

论文

Staurosporine诱导人乳腺癌MCF7/GFP-Bax非受体依赖途径的细胞凋亡对Bax自胞浆至线粒体转移定位的影响

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摘要:

以建立的人乳腺癌MCF7 细胞GFP-Bax稳定表达细胞株(MCF7/GFP-PBax), 观察staurosporine(STS)诱导的非受体途径的细胞凋亡对Bax自胞浆转移定位于线粒体的影响。荧光显微镜观察凋亡细胞Bax从细胞浆至线粒体转移和细胞核染色体断裂, 检测STS诱导细胞凋亡的量效和时效关系。免疫荧光法观察GFP-Bax从细胞浆转移至线粒体与定位、细胞色素c(cytochrome c, Cyt-c)释放和Annexin V染色。MTT法测定STS的细胞毒作用, TMRE观测对细胞线粒体膜电位(ΔΨm)与功能的影响。Western blotting方法分析STS诱导的细胞凋亡的信号转导途径和作用机制。结果STS可明显促进Bax从细胞浆转移至线粒体与定位、细胞色素c释放, Western blotting显示JNK特异抑制剂SP600125可抑制STS诱导的细胞pJNK表达, 表明STS作用机制与激活JNK信号通路相关。

关键词: staurosporine 细胞凋亡 GFP-Bax 线粒体

Effect of staurosporine induced apoptosis of MCF7/GFP-Bax stable cell line on Bax translocation from cytosol into mitochondria

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Abstract:

To investigate Bax translocation from cytosol into mitochondria induced by staurosporine (STS) in GFP-Bax-tagged MCF7 stable cell line, the viability was measured by MTT method. Bax translocation from cytosol into mitochondria was investigated under the fluorescence microscope. The dose-effect and time-course relationships were also observed and the percentage of GFP-Bax punctuate cells were calculated. Immunofluoresence method was used to observe Bax translocation to mitochondria, Cyt-c release from mitochondria and Annexin V label. The TMRE assay was used to investigate membrane pertential (ΔΨm) and function of mitochondria. Western blotting was used to observe the mechanism of apoptosis induced by STS. The results showed that STS can induce Bax translocation from cytoplasm to mitochondria, Cyt-c release from mitochondria and Annexin V label. The Western blotting analysis presented the inhibitory effect on apoptosis induced by STS of SP600125 which is a specific JNK inhibitor. The study revealed the mechanism of STS induced apoptosis associated with JNK activated pathway.

Keywords: apoptosis GFP-Bax mitochondria staurosporine

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