

shRNA沉默CCAT2对非小细胞肺癌耐顺铂细胞A549的影响*

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【摘要】目的 研究短发夹RNA(short hairpin RNA, shRNA)沉默结肠癌相关转录因子2(colon cancer associated transcript 2, CCAT2)对非小细胞肺癌耐顺铂(cisplatin, DDP)细胞株(A549/DDP)的增殖、侵袭和凋亡以及体内肿瘤形成的影响。**方法** shRNA-CCAT2(sh-CCAT2)或shRNA-阴性对照(shRNA-NC)转染A549/DDP细胞(并设未经处理的A549/DDP细胞为对照组),通过实时荧光定量PCR(quantitative real-time PCR, qRT-PCR)检测3组A549/DDP细胞CCAT2 mRNA表达;通过MTT实验检测3组A549/DDP细胞经过不同质量浓度(0~8 mg/L)DDP处理后的增殖能力变化,并依此选定2 mg/L DDP进行后续实验。采用克隆形成实验、流式细胞法、Transwell实验检测2 mg/L DDP处理对各组细胞(并设未经处理的A549/DDP细胞为对照组)增殖、凋亡、侵袭的影响,用Western blot检测各组细胞增殖标记蛋白〔Ki67蛋白、增殖细胞核抗原(proliferating cell nuclear antigen, PCNA)〕、凋亡标记蛋白(Caspase-3、Caspase-9)、侵袭标记蛋白〔血管内皮生长因子(vascular endothelial growth factor, VEGF)、基质金属蛋白酶-14(matrix metalloproteinase-14, MMP-14)〕蛋白的表达。裸鼠皮下注射A549/DDP细胞、转染shRNA-NC和转染sh-CCAT2的A549/DDP细胞,每3 d腹腔注射DDP 2 mg/kg,连续给药7次,另设对照组(皮下注射A549/DDP细胞,腹腔注射等量生理盐水)。检测每5 d肿瘤体积,共30 d。30 d后处死取肿瘤组织,RT-PCR检测CCAT2 mRNA表达, TUNEL染色检测肿瘤细胞凋亡。**结果** 与对照组和shRNA-NC转染组相比, sh-CCAT2转染A549/DDP细胞后,细胞CCAT2 mRNA表达降低($P<0.01$), 2~8 mg/L DDP作用后细胞增殖抑制更明显($P<0.01$)。与对照组相比, DDP处理的3组细胞克隆形成减少,增殖标记蛋白Ki67和PCNA表达减少($P<0.01$);细胞凋亡率增加,凋亡标记蛋白Caspase-3和Caspase-9表达增多($P<0.01$);细胞侵袭数目减少,侵袭标记蛋白VEGF和MMP-14表达降低($P<0.01$); DDP处理的3组细胞中, sh-CCAT2转染的细胞上述表现最为明显($P<0.01$)。体内实验显示,与对照组相比, 3个DDP组肿瘤体积减小, 30 d时差异有统计学意义($P<0.01$);肿瘤组织CCAT2 mRNA表达减少($P<0.01$),细胞凋亡增加($P<0.01$)。3个DDP组中,接种sh-CCAT2转染的A549/DDP细胞组上述表现最为明显($P<0.01$)。**结论** sh-CCAT2可以抑制A549/DDP细胞的增殖、诱导细胞凋亡和降低细胞的侵袭能力,从而抑制A549/DDP细胞生长。

【关键词】 CCAT2 非小细胞肺癌 顺铂

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【Abstract】 Objective To investigate the effects of short hairpin RNA (shRNA) on the proliferation, invasion, apoptosis and tumor formation of non-small cell lung cancer cisplatin-resistant cell line (A549/DDP) via silencing of colon cancer associated transcript 2 (CCAT2). **Methods** TA549/DDP cells were transfected with shRNA-CCAT2 (sh-CCAT2) or shRNA-negative control (shRNA-NC), and untransfected A549/DDP cells were used as the control group. CCAT2 mRNA expression in three groups of A549/DDP cells was detected by quantitative real-time PCR (qRT-PCR). The proliferation of three groups of A549/DDP cells treated with different mass concentrations of DDP (0-8 mg/L) was detected by MTT. According to the proliferation experiment results, 2 mg/L was selected as DDP concentration for subsequent experiments. The effects of 2 mg/L DDP treatment on the proliferation, apoptosis, and invasion ability of each group of cells (with untreated A549/DDP cells as the control group) were tested by clone formation experiments, flow cytometry analysis and Transwell experiments. The expression levels of cell proliferation marker proteins (Ki67, PCNA), apoptosis marker proteins (Caspase-3, Caspase-9) and invasion marker proteins (VEGF, MMP-14) were detected by Western blot. Nude mice were injected subcutaneously with A549/DDP cells, A549/DDP cells transfected with shRNA-NC or A549/DDP cells transfected with sh-CCAT2. DDP was intraperitoneally injected at the concentration of 2 mg per kilogram of mice body weight totally for 7 times with an interval of 3 d. A control group was injected subcutaneously with A549/DDP cells, and an equal volume of normal saline instead of DDP was injected intraperitoneally. The tumor volume was detected every 5 d for a total of 30 d. Mice were sacrificed and tumor tissues were taken out 30 d later. CCAT2 mRNA

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expression level in tumor tissues was detected by RT-PCR, and tumor cell apoptosis was detected by TUNEL staining.

