沉默circCCDC66靶向miR-129-5p对食管癌细胞Eca-109增殖及凋亡的影响

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摘要 该文探讨了沉默circCCDC66对食管癌细胞Eca-109增殖及凋亡的影响及其可能作用机制。qRT-PCR法与Western blot法分别检测食管癌组织、癌旁组织中circCCDC66、miR-129-5p、HMGB1的表达量; 体外培养人食管癌细胞Eca-109, 将si-NC、si-circCCDC66、miR-NC、miR-129-5p mimic、si-circCCDC66+miR-129-5p inhibitor分别转染至Eca-109细胞; qRT-PCR法与Western blot法分别检测细胞中circCCDC66、miR-129-5p、HMGB1的表达量; CCK-8法、平板克隆形成实验与流式细胞术分别检测细胞增殖、集落形成数及细胞凋亡率; 双荧光素酶报告实验验证circCCDC66与miR-129-5p的靶向关系, 以及miR-129-5p与HMGB1的靶向关系。食管癌组织中circCCDC66、HMGB1的表达量高于癌旁组织(P<0.05), miR-129-5p的表达量低于癌旁组织(P<0.05); 转染si-circCCDC66或转染miR-129-5p mimic后miR-129-5p的表达量、细胞凋亡率、细胞增殖抑制率升高(P<0.05), 而HMGB1蛋白水平降低(P<0.05), 集落形成数减少(P<0.05); circCCDC66可靶向调控miR-129-5p的表达, *HMGB1*是miR-129-5p的靶基因; 共转染si-circCCDC66和miR-129-5p inhibitor可降低转染si-circCCDC66对Eca-109细胞增殖、集落形成、凋亡的影响。沉默circCCDC66可通过靶向调控miR-129-5p/*HMGB1*表达而降低食管癌细胞增殖、克隆形成能力, 并可诱导细胞凋亡。

关键词 食管癌; circCCDC66; miR-129-5p; 细胞增殖; 凋亡

Effect of Silencing circCCDC66 on the Proliferation and Apoptosis of Esophageal Cancer Cells Eca-109 by Targeting miR-129-5p

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Abstract In this paper, the effect of silencing circCCDC66 on the proliferation and apoptosis of Eca-109 in esophageal cancer cells and its possible mechanism were discussed. The expression of circCCDC66, miR-129-5p and HMGB1 in esophageal cancer tissues and adjacent tissues were detected by qRT-PCR and Western blot. Human esophageal cancer cells Eca-109 were cultures *in vitro*, si-NC, si-circCCDC66, miR-NC, miR-129-5p mimic, si-circCCDC66+miR-129-5p inhibitor were transfected into Eca-109 cells, respectively. The expression of circCCDC66, miR-129-5p and HMGB1 in cells were detected by qRT-PCR and Western blot, respectively. Cell proliferation, colony formation and apoptosis rate were detected by CCK-8 assay, plate clone formation as-

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say and flow cytometry, respectively. The targeting relationship between circCCDC66 and miR-129-5p, and the targeting relationship between miR-129-5p and HMGB1 were verified by dual luciferase report experiment. The expression levels of circCCDC66 and HMGB1 in esophageal cancer tissues were higher than those in paracancer tissues (P<0.05), while the expression of miR-129-5p was lower than those in paracancer tissues (P<0.05). After transfection with si-circCCDC66 or miR-129-5p mimic, the expression of miR-129-5p, the rate of apoptosis, and the inhibition rate of cell proliferation were increased (P<0.05), while the protein level of HMGB1 and the number of colonies formed were decreased (P<0.05). circCCDC66 could target and regulate the expression of miR-129-5p, and *HMGB1* is the target gene of miR-129-5p. Co-transfection of si-circCCDC66 and miR-129-5p inhibitor could reduce the effect of transfection of si-circCCDC66 on the proliferation, clone formation and apoptosis of Eca-109 cells. Silencing circCCDC66 can reduce the proliferation and clonal formation ability of esophageal cancer cells and induce cell apoptosis by targeting the expression of miR-129-5p/*HMGB1*.

Keywords esophageal cancer; circCCDC66; miR-129-5p; cell proliferation; apoptosis

环状RNA(circular RNA, circRNA)具有稳定性与组织特异性, 研究表明circRNA在食管癌中表达上调或下调, 并可通过充当微小RNA(miRNA)的海绵分子而调节食管癌细胞增殖、凋亡等生物学行为[1-4]。最近我们注意到circCCDC66在人类肿瘤中过表达, 特别是在胃肠道恶性肿瘤中[5-6]。circCCDC66起源于*CCDC66*基因的8、9外显子, 位于chr3:56626997-56628056位点, 全长468 nt。YANG等[5]报道circCCDC66可促进胃癌的增殖和侵袭。对于结肠癌, HSIAO等[6]注意到circCCDC66促进了结肠癌细胞的生长和转移。但circCCDC66在食管癌中的表达及其可能作用机制尚未可知。Starbase预测显示circCCDC66与miR-129-5p存在结合位点, 研究表明, 食管鳞状细胞癌中miR-129-5p呈低表达, 上调miR-129-5p可抑制细胞增殖及侵袭[7]。Targetscan预测显示miR-129-5p与高迁移率族蛋白B1(high mobility group protein B1, *HMGB1*)存在互补的核苷酸位点。抑制HMGB1表达可诱导食管癌细胞凋亡[8]。因此, 本研究主要探讨circCCDC66是否通过调节miR-129-5p/HMGB1表达来影响食管癌的发生及发展。

