

紫杉醇对人骨肉瘤细胞凋亡及对bcl-2、bax基因表达的影响

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Effect of Paclitaxel on Apoptosis and bcl-2, bax Gene Expression in Human Osteosarcoma Cell

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- 摘要
- 参考文献
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摘要 目的 了解紫杉醇诱导人骨肉瘤细胞的凋亡效果,以及凋亡与bcl-2和bax表达之间的关系。方法 用不同浓度的紫杉醇作用于骨肉瘤细胞MG63。采用胎盼蓝染色法,于细胞计数板进行活细胞率检测计算。采用光镜和电子显微镜对细胞形态改变进行观测,采用原位缺口末端标记凋亡检测法(TUNEL)和流式细胞术(Annexin-V-FITC&PI)对细胞凋亡进行检测。采用免疫组织化学法对凋亡调节基因bcl-2和bax表达进行检测。结果 骨肉瘤活细胞率随着紫杉醇作用时间延长和浓度增高而降低。紫杉醇诱导骨肉瘤细胞MG63凋亡表现为典型的凋亡特征,包括:细胞形态学改变,细胞染色质浓聚,染色质新月形变,胞核碎裂等。TUNEL法检测到了细胞DNA片段的断裂。随着细胞凋亡的发生,开始出现G₂/M期阻滞。紫杉醇可以降低凋亡相关基因bcl-2的表达和增加bax基因的表达。结论 紫杉醇可以通过时间依赖性和剂量依赖性方式,促使细胞G₂/M期阻滞和抑制细胞有丝分裂,从而诱导人骨肉瘤细胞凋亡。这种凋亡可能受到凋亡相关基因bcl-2低表达和bax高表达的调控。

关键词: 紫杉醇 凋亡 骨肉瘤 bcl-2 bax

Abstract: Objective To investigate the apoptosis in human osteosarcoma cells induced by paclitaxel, and the relationship between this apoptosis and expression of bcl-2 and bax. Methods MG63 OS cells were treated with various concentrations of paclitaxel. Proliferation was determined by cell count in a cytometer chamber. Viability was assessed by trypan blue dye exclusion. The cell morphologic alterations were visualized using light and transmitting electron microscope. The percentage of apoptosis of osteosarcoma cell MG63 was measured by TdT-mediated dUTP Nick End Labeling technique (TUNEL) staining method and flow cytometry using Annexin V/PI double staining method after 0, 24, 48, 72 and 96 hours of culture in the presence or absence of paclitaxel. The expression of apoptosis-regulated gene bcl-2 and bax were detected using immunohistochemical staining. Results A time-dependent and dose-dependent cell growth inhibition was shown after exposure to paclitaxel. Paclitaxel induced MG63 cells to undergo apoptosis with typical apoptosis characteristics, including morphological changes of chromatin condensation, chromatin crescent formation, nucleus fragmentation. The DNA-cleavage was detected by using TUNEL assay. The cells treated with paclitaxel showed initially G₂/M phase arrest, which was followed by apoptosis. Paclitaxel could reduce the expression of apoptosis-regulated gene bcl-2 and improved the expression of apoptosis-regulated gene bax. Conclusion Paclitaxel is able to induce the apoptosis in human osteosarcoma cells through the initiation of G₂/M phase arrest and inhibiting mitosis in both a time-dependent and dose-dependent manner. This apoptosis maybe mediated by down-expression of apoptosis-regulated gene bcl-2 and up-expression of apoptosis-regulated gene bax.

Key words: Paclitaxel Apoptosis Osteosarcoma bcl-2 bax

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