



人食管鳞癌EC9706细胞线粒体DNA与凋亡的关系

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Relationship between mtDNA and Apoptosis in Esophageal Squamous Cell Carcinoma

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摘要 目的: 建立人食管鳞癌EC9706细胞的无线粒体DNA (p°) 细胞, 探讨食管癌线粒体DNA与凋亡的关系。方法: 在细胞培养液中加入EB 50 μ g/ml、尿嘧啶50 μ g/ml、丙酮酸100 μ g/ml, 进行连续传代培养, 获得完全缺失mtDNA的细胞 (p° 细胞); 运用实时荧光定量PCR技术, 检测EB处理后不同时间的人食管鳞癌细胞EC9706 mtDNA的拷贝数, 并采用琼脂糖凝胶电泳对mtDNA进行定性检测; 采用TUNEL染色和流式细胞技术, 检测EB处理后不同时间人食管鳞癌细胞EC9706的凋亡情况。结果: 成功建立了人食管鳞癌细胞EC9706的 p° 细胞, 经实时荧光定量PCR鉴定, 发现在EB存在下, 随着细胞分裂, mtDNA拷贝数进行性减少, 直到12天, mtDNA完全丢失; 流式细胞术检测结果显示, EC9706细胞EB处理后, 第4天、8天及12天细胞凋亡率(%)分别为(2.78 \pm 1.04)、(11.68 \pm 1.85)、(26.62 \pm 1.06), 与对照组相比, 差异均有统计学意义($P < 0.05$); TUNEL检测结果与上述一致, 从第4天到第12天凋亡也逐渐增加。结论: 成功建立了EC9706 p° 细胞。随着EC9706细胞mtDNA拷贝数量的逐渐减少, 细胞凋亡率逐渐增加, 表明mtDNA在诱导细胞凋亡中起着一定调控作用, 提示选择性地诱导食管癌细胞mtDNA损伤, 使食管癌细胞mtDNA拷贝数量明显减少, 进而诱导细胞凋亡, 可望成为食管癌生物治疗的一个新靶点。

关键词: 食管鳞癌 线粒体DNA 凋亡

Abstract: Objective: To establish the p° cells of the human esophageal carcinoma cells EC9706 and investigate the relationship between mtDNA copies and apoptosis. Methods: Cells deficient mtDNA (p° cells) were acquired from ESCC cell lines EC9706 through continuous passage culture in the RPMI 1640 supplemented with 50 μ g/ml EB, 50 μ g/ml uridine and 100 μ g/ml pyruvate. MtDNA copies of the two cell lines were detected at different time using the real-time fluorescence quantitative PCR after treated by EB. PCR products were validated by agarose gel electrophoresis; Apoptosis of ESCC cell lines EC9706 were analyzed using TUNEL staining and flow cytometry at different time after treated by EB. Results: The p° cells of ESCC cell lines EC9706 were successfully established. The Results: identified by the real-time fluorescence quantitative PCR indicated that mtDNA copies decreased progressively with the increasing in times of cell division in the presence of EB and mtDNA was disappear until 12 days. Apoptosis analysis was performed during the culture of p° cells of EC9706 after the cells were treated with EB on the 4th, 8th and 12th day. The Results: detected by flow cytometry indicated that apoptosis was increased gradually from the 4th day to 12th day. Apoptotic rates(%) were 2.78 \pm 1.04, 11.68 \pm 1.85 and 26.62 \pm 1.06 in the cells

EC9706. The apoptosis detected by TUNEL was increased gradually from the 4th day to 12th day. Conclusion: Establishment of the p^o cells of ESCC cell lines EC9706 offers new tools for research on the relationship between mitochondrial DNA and esophageal carcinoma. Apoptotic rate of EC9706 cells was increased gradually with the decrease of mtDNA copies. The Results: suggest that mtDNA may participate in the inducement of apoptosis and mtDNA lesions induced selectively could lead to mtDNA copies obvious decrease and further induce cell apoptosis. This is wished to become a new target for biotherapy of esophageal carcinoma.

Key words: Esophageal squamous cell carcinoma (ESCC) Mitochondrial DNA Apoptosis

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