Results Compared with the control group and the shRNA-NC transfection group, the expression level of CCAT2 mRNA was decreased in sh-CCAT2 transfected A549/DDP cells ($P<0.01$). The decrease degree of cell proliferation was more pronounced after treating with 2 to 8 mg/L of DDP ($P<0.01$). Compared with the control group, in the three groups that treated with DDP, the formation of clones and the expression of proliferation marker proteins Ki67 and PCNA were reduce ($P<0.01$), while the rate of apoptosis and the expression of apoptosis marker proteins Caspase-3 and Caspase-9 were increased ($P<0.01$). Also, the number of invasion cell and the expression of invasion marker proteins VEGF and MMP-14 were reduced in the three groups that treated with DDP ($P<0.01$). Among the three groups of DDP-treated cells, the changes in sh-CCAT2 transfected cells was the most obvious ($P<0.01$). Compared with the control group, the tumor volume of the three DDP treatment groups was smaller and the differences were statistically significant at 30 d ($P<0.01$). The expression of CCAT2 mRNA was decreased in tumor tissues ($P<0.01$), while apoptosis increased ($P<0.01$). Among the three DDP treatment groups, the A549/DDP cell group transfected with sh-CCAT2 showed the most notable changes ($P<0.01$).

Conclusion sh-CCAT2 can inhibit the proliferation of A549/DDP cells, induce apoptosis and reduce the cell invasion ability, thereby inhibiting the growth of A549/DDP cells.

【Key words】 CCAT2 Non-small cell lung cancer Cisplatin

肺癌是最常见的肺原发性恶性肿瘤,在所有癌症中死亡率最高,而其中非小细胞肺癌约占所有肺癌患者的85%^[1-2]。非小细胞肺癌易发生转移,一般确诊时已是癌症晚期,目前治疗效果有限,主要采用顺铂(cisplatin, DDP)进行化疗,但由于铂类药物在临床使用上存在较为严重的耐药性,导致肺癌治疗更加困难,研究显示,非小细胞肺癌5年生存率仅15%,预后极差^[3-4]。为了提高肺癌患者的预后,开发新的药物或增强非小细胞肺癌细胞的DDP敏感性尤为迫切。长链非编码RNA是一种新型肿瘤生物标志物,可为癌症的早期诊断和治疗提供一种新策略^[5]。而结肠癌相关转录因子2(colon cancer associated transcript 2, CCAT2)是在结肠癌发现的一个长链非编码RNA,由染色体8q24这一保守区转录而来,其与结肠癌、乳腺癌和肺癌的发生和发展有关^[6]。已有研究表明,CCAT2是一种非小细胞肺癌细胞中特异性表达的长链非编码RNA,可以促进非小细胞肺癌细胞增殖和侵袭,并且以小干扰RNA(small interfering RNA, siRNA)干扰CCAT2能够抑制非小细胞肺癌细胞系的体外增殖和侵袭^[7]。但是沉默CCAT2能否抑制非小细胞肺癌耐药细胞株的增殖和侵袭还不清楚。本研究利用短发夹RNA(short hairpin RNA, shRNA)沉默CCAT2,研究其对非小细胞肺癌耐药细胞株的增殖、侵袭和凋亡的影响,以及对体内肿瘤形成的影响,为后续非小细胞肺癌的分子治疗和化疗相结合提供参考依据。

1 材料与方法

1.1 细胞系及主要试剂

耐DDP人肺癌细胞株A549/DDP购自上海慧颖生物科技有限公司; shRNA-CCAT2(sh-CCAT2)和shRNA无义

序列阴性对照(shRNA-NC)及相关引物序列由上海生物工程有限公司设计并合成;热灭活胎牛血清、青霉素、链霉素、RPMI-1640培养液购自美国Gibco公司; DDP购自西安凯萌生物技术有限公司;转染试剂盒lipofectamine 3000购自Thermo公司; Trizol试剂购自美国Life Technologies公司, BCA试剂盒、MTT试剂盒购自北京索宝科技有限公司; Ki67、增殖细胞核抗原(proliferating cell nuclear antigen, PCNA)、Caspase-3、Caspase-9、血管内皮生长因子(vascular endothelial growth factor, VEGF)、基质金属蛋白酶-14(matrix metalloproteinase-14, MMP-14)抗体购自碧云天生物科技有限公司; ECL发光底物购自上海慧颖生物科技有限公司; GIMESA染色液 购自北京中生瑞泰科技有限公司, TNUEL检测试剂盒购自武汉博士德生物工程公司, 裸鼠购自四川大学华西医院基因工程小鼠中心。CF×96实时定量PCR仪购自Bio-Rad公司。

