circEPSTI1通过靶向调控miR-216b-5p调节胃癌细胞顺铂耐药性

郭海龙 马晔 王晓耘 史佩东 万伯顺*

(上海市嘉定区中心医院, 普外科, 上海 201800)

摘要 该研究的目的是为了观察circEPSTI1是否通过靶向调控miR-216b-5p对胃癌细胞顺铂耐药性产生影响, 并探讨其可能的作用机制。采用实时荧光定量聚合酶链反应(quantitative real-time polymerase chain reaction, qRT-PCR)检测胃癌组织、癌旁组织、人胃黏膜细胞GES-1、人胃癌细胞HGC27与胃癌耐药性细胞HGC27/DDP中circEPSTI1与miR-216b-5p的表达情况; 使用双荧光素酶报告实验验证circEPSTI1与miR-216b-5p的靶向调控关系; 以HGC27/DDP细胞为研究对象, 实验分组: si-NC组、si-circEPSTI1组、si-circEPSTI1+anti-miR-NC组、si-circEPSTI1+anti-miR-216b-5p组; 采用CCK-8法、平板克隆形成实验、流式细胞术与Transwell实验分别检测细胞增殖、克隆形成、凋亡、迁移及侵袭; Western blot检测Cleavea-caspase 3、Cleavea-caspase 9、E-cadherin、N-cadherin蛋白表达情况。结果显示, 与癌旁组织相比, 胃癌组织中的circEPSTI1表达上调(P<0.05), miR-216b-5p表达下调(P<0.05); 与GES-1细胞相比, HGC27细胞、HGC27/DDP细胞的circEPSTI1表达上调(P<0.05), miR-216b-5p表达下调(P<0.05); 与HGC27细胞相比, HGC27/DDP细胞的circEPSTI1表达上调(P<0.05), miR-216b-5p表达下调(P<0.05); miR-216b-5p过表达可降低wt-circEPSTI1的荧光素酶活性(P<0.05), 未影响mut-circEPSTI1的荧光素酶活性; 与si-NC组相比, si-circEPSTI1组细胞增殖抑制率、凋亡率和Cleavea-caspase 3、Cleavea-caspase 9、E-cadherin蛋白表达上调(P<0.05), 克隆形成数、迁移及侵袭细胞数减少(P<0.05), N-cadherin蛋白表达下调(P<0.05); 与si-circEPSTI1+anti-miR-NC组相比, si-circEPSTI1+anti-miR-216b-5p组细胞增殖抑制率、凋亡率和Cleavea-caspase 3、Cleavea-caspase 9、E-cadherin蛋白表达下调(P<0.05), 克隆形成数、迁移及侵袭细胞数增加(P<0.05), N-cadherin蛋白表达上调(P<0.05)。结果表明, circEPSTI1通过靶向抑制miR-216b-5p表达促进胃癌耐药性细胞增殖、克隆形成、迁移及侵袭, 抑制胃癌耐药性细胞凋亡, 从而增强胃癌细胞对DDP的耐药性, 该机制可能与改变凋亡相关蛋白表达情况和逆转上皮–间质转化过程有关。

关键词 胃癌; 顺铂耐药性; circEPSTI1; miR-216b-5p; 细胞增殖; 迁移; 侵袭

CircEPSTI1 Regulates Cisplatin Resistance of Gastric Cancer Cells by Targeting miR-216b-5p

GUO Hailong, MA Ye, WANG Xiaoyun, SHI Peidong, WAN Boshun*

(*General Surgery, Jiading District Central Hospital Affiliated Shanghai University of Medicine & Health Sciences, Shanghai 201800, China*)

收稿日期: 2023-02-17 接受日期: 2023-04-03

上海市嘉定区区科委项目(批准号: JDKW-2018-W14)资助的课题

*通讯作者。Tel: 13901926643, E-mail: wanboshun@sina.com

Received: February 17, 2023 Accepted: April 3, 2023

This work was supported by the Program of Shanghai Jiading District Science and Technology Commission (Grant No.JDKW-2018-W14)

