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论著

地钱素C衍生物F41对子宫颈癌HeLa细胞凋亡的影响

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摘要 目的 对地钱素C(MC)羟基衍生物进行筛选, 寻找具有高抗肿瘤活性的MC衍生物, 并初步探讨其抗肿瘤的细胞与分子生物学机制。方法 用MTT法观察56种MC羟基衍生物对子宫颈癌HeLa细胞的毒性作用, 筛选其中细胞毒作用最强的MC衍生物, 并比较其与MC对HeLa细胞存活的抑制作用。用倒置显微镜、DAPI染色和DNA梯带检测其对细胞凋亡的影响。用流式细胞术检测其对细胞周期的影响。用Western印迹法检测其对细胞周期和凋亡相关蛋白表达的影响。结果 在56种MC衍生物中, F41对HeLa细胞毒性最强, 抑制HeLa细胞存活的IC_{50}为(11.31±2.13)μmol·L^{-1}, 明显低于MC(IC_{50}为(17.19±3.28)μmol·L^{-1})(P<0.01)。F41和MC与HeLa细胞作用24, 48和72 h, F41对HeLa细胞存活的抑制作用均明显强于MC(P<0.05)。形态学和DNA梯带检测显示, F41处理后, HeLa细胞皱缩变小, 有空泡和凋亡小体出现, 胞核浓缩变小, DNA电泳呈梯状条带。流式细胞分析显示, F41 15 μmol·L^{-1}处理HeLa细胞24 h, G_2/M期细胞占总细胞的比例为(43.8±3.0)%, 明显高于对照组的(13.1±1.6)%(P<0.01); G_1期细胞比例为(34.8±3.8)%, 明显低于对照组的(63.6±5.5)%(P<0.01)。Western免疫印迹结果表明, F41可使HeLa细胞内磷酸化细胞周期蛋白依赖性蛋白激酶1水平降低, 细胞周期蛋白B_1和 P53蛋白表达增多。结论 F41可诱导HeLa细胞凋亡, 抑制HeLa细胞分裂, 其抗肿瘤活性可能强于MC。

关键词 地钱素C 羟基衍生物 细胞周期 细胞凋亡
分类号 R966

Effect of marchantin C derivative F41 on apoptosis of human cervical cancer HeLa cells

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Abstract

OBJECTIVE To select a derivative of marchantin C (MC) with the highest antitumor activity by screening hydroxyl derivatives of MC and investigate the cellular and molecular biological mechanisms of its antitumor action. **METHODS** Fifty-six kinds of MC hydroxyl derivatives were screened using human cervical cancer HeLa cells by MTT assay in order to find the MC derivative with the stronger cytotoxicity. The inhibitory effect of the MC derivatives on the growth of HeLa cells was compared with that of MC by MTT assay. An inverted microscope, DAPI staining, and apoptosis-DNA ladder assay were used to observe its effects on apoptosis. Its influences on cell cycle were detected by flow cytometry. The influences on expression of proteins related to cell cycle and apoptosis were detected using Western blotting. **RESULTS** F41 showed the strongest cytotoxicity to HeLa cells among the 56 kinds of MC derivatives. The IC_{50} of HeLa cells exposed to F41 and MC were 11.3±2.1 and (17.2±3.3)μmol·L^{-1}, respectively, which had obvious difference (P<0.01). The MTT assay showed that when HeLa cells were treated with F41 and MC for 24, 48, and 72 h, the growth inhibitory rate of HeLa cells treated with F41 was higher than that with MC (P<0.05). Morphological observations and DNA ladder assay indicated that after HeLa cells were treated with F41, they shrank and became smaller, vacuoles and apoptotic bodies appeared, the nuclei condensed and became smaller, and a typical ladder pattern of DNA fragmentation in them was observed. Flow cytometry analysis showed that after HeLa cells were treated with F41 15 μmol·L^{-1} for 24 h, the percentage of HeLa cells at G_2/M phase was (43.8±3.0)%, much more than (13.1±1.6)% in control cells (P<0.01) while the percentage of HeLa cells at G_1 phase was (34.8±3.8)%, much less than (63.6±5.5)% in control cells (P<0.01). Western blotting results demonstrated that F41 could reduce the level of phophorylated cyclin-dependent protein kinase 1 and increase the expression of cyclin B_1 and P53 in HeLa cells. **CONCLUSION** F41 can promote HeLa cell apoptosis and inhibit their division and has much stronger cytotoxicity against HeLa cells than MC.

Key words marchantin C hydroxyl derivatives cell cycle apoptosis

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