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论著

ING4基因的克隆及其诱导HeLa细胞凋亡的实验研究

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摘要

目的：分离小鼠ING4基因并构建真核表达载体pcDNA3.0-ING4, 观察ING4基因转染人宫颈癌HeLa细胞后对细胞凋亡的影响。

方法:应用RT-PCR技术从小鼠肝组织中扩增得到ING4cDNA。利用DNA重组技术构建真核表达载体pcDNA3.0-ING4并用双酶切、PCR、基因测序进行鉴定,然后利用阳离子脂质体lipofectamine将其转染HeLa细胞，用RT-PCR检测ING4基因的表达情况，应用荧光显微镜（Hoechst33258染色）、激光扫描共聚焦显微镜和流式细胞仪观察细胞凋亡情况。

结果:RT-PCR产物为约 750 bp 的条带,构建的真核表达载体pcDNA3.0-ING4经双酶切、PCR鉴定均出现750 bp 大小的条带，基因测序正确, Hoechst33258染色发现pcDNA3.0-ING4转染HeLa细胞的凋亡率（21.25%）明显高于对照组pcDNA3.0转染HeLa细胞的凋亡率（8.91%,P<0.01）。共聚焦显微镜也观察到了典型的细胞凋亡。细胞周期检测显示S期细胞所占百分数pcDNA3.0-ING4转染组高于pcDNA3.0转染组。

结论:分离得到了小鼠ING4基因并成功构建真核表达载体pcDNA3.0-ING4, 初步研究发现该基因转染HeLa细胞后可促使细胞凋亡。

关键词 基因,ING4 细胞转染 HeLa细胞

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Molecular cloning of ING4 gene and ING4-induced apoptosis in HeLa cells

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Abstract

<P><FONT face=Verdana>AIM: To isolate a gene encoding mouse ING4, construct pcDNA3.0-ING4 recombinant eukaryotic expression plasmid and investigate its effects on HeLa cells in vitro.
METHODS: The mouse ING4cDNA was amplified by RT-PCR from mouse liver. The eukaryotic expression vector pcDNA3.0-ING4 was constructed by DNA recombination technique. The recombinant plasmid pcDNA3.0-ING4 was identified by PCR, restriction enzyme digestion and DNA sequence analysis, then was transfected into HeLa cells by lipofectamine. The expression was determined by RT-PCR. Apoptosis was detected by fluorescence microscope with Hoechst33258 staining and laser scanning confocal microscope. Cell cycle distribution was measured with flow cytometry.
RESULTS: RT-PCR product was about 750 bp specific fragment. Analysis by restricting enzyme digestion and PCR of pcDNA3.0-ING4 recombiant plasmid showed that results were about 750 bp, DNA sequencing revealed that ING4 cloning were successful. With Hoechst fluorescence staining, we found that the percentage of apoptotic rate in HeLa cells transfected with pcDNA3.0- ING4 (21.25%) was higher than that in HeLa cells transfected with pcDNA3.0 (8.91%,P<0.01). Apoptosis was also detected by laser scanning confocal microscope. Cell cycle analysis reavealed the cell number in S phase of HeLa cells transfected with pcDNA3.0- ING4 increased.
CONCLUSION: The gene encoding mouse ING4 and construction of pcDNA3.0- ING4 eukaryotic expression vector were successfully obtained, ING4 could enhance apoptosis in HeLa cells.</FONT></P>

Key words Genes ING4 Cell transfection HeLa cells

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