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Oxidative stress sensitivity of cancer cells could be altered by plasma membrane status

Oxidative stress is defined as “A status that the balance between the oxidation reaction and the antioxidant reaction is lost in our body, and that the oxidation state is dominant.” Oxidative stress is generally adverse for our bodies because it can impair DNA, protein, and lipids. Oxidative stress is caused by reactive oxygen species (ROS) such as active oxygen (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (•OH), and singlet oxygen ($^1O_2$), which are generated as a result of electron reduction of oxygen.

ROS are key molecules in maintaining cell proliferation, inflammation, and cell death. ROS protect us by killing bacteria that invade our bodies, but these are also involved in aging and may cause diseases such as Parkinson’s disease, Alzheimer’s disease, cancer, diabetes mellitus, and periodontal disease. In addition, when oxidative stress, such as due to radiation or drugs, is externally applied, the amount of intracellular ROS increases or cell membrane lipid peroxidization occurs and eventually results in cell death. The primary site of ROS generation *in vivo* is mitochondria. It has been revealed that ROS produced in mitochondria exhibit biological defense, as mentioned above.

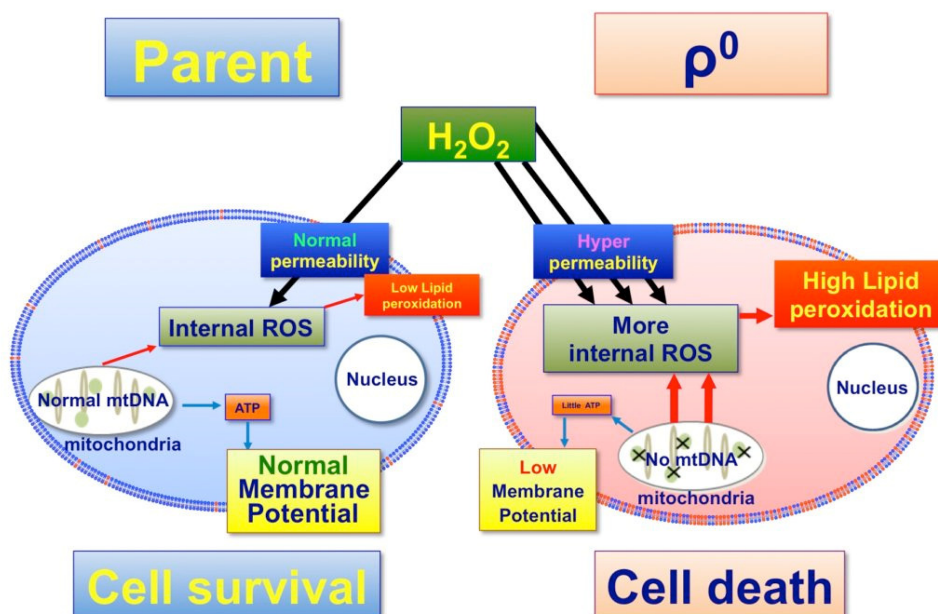

Fig. 1. The scheme of relationship between H2O2 and changes in plasma membrane peroxidation status in parent and ρ^0 cells. The red membrane lipids indicate lipoperoxide. Dysfunction of mitochondria in ρ^0 cells could reduce the plasma membrane potential through the production of ATP or accelerate membrane lipid peroxidation status, which could cause cell death by external H_2O_2 administration. The enhancement of lipid peroxidation in ρ0 cells might be explained by the increase in internal ROS via mitochondrial dysfunction due to depleted mtDNA (complete lack of mtDNA). (Tomita K. et al., J. Tohoku Med. Pharm. Univ. **64** 49-55 2017

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Mitochondria have their own DNA (mtDNA) that partly encodes oxidative phosphorylation component proteins, and mitochondrial dysfunction occurs when mtDNA is impaired. Therefore, mtDNA impaired cells can serve as valuable cell models for studying oxidative stress and overcoming ROS-derived diseases. In addition, H_2O_2 is recognized as a sensitizer of anti-cancer therapy. A further understanding of H_2O_2 action mechanisms in cell models is necessary for the development of new anti-cancer therapeutic agents, protocols, or strategies.

To analyze the mechanism of oxidative stress and ROS-derived diseases, mtDNA depleted cells (ρ^0 cells) were developed by treatment with low-dose ethidium bromide. We established two ρ^0 cell lines, namely cervical cancer (HeLa) and tongue squamous cell carcinoma (SAS).

We investigated whether established ρ^0 cells contain mtDNA, extracted RNA from the cells, and performed quantitative PCR (qPCR) for ND1, which is an mtDNA-encoded gene. Complete ND1 expression silencing was observed in ρ^0 cells. Next, we tested H_2O_2 sensitivity. Higher sensitivity was seen in ρ^0 cells than in their parental cells. We also investigated the activity of catalase, which is an enzyme that decomposes H_2O_2 in cells. In ρ^0 cells, higher catalase activity was observed despite the higher H_2O_2 sensitivity. The expression of other antioxidative enzyme genes was investigated, but their expression seemed unrelated to H_2O_2 sensitivity. We further investigated cell surface plasma membrane status using $DiBAC_4(3)$, a potential-dependent fluorescence dye. The fluorescence intensities were statistically lower in ρ^0 cells with plasma membrane potential. We confirmed the internal amount of H_2O_2 after H_2O_2 administration and found that H_2O_2 membrane permeability was facilitated in ρ^0 cells. We also found that the internal amount of H_2O_2 was higher in ρ^0 cells than in the parent cells after 1h of H_2O_2 administration.

Our results suggest that changes in the plasma membrane status induced by increased internal ROS generation provide a hyper-permeability of H_2O_2 in ρ^0 cells after H_2O_2 administration. The extracellular H_2O_2 administration to ρ^0 cells may lead to higher internal ROS generation including the external H_2O_2 permeating the cell. The lipid peroxidation of plasma membranes induced by internal ROS (including the external permeated H_2O_2) will eventually result in cell death.

These results suggest a new anti-cancer therapeutic protocol if the membrane peroxidation status can be changed using selective anti-cancer agents or mitochondrial functional inhibitors. In future, we will further investigate how membrane status may be used to enhance the therapeutic effect on cancer.

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