*Corresponding author. Tel: +86-13901926643, E-mail: wanboshun@sina.com

Abstract The objective was to observe the effect of circEPSTI1 on cisplatin resistance of gastric cancer cells by targeting miR-216b-5p and to explore its possible mechanism. The qRT-PCR (quantitative real-time polymerase chain reaction) was used to detect the expression of circEPSTI1 and miR-216b-5p in gastric cancer tissues, adjacent tissues, human gastric mucosal cells GES-1, human gastric cancer cells HGC27 and gastric cancer drug-resistant cells HGC27/DDP. The dual luciferase reporting assay was used to verify the targeted regulatory relationship between circEPSTI1 and miR-216b-5p. HGC27/DDP cells were selected as the research object and divided into four groups: the si-NC group, the si-circEPSTI1 group, the si-circEPSTI1+anti-miR-NC group, and the si-circEPSTI1+anti-miR-216b-5p group. Cell proliferation, clonal formation, apoptosis, migration, and invasion were detected by CCK-8 assay, plate clonal formation assay, flow cytometry, and Transwell assay, respectively. Western blot was used to detect the protein expression of Cleavea-caspase 3, Cleavea-caspase 9, E-cadherin, and N-cadherin. Compared with adjacent tissues, the results showed that circEPSTI1 expression in gastric cancer tissues was up-regulated (P<0.05) and miR-216b-5p expression was down-regulated (P<0.05). Compared with GES-1 cells, circEPSTI1 expression in HGC27 cells and HGC27/DDP cells was up-regulated (P<0.05), while miR-216b-5p expression was down-regulated (P<0.05). Compared with HGC27 cells, circEPSTI1 expression in HGC27/DDP cells was up-regulated (P<0.05) and miR-216b-5p expression was down-regulated (P<0.05). Overexpression of miR-216b-5p could reduce the luciferase activity of wt-circEPSTI1 (P<0.05), but did not affect the luciferase activity of mut-circEPSTI1. Compared with the si-NC group, cell proliferation inhibition rate, apoptosis rate, Cleavea-caspase 3, Cleavea-caspase 9, and E-cadherin protein expression in the si-circEPSTI1 group were up-regulated (P<0.05). The clone formation numbers, migration, and invasion cells were decreased (P<0.05), and the expression of N-cadherin protein was down-regulated (P<0.05). Compared with the si-circEPSTI1+anti-miR-NC group, cell proliferation inhibition rate, apoptosis rate, Cleavea-caspase 3, Cleavea-caspase 9, and E-cadherin expression were down-regulated in the si-circEPSTI1+anti-miR-216b-5p group (P<0.05). The number of clone formation, migration, and invasion cells increased (P<0.05), and the expression of N-cadherin protein was up-regulated (P<0.05). The results showed that circEPSTI1 promoted the proliferation, clone formation, migration, and invasion, and inhibited the apoptosis in drug-resistant gastric cancer cells by targeting the expression of miR-216b-5p, thus promoting the drug resistance of gastric cancer cells to DDP. This mechanism may be related to the change of apoptosis-related protein expression and the reversal of the epithelial-mesenchymal transformation process.

Keywords gastric cancer; cisplatin resistance; circEPSTI1; miR-216b-5p; cell proliferation; migration; invasion

胃癌(gastric cancer, GC)是我国发病率与死亡率较高的恶性肿瘤, 其早期症状较为隐匿, 大部分患者确诊时已处于胃癌中晚期, 而针对胃癌中晚期患者主要采用以铂类药物为基础的化疗, 但部分患者产生顺铂耐药性导致治疗效果降低[1-2]。环状RNA(circular RNA, circRNA)异常表达与胃癌等多种肿瘤相关, 并可能作为胃癌早期诊断及评估患者预后的潜在生物学标志物, 但circRNA与胃癌顺铂耐药性间的相关研究尚未明确[3-4]。circEPSTI1在非小细胞肺癌细胞中的表达上调, 并促进癌细胞增殖[5]。但circEPSTI1与胃癌细胞顺铂耐药性的关系及可能机制未见报道。Starbase预测circEPSTI1与miR-216b-5p可能存在靶向关系。报道显示, miR-216b在胃癌细胞中的表达下调, 上调其表达可促进胃癌细胞凋亡从而增强胃癌细胞顺铂敏感性[6]。但miR-216b-5p是否在胃癌细胞顺铂耐药性中发挥作用? 如存在作用, 其可能机制等问题均无明确研究。为此, 本研究拟探讨circEPSTI1参与胃癌细胞顺铂耐药性过程是否与靶向调控miR-216b-5p相关, 现将结果报道如下。

1 研究材料及方法

1.1 研究组织、细胞及试剂

收集2020年04月—2020年06月于我院就诊、经

经病理学诊断确诊为胃癌并接受手术切除的52例胃癌患者的癌组织及>5 cm处的癌旁组织标本，其中男性32例，女性20例，年龄51~67岁，平均年龄为(55.14±5.02)岁。患者或家属均了解本研究的目的、意义及大致的研究过程，均签署知情同意书。本研究经本院医学伦理委员会批准(批准号：2022K24)，符合《世界医学协会赫尔辛基宣言》相关要求。

人胃黏膜细胞GES-1、人胃癌细胞HGC27均购自美国菌种保藏中心；顺铂购自山东齐鲁制药公司；反转录与荧光定量聚合酶链反应试剂、Trizol试剂均购自美国Thermo Fisher公司；胎牛血清、DMEM培养液均购自上海碧云天生物技术有限公司；Lipofectamine™ 3000 Transfection Reagent转染试剂购自美国Invitrogen公司；si-NC、miR-NC、anti-miR-NC、si-circE、PSTI1、anti-miR-216b-5p与miR-216b-5p mimics均购自广州锐博生物科技有限公司；荧光素酶活性检测试剂盒、突变型载体mut-circEPSTI1与野生型载体wt-circEPSTI1均购自美国Promega公司；兔抗人Cleavea-caspase 3、Cleavea-caspase 9抗体购自武汉艾美捷科技有限公司；兔抗人E-cadherin、兔抗人N-cadherin、GAPDH均购自美国CST公司；HRP标记的山羊抗兔IgG二抗、0.25%胰蛋白酶均购自美国Abcam公司；细胞凋亡检测试剂盒、CCK-8试剂、Matrigel基质胶与Transwell小室均购自北京索莱宝科技有限公司。

