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基础研究

香加皮杠柳苷对MCF-7细胞周期及$p21^{WAF1/CIP1}$表达的影响

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Effects of Periplocin from Cortex Periplocae on Cell Cycle of MCF-7 Cells and Expression of $p21^{WAF1/CIP1}$

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摘要 目的：研究香加皮杠柳苷（CPP）对人乳腺癌MCF-7细胞周期及$p21^{WAF1/CIP1}$表达的影响，探讨其抗肿瘤作用及作用机制。方法：采用MTT法检测不同浓度CPP（1.25、2.50、5.00、10.00、20.00 ng/ml）作用不同时间（24、48、72 h）对MCF-7细胞的增殖抑制作用；应用流式细胞术分析不同浓度CPP （2.50、5.00、10.00 ng/ml）分别作用于MCF-7细胞6、12、24、48、72 h对肿瘤细胞周期的影响；并采用RT-PCR和免疫细胞化学技术检测细胞周期相关因子$p21^{WAF1/CIP1}$的表达。结果：CPP能明显抑制MCF-7细胞的增殖，并呈浓度及时间依赖性，作用于MCF-7细胞48 h的IC_{50}为（4.88±0.16）ng/ml。流式细胞术结果显示，CPP作用MCF-7细胞24 h时，G_0/G_1期细胞明显增多，而S期和G_2/M期细胞显著减少，与对照组相比差异有统计学意义（$P<0.05$），其中5.00 ng/ml组G_0/G_1期细胞由对照组的（49.33±3.25）%升高至（79.47±2.40）%，S期和G_2/M期细胞由对照组的（28.47±1.59）%和（22.20±2.09）%分别下降至（10.13±3.26）%和（10.40±1.41）%。经CPP处理的MCF-7细胞中$p21^{WAF1/CIP1}$ mRNA的表达明显增强，$p21^{WAF1/CIP1}$/β-actin光吸度比值与对照组相比明显增高（$P<0.05$）。免疫细胞化学结果显示，CPP组MCF-7细胞中$p21^{WAF1/CIP1}$ 蛋白的表达随作用浓度的增加而增强，其中10.00 ng/ml组肿瘤细胞$p21^{WAF1/CIP1}$的表达呈强阳性。结论：香加皮杠柳苷（CPP）具有显著的体外抗肿瘤作用，且有效剂量很小，其IC_{50}仅为（4.88±0.16）ng/ml。CPP可使MCF-7细胞发生G_0/G_1期阻滞，并可使细胞周期相关基因$p21^{WAF1/CIP1}$的mRNA及蛋白表达增强，提示阻滞细胞周期可能是CPP体外抗肿瘤的作用机制之一。

关键词： 香加皮杠柳苷 乳腺癌 抗肿瘤 细胞周期

Abstract： Objective：To study the effect of Periplocin from Cortex Periplocae (CPP) on cell cycle of MCF-7 cells and expression of $p21^{WAF1/CIP1}$, and demonstrate the possible mechanism of anti-tumor. Methods：Inhibitory effects of CPP in different concentrations (1.25, 2.50, 5.00, 10.00, 20.00 ng/ml) and different time (24, 48, 72 h) on proliferation of MCF-7 were detected by MTT. The changes of cell cycle of tumor cells treated with CPP under different concentrations (2.50, 5.00, 10.00ng/ml) and various time (6, 12, 24, 48, 72 h) were detected using flow cytometry,respectively. Expression of cell cycle associated gene $p21^{WAF1/CIP1}$ was assessed by semi-quantitative RT-PCR and SP immunocytochemistry method. Results：A dose and time-dependent proliferation inhibition of CPP was demonstrated in MCF-7 with IC_{50} value (for 48h) of (4.88±0.16) ng/ml. Compared to control group, the number of G_0/G_1 phase cells increased markedly, but which of S and G_2/M phase cells decreased, after treatment with CPP for 24 hours, the difference was significant ($P<0.05$); in 5.00 ng/ml group, the proportion of tumor cells in the G_0/G_1 phase was increased from (49.33±3.25)% to (79.47±2.40)%, the proportion of S and G_2/M phase cells was decreased from (28.47±1.59)% and (22.20±2.09)% to

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(10.13±3.26)% and (10.40±1.41)%,respectively. $p21^{WAF1/CIP1}$ mRNA level increased obviously in MCF-7 cells exposed to CPP, the ratio of $p21^{WAF1/CIP1}$ to β-actin was much higher, compared with that in control group ($P<0.05$). The result of immunocytochemistry indicated that $p21^{WAF1/CIP1}$ protein expression increased obviously in MCF-7 cells exposed to CPP in concentration-dependent manner. $p21^{WAF1/CIP1}$ protein showed stronger positive staining in cells with 10.00 ng/ml CPP treatment. Conclusion：CPP has marked anti-tumor effect in vitro, its curative dose is small, the IC_{50} was (4.88±0.16)ng/ml. CPP could hinder the cell cycle of MCF-7 cells at G_0/G_1 phase. CPP elevated mRNA and protein expression of cell cycle associated gene $p21^{WAF1/CIP1}$. The result suggested that blocking the cell cycle may be one of its anti-tumor mechanisms in vitro.

Key words： Periplocin from Cortex Periplocae　Breast cancer　Antitumor　Cell cycle

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