1 材料与方法

1.1 材料

1.1.1 组织 收集2019年6月—2020年11月于本院进行手术治疗的食管癌患者的癌组织(n=27)及其相应癌旁组织(n=27)标本, 置于–80 °C超低温冰箱内保存, 其中女10例, 男17例, 年龄在50至67岁之间, 平均年龄(55.38±4.11)岁。所有参与者都签署了知情同意书。本研究经绵阳市人民医院伦理委员会审批, 其批准号为2019051502。

1.1.2 主要试剂 人食管癌Eca-109细胞购自美国ATCC; CCK-8试剂购自上海碧云天生物技术有限公司; Annexin -VFITC/PI凋亡检测试剂盒购自北京索莱宝科技有限公司; RNA提取试剂购自北京全式金生物技术有限公司; Lipofectamine 2000购自美国Invitrogen公司; 反转录试剂、荧光定量PCR试剂购自美国ThermoFisher公司; circCCDC66小干扰RNA(si-circCCDC66 1, 5′-CAA UUA GAG CAU CAG GAA A-3′; si-circCCDC66 2, 5′-GAG CAU CAG GAA ACA GUA C-3′; si-circCCDC66 3, 5′-CAA UUA GAG CAU CAG GAA ATT-3′)及其阴性对照(si-NC, 5′-UUC UCC GAA CGU GUC ACG UTT-3′)、miR-129-5p 模拟物(mimic)、miR-NC、miR-129-5p 抑制剂(inhibitor)购自广州锐博生物科技有限公司; HRP标记的山羊抗兔IgG二抗购自美国Abcam公司; 兔抗人HMGB1多克隆抗体购自北京冬歌博业生物科技有限公司。

1.2 方法

1.2.1 RNase R和放线菌素D处理 总RNA(2 μg)与3 U/mg的RNase R在37 °C孵育15 min。采用qRT-PCR检测circCCDC66等RNA的表达情况。用放线菌素D或DMSO处理食管癌Eca-109细胞, 评价circCCDC66及其线性基因*CCDC66*的稳定性。采用qRT-PCR检测RNA的稳定性。

1.2.2 实验分组 用不含血清的培养基稀释Lipofectamine 2000和不含血清的培养基分别稀释si-NC、si-circCCDC66、miR-NC、miR-129-5p mimic、si-

circCCDC66+miR-129-5p inhibitor, 培养5 min, 转染物稀释液与转染试剂充分混匀后室温孵育20 min, 同时将Eca-109细胞(2×10^{5}个/mL)按照每孔100 μL接种于6孔板中, 将上述混合液按照每孔200 μL均匀加入6孔板的细胞中, 培养48 h, 分别记为si-NC组、si-circCCDC66组、miR-NC组、miR-129-5p mimic组、si-circCCDC66+miR-129-5p inhibitor组。同时将正常培养的Eca-109细胞记为control组。

1.2.3 qRT-PCR检测circCCDC66、miR-129-5p表达情况 用Trizol试剂在食管癌细胞和组织中提取RNA, 反转录后进行PCR扩增反应。反应条件: 95 °C预变性2 min; 95 °C变性30 s, 60 °C退火30 s, 72 °C延伸30 s, 共40个循环。应用罗氏LightCycler480荧光定量PCR仪检测Ct值并采用$2^{-\Delta\Delta Ct}$法计算其相对表达。所用引物序列为: circCCDC66正向5′-TCT CTT GGA CCC AGC TCA G-3′, 反向5′-TGA ATC AAA GTG CAT TGC CC-3′; miR-129-5p正向5′-CGG CGG TTT TTT GCG GTC TGG GCT-3′; 反向5′-AGC CCA GAC CGC AAA AAA CCG CCG-3′; *GAPDH*正向5′-GGA GCG AGA TCC CTC CAA AAT-3′, 反向5′-GGC TGT TGT CAT ACT TCT CAT GG-3′; *U6*正向5′-CTC GCT TCG GCA GCA CA-3′, 反向5′-AAC GCT TCA CGA ATT TGC GT-3′。

1.2.4 CCK-8检测Eca-109细胞增殖 将Eca-109细胞(2×10^{4}个/mL)接种至96孔板(100 μL/孔)中, 并置于37 °C、5% CO_2培养2天。检测前2 h, 在孔中添加10 μL CCK-8溶液, 在450 nm波长处记录光密度(*D*)值。

1.2.5 平板克隆形成实验 首先将细胞接种到6孔板中, 每孔500个细胞, 培养14天。随后, 用甲醇在室温下固定细胞20 min, 用1%结晶紫染色15 min。去除染色液后, 将形成的菌落晾干, 显微镜下观察并统计克隆形成数。

1.2.6 流式细胞术检测Eca-109细胞凋亡率 严格按照Annexin V-FITC/PI凋亡检测试剂盒, 用5 μL Annexin V-FITC与5 μL PI在黑暗中室温下孵育转染细胞10 min。采用FACS Calibur流式细胞仪评估细胞凋亡情况。

1.2.7 双荧光素酶报告实验证实miR-129-5p与circCCDC66、*HMGB1*的靶向关系 Starbase预测显示circCCDC66与miR-129-5p存在结合位点, Targetscan预测显示miR-129-5p与HMGB1存在结合位点, 由美国Promega公司设计合成含有circCCDC66与miR-129-5p结合位点的野生型载体WT-circCCDC66与含有其突变位点的突变型载体MUT-circCCDC66, 同时设计合成含有miR-129-5p与*HMGB1*结合位点的野生型载体WT-HMGB1与含有其突变位点的突变型载体MUT-HMGB1, 用脂质体转染法将WT-circCCDC66、MUT-circCCDC66、WT-HMGB1、MUT-HMGB1分别与miR-NC或miR-129-5p mimic共转染至Eca-109细胞, 于37 °C、5% CO_2体积分数培养箱内继续培养48 h后, 检测其荧光素酶活性。