1.2 方法

1.2.1 *构建耐药性胃癌细胞HGC27/DDP*[7] 将HGC27细胞接种于含胎牛血清的DMEM培养基，置于5% CO_2、37 °C培养箱内培养，2~3天换液1次，当细胞生长汇合度约80%时，以0.25%胰蛋白酶消化，传代培养。用0.1 μg/mL顺铂的培养基培养HGC27细胞，采用逐步增加顺铂浓度由5 μg/mL至10 μg/mL，连续培养14天，HGC27细胞可存活于含有浓度为10 μg/mL顺铂的培养基中，即获得耐药性胃癌细胞HGC27/DDP。

1.2.2 *实验分组* 将HGC27/DDP细胞以10^4个/孔接种于6孔板，待细胞生长汇合度达到80%时进行转染，将si-NC、si-circEPSTI1以Lipofectamine™ 3000 Transfection Reagent转染试剂分别转染至HGC27/DDP细胞，记为si-NC组、si-circEPSTI1组。将si-circEPSTI1和anti-miR-NC、si-circEPSTI1和anti-miR-216b-5p以Lipofectamine™ 3000 Transfection Reagent转染试剂分别转染至HGC27/DDP细胞，记为si-circEPSTI1+anti-miR-NC组、si-circEPSTI1+anti-miR-216b-5p组。

1.2.3 *实时荧光定量聚合酶链反应*(quantitative real-time polymerase chain reaction, qRT-PCR)*检测circEPSTI1、miR-216b-5p表达情况* 按Trizol试剂说明书步骤提取癌旁组织、胃癌组织、GES-1细胞、HGC27细胞、HGC27/DDP细胞总RNA，以紫外分光光度计测定以上组织或细胞总RNA纯度。逆转录反转录体系(20 μL)：2 μL 5× gDNA缓冲液、2 μL 10× King逆转录缓冲液、1 μL FastKing逆转录酶混合液、2 μL FQ-逆转录引物混合液、2 μg RNA，以RNase-Free ddH_2O补足。反应条件：42 °C 15 min、95 °C 3 min。cDNA作为模板行qRT-PCR扩增：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，循环40次。以LightCycler480型荧光定量PCR仪(Roche公司)检测并采用$2^{-\Delta\Delta Ct}$法进行定量分析circEPSTI1、miR-216b-5p相对表达水平，*GAPDH*和*U6*为内参。引物序列如下。circEPSTI1正向引物(5′→3′) AAG CTG AAG AAG CTG AAC TC，反向引物(5′→3′) GTG TAT GCA CTT GTG TAT TGC；miR-216b-5p正向引物(5′→3′) TCT GAG AAA TCT CTG CAG GC，反向引物(5′→3′) CTC AAC TGG TGT CGT GGA G；*GAPDH*正向引物(5′→3′) ACC AGG TGG TCT CCT CTG ACT T，反向引物(5′→3′) AAG TGG TCG TTG AGG GCA AT；*U6*正向引物(5′→3′) CTC GCT TCG GCA GCA CA，反向引物(5′→3′) AAC GCT TCA CGA ATT TGC GT。

1.2.4 *双荧光素酶报告实验检测circEPSTI1与miR-216b-5p的靶向关系* 以基因突变技术，将circEPSTI1预测结合miR-216b-5p的位点进行突变，将circEPSTI1结合miR-216b-5p的突变位点克隆至pGL3质粒，人工构建突变型载体mut-circEPSTI1。将circEPSTI1结合miR-216b-5p位点以分子克隆法克隆至pGL3质粒，人工构建野生型载体wt-circEPSTI1。将上述载体以Lipofectamine™ 3000 Transfection Reagent转染试剂分别与miR-NC或miR-216b-5p mimics共转染HGC27/DDP细胞，37 °C孵育24 h，检测细胞相对荧光素酶活性。

1.2.5 *CCK-8实验检测细胞增殖* 将各组HGC27/DDP细胞以1 000个/孔接种到96孔板，加入CCK-8 10 μL/孔，置于5% CO_2、37 °C培养箱内培养2 h，以

酶标仪检测各孔吸光度(D)值, 波长为450 nm, 根据D值计算细胞增殖抑制率, 细胞增殖抑制率=[($D_{对照组}-D_{实验组}$)/($D_{对照组}-D_{空白组}$)]×100%。

1.2.6 平板克隆形成实验检测细胞克隆形成数 将各组HGC27/DDP细胞以500个/孔接种到6孔板, 置于5% CO_2、37 °C培养箱内培养至出现细胞克隆团, 吸去培养基, 4 °C下多聚甲醛固定2 h, 加入1%结晶紫染色液染色, 记录各组细胞克隆形成数。

1.2.7 流式细胞术检测细胞凋亡率 将各组HGC27/DDP细胞调整为浓度2×10^{5}个/孔, 以胰蛋白酶消化3~5 min, 3 000 r/min转速离心5 min后弃上清, 加入PBS溶液悬浮细胞, 加入5 μL Annexin V-FITC与5 μL PI, 37 °C避光孵育15 min, 以流式细胞仪检测各组细胞凋亡率。

1.2.8 Transwell实验检测细胞侵袭及迁移 侵袭实验: 将各组HGC27/DDP细胞以1×10^{5}个/孔接种于Matrigel基质胶稀释液的Transwell上室, 下室加入600 μL含有10%胎牛血清的培养液, 37 °C培养48 h, 甲醇室温固定20 min, 1%结晶紫染色液室温染色20 min, 镜下记录侵袭细胞数。迁移实验: 将各组HGC27/DDP细胞以1×10^{5}个/孔直接接种于Transwell上室, 后续同侵袭实验。

