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论著

氧化应激诱导HepG2肝癌细胞凋亡的研究(英)

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摘要 目的：直接暴露细胞于活性氧能诱导发生凋亡，本文研究氧化应激诱导HepG2肝癌细胞的死亡及其机制。

方法：暴露细胞于2 mmol/L过氧化氢产生氧化应激，用DNA凝胶电泳检测细胞凋亡，用荧光染色法检测细胞线粒体膜电位变化，Western blotting检测细胞浆中细胞色素c变化，fluorometric assay kit检测caspase活性变化。

结果：氧化应激作用于HepG2细胞后12 h开始发生凋亡；氧化应激作用后4 h，细胞线粒体膜电位明显下降；胞浆中细胞色素c浓度呈时间依赖性增高；氧化应激作用8 h、12 h后细胞内caspase-3、caspase-9活性分别升高6.7及3.6倍，但caspase-8活性无变化。

结论：氧化应激能诱导HepG2肝癌细胞发生凋亡，其途径与线粒体通路及caspase激活有关。

关键词 细胞凋亡； HepG2细胞； 线粒体； 细胞色素C 半胱氨酸天冬氨酸蛋白酶； 氧化性应激

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Oxidative stress induces apoptosis in HepG2 cells

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Abstract

<FONT face=Verdana>AIM:
Direct exposure of cells to reactive oxygen species can induce apoptosis. In this study we investigate how oxidative stress induces cell death in HepG2 cells and characterize the molecular events involved.
METHODS: Oxidative stress was created by exposing HepG2 cells to 2 mmol/L H 2O 2. Apoptosis was determined by analysis of DNA fragmentation by agarose gel electorphoresis. The mitochondrial membrane potential was analyzed using DePsipher fluorescent staining and the expression of cytochrome c in the cytosolic fraction was measured by Western blotting analysis. The caspase activity was detected using fluorometric assay kit by a fluorescence microplate reader.
RESULTS:
When HepG2 cells were treated with 2 mmol/L H 2O 2, the cells displayed DNA fragmentation, a typical feature of apoptosis, after 12 h. The mitochondrial membrane potential appeared different in two group of cells. H 2O 2-treated cells appeared green fluorescence as early as 4 h, which represents de-energized mitochondria, the untreated cells appeared red fluorescence, a feature of mitochondria with intact membrane potential. In treated cells, the expression of cytochrome c increased and accumulated in cytosolic fraction with treatment time, caspase-3 activity increased by 6.7-fold (P<0.01) at 8 h and caspase-9 activity increased by 3.6-fold (P<0.01) at 12 h, respectively, however, the activity of caspase-8 remained unchanged.
CONCLUSION: These findings suggest that oxidative stress can induce apoptotic cell death in HepG2 cells, and the mechanism is related to mitochondrial pathway, which activates caspase-9 and-3, but not caspase-8.</FONT>

Key words Apoptosis HepG2 cells Mitochondria Cytochrome C Caspases Oxidative stress

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