biochem, 2019, 83(9): 1703-8.