1.2.9 Western blot检测细胞Cleavea-caspase 3、Cleavea-caspase 9、E-cadherin、N-cadherin蛋白表达 将各组HGC27/DDP细胞以RIPA裂解液裂解细胞并提取细胞总蛋白, 以BCA法检测蛋白纯度。通过SDS-PAGE将40 mg各组HGC27/DDP细胞的总蛋白转至PVDF膜, 脱脂奶粉室温封闭1.5 h, 分别加入Cleavea-caspase 3(稀释比例1:800)、Cleavea-caspase 9(稀释比例1:800)、E-cadherin(稀释比例1:1 000)、N-cadherin(稀释比例1:1 000)一抗与内参GAPDH(稀释比例1:3 000), 4 °C孵育24 h, 加入HRP标记的二抗稀释液(稀释比例1:5 000), 37 °C孵育1 h, ECL显色, 以Quantity One软件计算Cleavea-caspase 3、Cleavea-caspase 9、E-cadherin、N-cadherin条带的灰度值。

1.3 统计学处理

采用SPSS 21.0分析数据, 计量资料, 如Cleavea-caspase 3、Cleavea-caspase 9、E-cadherin、N-cadherin蛋白表达等, 以($\bar{x}\pm s$)表示, 多组间比较采用单因素方差分析, 组间两两比较采用LSD-t或独立样本t检验, Pearson相关性分析胃癌患者肿瘤组织中circEPSTI1与miR-216b-5p的相关性, 用GraphPad Prism 7软件绘制相关图。P<0.05为差异具有统计学意义。

2 结果

2.1 circEPSTI1和miR-216b-5p在胃癌组织、细胞中的表达

与癌旁组织相比, 胃癌组织中circEPSTI1表达上调(P<0.05), miR-216b-5p表达下调(P<0.05), 见图1A和图1B; 胃癌组织中circEPSTI1和miR-216b-5p表达具有显著负相关性相关性(P<0.05, 图1C); 与HGC27细胞相比, 顺铂在耐药性胃癌细胞HGC27/DDP中的IC_{50}值显著升高(P<0.05), 说明耐药性胃癌细胞HGC27/DDP构建成功(图1D); 与GES-1细胞相比, HGC27细胞、HGC27/DDP细胞的circEPSTI1表达上调(P<0.05), miR-216b-5p表达下调(P<0.05); 与HGC27细胞相比, HGC27/DDP细胞的circEPSTI1表达上调(P<0.05), miR-216b-5p表达下调(P<0.05), 见图1E和图1F。

2.2 circEPSTI1和miR-216b-5p转染效率

与si-NC组相比, si-circEPSTI1组circEPSTI1表达下调(P<0.05), miR-216b-5p表达上调(P<0.05); 与anti-miR-NC组相比, anti-miR-216b-5p组miR-216b-5p表达下调(P<0.05); 见图2。

2.3 circEPSTI1靶向miR-216b-5p

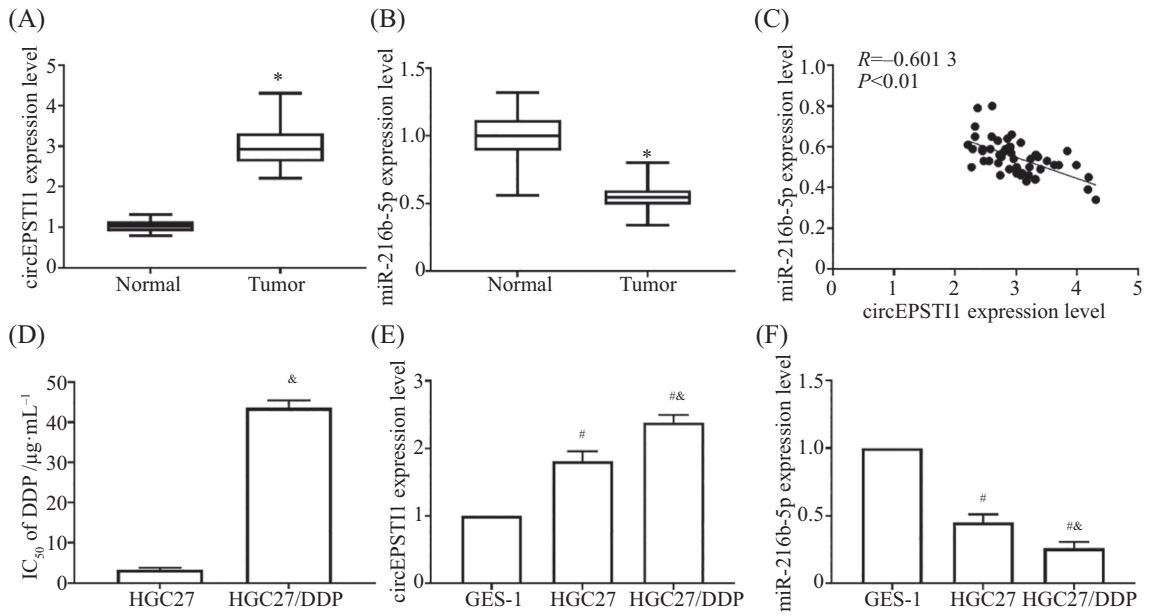
circEPSTI1和miR-216b-5p存在互补序列(图3)。与miR-NC组相比, miR-216b-5p组相对荧光素酶活性降低(P<0.05), miR-216b-5p组与miR-NC组的相对荧光素酶活性差异无统计学意义(P>0.05), 见表1。