1.2 细胞培养及转染

将A549/DDP细胞菌株置于含10%热灭活胎牛血清、青霉素(100 U/mL)、链霉素(100 U/mL)的RPMI-1640培养液中,在体积分数为5% CO₂、37 °C孵箱中培养。将细胞随机分为对照组、shRNA-NC组和sh-CCAT2组。细胞培养24 h后,根据转染试剂盒说明将构建的shRNA-NC(20 ng/μL)和sh-CCAT2(20 ng/μL)分别转染入A549/DDP细胞。对照组细胞不进行转染处理。

1.3 转染细胞CCAT2 mRNA表达检测

采用实时荧光定量PCR(qRT-PCR)检测CCAT2 mRNA表达。取转染5 d后的各组A549/DDP细胞,利用Trizol试剂提取细胞总RNA,用反转录试剂盒合成cDNA, qRT-PCR仪进行基因片段扩增。PCR引物序列: CCAT2 F: 5'- CCCTGGTCAAATTAACCT-3', R: 5'-

TTATTCGTCCCTCTGTTTATGGAT-3'。反应条件: 95 ℃ 预变性3 min, 然后95 ℃ 10 s, 57 ℃ 30 s, 65 ℃ 30 s, 总共40个循环。最后72 ℃延伸2 min。以甘油醛-3-磷酸脱氢酶(GAPDH)为内参, 计算 ΔC_t , 以 $2^{-\Delta\Delta C_t}$ 表示CCAT2 mRNA的表达量, 并以对照组CCAT2 mRNA表达为1, 计算其他组相对于对照组的表达量。

1.4 MTT检测sh-CCAT2对不同质量浓度DDP处理细胞增殖的影响

A549/DDP细胞分为DDP组(不同质量浓度DDP处理A549/DDP细胞)、DDP+shRNA-NC组(不同质量浓度DDP处理shRNA-NC转染的A549/DDP细胞)和DDP+sh-CCAT2组(不同质量浓度DDP处理sh-CCAT2转染的A549/DDP细胞), 以各组细胞未加DDP处理前为自身对照。DDP不同质量浓度分别为2、4、6、8 mg/L。将各组A549/DDP细胞(A549/DDP细胞、shRNA-NC转染的A549/DDP细胞、sh-CCAT2转染的A549/DDP细胞)以每孔1 000个细胞接种于96孔板, 培养24 h后加入上述不同质量浓度DDP, 培养3 d后, 每孔加20 μ L MTT溶液(PBS配制5 mg/mL, pH=7.4)继续孵育4 h, 终止培养, 弃上清。每孔加150 μ L DMSO, 振荡10 min, 酶标仪测定490 nm波长处吸光度(A)值。重复实验3次。按常规计算细胞存活率, 以存活率间接反映细胞增殖能力。选择合适的DDP质量浓度进行后续实验。

1.5 sh-CCAT2对A549/DDP细胞增殖、侵袭和凋亡的影响

1.5.1 实验分组及处理 将A549/DDP细胞分为对照组(未处理的A549/DDP细胞)、DDP组(DDP 2 mg/L处理A549/DDP细胞)、DDP+shRNA-NC组(DDP 2 mg/L处理shRNA-NC转染的A549/DDP细胞)和DDP+sh-CCAT2组(DDP 2 mg/L处理sh-CCAT2转染的A549/DDP细胞)。

1.5.2 克隆形成实验 取对数期生长的A549/DDP细胞, 按分组接种于完全培养基, 200个细胞/孔, 体积分数为5% CO₂, 37 ℃培养2周, 用Gimesa染色, 在200倍显微镜下任意选取5个视野, 观察每视野中细胞克隆数目。

1.5.3 Western blot检测增殖、凋亡、侵袭标记蛋白的表达 采用Western blot检测增殖标记蛋白(Ki67和PCNA)、凋亡标记蛋白(Caspase-3和Caspase-9)、侵袭标记蛋白(VEGF和MMP-14)的表达。用RIPA裂解液提取培养3 d的各组细胞总蛋白。用BCA试剂盒检测总蛋白浓度, 10% SDS-PAGE分离蛋白后用半干转膜仪转移蛋白质至PVDF膜。用5%脱脂牛奶室温封闭蛋白2 h, 随后加入一抗(Ki67, 1 : 1 000; PCNA, 1 : 1 000; cleaved Caspase-3, 1 : 1 000; cleaved Caspase-9, 1 : 1 000; VEGF, 1 : 1 000;

MMP-14 1 : 1 000)于4 ℃封闭过夜, 第二天加入对应二抗室温封闭1 h, 最后滴ECL曝光。以GAPDH为内参对照。图像采用Bio-Rad凝胶成像系统拍照并测定各显色条带的灰度值, 以目的条带与GAPDH灰度值的比值为目的蛋白的相对表达量。

1.5.4 流式检测A549/DDP细胞的体外凋亡能力 将各组A549/DDP细胞培养24 h后, 用胰酶消化, 3 500 $\times$ g离心5 min, 再按每毫升10⁶个细胞的浓度重悬。细胞悬液中加入5 mL Annexin-V-FITC和5 mL PI, 在黑暗的房间里孵化15 min, 然后用流式细胞仪分析细胞凋亡情况。细胞凋亡率(%)=早期细胞凋亡率(%) + 晚期细胞凋亡率(%)。将各组A549/DDP细胞培养24 h后, 胰酶消化, 3 500 $\times$ g, 离心5 min, 细胞再按1 $\times$ 10⁶ mL⁻¹浓度重悬。细胞悬液中加入5 mL Annexin-V-FITC和5 mL PI, 在黑暗的房间里孵化15 min, 然后用流式细胞仪分析细胞凋亡情况。细胞凋亡率(%)=早期细胞凋亡率(%) + 晚期细胞凋亡率(%)。

