Sensitivity for hydrogen peroxide in ρ^0 cells and involvement of membrane status

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Mitochondria have been recognized as “Powerhouse of the Cell” for a long time. Lately, mitochondria have also been shown as the primary site of reactive oxygen species (ROS) generation and to be involved in apoptosis. Mitochondria have its own DNA (mtDNA) and mtDNA encodes 13 mitochondrial structure protein. It has been shown that mtDNA damage by ROS causes various types of diseases such as cancer. Therefore, these damaged cells will provide valuable cell models to study oxidative stress and overcome ROS derived diseases. We established mtDNA depleted ρ^0 cells from HeLa and SAS cell lines and investigated the effect of ROS, especially hydrogen peroxide (H_2O_2) sensitivity. First, we performed H_2O_2 treatment on HeLa and SAS ρ^0 cells at a concentration of 0 to 100 μ M. Next, we investigated catalase gene expression and catalase enzyme activity. As a result, ρ^0 cells showed high sensitivity to H_2O_2 compared with its parental cells, even though the catalase activity of ρ^0 cells were up-regulated. We investigated cell membrane potential by DiBAC4 (3), lipid peroxidation by HNE immunostaining, endogenous H_2O_2 amount by HYDROP and uptake of H_2O_2 by stable isotope-containing H_2O_2 . The result showed that the membrane potential of ρ^0 cells was lower than their parental cells, the amount of HNE was elevated in ρ^0 cells as compared with their parental cells, internal H_2O_2 amount was significantly increased only in ρ^0 cells after 50 μ M H_2O_2 treatment for 1 h and uptake of H_2O_2 were increased in ρ^0 cells as compared with their parental cells. These changes in the membrane state, particularly lipid peroxidation, occurred in ρ^0 cells as compared with their parental cells, leads to increase membrane permeability of H_2O_2 . And the timing of the intracellular H_2O_2 concentration will be advanced. In conclusion, ρ^0 cells are considered to be more susceptible to H_2O_2 than their parental cells because the membrane statuses change.