1.2.8 Western blot评估HMGB1蛋白表达情况 用蛋白裂解液提取癌旁组织、食管癌组织与各组Eca-109细胞总RNA, 每孔道加入40 μg蛋白样品。蛋白质用10% SDS-PAGE分离, 转移至PVDF膜上。PVDF膜在室温下用脱脂牛奶封闭2 h, 然后在4 °C下与HMGB1一抗(1:800)与内参GAPDH抗体(1:1 000)孵育过夜。随后, 将膜与二抗(1:5 000)浸泡1 h。最后, 应用ImageJ软件分析各蛋白水平。

1.3 统计学分析

所有统计分析均采用SPSS 21.0软件进行, 符合正态分布的计量资料表示为均数±标准差($\bar{x}\pm s$)。Person相关性检验分析食管癌中circCCDC66、miR-129-5p、HMGB1的相关性。两组间差异比较行独立样本*t*检验, 多组间差异比较行单因素方差分析, 两两比较行LSD-*t*检验。$P<0.05$为差异具有统计学意义。

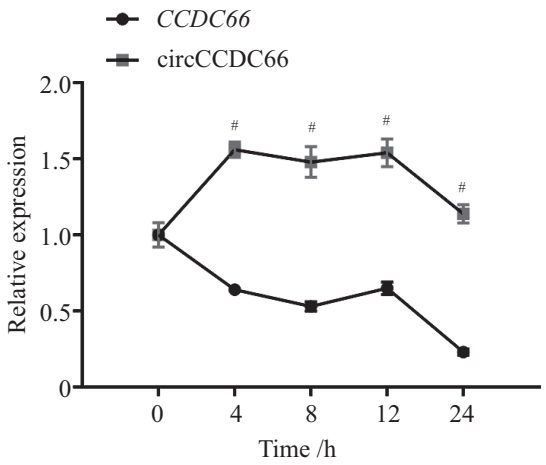
2 结果

2.1 食管癌中circCCDC66的稳定性

由于圆形异构体的高稳定性, 在Eca-109细胞中, circCCDC66的半衰期超过24 h(图1)。此外, 与Mock组相比, RNase R组*CCDC66* mRNA的相对丰度明显降低, circCCDC66的相对丰度基本没有变化, 证实了circCCDC66是环状的(图2)。我们发现circCCDC66由于其圆形结构, 比*CCDC66*更稳定。这些结果表明, circCCDC66是一个稳定的circRNA, 且在食管癌细胞中表达。

2.2 食管癌中circCCDC66、miR-129-5p、HMGB1的表达情况

食管癌组织与癌旁组织比较, circCCDC66、HMGB1表达水平升高, miR-129-5p表达水平下降($P<0.05$, 图3)。相关性分析结果显示, 食道癌中

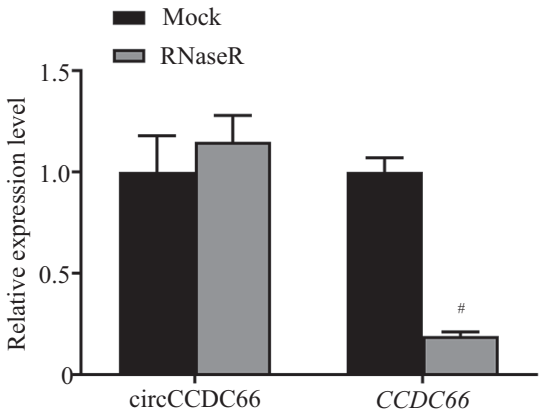


#*P*<0.05, 与*CCDC66*相比。

#*P*<0.05 compared with *CCDC66*.

图1 qRT-PCR检测经放线菌素D处理的Eca-109细胞在指定时间点的circCCDC66和*CCDC66* mRNA丰度

Fig.1 mRNA abundance of circCCDC66 and *CCDC66* in Eca-109 cells treated with actinomycin D was detected by qRT-PCR



#*P*<0.05, 与Mock相比。

#*P*<0.05 compared with Mock.

图2 qRT-PCR检测加RNase R和不加RNase R处理后Eca-109细胞中circCCDC66和*CCDC66* mRNA的相对丰度

Fig.2 The relative abundance of circCCDC66 and *CCDC66* mRNA in Eca-109 cells treated with and without RNase R was determined by qRT-PCR

circCCDC66与miR-129-5p表达呈负相关(*r*=–0.655, *P*<0.05), miR-129-5p与HMGB1表达呈负相关(*r*=–0.756, *P*<0.05)。