1.5.5 Transwell检测A549细胞的体外侵袭能力 将各组A549/DDP细胞以无血清培养液培养24 h后, 用0.25%胰蛋白酶消化细胞并传代接种于用Matrigel预处理的Transwell小室中, 细胞密度为3 $\times$ 10⁵ mL⁻¹。小室上层加入无血清培养液培养细胞, 下层则加入含血清的正常培养液。48 h后用无菌棉签擦去小室上层细胞, 下层细胞结晶紫染色后计数统计。倒置显微镜下随机选择5个高倍视野(HP, $\times$ 400)拍照, 计算每个HP下的细胞数, 实验重复3次。

1.6 体内实验

将24只裸鼠随机分为DDP组、DDP+shRNA-NC组、DDP+sh-CCAT2组和对照组, 每组6只。DDP组为裸鼠皮下注射未处理的A549/DDP细胞并腹腔给药DDP; DDP+shRNA-NC组为裸鼠皮下注射shRNA-NC转染的A549/DDP细胞并腹腔给药DDP; DDP+sh-CCAT2组为皮下注射sh-CCAT2转染的A549细胞并腹腔给药DDP; 对照组为裸鼠皮下注射未处理的A549/DDP细胞, 相应时间腹腔注射等量生理盐水。各组裸鼠皮下注射A549/DDP细胞密度为2 $\times$ 10⁵ mL⁻¹, 腹腔注射给药为每3 d给药1次, 每次注射DDP 2 mg/kg, 连续给药7次。每5 d测量1次肿瘤体积, $V = \text{长} \times \text{宽}^2 / 2 (\text{mm}^3)$ 。绘制肿瘤生长曲线。30 d后处死裸鼠, 取肿瘤组织标本, 部分进行qRT-PCR检测CCAT2 mRNA表达, 部分进行TUNEL染色检查细胞凋亡。

CCAT2 mRNA表达检测: 提取肿瘤组织总RNA, 按试剂盒说明操作。样品均取1 μ g总RNA进行DNase I 处理, 逆转录依试剂盒说明操作。按1.3进行CCAT2 mRNA表达检测。TUNEL染色: 肿瘤组织常规固定、切片, 玻片预先用多聚赖氨酸处理以防止脱片。切片脱蜡入水后按试

剂盒内使用说明操作, DAB染色, 常规脱水、透明、封片, 光镜下检查细胞凋亡。棕黄色的为凋亡细胞, 细胞凋亡率= 凋亡细胞数/总细胞数 $\times 100\%$ 。

1.7 统计学方法

数据以 $\bar{x} \pm s$ 表示。组间比较采用 t 检验分析, $P < 0.05$ 为差异有统计学意义。

2 结果

2.1 shRNA沉默CCAT2检测

见图1。结果表明: 与对照组比较, shRNA-NC组A549/DDP细胞CCAT2 mRNA的表达差异无统计学意义, 而sh-CCAT2组细胞CCAT2 mRNA的表达降低($P < 0.01$), 表明转染成功, shRNA沉默了CCAT2 mRNA表达。

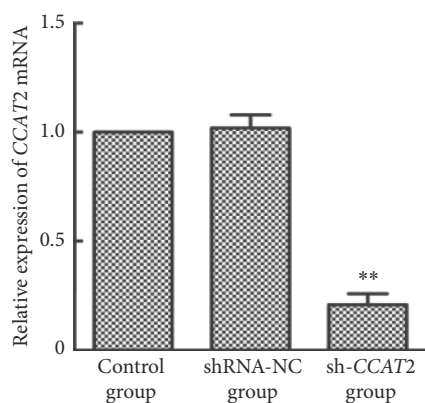


图1 qRT-PCR检测CCAT2 mRNA的表达($n=3$)

Fig 1 CCAT2 mRNA expression was detected by qRT-PCR ($n=3$)

** $P < 0.01$, vs. control group and shRNA-NC group

2.2 MTT检测sh-CCAT2对不同质量DDP处理的细胞增殖能力的影响

MTT实验结果(图2)显示, 以未加DDP时(对照)细胞存活率为100%, 各组细胞随着DDP质量浓度的升高, 细胞存活率逐渐降低, 说明DDP可以抑制A549/DDP增殖, 其中, 2 mg/L DDP处理细胞时, 对DDP组和DDP+shRNA-NC组的A549/DDP细胞存活率无明显影响, 但sh-CCAT2组细胞存活率下降($P < 0.01$), 因此, 后续实验选择用2 mg/L DDP处理细胞; 相同DDP质量浓度下, DDP组和DDP+shRNA-NC组比较, 细胞存活率差异无统计学意义, 而DDP+sh-CCAT2组与DDP组和DDP+shRNA-NC组比较, 细胞存活率降低($P < 0.01$), 说明shRNA沉默CCAT2可抑制细胞增殖。

