Thyrocytes are particularly well protected against oxidative stress induced by H2O2

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Background:
H2O2 produced in large quantities in the thyroid may play a role in the pathogenesis of thyroid nodules and cancer. In vitro, moderate amounts of H2O2 are able to cause similar DNA damage compared to irradiation and even to induce RET/PTC rearrangements.

Objective and hypotheses:
We compared the defence mechanisms against H2O2 and irradiation in human thyrocytes, T-cells and other cell types.

Method:
Human thyrocytes in primary culture were compared to other cell types: human T-cells in primary culture, a human thyroid epithelial cell line (Nthy-ori 3-1), non-transformed rat fibroblasts (F208) and a human myeloid cell line (PLB-XGCD) in terms of ability to degrade H2O2, glutathione peroxidase (GPx) activity, heme oxygenase-1 (HMOX1) expression, cell survival and capacity to repair DNA damage after H2O2 exposure or irradiation (O2-17 source).

H2O2 was measured in the medium by a sensitive fluorimetric assay. Cells were incubated overnight with BSO (Buthionine-sulfoximine, an indirect inhibitor of Glutathione peroxidase) before H2O2 or irradiation treatment. GSH peroxidase activity was measured for each cell type. qPCR were performed on cells after different treatments (H2O2, irradiation) to study regulation of the Heme oxygenase 1 (HMOX1) transcription. Alkaline COMET assay was used to measure total DNA damage after treatment with radiation or H2O2. Survival test were evaluated by MTS/PMS test and by FACs analysis.

Results

1) Thyrocytes are particularly efficient to degrade H2O2: Clearance of extracellular H2O2 added in the medium of thyrocytes and T-cells: 300.000 cells

2) Glutathione peroxidase protects thyrocytes against DNA damage induced by H2O2: Effect of BSO on global DNA damage

3) Comparison of GPx enzyme activity in different cell lines

4) H2O2 induces the expression of another antioxidant enzyme in the thyrocytic: the Heme oxygenase 1: RT-qPCR of HMOX1

5) How fast DNA damage repaired after H2O2 and irradiation treatment?

Conclusion:
Thyrocytes rapidly degraded extracellular H2O2 and presented a low mortality rate after H2O2 exposure. Thyrocytes had the highest basal GPx activity which was stimulated by H2O2. This effect was mimicked by tyrosine phosphatase inhibitor treatment. Expression of HMOX1 mRNA was up-regulated by H2O2 in thyrocytes but not in the other cells. HMOX1 expression and GPx activity were unchanged after irradiation in all tested cell types. DNA damage caused by irradiation was more rapidly repaired than that caused by H2O2 in all investigated cells. T-cells did not repair DNA damage caused by H2O2.

Thyrocytes have developed multiple mechanisms of protection against oxidative stress induced by H2O2. Our results suggest that deficiency of one of these mechanisms could promote the appearance of sporadic thyroid cancer. Due to their extreme sensitivity to H2O2, T-cells are probably not a good surrogate tissue to study individual susceptibility to H2O2.

References