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论著

腺病毒介导反义c-myc联合咖啡因在骨肉瘤化疗中增效作用的实验研究

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摘要 目的： 构建重组反义c-myc腺病毒并探讨其与咖啡因对人骨肉瘤MG-63细胞顺铂化疗的影响。

方法: 应用基因重组技术，将约750 bp的人c-myc cDNA反向克隆到腺病毒载体，构建表达反义c-myc的重组腺病毒（Ad-Asc-myc），与咖啡因、顺铂单独或联合体外作用于人骨肉瘤MG-63细胞，采用蛋白免疫印迹（Western blotting）、MTT、流式细胞仪（FCM）、透射电镜等检测c-Myc蛋白、bcl-2、bax、E_2F-1等基因表达、瘤细胞体外增殖抑制、凋亡及细胞周期，分析Ad-Asc-Myc、咖啡因对人骨肉瘤MG-63细胞顺铂化疗的影响。

结果: 成功构建Ad-Asc-myc，滴度可达2×10^{12}pfu/L，体外转染MG-63细胞48 h后，可降低c-Myc蛋白表达，并抑制其体外增殖；Ad-Asc-myc、2.0 mol/L咖啡因分别与浓度为2.0、5.0 mg/L顺铂共同作用后 ，可增加顺铂对MG-63细胞的体外增殖抑制率；Ad-Asc-myc联合2.0 mol/L咖啡因能明显加强顺铂的体外抗肿瘤作用，凋亡相关基因bcl-2基因表达下降，bax表达上升，而E 2F-1表达无明显变化；FCM检测显示Ad-Asc-myc的转染可诱导骨肉瘤细胞凋亡，并增加顺铂诱导瘤细胞凋亡作用；单独咖啡因不能诱导瘤细胞凋亡，但能增加顺铂诱导瘤细胞凋亡作用；Ad-Asc-myc联合2.0 mol/L咖啡因能明显加强顺铂诱导瘤细胞凋亡作用。细胞周期分析显示顺铂作用后瘤细胞出现S期阻滞，而咖啡因则能逆转这种阻滞；Ad-Asc-myc转染的骨肉瘤细胞出现G_2/M期阻滞。

结论: 腺病毒介导反义c-myc联合咖啡因能明显增强顺铂对人骨肉瘤MG-63细胞的诱导凋亡及化疗作用。

关键词 骨肉瘤 基因,c-myc 咖啡因 腺病毒

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Enhancement effect of adenovirus mediated antisense c-myc gene and caffeine on chemotherapy of osteosarcoma cells to cisplatin

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Abstract

<FONT face=Verdana>AIM: The cancer biology has showed that overexpression of oncogenes is responsible for the progression of human malignancies,antisense technology can block a certain gene expression.Caffeine has enhancement effect on chemotherapy of osteosarcoma cells to cisplatin,we constructed the recombinant adenovirus (Ad-Asc-myc) encoding antisense c-myc fragment and investigated its effect on the in vitro sensitivity of osteosarcoma MG-63 cells to cisplatin.
METHODS: The recombinant adenovirus (Ad-Asc-myc) encoding antisense c-myc fragment was constructed by cloning c-myc cDNA of about 750 base pairs in a reverse direction into adenovirus vector.Ad-Asc-myc and caffeine was used respectively or together to co-operate with cisplatin to treat the osteosarcoma MG-63 cells in vitro,and Western blotting,MTT,flow cytometry (FCM),electron microscope were used to evaluate expression of c-Myc protein,tumor cell proliferation in vitro,apoptosis and cell cycle analysis.
RESULTS: Ad-Asc-myc was obtained with the titer of 2×10¹² pfu/L.Ad-Asc-myc down-regulated the expression of c-Myc protein,Ad-Asc-myc or caffeine enhanced the effects of 2.0,5.0 mg/L cisplatin on MG-63 cells.Moreover,Ad-Asc-myc combined with caffeine significantly enhanced this effects,not only on cisplatin-induced apoptosis,but also on tumor cells proliferation in vitro.The expression of bcl-2 was downregulated,bax were upregulated,while there was no change in the expression of E2F-1.FCM analysis showed that cisplatin treatment induced a block in S

phase,and caffeine reversed this block and speeded up the progression of cells out of the S phase.Ad-Asc-myc induced obvious G₂/M phase arrest in transfected cells.
CONCLUSION: Ad-Asc-myc combined with caffeine may enhance apoptosis-induced and chemotherapy effects of osteosarcoma MG-63 cells to cisplatin.</FONT>

Key words Osteosarcoma Genes c-myc Caffeine Adenovirus

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