2.3 sh-CCAT2对A549/DDP细胞增殖及增殖标记蛋白表达的影响

克隆形成实验结果(图3A)表明, DDP组与对照组比

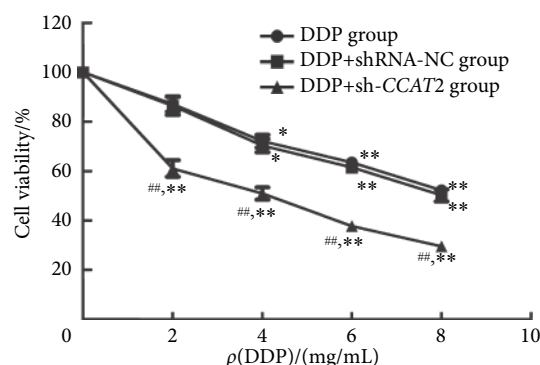


图2 sh-CCAT2对DDP处理的细胞增殖能力的影响($n=3$)

Fig 2 Effect of sh-CCAT2 on cell proliferation of DDP-treated cells ($n=3$)

* $P < 0.05$, ** $P < 0.01$, vs. control (DDP 0 mg/L); ## $P < 0.01$, vs. DDP group and DDP+shRNA-NC group

较, A549/DDP细胞克隆形成减少($P < 0.01$), DDP组和DDP+shRNA-NC组比较, 克隆形成变化不明显($P > 0.05$), DDP+sh-CCAT2组克隆形成较DDP组和DDP+shRNA-NC组减少($P < 0.01$), 说明shRNA干扰CCAT2抑制细胞增殖, 与MTT检测结果相一致。

Western blot检测结果(图3B)显示, DDP组与对照组比较, 细胞增殖标记蛋白Ki67和PCNA蛋白表达减少($P < 0.01$), DDP组和DDP+shRNA-NC组细胞Ki67和PCNA蛋白表达差异无统计学意义, DDP+sh-CCAT2组较DDP组和DDP+shRNA-NC Ki67和PCNA蛋白表达减少($P < 0.01$)。

2.4 sh-CCAT2对DDP处理的细胞凋亡及凋亡标记蛋白表达的影响

流式检测(图4A)发现, DDP组与对照组比较, 细胞凋亡率增加($P < 0.01$), DDP组和DDP+shRNA-NC组比较, 细胞凋亡率无变化($P > 0.05$), DDP+sh-CCAT2组较DDP组和DDP+shRNA-NC组细胞凋亡率增加($P < 0.01$), 说明sh-CCAT2可诱导DDP处理的A549/DDP细胞凋亡。

Western blot检测(图4B)结果显示, DDP组与对照组比较, 凋亡标记蛋白Caspase-3和Caspase-9表达增多($P < 0.01$), DDP组和DDP+shRNA-NC组比较, 凋亡标记蛋白Caspase-3和Caspase-9表达量差异无变化($P > 0.05$), DDP+sh-CCAT2组与DDP组、DDP+shRNA-NC组比较, Caspase-3和Caspase-9蛋白表达量增多($P < 0.01$)。

2.5 sh-CCAT2对DDP处理细胞侵袭能力及标记蛋白表达的影响

Transwell检测实验结果(图5A)显示, DDP组与对照组比较, 细胞侵袭数目减少($P < 0.01$), DDP组和DDP+shRNA-NC组比较, 细胞侵袭数目相近($P > 0.05$), DDP+sh-CCAT2组与DDP组和DDP+shRNA-NC组比较, 细胞侵袭