2.4 circEPSTI1和miR-216b-5p对胃癌耐药HGC27/DDP细胞增殖、凋亡及克隆形成数的影响

与si-NC组相比, si-circEPSTI1组的细胞增殖抑制率、凋亡率增高(P<0.05), 克隆形成数降低(P<0.05); 与si-circEPSTI1+anti-miR-NC组相比, si-circEPSTI1+anti-miR-216b-5p组的细胞增殖抑制率、凋亡率降低(P<0.05), 克隆形成数增高(P<0.05); 见图4、表2。

2.5 circEPSTI1和miR-216b-5p对胃癌耐药HGC27/DDP细胞迁移、侵袭的影响

与si-NC组相比, si-circEPSTI1组的迁移、侵袭细胞数减少(P<0.05); 与si-circEPSTI1+anti-miR-NC

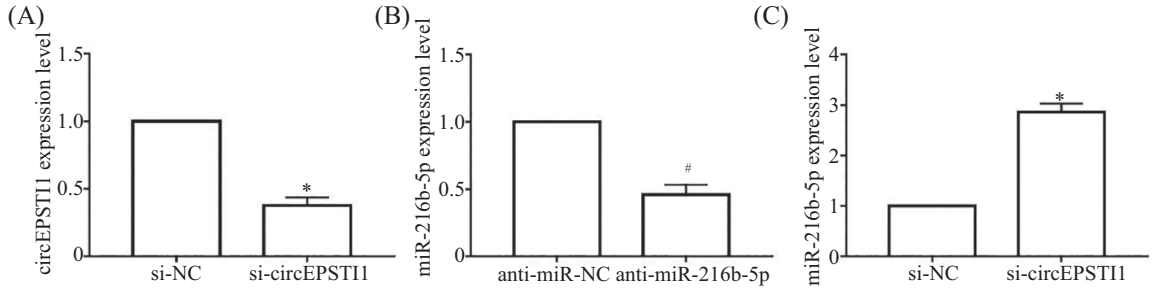


A: 胃癌组织中circEPSTI1表达上调(n=52); B: 胃癌组织中miR-216b-5p表达下调(n=52); C: 胃癌组织中circEPSTI1和miR-216b-5p表达相关性(n=52); D: 顺铂在胃癌细胞中的IC_{50}值(n=9); E: 胃癌细胞的circEPSTI1表达(n=9); F: 胃癌细胞的miR-216b-5p表达(n=9)。*P<0.05, 与癌旁组织比; #P<0.05, 与GES-1细胞比; &P<0.05, 与HGC27细胞比。

A: circEPSTI1 expression was up-regulated in gastric cancer tissues (n=52); B: down-regulated expression of miR-216b-5p in gastric cancer tissues (n=52); C: the correlation between circEPSTI1 and miR-216b-5p expression in gastric cancer tissues (n=52); D: IC_{50} value of cisplatin in gastric cancer cells (n=9); E: circEPSTI1 expression in gastric cancer cells (n=9); F: expression of miR-216b-5p in gastric cancer cells (n=9). *P<0.05 compared with adjacent tissues; #P<0.05 compared with GES-1 cells; &P<0.05 compared with HGC27 cells.

图1 circEPSTI1和miR-216b-5p的表达

Fig.1 Expression of circEPSTI1 and miR-216b-5p



A: 沉默circEPSTI1处理后circEPSTI1的表达(n=9); B: 抑制miR-216b-5p处理后miR-216b-5p的表达(n=9); C: 沉默circEPSTI1处理后miR-216b-5p的表达(n=9)。*P<0.05, 与si-NC组比; #P<0.05, 与anti-miR-NC组比。

A: circEPSTI1 expression after circEPSTI1 silencing (n=9); B: miR-216b-5p expression was inhibited after treatment with miR-216b-5p (n=9); C: miR-216b-5p expression was silenced after circEPSTI1 treatment (n=9). *P<0.05 compared with si-NC group; #P<0.05 compared with anti-miR-NC group.

图2 circEPSTI1和miR-216b-5p的表达

Fig.2 Expression of circEPSTI1 and miR-216b-5p

wt-circEPSTI1 5′ gaagcuggGCUUACAGAGAUUc 3′
miR-216b-5p 3′ aguguaaaCGGACGUCUCUAAa 5′
mut-circEPSTI1 5′ gaagcuggGCUUAACCGACAGc 3′

红色表示点突变后的碱基序列。

Red indicated the base sequence after the point mutation.

