Analysis of hydrogen peroxide resistance mechanism on hydrogen peroxide resistant cancer cells.

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<purpose>

Hydrogen peroxide is known as one of ROS which gives oxidative stress to cells and induces apoptosis. However, details of the mechanism for cancer cells by hydrogen peroxide is still unknown. We have established "hydrogen peroxide resistant (HR) cancer cells" that are resistant to high concentration hydrogen peroxide. The mechanism of resistance to hydrogen peroxide acquired by HR cancer cells has not yet been elucidated, however if the mechanism becomes clear, it could be applied to cancer treatment. In this study, therefore, we aimed to elucidate its hydrogen peroxide resistance mechanism and carried out the following experiment.

<method>

Cell lines that continued to survive against graded hydrogen peroxide treatment of HeLa (up to 70 μ M) and SAS (up to 35 μ M) were subjected to hydrogen peroxide at the concentrations of 0, 25, 50, 75 and 100 μ M respectively, and the cell viability was examined by WST assay. Subsequently, the endogenous catalase enzymatic activity of HR cancer cells was measured using Catalase Assay Kit (SIGMA). Furthermore, lipid peroxidation of HR cancer cells was analyzed by immunofluorescence using 4-hydroxynonenal (HNE) and 5-lipoxygenase (5-LOX) antibody. HNE is typical lipid peroxidation marker and 5-LOX is known as lipid peroxidase.

<results and discussion>

In HeLa and SAS parental cells, they survived to the extent of 25 μ M by hydrogen peroxide treatment. On the other hand, stepwise hydrogen peroxide-treated cells survived up to 100 μ M (HeLa) and 50 μ M (SAS), showing resistance to hydrogen peroxide. Analysis of catalase enzyme activity showed significant increase in HeLa HR cells compared with the HeLa parent, but there were no significant differences in SAS cells.

Furthermore, HNE and 5-LOX expression levels in HR cells were significantly decreased compared with the parental cells by immunofluorescent staining.

As mentioned above, the membrane lipid peroxidation is regulated by the expression of 5-LOX rather than a catalase activity. The decreased 5-LOX expression may suppress lipids peroxidation in plasma membrane and lead to retention viability to hydrogen peroxide in HR cells.