中国药理学与毒理学杂志 2012 26 (4): 482-488 ISSN: 1000-3002 CN: 11-1115/R

论著

线粒体DNA缺失A549细胞与其母本细胞核蛋白质组差异分析

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收稿日期 2011-6-1 修回日期 2012-3-19 网络版发布日期 2012-8-21 接受日期

摘要 目的 比较线粒体DNA(mtDNA)缺失A549细胞(Rho^{0}细胞)与其母本细胞(Rho$^+$细胞)核蛋白表达谱, 并探讨细胞核对线粒体功能缺陷的应答反应。方法 二维凝胶电泳(2-DE)和表面增强激光法解吸电离-飞行时间(SELDI-TOF)蛋白芯片测定Rho^{0}细胞和Rho$^+$细胞核蛋白表达谱,基质辅助激光解吸电离-飞行时间(MALDI-TOF)质谱结合数据库检索鉴定差异表达的蛋白点,Western印迹法测定核磷蛋白和P53表达,激光共聚焦显微镜测定线粒体膜电位。结果 2-DE显示Rho^{0}细胞核中11个蛋白点表达下调,21个蛋白点表达上调。基于NP20蛋白质芯片的SELDI-TOF质谱分析发现4个蛋白质峰在Rho^{0}细胞核中明显下降。其中1个表达下调的蛋白点被鉴定为eIF-6,4个表达上调的蛋白点被鉴定为核磷蛋白, SFRS1, SFRS3和hnRNP G。Western印迹实验结果显示, Rho^{0}细胞中核磷蛋白表达增加。P53和线粒体膜电位(MMP)测定结果显示, Rho^{0}细胞中P53表达高于Rho$^+$细胞,两种细胞MMP基本一致。结论 mtDNA缺失诱导了细胞核蛋白质组改变。Rho^{0}细胞可以作为研究线粒体与核交互作用的模型。

关键词 核蛋白质组 基因,*p53* 膜电位,线粒体

分类号 R963

Comparative analysis of nuclear proteomes in mitochondrial DNA-depleted A549 cells and their parental cells

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Abstract

OBJECTIVE To investigate the nuclear proteomes in mitochondrial DNA (mtDNA)-depleted A549 cells (Rho^0 cells) and their parental cells (Rho$^+$ cells), and to learn more about the nuclear responses to mitochondrial dysfunction. METHODS The nuclear proteomes of Rho^0 and Rho$^+$ cells were characterized by two dimensional electrophoresis (2-DE) and SELDI-TOF ProteinChip technologies, the differentially expressed protein-spots were identified by MALDI-TOF mass spectrum (MS), the nucleophosmin and P53 expression were detected by Western blotting assay, and the mitochondrial membrane potential (MMP) was measured by the laser scanning confocal microscope. RESULTS 2-DE results showed 11 protein-spots were down-regulated and 21 protein-spots were up-regulated in Rho^0 cell nuclei. SELDI-TOF MS analysis with NP20 ProteinChips revealed 4 protein-peaks decreased in Rho^0 cell nuclei. One down-regulated protein-spot was identified as eIF-6, and 4 up-regulated protein-spots were identified as nucleophosmin, SFRS1, SFRS3 and hnRNP G, respectively. The increased expression of nucleophosmin in Rho^0 cells was verified by Western blotting. Based on the clues from proteomic analysis, P53 expression in Rho^0 cells was higher than in Rho$^+$ cells, and MMP was consistent in Rho$^+$ and Rho^0 cells. CONCLUSIONmtDNA-depletion induces nuclear proteome alteration. Rho^0 cells can be used as a model to study the cross-talk between mitochondrion and nucleus.

Key words nuclear proteomics genes *p53* membrane potential mitochondrial

DOI: 10.3867/j.issn.1000-3002.2012.04.003

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