图3 circEPSTI1和miR-216b-5p的互补序列

Fig.3 Complementary sequences of circEPSTI1 and miR-216b-5p

表1 双荧光素酶报告实验

Table 1 Dual luciferase reporter assay

分组 Group	wt-circEPSTI1	mut-circEPSTI1
miR-NC	0.99±0.05	0.98±0.08
miR-216b-5p	0.34±0.05*	0.99±0.09
t	25.577	0.249
P	<0.05	0.806

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

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

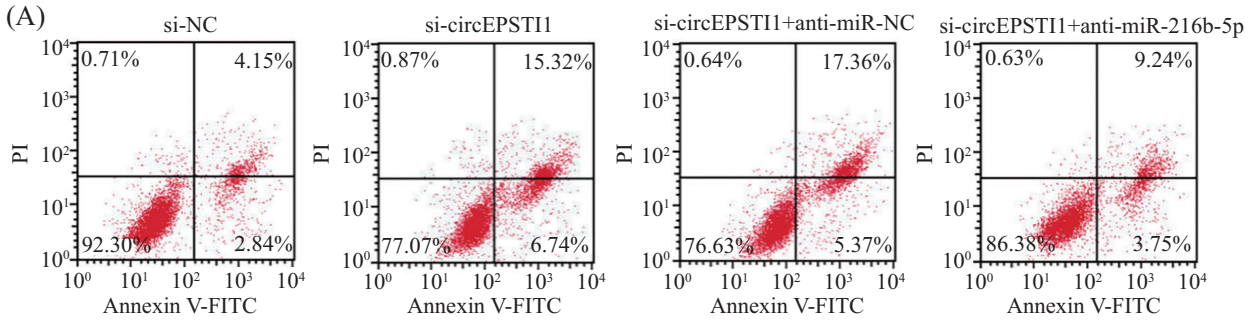


图4 circEPSTI1和miR-216b-5p对胃癌耐药HGC27/DDP细胞凋亡的影响

Fig.4 Effects of circEPSTI1 and miR-216b-5p on apoptosis of drug-resistant HGC27/DDP cells

表2 circEPSTI1和miR-216b-5p对胃癌耐药HGC27/DDP细胞增殖和凋亡的影响

Table 2 Effects of circEPSTI1 and miR-216b-5p on proliferation and apoptosis of drug-resistant HGC27/DDP cells

分组 Group	抑制率/% Inhibition rate /%	凋亡率/% Apoptosis rate /%	克隆数 Clone number
si-NC	0.00±0.00	7.09±0.34	116.22±6.73
si-circEPSTI1	47.88±2.22*	22.42±1.34*	58.22±4.44*
si-circEPSTI1+anti-miR-NC	47.46±2.42	22.69±1.64	56.67±2.98
si-circEPSTI1+anti-miR-216b-5p	24.20±2.14#	13.25±0.90#	100.44±4.88#
F	1 216.476	382.341	333.464
P	<0.01	<0.01	<0.01

*P<0.05, 与si-NC组比; #P<0.05, 与si-circEPSTI1+anti-miR-NC组比。$\bar{x}\pm s$, n=9。

*P<0.05 compared with si-NC group; #P<0.05 compared with si-circEPSTI1+anti-miR-NC group. $\bar{x}\pm s$, n=9.

组相比, si-circEPSTI1+anti-miR-216b-5p组迁移、侵袭细胞数增加(P<0.05); 见表3。

2.6 circEPSTI1和miR-216b-5p对胃癌耐药HGC27/DDP细胞的相关蛋白表达的影响

与si-NC组相比, si-circEPSTI1组Cleavea-caspase 3、Cleavea-caspase 9、E-cadherin蛋白表达上调(P<0.05), N-cadherin蛋白表达下调(P<0.05); 与si-circEPSTI1+anti-miR-NC组相比, si-circEPSTI1+anti-miR-216b-5p组Cleavea-caspase 3、Cleavea-caspase 9、E-cadherin蛋白表达下调(P<0.05), N-cadherin蛋白表达上调(P<0.05); 见图5、表4。

3 讨论

circRNA是前体RNA通过反向剪接形成的闭合环状RNA分子, 其具有多种生物学功能, 并可调节细胞增殖、凋亡等生物学行为[8]。circRNA可作为miRNA的海绵分子而靶向mRNA从而调控细胞生物学过程, 此外, circRNA还具有翻译蛋白的功能[9]。既往报道显示, circRNA在胃癌细胞顺铂耐药性中表达异常, 通过调控miRNA/mRNA分子轴参与胃癌细

表3 circEPSTI1和miR-216b-5p对胃癌耐药HGC27/DDP细胞迁移和侵袭的影响

Table 3 Effects of circEPSTI1 and miR-216b-5p on migration and invasion of drug-resistant HGC27/DDP cells

分组 Group	迁移数 Migration cell number	侵袭数 Invasion cell number
si-NC	233.44±14.83	173.56±8.07
si-circEPSTI1	112.78±6.37*	90.00±5.94*
si-circEPSTI1+anti-miR-NC	117.11±6.28	86.56±4.92
si-circEPSTI1+anti-miR-216b-5p	192.67±8.65#	145.22±10.20#
F	335.122	286.762
P	<0.01	<0.01

*P<0.05, 与si-NC组比; #P<0.05, 与si-circEPSTI1+anti-miR-NC组比。$\bar{x}\pm s$, n=9。

*P<0.05 compared with si-NC group; #P<0.05 compared with si-circEPSTI1+anti-miR-NC group. $\bar{x}\pm s$, n=9.

