

RESISTANCE TO CERAMIDE-INDUCED APOPTOSIS IN THE MITOCHONDRIAL DNA-DEPLETED C6 GLIOMA CELLS

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Mitochondria is one of the master regulators in apoptosis signaling pathway. Mitochondrial DNA (mtDNA)-depleted cell line has lower sensitivity to apoptosis which inducing by TRAIL (TNF-related apoptosis-inducing ligand). In this study, we created C6 glioma cells with mtDNA depletion by treatment with ethidium bromide (EtBr) and zalcitabine (ddC) for 72 hours. The mtDNA copy numbers were determined from cells treated with EtBr and ddC followed removing incubated reagent 72 hours. MtDNA copy numbers were determined by with real-time polymerase chain reaction (real-time PCR) and listed as followed list: control: 100%, 1 μ M EtBr: 52%, 25 μ M EtBr: 13%, 5 μ M ddC: 25%, re-1 μ M EtBr: 92%, re-25 μ M EtBr: 52%, re- 5 μ M ddC: 51%. We counted cell number for cell growth rate. The cell viabilities were also analyzed by MTS assay. Mitochondrial membrane potential ($\Delta\Psi$) analyzed by JC-1 staining indicated that EtBr groups had lowest $\Delta\Psi$. Contents of cellular ATP generation were determined represented that ditacted lower mtDNA copy number was not direct proportion to ATP generation. Estimated mitochondrial electron transport chain (ETC) proteins (ND6, Core 2 and F1 α) expression with Western blotting, the result indicated that cells harboring lower mtDNA copy number had lower mtDNA encoded protein, but mtDNA copy number restores and more higher than control cell after all drugs were removed. To confirme the influence of mtDNA depleting to cells, we observed the Cytochrome *c* (cyt *c*) translocation with confocol microscopy and calculated apoptotic proportion with flow cytometry. The result showed only the 25 μ M EtBr treatment group has conspicuous effect of cyt *c* translocation and higher apoptotic ratio. The cell apoptosis induced with 25 μ M ceramide showed that mtDNA-depleted cells were not susceptible for ceramide treatment. Moreover, cells incubated with 3 μ M cyclosporine A (MPT pore inhibitor, CsA) for 30 min before 25 μ M ceramide treatment showed lower apoptosis proportion occurred in all groups, this data indicated the cell apoptosis was induced by ceramide. According to our results, we suggest that decreased mtDNA copy number affected cell physiology and resisted to ceramide-inducing apoptosis.

Key words:

mtDNA copy number, EtBr, ddC, Cytochrome *c*, apoptosis, ceramide