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MEETING ABSTRACT

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12-Oxo-chenodeoxycholic acid potentiates doxorubicin-induced oxidative stress through Nrf2 axis in breast adenocarcinoma cells

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Background: As a transcription factor, nuclear factor E2-like factor 2 (Nrf2) controls the expression of genes encoding cytoprotective proteins, including antioxidant enzymes counteracting oxidative and electrophilic stress to maintain redox homeostasis. Aberrant activetion of Nrf2 in malignant cells promotes high expression of cytoprotective proteins, which can decrease the efficacy of antineoplastic agents used for chemotherapy. The aim of this study was to analyse the expression of *NRF2* gene as well as antioxidative system genes in a human breast adenocarcinoma cell line (MCF-7) treated with doxorubicin and the bile acid 12-oxo-chenodeoxycholic acid (12-monoketocholic acid, 12MKC).

Methods: The MCF-7 cell line was maintained in required microenvironmental conditions until confluence was reached. Cells were afterwards treated with 0.25 µM of doxorubicin (D group) or co-treated with 0.25 µM doxorubicin and 25 µM 12MKC (DM group). Following 24 h of incubation, cells were collected, RNA was isolated and transcribed into cDNA. The expression of the genes for Nrf2 (*NRF2*), superoxide dismutase (*SOD*), catalase (*CAT*), and β-actin (*ACTB*) as a housekeeping gene, was determined using RT-qPCR. Gene expression was analysed using comparative $2^{-\Delta\Delta C_T}$ method and statistical analysis was performed using Anova and Tukey's post-hoc test.

Results: Compared to untreated group of cells, treatment of MCF-7 cells reduced expression of *NRF2* both in the D and in the DM group: 2.74 ± 0.57 ($p < 0.001$) and 1.74 ± 0.59 ($p = 0.014$), respectively. Expression of *SOD* was also repressed in the D and in the DM group: 3.68 ± 0.78 ($p < 0.001$) and 1.11 ± 0.37 ($p < 0.001$), respectively. On the other hand, the expression of *CAT* was induced in the D group 1.50 ± 0.34-fold ($p > 0.05$), whereas supressed in the DM group 1.15 ± 0.39-fold ($p > 0.05$), compared to control.

Discussion: Oncogene-induced mutations with gain of function of Nrf2 promote both ROS detoxification and tumorigenesis, whereas suppression of Nrf2 in neoplastic cells and alters redox homeostasis of malignant cells. Through suppression of *NRF2*, *SOD* and *CAT* genes, 12MKC exerts potential to impinge cellular antioxidative defence at the transcriptional level in MCF-7 cells treated with doxorubicin, with potential favourable effects in terms of therapeutic outcome.

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