[1]李绍平,卢佳,马小洁,等.华蟾素对神经母细胞瘤SH-SY5Y细胞凋亡的影响[J].第三军医大学学报,2014,36(12):1260-1263.

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# 华蟾素对神经母细胞瘤SH-SY5Y细胞凋亡的影响(PDF)

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Title: Cinobufacini-induced apoptosis in human neuroblastoma SH-SY5Y cells

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关键词: 华蟾素; 人神经母细胞瘤; 凋亡; 线粒体

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摘要: 目的 观察华蟾素对人神经母细胞瘤SH-SY5Y细胞凋亡的影响，并探讨其作用机制。 方法 采用巯基罗丹明B（SRB）法检测华蟾素对SH-SY5Y细胞的抑制率，流式细胞术检测细胞凋亡率，荧光探针二乙酸二氯荧光素盐(DCFH-DA)法检测细胞内活性氧（reactive oxygen species，ROS）水平；罗丹明123（Rhodamine 123）染色法检测细胞线粒体膜电位；Western blot检测胞质细胞色素C（Cyt-C）的表达；ELISA检测Caspase-3 和 Caspase-9 酶活性。 结果 华蟾素对SH-SY5Y细胞增殖具有明显抑制作用，且表现出剂量和时间依赖性。流式细胞术检测对照组及各浓度组凋亡率分别为（3.12±0.91）%、（17.65±4.28）%、（29.14±6.72）%、（50.01±9.26）%，与对照组比较，华蟾素组的凋亡率均显著性增高（$P<0.01$）；华蟾素能够呈浓度依赖的增加细胞内ROS水平（$P<0.01$），降低线粒体膜电位（$P<0.01$），增加胞质中Cyt-C的表达（$P<0.01$），同时增加Caspase-3 和 Caspase-9 酶活性。 结论 华蟾素能够通过诱导SH-SY5Y细胞产生凋亡而发挥增殖抑制作用，其作用机制可能与激发线粒体凋亡途径有关。

Abstract: Objective To investigate apoptotic effects of cinobufacini on human neuroblastoma SH-SY5Y cells and its relevant mechanism. Methods Effects of cinobufacini on SH-SY5Y cell proliferation were tested by SRB assay. Cell apoptosis of SH-SY5Y was measured by flow cytometry. The levels of reactive oxygen species (ROS) in the cells were analyzed by ROS-sensitive fluorometric probe DCFH-DA assay and mitochondria transmembrane potential was revealed by rhodamine123 staining. The expression of cytosolic Cyt-C was analyzed by

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Western blotting. Caspase-3 and caspase-9 activities were detected by ELISA. Results Cinobufacini inhibited the proliferation of SH-SY5Y cells in a dose- and time-dependent manner. Flow cytometry results showed that the apoptotic rates in the control group and the cinobufacini-treated groups (cinobufacini concentration 0.125, 0.25, and 0.5 μg/mL) were (3.12±0.91)%, (17.65±4.28)%, (29.14±6.72)%, and (50.01±9.26)%, respectively. Compared with the control group, the cinobufacini-treated cells showed increased apoptotic rate ($P$<0.01). Cinobufacini, in a dose-dependent manner, increased the ROS levels ($P$<0.01), decreased mitochondria transmembrane potential ($P$<0.01), enhanced cytosolic Cyt-C expression ($P$<0.01) in SH-SY5Y cells, and increased caspase-3 and caspase-9 activities. Conclusion Cinobufacini can inhibit the proliferation of SH-SY5Y cells by inducing cell apoptosis, which may involves the stimulation of mitochondrial apoptotic pathway.

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