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APE1基因沉默增强骨肉瘤U2-OS细胞硼替佐米治疗敏感性的实验研究

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APE1 Gene Silencing Promotes the Sensitivity of Osteosarcoma U2-OS Cells to Bortezomib

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摘要 探讨脱嘌呤/脱嘧啶核酸内切酶1 (APE1) 基因沉默对蛋白酶体抑制剂硼替佐米 (bortezomib, PS-341) 抑制骨肉瘤U2-OS细胞增殖作用的影响及其生物学机制。方法: 将APE1特异性shRNA的重组质粒, 稳定转染人骨肉瘤U2-OS细胞, 采用聚合酶链反应和免疫印迹法检测转染前后U2-OS细胞中APE1的表达, 采用四甲基偶氮唑盐法观察PS-341和APE1-siRNA对人骨肉瘤U2-OS细胞生长的抑制作用, 采用免疫印迹法检测PS-341和APE1-siRNA对U2-OS细胞中APE1和胞核NF-κB的表达的影响。结果: 细胞稳定转染APE1-siRNA重组质粒后, APE1 mRNA和蛋白表达分别下降约46.1%和62.6%, MTT法检测U2-OS细胞增殖受到抑制。转染前后U2-OS细胞PS-341的IC50值分别为371.54 nmoL/L与109.64 nmoL/L, 两者比较差异有统计学意义 (P<0.01)。Western blot结果显示PS-341和APE1-siRNA均抑制U2-OS细胞胞核中NF-κB的表达, 两者联合应用抑制效果更明显。结论: APE1-shRNA质粒转染骨肉瘤U2-OS细胞后, 肿瘤细胞的增殖率降低, 对PS-341抑制U2-OS细胞的增殖具有协同作用。推测其生物学机制可能与下调胞核NF-κB蛋白表达有关。

关键词: 骨肉瘤 蛋白酶体抑制剂 脱嘌呤脱嘧啶核酸内切酶1 NF-κB B蛋白

Abstract: To investigate the effects of apurinic / apyrimidinic endonuclease 1 (APE1) on the inhibitory action of bortezomib on human osteosarcoma U2-OS cells and the underlying biological mechanisms. Methods: An shRNA plasmid that targets APE1 was constructed and transfected into U2-OS cells. The mRNA and protein levels of APE1 were detected via reverse transcription polymerase chain reaction and Western blot analysis. The inhibition of cell proliferation induced by PS-341 and APE1-siRNA was examined with an 3- (4, 5-dimethylthiazol-2-yl) 2, 5-diphenyl tetrazolium bromide assay. The change in nuclear NF-κB and APE1 expression induced by PS-341 and APE1 in osteosarcoma U2-OS cells was examined using Western blot analysis. Results: The APE1-shRNA expression plasmid was successfully constructed and transfected into U2-OS cells. The expression inhibition rate was about 47.6 % at the mRNA level, and was about 62.6 % at the protein level. Osteosarcoma cell proliferation was inhibited, as indicated by the MTT analysis. The median inhibitory concentration of PS-341 was 371.54 nmoL/L before APE1-shRNA transfection, which significantly decreased to 109.64 nmoL/L after APE1-shRNA transfection (P < 0.01). The Western blot analysis indicated that both PS-341 and APE1-siRNA downregulated nuclear NF-κB protein expression in the U2-OS cells. The effect was more significant than that of combination of the above two. Conclusion: After APE1-shRNA plasmid transfection into the osteosarcoma U2-OS cells, APE1 expression was inhibited at the protein and mRNA levels. The osteosarcoma cell proliferation rate was also decreased, and the PS-341 inhibitory effect on the osteosarcoma cells was promoted. The biological mechanisms may be related to the downregulation of nuclear NF-κB expression.

Key words: Osteosarcoma Proteasome inhibitor Apurinic / Apyrimidinic Endonuclease 1 NF-κB protein

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