粒線體 DNA 匱乏細胞之建立及其運用之研究

Establishment of mtDNA-depletion cells and further utilization

中文摘要

本實驗藉由添加 ethidium bromide (100 ng/mL)或 RNA 干擾技術(RNA interference，RNAi)，抑制調控粒線體轉錄和複製之基因[包括:粒線體轉錄因子 A(mtTFA)和 DNA 聚合酶　(POLG)]，主要目的是希望建立粒線體 DNA 匱乏(mitochondrial DNA depletion)細胞株，進一步將粒線體 DNA 匱乏細胞與粒線體疾病病人身上所得之細胞進行融合，得到融合細胞株(cybrid cell)，作爲研究粒線體 DNA 缺陷之工具。在 RNAi 抑制方法上，分別以長期穩定(stable)及短暫(transient)抑制兩部分進行。實驗結果顯示以 ethidium bromide 處理和 RNAi 抑制方法所得細胞株之生長速度較控制組(wild-type)慢，有些細胞株之外觀形態也由原本菱狀改變成紡綞纖維狀，利用流式細胞儀分析，結果顯示，實驗組有大部分之細胞株出現細胞凋亡現象。而以反轉錄聚合酶連鎖反應(RT-PCR)和同步定量聚合酶連鎖反應(real-time quantitative PCR)監測 mtTFA 之 mRNA 和 mtDNA copy numbers 表現量，發現處理 ethidium bromide (100 ng/mL)第 6 天 mtDNA copy numbers 降低 60%，第 16 天以後降低 80-90%。在體外(in vitro)短暫表現 RNAi 的方法上，發現將 mtTFA 之 siRNA 送入細胞後 36 hr 約抑制 60-70%左右，在 mtDNA copy numbers 表現量測定上也得到相同結果，約抑制 70-80%左右；而在長期穩定表現 RNAi 抑制方面，從選殖到穩定細胞株(stable clones)約 2 週後 mtTFA 基因表現量抑制 50%，5-6 週後則抑制 80-90%，mDNA copy numbers 下降 100-1000 倍。在蛋白質分析方面則以西方墨點法(western blot)定量一些分別由染色體或粒線體 DNA 負責製造的蛋白質，以偵測於形成粒線體 DNA 匱乏細胞株之過程中，粒線體呼吸鏈酵素群蛋白質表現的變化。實驗結果顯示以 ethidium bromide 處理細胞，可得到粒線體 DNA 匱乏之細胞，同時利用 RNAi 抑制 mtTFA 之基因表現，可達抑制粒線體 DNA 複製之效果，進而得到粒線體 DNA 匱乏細胞，也證實 mtTFA 確實參與粒線體 DNA 的複製工作。

英文摘要

The aim of this study was to construct mitochondrial DNA-depletion cell lines by RNA interference or adding ethidium bromide (100 ng/mL). The mtDNA-depletion cell lines can be further used to fuse with primary cultured cells from the patients. We expect to use the cybrid cells for the research to mimic mtDNA depletion and mutation. The study of RNAi to inhibit mtDNA was divided into two parts: stable and transient inhibition. The appearance of the treated cell lines showed longer and

narrower than the control group (143B TK- wild-type) and the growth was also slower. The cell lines of the experiment group went on apoptosis through flow cytometry analysis. mtTFA gene expression and mtDNA copy numbers were determined by RT-PCR and real-time quantitative PCR. mtDNA copy number decreased about 60% at the 6th days and 80~90% at the 16th days after adding ethidium bromide. In vitro study, transient transfection with RNAi inhibited 60~70% of mtTFA expression at 36th hrs. The same outcome was also determined in mtDNA copy numbers showed 70~80% inhibition effect. In the stable transfection, the effect was obvious at two weeks after selecting the stable clones. The mtTFA gene expression decreased about 50% at selected time and 80-90% at 5~6 weeks later, and the mtDNA copy number decreased 100-1000 fold in the selected clones compared with wide-type. In the protein assay, western blot was used to quantify the proteins encoded by mtDNA for measuring the expression leves of mitochondrial respiratory chain complex during the process of forming mtDNA-depletion cell lines. In conclusion, we get several mtDNA-depletion cell lines by adding ethidium bromide or blocking mtTFA gene expression with RNAi