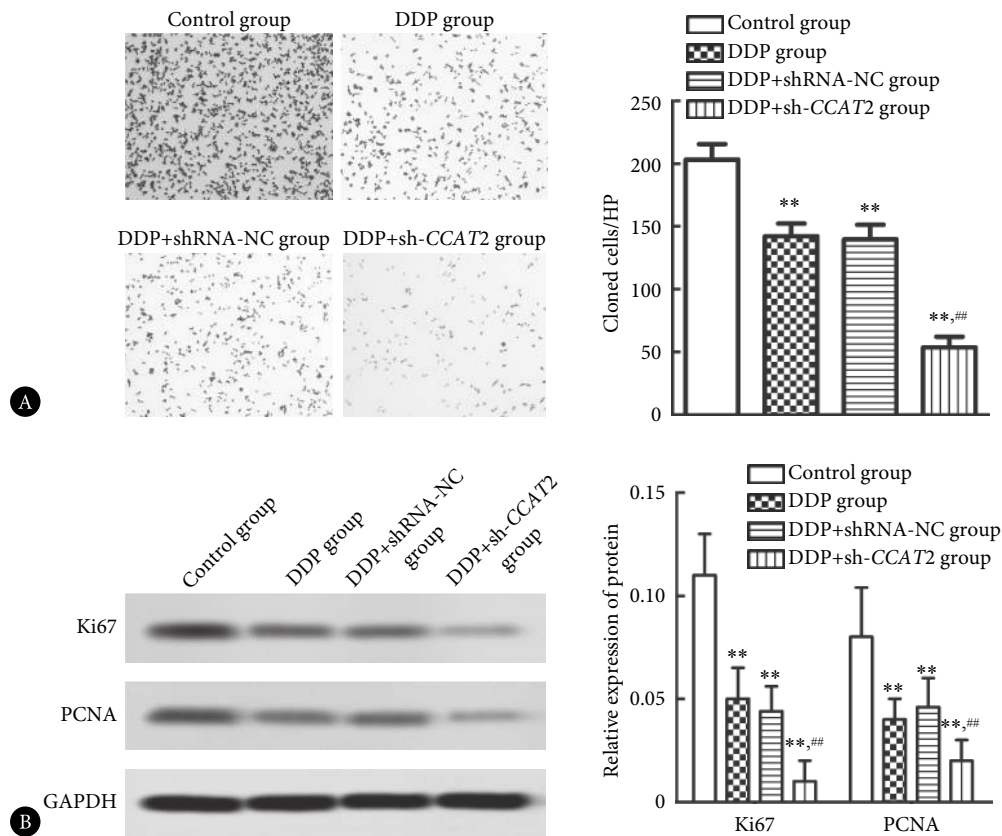


图 3 各组细胞克隆形成 (A, ×200) 和 Western blot 检测 Ki67、PCNA 蛋白的表达 (B)

Fig 3 Cellular clone formation (A, ×200) and the expression of Ki67 and PCNA proteins (B) in each group by Western blot assay

** $P < 0.01$, vs. control group; ## $P < 0.01$, vs. DDP+shRNA-NC group and DDP group

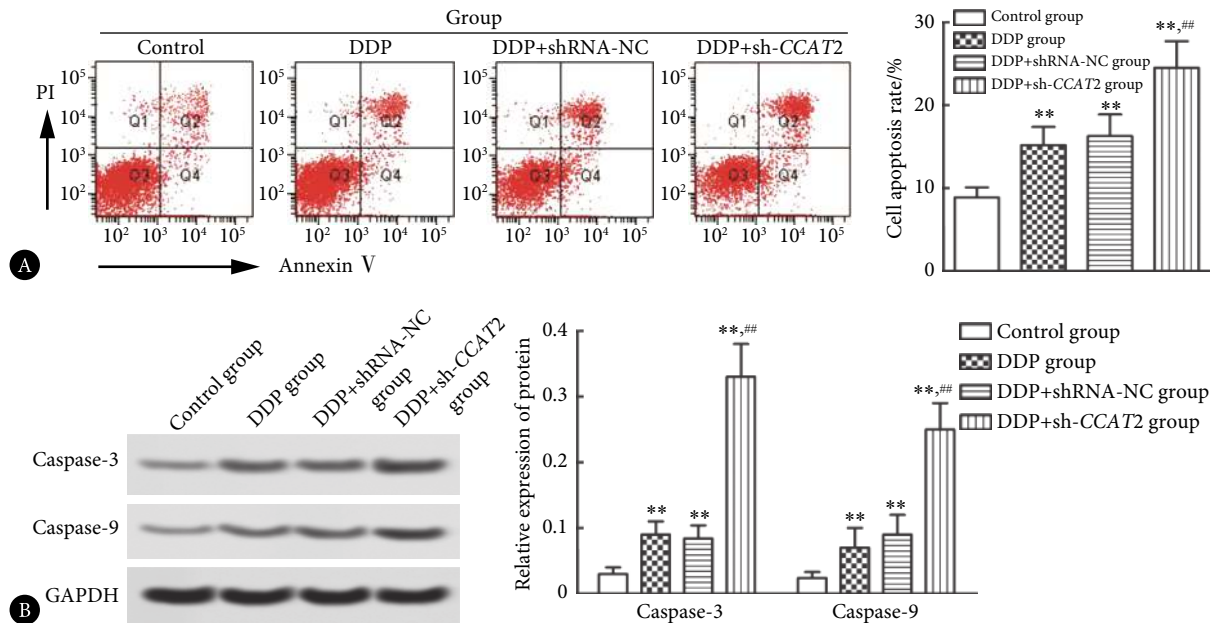


图 4 流式细胞法检测各组细胞凋亡 (A) 和 Western blot 检测 Caspase-3、Caspase-9 蛋白的表达 (B)

Fig 4 Apoptosis rate detected by flow cytometry (A) and protein expressions of Caspase-3 and Caspase-9 by Western blot (B)

** $P < 0.01$, vs. control group; ## $P < 0.01$, vs. DDP+shRNA-NC group and DDP group

数目减少 ($P < 0.01$), 说明 sh-CCAT2 可降低耐顺铂 A549 细胞的侵袭能力。

Western blot 检测实验结果 (图 5B) 表明, DDP 组与对照组比较, 侵袭标记蛋白 VEGF 和 MMP-14 表达量降低

($P < 0.01$), DDP组和DDP+shRNA-NC组比较, 细胞VEGF和MMP-14蛋白表达差异小($P > 0.05$), DDP+sh-CCAT2组与DDP组和DDP+shRNA-NC组比较, VEGF和MMP-14蛋白表达减少($P < 0.01$), 说明sh-CCAT2可降低A549/DDP的侵袭能力。