2.3 沉默circCCDC66对Eca-109增殖、凋亡的影响

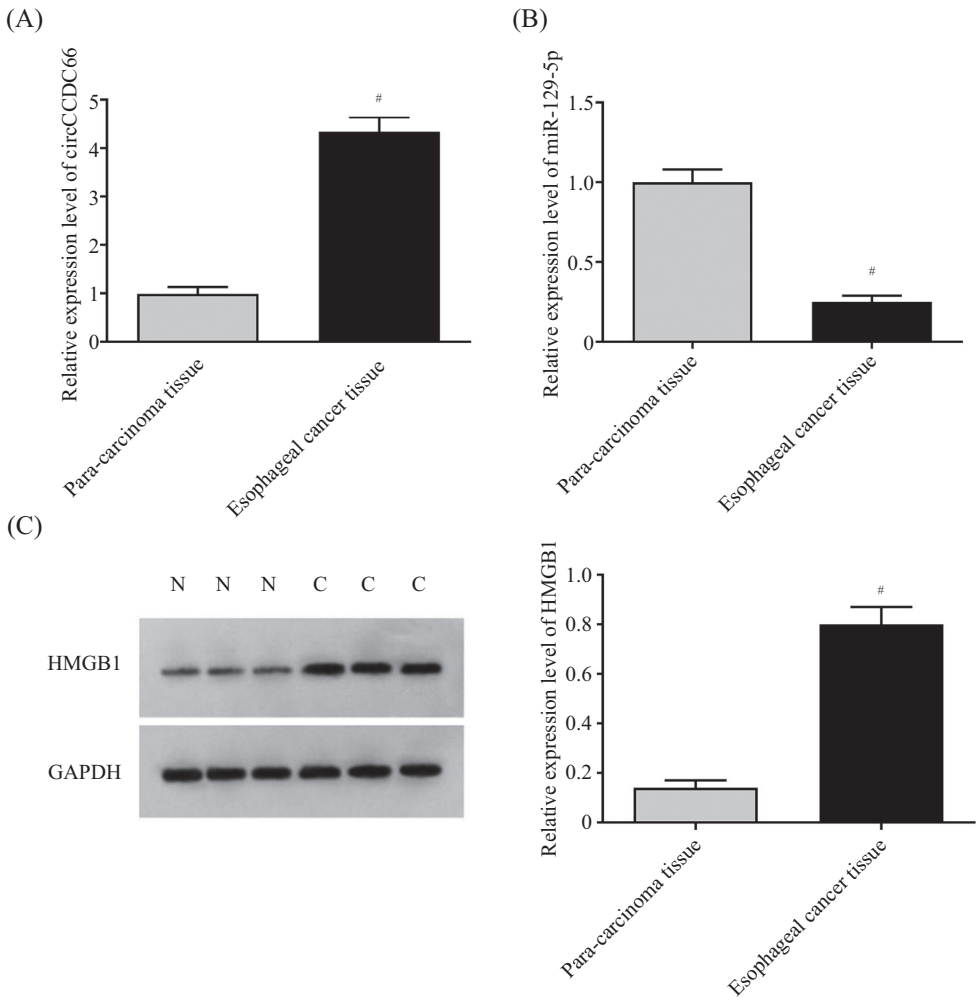
与si-NC组比较, si-circCCDC66 1组、si-circCCDC66 2组、si-circCCDC66 3组circCCDC66表达量下降(*P*<0.05, 图4)。由图4和图5可知, si-circCCDC66组与control组、si-NC组比较, miR-129-5p的表达水平升高(*P*<0.05), HMGB1蛋白表达水平降低(*P*<0.05), 细胞增殖抑制率升高(*P*<0.05), 集落形成数减少(*P*<0.05), 细胞凋亡率升高(*P*<0.05)。

2.4 circCCDC66靶向miR-129-5p及miR-129-5p靶向HMGB1

Starbase预测显示miR-129-5p是circCCDC66的可能靶点(图6A)。与miR-NC组比较, miR-129-5p组WT-circCCDC66细胞的相对荧光素酶活性下降(*P*<0.05, 图6B)。Targetscan预测显示miR-129-5p与HMGB1存在互补的核苷酸位点(图6C)。与miR-NC组比较, miR-129-5p组WT-HMGB1细胞相对荧光素酶活性下降(*P*<0.05, 图6B)。

2.5 miR-129-5p过表达对Eca-109增殖、凋亡的影响

由图7可知, miR-129-5p mimic组与control组、

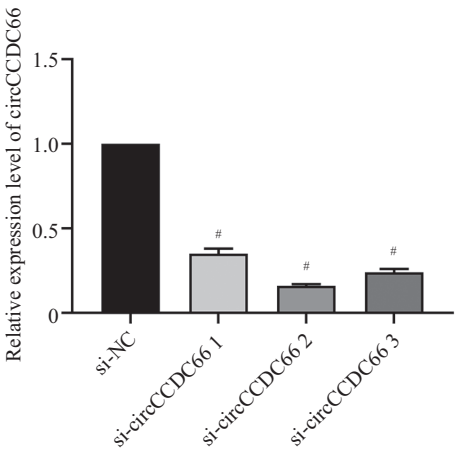


A: 食管癌中circCCDC66表达情况比较; B: 食管癌中miR-129-5p表达情况比较; C: 食管癌中HMGB1蛋白表达情况比较; #P<0.05, 与癌旁组织相比。C: 癌组织; N: 癌旁组织。

A: comparison of circCCDC66 expression in esophageal cancer; B: comparison of miR-129-5p expression in esophageal cancer; C: comparison of HMGB1 protein expression in esophageal cancer; #P<0.05 compared with para-carcinoma tissue. C: carcinoma tissue; N: para-carcinoma tissue.

