

RESPONSE TO OXIDATIVE STRESS ELICITED BY PATHOGENIC MUTATIONS OF MITOCHONDRIAL DNA: IMPLICATIONS IN THE PATHOPHYSIOLOGY OF MITOCHONDRIAL DISEASES

Yau-Huei Wei^{1,3}, Yi-Shing Ma¹, Yin-Chiu Chen¹, Chi-Hung Lin², and Chun-Yi Chien²

¹Institute of Biochemistry and Molecular Biology, and ²Institute of Microbiology and Immunology, School of Life Sciences, National Yang-Ming University, and ³Department of Medicine, Mackay Medical College, Taipei, Taiwan

Most pathogenic mitochondrial DNA (mtDNA) mutations impair respiratory function and generate more reactive oxygen species in affected cells. It was hypothesized that oxidative stress affects gene expression of cells harboring mtDNA mutations. We found that Mn-SOD expression was increased whereas other antioxidant enzymes were not changed in skin fibroblasts of patients with mitochondrial diseases such as CPEO, MELAS and MERRF syndromes. The imbalanced expression of antioxidant enzymes resulted in the overproduction of H₂O₂ and enhanced oxidative stress and increased oxidative damage in affected cells. By using cDNA microarray, RT-PCR, and Western blot analysis we identified genes with altered expressions in MERRF skin fibroblasts. Expression levels of a cluster of genes related to oxidative stress response were increased, and other clusters of genes involved in energy metabolism and protein synthesis were down-regulated. MnSOD, matrix metalloproteinases, and protein kinase C- δ were found to be up-regulated in skin fibroblasts harboring MERRF-specific A8344G mtDNA mutation. The basal levels of expression of these genes in MERRF skin fibroblasts were higher than those of controls and were further up-regulated by H₂O₂ and these effects were inhibited by N-acetylcysteine. Using confocal microscopy and time-lapse microphotographic techniques, we observed dramatic derangement of the mitochondrial network and slower mitochondrial movement in the MERRF and MELAS skin fibroblasts. These defects could be alleviated by treatment of patients' skin fibroblasts with pyruvate or N-acetylcysteine. The ATP content was increased and the intracellular level of H₂O₂ was decreased in skin fibroblasts of MERRF patients after addition with 100 g/ml pyruvate or 1 mM N-acetylcysteine. Recently, we found that oxidative damage to mitochondrial proteins containing iron-sulfur clusters in human cells harboring A8344G mutation of mtDNA play a role in the bioenergetic defect of MERRF syndrome. Taken together, we suggest that mtDNA mutation-elicited oxidative stress and oxidative damage as well as alterations in the expression of distinct clusters of genes may play an important role in the derangement of mitochondrial network and dynamics that result in bioenergetic breakdown in the affected tissues of patients with mitochondrial diseases such as MERRF syndrome. The role of oxidative stress and oxidative damage in the pathophysiology of mitochondrial diseases and the potential of antioxidants therapy for the treatment of these overt diseases will be discussed.