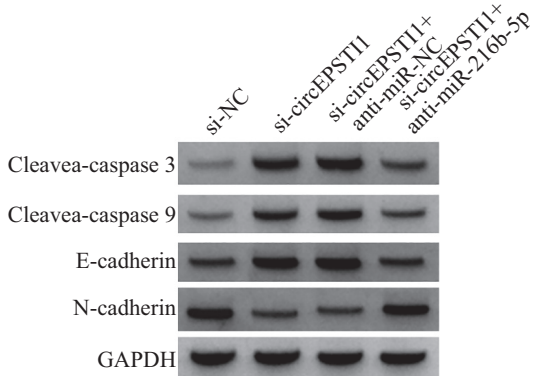


图5 胃癌耐药HGC27/DDP细胞的相关蛋白表达

Fig.5 Expression of related proteins in drug-resistant HGC27/DDP cells

表4 胃癌耐药HGC27/DDP细胞的相关蛋白表达

Table 4 Expression of related proteins in drug-resistant HGC27/DDP cells

分组 Group	Cleavea-caspase 3	Cleavea-caspase 9	E-cadherin	N-cadherin
si-NC	0.17±0.03	0.25±0.03	0.22±0.03	0.68±0.06
si-circEPSTI1	0.66±0.06*	0.74±0.06*	0.57±0.04*	0.31±0.04*
si-circEPSTI1+anti-miR-NC	0.65±0.06	0.74±0.07	0.57±0.05	0.29±0.04
si-circEPSTI1+anti-miR-216b-5p	0.33±0.03#	0.34±0.03#	0.29±0.03#	0.58±0.04#
F	235.833	235.427	206.797	163.000
P	<0.01	<0.01	<0.01	<0.01

*P<0.05, 与si-NC组比; #P<0.05, 与si-circEPSTI1+anti-miR-NC组比。$\bar{x}\pm s$, n=9。

*P<0.05 compared with si-NC group; #P<0.05 compared with si-circEPSTI1+anti-miR-NC group. $\bar{x}\pm s$, n=9.

胞顺铂耐药性过程[10-11]。

circEPSTI1在卵巢癌组织或细胞系中呈高表达, 并可促进卵巢癌细胞增殖及转移[12]。circEPSTI1在口腔鳞状细胞癌组织及细胞中的表达上调, 促进癌细胞增殖、侵袭[13]。但circEPSTI1在胃癌顺铂耐药性中的可能机制尚不明确。本研究中, 胃癌组织与细胞的circEPSTI1表达上调, 而胃癌耐药性细胞的circEPSTI1表达量高于胃癌细胞, 提示circEPSTI1在胃癌耐药性细胞中的表达上调, 参与胃癌细胞耐药性过程。本研究中, 干扰circEPSTI1表达, 胃癌耐药性细胞的增殖抑制率增高, 克隆形成数降低, 提示干扰circEPSTI1表达可抑制胃癌耐药性细胞增殖及克隆形成。caspase 9作为caspase级联反应上游蛋白, 其被激活后可形成Cleavea-caspase 9, 并可进一步激活凋亡执行因子caspase 3形成Cleavea-caspase 3, 促进细胞凋亡[14]。本研究中, 干扰circEPSTI1表达, 细

胞凋亡率、Cleavea-caspase 3及Cleavea-caspase 9蛋白表达上调, 提示干扰circEPSTI1表达可促进胃癌耐药性细胞凋亡。上皮–间质转化(epithelial to mesenchy-mal transition, EMT)包括E-cadherin、N-cadherin。E-cadherin表达下调以及N-cadherin表达上调, 有利于EMT转化, 而EMT转化又可促进细胞转移[15]。本研究中, 干扰circEPSTI1表达, 胃癌耐药性细胞迁移、侵袭细胞数同步降低, E-cadherin表达上调, N-cadherin表达下调, 以上结果提示干扰circEPSTI1表达有助于逆转EMT转化, 进而抑制胃癌耐药性细胞迁移、侵袭。

本研究通过双荧光素酶报告实验证实circEPSTI1基因与miR-216b-5p存在结合位点, 可作为miR-216b-5p的海绵分子。报道显示, miR-216b-5p在结直肠癌细胞中呈低表达, 上调miR-216b-5p可抑制结直肠癌细胞内的糖酵解反应[16]。miR-216b-5p过表达可抑制乳腺癌细胞增殖[17]。miR-216b-5p在上皮性卵巢癌组织中表达下调, 可作为评估患者预后的潜在标志物[18]。本研究中, 胃癌组织与胃癌细胞的miR-216b-5p表达均为下调, 胃癌耐药性细胞的miR-216b-5p表达显著低于胃癌细胞, 提示circEPSTI1可能靶向调控miR-216b-5p表达参与胃癌细胞顺铂耐药性过程。为进一步验证circEPSTI1/miR-216b-5p分子轴在胃癌细胞顺铂耐药性中的作用机制, 本研究中, 下调miR-216b-5p表达可缓解干扰circEPSTI1对胃癌耐药性细胞增殖、迁移、侵袭、克隆形成的抑制, 及对细胞凋亡的促进, 提示circEPSTI1可通过充当c的海绵分子而促进胃癌细胞顺铂耐药性的发生。

然而该研究尚有一定局限性, circRNA可能同时调控多个miRNA, circEPSTI1的下游靶向miRNA以及circEPSTI1的潜在靶基因仍需进一步研究。此外, circEPSTI1在胃癌耐药性细胞中表达上调的调控机制还不清楚。本次研究所获得的数据是基于有限的体外细胞实验, 考虑到本研究的不足, 我们会在未来的实验中使用动物模型以验证这些结论。