图3 食管癌中circCCDC66、miR-129-5p、HMGB1的表达

Fig.3 Expression of circCCDC66, miR-129-5p and HMGB1 in esophageal cancer

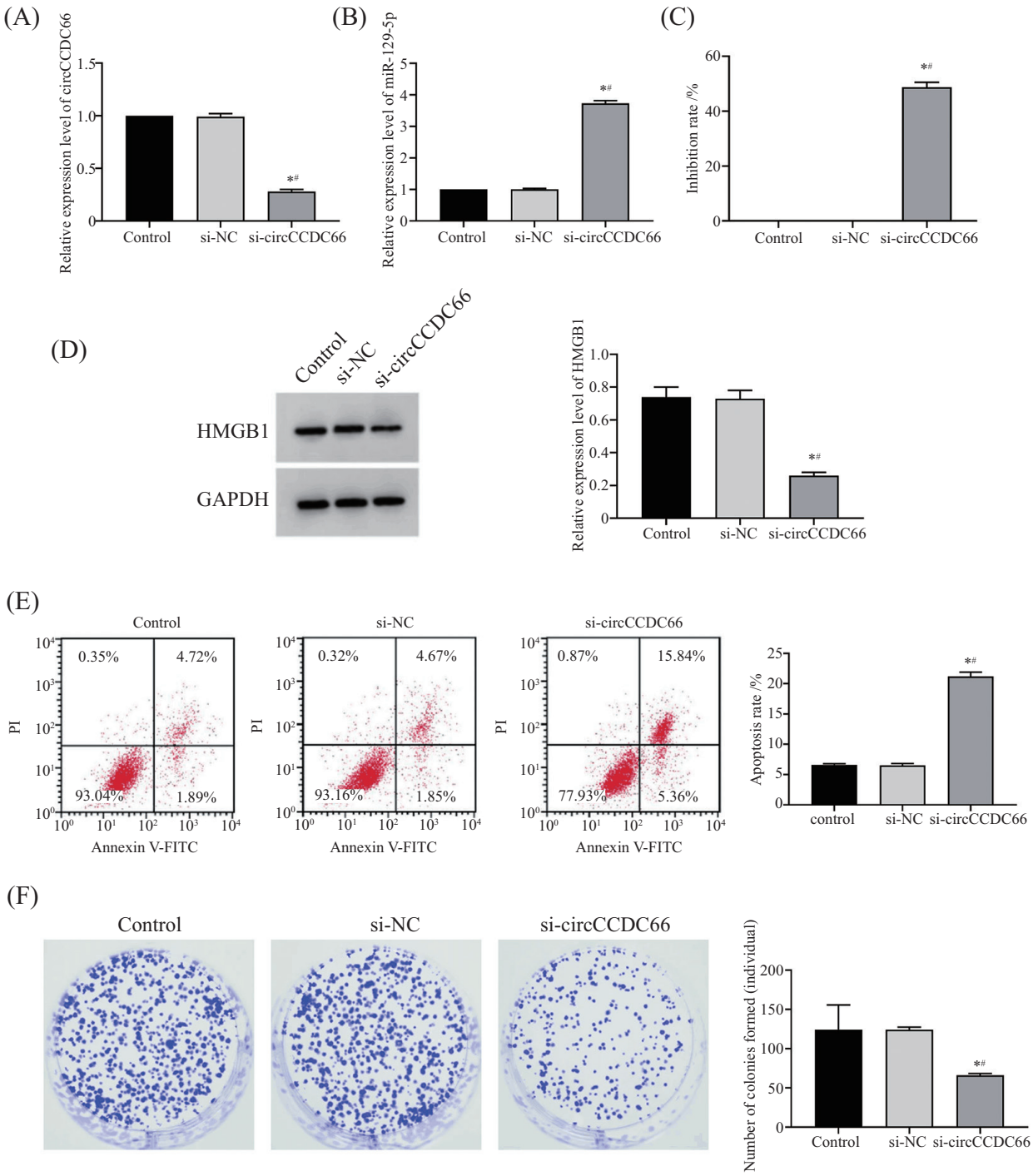


#P<0.05, 与si-NC组相比。

#P<0.05 compared with si-NC group.

图4 沉默circCCDC66处理后沉默circCCDC66表达的检测

Fig.4 Detection of silenced circCCDC66 expression after silenced circCCDC66 treatment

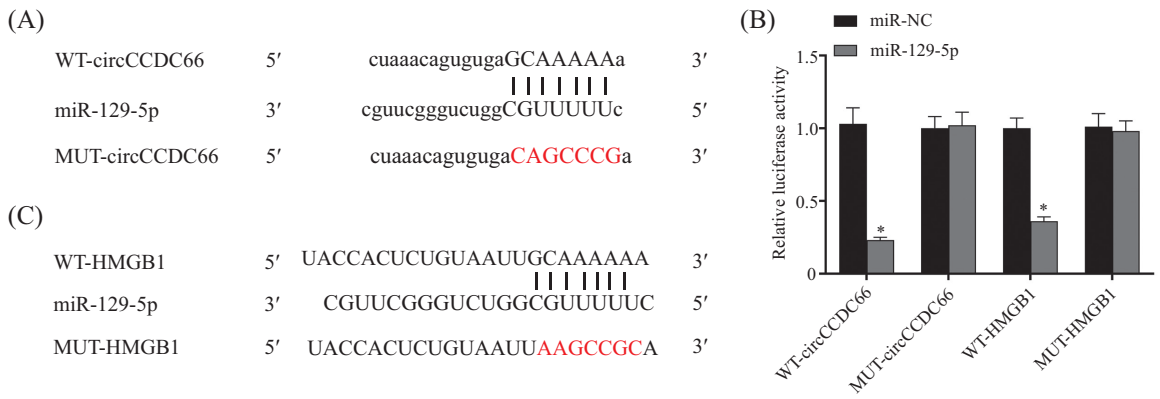


A: 沉默circCCDC66对Eca-109细胞circCCDC66表达的影响; B: 沉默circCCDC66对Eca-109细胞miR-129-5p表达的影响; C: 沉默circCCDC66对Eca-109细胞增殖抑制率的影响; D: 沉默circCCDC66对Eca-109细胞HMGB1蛋白表达的影响; E: 沉默circCCDC66对Eca-109细胞凋亡的影响; F: 沉默circCCDC66对Eca-109细胞克隆形成的影响; *P<0.05, 与control组相比; #P<0.05, 与si-NC组相比。

