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论著

雪胆素乙通过破坏微丝结构和促进p21^{Cip1}表达抑制 前列腺癌PC-3细胞的增殖

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摘要 目的 分析雪胆素乙(CuⅡb)对人前列腺癌PC-3细胞增殖及细胞周期的影响,探讨其作用机制。方法 MTS法检测CuⅡb 0.064~200 μmol·L^{-1}作用48 h后PC-3细胞增殖;CuⅡb 2和20 μmol·L^{-1}作用24 h,相差显微镜观察细胞形态;CuⅡb 2和20 μmol·L^{-1}作用48 h,流式细胞术分析细胞周期分布;免疫荧光染色分析CuⅡb 20 μmol·L^{-1}分别作用1, 4和24 h后微丝和微管细胞骨架变化;免疫印迹法测定CuⅡb 20 μmol·L^{-1}分别作用1, 4和24 h后G肌动蛋白、F肌动蛋白、p21^{Cip1}及细胞周期蛋白A, B1, E和D1的表达。结果 CuⅡb以浓度依赖方式抑制PC-3细胞的增殖(r=0.9817, P<0.05)。CuⅡb 20 μmol·L^{-1}作用48 h,使细胞周期阻滞于G_2/M期,从溶剂对照组的(27.7±1.5)%上升为(45.3±1.8)%(P<0.01),相差显微镜见细胞发生明显收缩。CuⅡb 20 μmol·L^{-1}作用24 h,使G肌动蛋白水平显著下降,F肌动蛋白发生严重聚集(P<0.05),而对微管只有轻微影响。与溶剂对照组相比,CuⅡb 20 μmol·L^{-1}作用24 h后,细胞p21^{Cip1}表达明显升高,细胞周期蛋白A表达显著下调,其他细胞周期蛋白表达上调(P<0.05)。结论 CuⅡb能明显抑制人前列腺癌PC-3细胞的增殖,其机制可能是通过诱导肌动蛋白聚集,破坏微丝骨架,促进抑癌因子p21^{Cip1}表达,阻滞细胞周期的进程。

关键词 雪胆素乙 前列腺癌细胞 肌动蛋白聚集 细胞周期阻滞
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Inhibitory effect of Cucurbitacin Ⅱb on proliferation of human prostate cancer PC-3 cells through disruption of microfilaments and upregulation of p21^{Cip1} expression

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Abstract

OBJECTIVE To explore effects of cucurbitacin Ⅱb (CuⅡb) on human prostate cancer PC-3 cells and its underlying mechanism. **METHODS** The proliferation of cells was detected by MTS assay after PC-3 cells were treated with CuⅡb 0.064-200 μmol·L^{-1} for 48 h. After treated with CuⅡb 2 and 20 μmol·L^{-1} for 24 h, cell morphologic changes were observed under phase contrast microscopy. After exposed with CuⅡb 2 and 20 μmol·L^{-1} for 48 h, cell cycle distribution was measured by flow cytometry using propidium iodide (PI) staining. When cultured with CuⅡb 20 μmol·L^{-1} for 1, 4 and 24 h dividedly, the changes of microfilament and microtubule structures were assessed by immunofluorescence staining. After separately treated with CuⅡb 20 μmol·L^{-1} for 1, 4 and 24 h, the protein expression levels of F-actin, G-actin, p21^{Cip1}, cyclin A, cyclin B1, cyclin D1 and cyclin E were detected by western blotting(P<0.05). **RESULTS** CuⅡb inhibited the proliferation of PC-3 cells in a

concentration-dependent manner. Cu Ⅱ b 20 μmol • L^{-1} increased the cell rates of G_2/M phase (tetraploid) to (45.3 ± 1.8)% from (27.7 ± 1.5)% in control group (P<0.01), indicating an arrest of cell cycle progression. The cells became shrunk after Cu Ⅱ b treatment. Meanwhile, Cu Ⅱ b 20 μmol • L^{-1} decreased G-actin levels and induced severe F-actin aggregation(P<0.05), but had minimal effect on the microtubules. In addition, Cu Ⅱ b 20 μmol • L^{-1} also elevated $p21^{Cip1}$ expression and downregulated