2.6 sh-CCAT2对细胞A549/DDP作用的体内实验

体内实验中各组裸鼠肿瘤生长曲线见图6A。结果表明: 随着接种时间增加, 各组裸鼠体内肿瘤体积增大;

DDP组与对照组比较, 裸鼠肿瘤体积减小($P < 0.01$), DDP组和DDP+shRNA-NC组比较, 肿瘤体积相近($P > 0.05$), DDP+sh-CCAT2组与DDP组和DDP+shRNA-NC组比较, 肿瘤体积减小($P < 0.01$), 说明sh-CCAT2可加强DDP抑制肿瘤生长。

qRT-PCR检测裸鼠肿瘤组织中CCAT2 mRNA表达结果(图6B)显示, DDP组与对照组比较, CCAT2 mRNA表达减少($P < 0.01$), DDP组和DDP+shRNA-NC组比较, CCAT2表

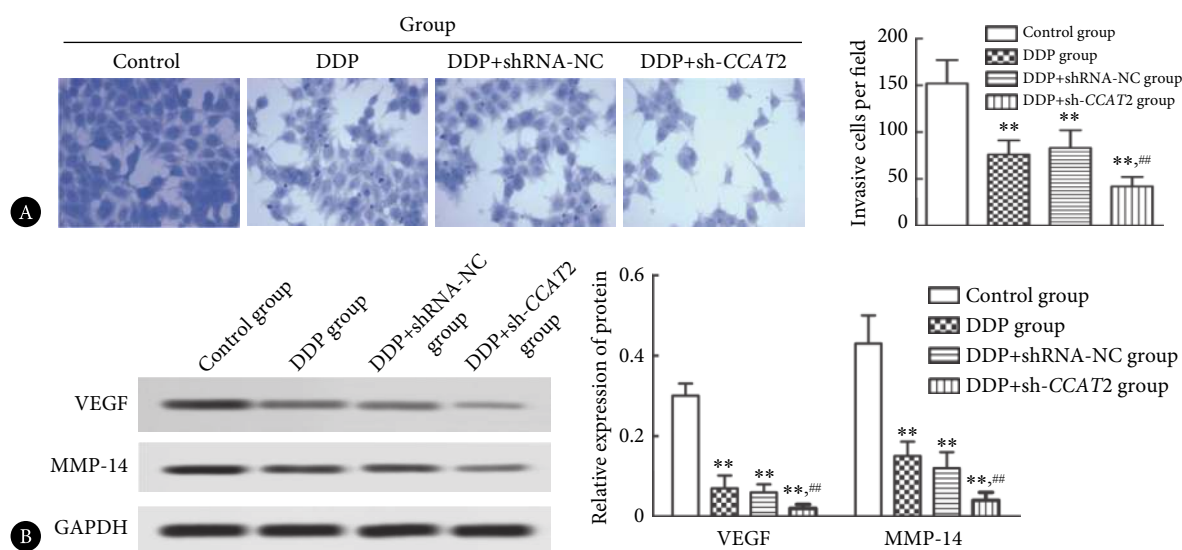


图5 用DDP处理A549/DDP细胞后, 以Transwell实验检测细胞侵袭能力(A, $\times 400$), Western blot检测VEGF、MMP-14蛋白表达(B)

Fig 5 Cell invasion detected by Transwell test (A, $\times 400$) and protein expressions of VEGF and MMP-14 by Western blot (B) in DDP-treated A549/DDP cells