A: the effect of circCCDC66 silencing on circCCDC66 expression in Eca-109 cells; B: the effect of silencing circCCDC66 on miR-129-5p expression in Eca-109 cells; C: silencing the effect of circCCDC66 on the proliferation inhibition rate of Eca-109 cells; D: the effect of silencing circCCDC66 on HMGB1 protein expression in Eca-109 cells; E: silencing the effect of circCCDC66 on apoptosis of Eca-109 cells; F: silencing the effect of circCCDC66 on the clonal formation of Eca-109 cells; *P<0.05 compared with the control group; #P<0.05 compared with si-NC group.

图5 沉默circCCDC66对Eca-109增殖、凋亡的影响

Fig.5 The effect of silencing circCCDC66 on proliferation and apoptosis of Eca-109

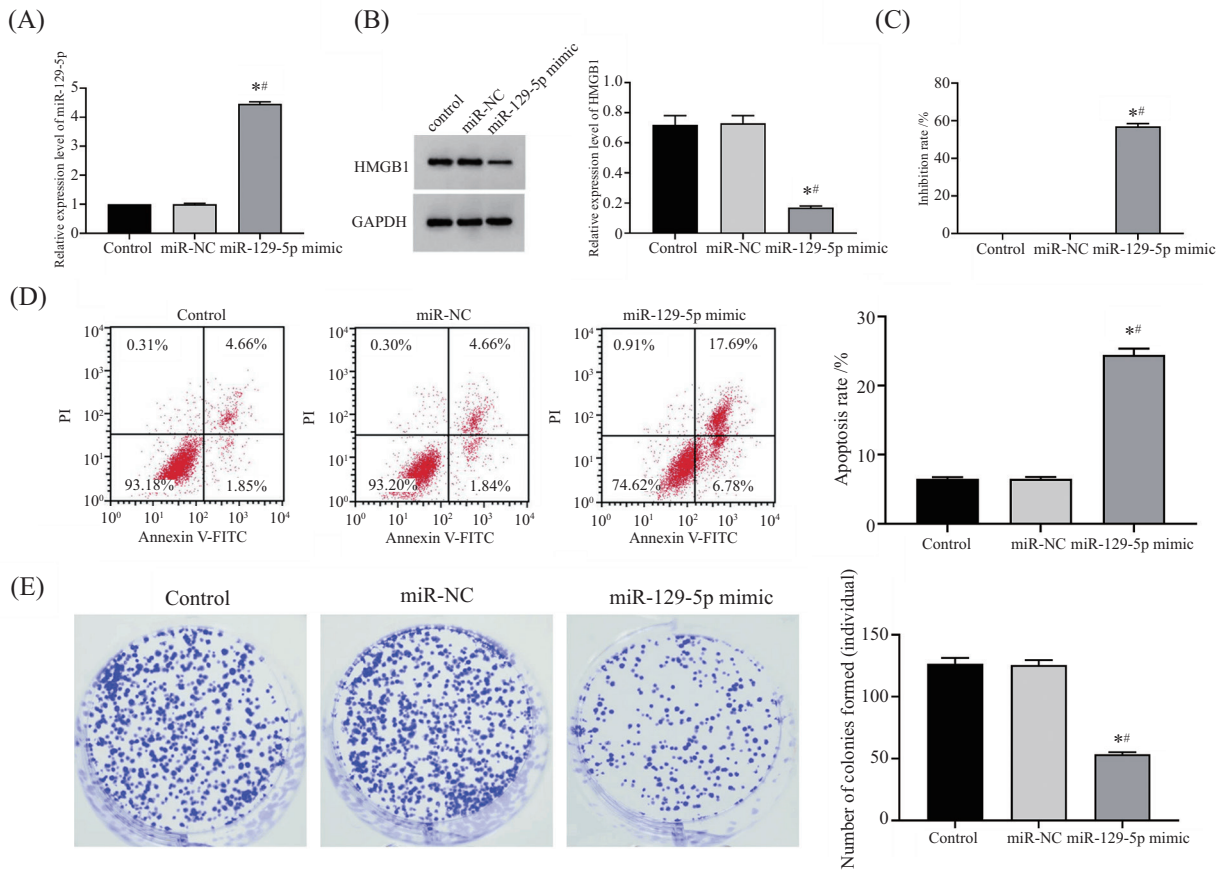


A: circCCDC66和miR-129-5p的互补序列; B: miR-129-5p和HMGB1的互补序列; C: 相对荧光素酶活性比较; 红色碱基为突变碱基; *P<0.05, 与miR-NC组比较。

A: complementary sequence of circCCDC66 and miR-129-5p; B: complementary sequence of miR-129-5p and HMGB1; C: comparison of relative luciferase activity; the red base is the mutant base; *P<0.05 compared with miR-NC group.

