Maintenance of mitochondrial DNA copy number and expression are essential for preservation of mitochondrial function and cell death

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摘要

Abstract

To examine whether a reduction in the mtDNA level will compromise mitochondrial biogenesis and mitochondrial function, we created a cell model with depleted mtDNA. Stable transfection of small interfering (si)RNA of mitochondrial transcription factor A (Tfam) was used to interfere with Tfam gene expression. Selected stable clones showed 60-95% reduction in Tfam gene expression and 50-90% reduction in cytochrome b (Cyt b) gene expression. Tfam gene knockdown clones also showed decreased mtDNA-encoded cytochrome c oxidase subunit I (COX I) protein expression. However, no significant differences in protein expression were observed in nuclear DNA (nDNA)-encoded mitochondrial respiratory enzyme subunits. The cell morphology changed from a rhombus-like to a spindle-like form as determined in clones with decreased expressions of Tfam, mtRNA, and mitochondrial proteins. The mitochondrial respiratory enzyme activities and ATP production in such clones were significantly lower. The proportions of mtDNA mutations including 8-hydroxy-2-deoxyguanosine (8-OHdG), a 4,977-bp deletion, and a 3,243-point mutation were also examined in these clones. No obvious increase in mtDNA mutations was observed in mitochondrial dysfunctional cell clones. The mitochondrial respiratory activity and ATP production ability recovered in cells with increased mtDNA levels after removal of the specific siRNA treatment. These experimental results provide direct evidence to substantiate that downregulation of mtDNA copy number and expression may compromise mitochondrial function and subsequent cell growth and morphology. J. Cell. Biochem. 103: 347-357, 2008.