



肿瘤防治研究 2012, Vol. 39 Issue (4): 394-399 DOI: 10.3971/j.issn.1000-8578.2012.04.007

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连翘叶乙醇提取物对人食管癌细胞增殖抑制作用的研究

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Ethanol Extract from Forsythia Suspensa Leaf Suppresses Human Esophageal Carcinoma Cells Growth

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摘要 目的

研究中药连翘叶乙醇提取物体外对食管癌细胞增殖的影响及对TE-13细胞凋亡的影响并探讨其作用机制。方法应用MTT法分析不同浓度连翘叶乙醇提取物(ethanol extract of Forsythia suspense leaf, FSEE)对人食管癌细胞TE-13、TE-1、Yes-2和 Eca-109增殖的影响;光学显微镜下观察经FSEE处理后细胞的形态学改变;Wright-Giemsa染色观察TE-13细胞凋亡的形态学变化;经Annexin V/PI双染后用流式细胞术检测 FSEE对TE-13细胞凋亡率的影响;用不同浓度(0.1、0.2、0.5mg/ml) FSEE作用于TE-13细胞24 h后,分析细胞PARP、Caspase 3、Caspase 8、Caspase 9蛋白表达的变化。RT-PCR法检测FSEE对TE-13细胞凋亡相关基因Bcl-2家族中Bcl-2、Bcl-xL、Bax mRNA表达的影响。结果FSEE对人食管癌细胞增殖具有显著抑制作用($P<0.05$);经FSEE处理24 h后,TE-13细胞出现明显的凋亡形态学改变,TE-13细胞凋亡率明显增高,与未处理组比较差异有统计学意义($P<0.01$);经0.1、0.2、0.5 mg/ml FSEE作用24 h后,TE-13细胞PARP、Caspase 3、Caspase 9蛋白出现裂解片段,并随药物浓度增加,裂解片段浓度升高,呈浓度依赖性,组间比较差异有统计学意义($P<0.01$)。经FSEE处理后,TE-13细胞Bax mRNA表达上调, Bcl-2和Bcl-xL mRNA表达水平下调。结论FSEE在体外可明显抑制食管癌细胞的增殖,诱导TE-13细胞凋亡,其机制可能与其通过Caspase依赖性的内源性途径诱导细胞凋亡有关。

关键词: 食管癌 FSEE TE-13 细胞凋亡 内源性凋亡途径

Abstract: Objective Effect of ethanol extract of Forsythia suspensa leaf on human esophageal carcinoma cells propagation and apoptosis of TE-13 were studied. Methods The suppressive effect of FSEE in different concentration and time on several human esophageal carcinoma cells were analyzed by MTT method, the effect on quantity and morphologic changes of esophageal carcinoma cells was observed with microscope, TE-13 cells were stained with Giemsa and its morphologic changes were investigated. After treatment with different concentration FSEE for 24 h, apoptotic rate of TE-13 cells were stained by Annexin V/PI and then invested by FCM, and protein expression of PARP, Caspase 3, Caspase 8 and Caspase 9 was detected by western blot. Effect of FSEE on mRNA level of Bcl-xL, Bcl-2, Bax in TE-13 cell was analyzed by RT-PCR. Results FSEE can significantly inhibit esophageal carcinoma cells proliferation ($P<0.05$), and this effect on TE-13 cells was in time and dose-dependent manner. By optical microscope, TE-13 cells showed typical cell apoptosis morphological changes. TE-13 cell apoptosis rate gradually increased in dose-dependent manner ($P<0.01$). As drug concentration (0.1, 0.2, 0.5 mg/ml) increased, PARP, Caspase3, Caspase9 proteins in TE-13 cells showed gradually cleavage fragments, but it was not found in Caspase8 protein. Expression of Bcl-2 and Bcl-xL mRNA in TE-13 cells is decreased and expression level of Bax mRNA is strengthen after treatment with 0.1, 0.2, and 0.5mg/ml FSEE for 24 h. Conclusion FSEE can significantly inhibit esophageal carcinoma cells proliferation in a dose-dependent manner in vitro. It also can induce TE-13 cell apoptosis, which suggesting that its antitumor mechanism may be related with endogenous apoptosis pathway.

Key words: Esophageal carcinoma FSEE TE-13 Apoptosis Endogenous apoptosis way

收稿日期: 2011-06-10;

引用本文:

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