图6 双荧光素酶报告实验

Fig.6 Double luciferase reporting experiments

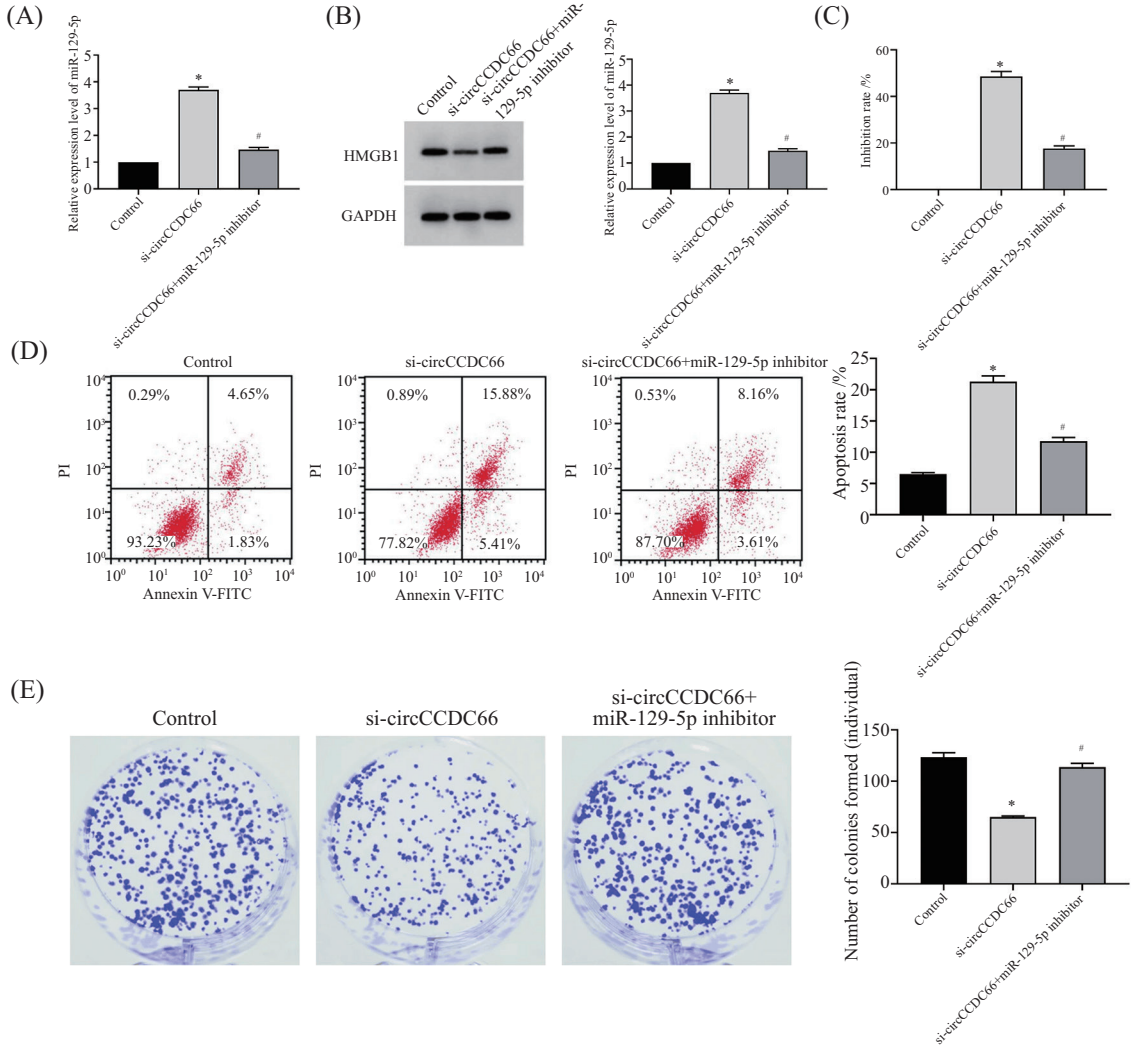


A: 过表达miR-129-5p对Eca-109细胞miR-129-5p表达的影响; B: 过表达miR-129-5p对Eca-109细胞HMGB1蛋白表达的影响; C: 过表达miR-129-5p对Eca-109细胞增殖抑制率的影响; D: 过表达miR-129-5p对Eca-109细胞凋亡的影响; E: 过表达miR-129-5p对Eca-109细胞克隆形成的影响; *P<0.05, 与control组相比; #P<0.05, 与miR-NC组相比。

A: the effect of overexpression of miR-129-5p on the expression of miR-129-5p in Eca-109 cells; B: the effect of overexpression of miR-129-5p on HMGB1 protein expression in Eca-109 cells; C: the effect of overexpression of miR-129-5p on proliferation inhibition rate of Eca-109 cells; D: the effect of overexpression of miR-129-5p on apoptosis of Eca-109 cells; E: the effect of overexpression of miR-129-5p on the clonal formation of Eca-109 cells; *P<0.05 compared with the control group; #P<0.05 compared with miR-NC group.