** $P < 0.01$, vs. control group; ## $P < 0.01$, vs. DDP+shRNA-NC group and DDP group

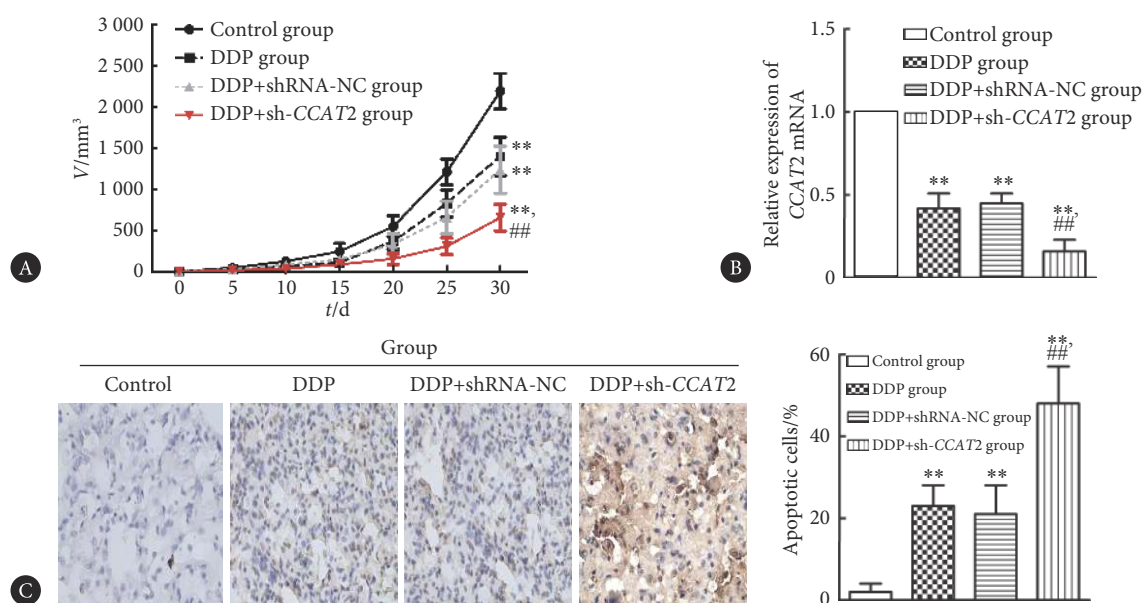


图6 sh-CCAT2对A549/DDP细胞作用后的小鼠体内实验($n=6$)

Fig 6 In vivo experiment of the effect of sh-CCAT2 on cell A549/DDP ($n=6$)

A: Tumor growth curve in mice; B: Expression of CCAT2 mRNA in tumor tissues of mice in each group by RT-PCR; C: Apoptosis in mice tumor tissue in each group by TUNEL staining, $\times 200$. ** $P < 0.01$, vs. control group; ## $P < 0.01$, vs. DDP+shRNA-NC group and DDP group

达量相近($P > 0.05$), DDP+sh-CCAT2组与DDP组和DDP+shRNA-NC组比较, CCAT2 mRNA表达进一步减少($P < 0.01$)。

裸鼠肿瘤组织TUNEL染色检测结果(图6C)显示, DDP组与对照组比较, 细胞凋亡率增加($P < 0.01$), DDP组和DDP+shRNA-NC组比较, 细胞凋亡率相近($P > 0.05$), DDP+sh-CCAT2组与DDP组和DDP+shRNA-NC组比较, 细胞凋亡率进一步增加($P < 0.01$)。

3 讨论

由于人口老龄化和空气污染不断加重, 恶性肿瘤发病率和死亡率逐年升高^[8-9]。肺癌是中国发生率和死亡率最高的恶性肿瘤, 并且其一直呈快速增长趋势^[10-12]。加强对肺癌治疗效果研究对人们健康至关重要。

恶性增殖是癌症细胞的基本特征, 也是癌症防治的关键, 而CCAT2与癌细胞的增殖是有着密切联系。研究发现^[13-15], CCAT2在卵巢癌、膀胱癌、前列腺癌中高表达, 沉默CCAT2可以抑制癌细胞的增殖。ZHAO等^[16]发现沉默CCAT2能够抑制非小细胞肺癌细胞的增殖, 但沉默CCAT2是否能够抑制耐DDP非小细胞肺癌细胞增殖并不清楚。本研究结果显示, shRNA沉默非小细胞肺癌耐DDP细胞A549的CCAT2后, 细胞的增殖明显减弱, 并且增殖标记蛋白Ki67和PCNA表达量减少, 说明shRNA沉默CCAT2对非小细胞肺癌耐DDP菌株增殖具有抑制作用。

细胞凋亡是机体维持自身稳定的一种基本生理机制, 癌症细胞存在着细胞凋亡失衡的现象, CCAT2在癌细胞的凋亡中起着重要作用。研究发现^[17-19], CCAT2可以抑制肝癌细胞、卵巢癌细胞的凋亡, 沉默CCAT2可以促进其凋亡。本研究发现: shRNA沉默非小细胞肺癌耐DDP细胞A549的CCAT2后, 细胞凋亡率增加, 凋亡标记蛋白Caspase-3和Caspase-9表达量明显增多, 这说明sh-CCAT2可诱导耐顺铂细胞的凋亡。

侵袭是癌症复发和预后不良的主要原因。研究发现^[20-22], 敲低CCAT2能够降低胰腺导管癌、神经胶质瘤、宫颈癌细胞侵袭能力。本研究结果表明, shRNA沉默非小细胞肺癌耐DDP细胞A549的CCAT2后, 细胞侵袭数目减少, 侵袭标记蛋白VEGF和MMP-14表达量降低, 说明sh-CCAT2能够降低耐顺铂A549细胞的侵袭能力。

癌症的发生发展与细胞的增殖、侵袭和凋亡息息相关, 而CCAT2与在细胞增殖、侵袭和凋亡中都起着重要作用。相比单一的实验外部环境, 机体内环境复杂多变。已有体内实验研究发现^[23-25], 沉默CCAT2会使神经胶质瘤、乳腺癌、胃癌生长受到抑制。本研究体内实验发现, DDP+sh-CCAT2组裸鼠比DDP组裸鼠肿瘤体积减小, 肿

瘤组织细胞凋亡率明显增加, 这说明sh-CCAT2可以抑制耐顺铂A549细胞的生长。

综上所述, sh-CCAT2可以抑制耐顺铂A549细胞的增殖、诱导凋亡和降低其侵袭能力, 从而抑制耐顺铂A549细胞生长, 这为肺癌的化疗和结合分子治疗提供了新思路, 为进一步研究CCAT2对耐顺铂A549细胞作用的分子机制及提高肺癌治疗效果提供参考。

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