综上所述, 胃癌组织与细胞中的circEPSTI1表达上调, miR-216b-5p表达下调, 且胃癌耐药性细胞的circEPSTI1表达量高于胃癌细胞, 而胃癌耐药性细胞的miR-216b-5p表达下调, 干扰circEPSTI1可促进miR-216b-5p表达抑制胃癌耐药性细胞增殖、迁移、侵袭、克隆形成, 并促进胃癌耐药性细胞凋亡, circEPSTI1可能作为逆转胃癌细胞顺铂耐药性的潜在靶点, 该机制可能与改变凋亡相关蛋白表达和逆转上皮–间质转化过程有关。

参考文献 (References)

[1] ZHANG Z, YU X, ZHOU B, et al. Circular RNA circ_0026359 enhances cisplatin resistance in gastric cancer via targeting miR-1200/POLD4 pathway [J]. Biomed Res Int, 2020, 2020(1): 5103272-82.

[2] YANG G, TAN J, GUO J, et al. Exosome-mediated transfer of Circ_0063526 enhances cisplatin resistance in gastric cancer cells via regulating miR-449a/SHMT2 axis [J]. Anticancer Drugs, 2022, 33(10): 1047-57.

[3] ROY S, KANDA M, NOMURA S, et al. Diagnostic efficacy of circular RNAs as noninvasive, liquid biopsy biomarkers for early detection of gastric cancer [J]. Mol Cancer, 2022, 21(1): 42.

[4] WANG X, ZHANG Y, LI W, et al. Knockdown of cir_RNA PVT1 elevates gastric cancer cisplatin sensitivity via sponging miR-152-3p [J]. J Surg Res, 2021, 261(1): 185-95.

[5] XIE Y, WANG L, YANG D. CircEPSTI1 promotes the progression of non-small cell lung cancer through miR-145/HMGB3 axis [J]. Cancer Manag Res, 2020, 12(1): 6827-36.

[6] 周京涛, 刘佳, 李亮, 等. miRNA-216b通过靶向调控Beclin1对顺铂诱导的胃癌细胞自噬及凋亡的影响[J]. 中国中西医结合消化杂志(ZHOU J T, LIU J, LI L, et al. Effect of miRNA-216b on autophagy and apoptosis of gastric cancer cells induced by cisplatin through targeted regulation of Beclin1 [J]. Chinese Journal of Integrated Traditional and Western Medicine on Digestion), 2021, 29(6): 398-405.

[7] ZHANG Q, MIAO Y, FU Q, et al. CircRNACCDC66 regulates cisplatin resistance in gastric cancer via the miR-618/BCL2 axis [J]. Biochem Biophys Res Commun, 2020, 526(3): 713-20.

[8] WANG C, LIU W R, TAN S, et al. Characterization of distinct circular RNA signatures in solid tumors [J]. Mol Cancer, 2022, 21(1): 63.

[9] WANG Y, WU C, DU Y, et al. Expanding uncapped translation and emerging function of circular RNA in carcinomas and noncarcinomas [J]. Mol Cancer, 2022, 21(1): 13.

[10] CHENG W, LUAN P, JIN X. circUBAP2 inhibits cisplatin resistance in gastric cancer via miR-300/KAT6B axis [J]. Anticancer Drugs, 2023, 34(1): 126-34.

[11] ZHANG J, ZHA W, QIAN C, et al. Circular RNA circ_0001017 sensitizes cisplatin-resistant gastric cancer cells to chemotherapy by the miR-543/PHLPP2 axis [J]. Biochem Genet, 2022, 60(2): 558-75.

[12] XIE J, WANG S, LI G, et al. circEPSTI1 regulates ovarian cancer progression via decoying miR-942 [J]. J Cell Mol Med, 2019, 23(5): 3597-602.

[13] WANG J, JIANG C, LI N, et al. The circEPSTI1/mir-942-5p/LTBP2 axis regulates the progression of OSCC in the background of OSF via EMT and the PI3K/Akt/mTOR pathway [J]. Cell Death Dis, 2020 , 11(8): 682-92.

[14] WANG Z, YU K, HU Y, et al. Schisantherin A induces cell apoptosis through ROS/JNK signaling pathway in human gastric cancer cells [J]. Biochem Pharmacol, 2020, 173(1): 113673-83.

[15] HANG C, YAN H S, GONG C, et al. MicroRNA-9 inhibits gastric cancer cell proliferation and migration by targeting neuropilin-1 [J]. Exp Ther Med, 2019, 18(4): 2524-30.

[16] SUN S, LI W, MA X, et al. Long noncoding RNA LINC00265 promotes glycolysis and lactate production of colorectal cancer through regulating of miR-216b-5p/TRIM44 axis [J]. Digestion, 2020, 101(4): 391-400.

[17] MENBARI M N, RAHIMI K, AHMADI A, et al. miR-216b-5p inhibits cell proliferation in human breast cancer by down-regulating HDAC8 expression [J]. Life Sci, 2019, 237(1): 116945-55.

[18] PEI M L, ZHAO Z X, SHUANG T. Dysregulation of lnc-SNHG1 and miR-216b-5p correlate with chemoresistance and indicate poor prognosis of serous epithelial ovarian cancer [J]. J Ovarian Res, 2020, 13(1): 144-54.