图7 过表达miR-129-5p对Eca-109增殖、凋亡的影响

Fig.7 The effect of overexpression of miR-129-5p on proliferation and apoptosis of Eca-109



A: 抑制miR-129-5p逆转了沉默circCCDC66对Eca-109细胞miR-129-5p表达的影响; B: 抑制miR-129-5p逆转了沉默circCCDC66对Eca-109细胞HMGB1蛋白表达的影响; C: 抑制miR-129-5p逆转了沉默circCCDC66对Eca-109细胞增殖抑制率的影响; D: 抑制miR-129-5p逆转了沉默circCCDC66对Eca-109细胞凋亡的影响; E: 抑制miR-129-5p逆转了沉默circCCDC66对Eca-109细胞克隆形成的影响; *P<0.05, 与control组相比; #P<0.05, 与si-circCCDC66组相比。

A: inhibition of miR-129-5p reversed the effect of circCCDC66 silencing on the expression of miR-129-5p in Eca-109 cells; B: inhibition of miR-129-5p reversed the effect of circCCDC66 on HMGB1 protein expression in Eca-109 cells; C: inhibition of miR-129-5p reversed the effect of circCCDC66 silencing on the proliferation inhibition rate of Eca-109 cells; D: inhibition of miR-129-5p reversed the effect of circCCDC66 on apoptosis of Eca-109 cells; E: inhibition of miR-129-5p reversed the effect of circCCDC66 silencing on the formation of Eca-109 cell clones; *P<0.05 compared with the control group; #P<0.05 compared with si-circCCDC66 group.

图8 抑制miR-129-5p可逆转沉默circCCDC66对Eca-109增殖、凋亡的检测

Fig.8 Inhibition of miR-129-5p can reverse the effects of circCCDC66 silencing on proliferation and apoptosis of Eca-109

miR-NC组比较, HMGB1蛋白水平降低(P<0.05), 细胞增殖抑制率升高(P<0.05), 集落形成数减少(P<0.05), 细胞凋亡率升高(P<0.05)。

2.6 抑制miR-129-5p对沉默circCCDC66处理的Eca-109增殖、凋亡的影响

由图8可知, 与si-circCCDC66组比较, si-circCCDC66+miR-129-5p inhibitor组HMGB1蛋白水平升高(P<0.05), 细胞增殖抑制率降低(P<0.05), 集落形成数增多(P<0.05), 细胞凋亡率降低(P<0.05)。

3 讨论

结直肠癌中circCCDC66的表达上调, 敲低circCCDC66的表达可阻碍结直肠癌的增殖、迁移侵袭, 并可促进细胞凋亡[9-10]。circCCDC66在胃癌组织与细胞系中表达水平升高, 其高表达量与患者肿瘤分期、淋巴转移有关[11]。甲状腺癌组织和细胞系

中circCCDC66的表达量升高, 沉默circCCDC66可抑制甲状腺癌细胞增殖、迁移、侵袭及糖酵解[12]。以上研究说明, circCCDC66在肿瘤细胞中高表达, 推测这与肿瘤细胞的高转移和高侵袭能力相关。但食管癌中circCCDC66的表达仍不明确。本研究发现, circCCDC66在食管癌组织中表达水平升高, 进一步研究发现, 沉默circCCDC66后食管癌细胞增殖抑制率、凋亡率升高, 而集落形成数减少, 提示沉默circCCDC66可抑制食管癌细胞增殖、克隆形成及促进细胞凋亡, 这与其在甲状腺癌中的功能一致[13], 说明circCCDC66在头颈部癌症中均发挥促癌作用。

本研究证实, circCCDC66可充当miR-129-5p的海绵分子, *HMGB1*是miR-129-5p的靶基因, 推断circCCDC66/miR-129-5p/HMGB1与食管癌发展密切相关。研究表明, miR-129-5p通过抑制COL1A1表达而抑制胃癌细胞侵袭和增殖[14]。miR-129-5p通过靶向ETV1抑制前列腺癌细胞增殖[15]。miR-129-5p通过靶向RSF1抑制结肠癌细胞增殖并促进凋亡[16]。HMGB1属于DNA结合非组蛋白, 其具有稳定核小体并具有转录功能, 并可参与神经轴突生长与肿瘤转移等生理病理过程[17]。lncRNA ZEB2-AS1通过抑制miR-204表达而促进HMGB1表达进而促进胰腺癌细胞生长和侵袭[18]。lncRNA KTN1-AS1通过靶向miR-505上调HMGB1促进胶质瘤细胞增殖[19]。本研究结果显示, 食管癌组织中miR-129-5p的表达量降低, 而HMGB1的表达量升高, 沉默circCCDC66可通过靶向促进miR-129-5p表达而抑制HMGB1表达, miR-129-5p过表达可通过靶向下调HMGB1表达降低食管癌细胞增殖、克隆形成能力, 提高细胞凋亡能力。此外, 抑制miR-129-5p表达可回复沉默circCCDC66抑制食管癌细胞增殖、克隆形成的作用及促进凋亡的作用。这提示circCCDC66可通过靶向调节miR-129-5p/HMGB1表达而促进食管癌细胞增殖及克隆形成, 进而抑制细胞凋亡。生物信息学预测和既往发表的文献显示, circCCDC66还有miR-370、miR-211-5p等靶miRNA分子[10,12], 说明在食管癌进展中circCCDC66可能通过其他miRNA发挥作用; 同时miR-129-5p的下游靶基因还有*ETV1*、*RSF1*[15-16], 这说明circCCDC66/miR-129-5p可能通过影响其他靶基因表达调控食管癌细胞进展。

综上所述, 食管癌组织中circCCDC66与HMGB1上调表达, miR-129-5p下调表达, 沉默circCCDC66可抑制食管癌细胞增殖、促进细胞凋亡, 这可能是通过提高miR-129-5p表达而降低HMGB1的表达实现的。但circCCDC66是否可通过调控其他miRNA/mRNA而发挥作用尚需进一步探究。另外, 本研究仅采用一种食管癌细胞株进行功能验证, 这是我们的不足之处, 未来将在其他食管癌细胞株中验证circCCDC66的功能和机制。

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