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Short communication

Changes in redox homeostasis in malignant cells induced by ursodeoxycholic acid and doxorubicin - transcriptional analysis

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Introduction

One of the hallmarks of cancer is the deregulation of cellular energetics and production of higher levels of reactive oxygen species (ROS). Cancer cells have adapted to the oxidative stress through various mechanisms allowing them to survive in hypoxia and to become drug resistant. This has given rise to different treatment strategies aiming to selectively enhance ROS production in cancer cells. One of the potential targets of antitumor therapy strategies is the nuclear factor erythroid 2–related factor 2 (Nrf2), a *cis*-active transcription factor that regulates the transcription of genes encoding antioxidant response elements (AREs) and genes of mechanisms involved in phase 2 and phase 3 of metabolism of xenobiotics. Nrf2 controls the basal and induced expression of an array of ARE–dependent genes to regulate the outcomes of oxidant exposure (Rojo de la Vega et al., 2018). Due to the amphiphilic structure, bile acids (BAs) are important promoters of the transport of pharmacologically active substances across biological membranes. In addition to the role of passive drug carriers, BAs are signaling molecules that regulate various aspects of cellular metabolism and homeostasis. Ursodeoxycholic acid (Udca) is a hydrophilic bile acid metabolite of chenodeoxycholic acid, formed by probiotic bacteria during intestinal biotransformation. Unlike hydrophobic BAs, Udca has long been considered a cytoprotective and tumor suppressive agent, with antioxidant and antiapoptotic properties (Pavlović et al., 2018).

The aim of this study was to assess the influence of the doxorubicin (Dox) and Udca on the changes in the expression of genes involved in antioxidant defense in breast adenocarcinoma cell line, MCF-7.

Materials and methods

Cell line and culture conditions - The cell line MCF-7 (ATCC® HTB-22TM) was cultivated in the Dulbecco's modified Eagle's medium (DMEM, Sigma-Aldrich ChemieGmbH, Munich, Germany) supplemented with 10% fetal calf serum (FCS, Sigma-Aldrich) supplemented with antibiotics (100 U/ml penicillin, 100 μg/ml streptomycin) (Sigma-Aldrich Chemie GmbH, Munich, Germany) at 37 °C and saturated air with 5% CO_2.

Drug treatment - Cells were seeded in T25 flasks at a density of $1{,}25x10^6$ cells/flask in DMEM supplemented medium and were cultured in a CO_2 incubator at 37 °C for at least 24 h. The culture medium was then replaced in experimental groups with fresh medium containing 0.25 μM of Dox or 0.25 μM of Doxplus 50 μM of Udca, and incubated for additional 24 h.

RNA isolation, cDNA synthesis, and reverse transcription quantitative PCR (RT-qPCR) - Total RNA was isolated from cultured cells using the GenElute Mammalian total RNA mini prep kit (Sigma-Aldrich) according to the manufacturer's instructions. RNA samples (1 μg) were reverse transcribed to cDNA using the Enhanced Avian First Strand Synthesis Kit (Sigma-Aldrich) according to the manufacturer's instructions. RT-qPCR was performed on StepOne Plus (Applied Biosystems) Real-Time PCR System using SYBR Green®. Relative transcript expression was determined using the $2^{-\Delta\Delta Ct}$ method, normalized to β-actin as a housekeeping gene. The qPCR primers used were: β-

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actin: F: 5'-gcaccacaccttctacaatg-3', r:tgcttgctgatccacatctg; *NRF2*: f: gtcagcgacggaaagagta, r: acctgggagtagttggca; *SOD* f: ctagcgagttatggcgac, r: gaatgtttattgggcgatc; *CAT*: f: cagcgaccagatgcagcac, r: atgcagagactcaggacg;*GR*: f: aacatcccaactgtggtcttcagc, r: ttggtaactgcgtgatacatcggg.

Statistical analysis - SPSS version 21 was used for statistical analysis. Data were expressed as mean ± standard deviation of the mean and analyzed using the paired student t test or one-way ANOVA, followed by Tukey post-hoc. A $p<0.05$ was considered statistically significant.

Results and discussion

Treatment of MCF-7 cells with Dox reduced *NRF2* gene expression 2.74±0.57 fold compared to the control group ($p<0.001$). Co-treatment with doxorubicin and Udca also significantly reduced *NRF2* expression compared to control, 1.94±0.42 fold ($p=0.004$). Co-treatment with doxorubicin and Udca increased the expression of this transcription factor as a positive regulator of protective cellular mechanisms including antioxidant protection, but the increase was not statistically significant compared to the group of cells treated with Dox alone. Dox treatment reduced *SOD* expression 3.68±0.78 fold ($p<0.001$), while dox and Udca co-treatment reduced *SOD* expression 4.78±1.03-fold ($p<0.001$). Treatment of MCF-7 cells with Dox increased *CAT* expression 1.50±0.34-fold compared to the control cells, however, no statistical significance was observed. Co-treatment with Dox and Udca increased *CAT* mRNA expression relative to the control cells ($p=0.004$), resulting in an increase in *CAT* expression of 2.12±0.49-fold. Dox treatment reduced *GR* expression by 3.26±0.71-fold ($p=0.002$) while co-treatment reduced expression by 19.8±4.30-fold compared to control ($p<0.001$). Due to genetic instability and metabolic reprogramming, malignant cells are characterized by a constitutively elevated level of pro-oxidative metabolism. Also, mutations in the gene encoding Keap1 result in increased expression of *NRF2* in malignant cells leading to increased transcription of antioxidant defense mechanisms and adaptation to elevated levels of oxidative stress. Studies reported an increase in *NRF2* expression in squamous cell carcinoma, adenocarcinoma, prostate cancer cell lines, and breast cancer cells, where *NRF2* has also been shown to be a common denominator for chemotherapy resistance (Syu, 2016). Thus, pharmacological reduction of *NRF2* expression in malignant cells is a modern concept of developing new therapeutic strategies in the treatment of oncology patients. Udca acid exhibits antioxidant and cytoprotective effects associated with various beneficial biochemical-physiological-pharmacological effects such as glycemic regulation and insulin sensitivity, obesity, hypertension, cardiomyocyte contractile dysfunction and liver disorders (Đanić et al., 2018; Stanimirov et al., 2015). However, the decrease in mRNA expression for the antioxidant enzymes *SOD* and *GR* in our study indicates the tissue specificity of the action of Udca. Reduction of *SOD* and *GR* mRNA expression in the context of dox-treated test cells has a positive effect with potential effects of chemo-sensitization. However, the mechanisms of interaction of Udca with doxorubicin need to be further elucidated to explain the obtained results.

Conclusion

This *in vitro* study indicates that Udca has the potential to modify the response of malignant cells to doxorubicin-induced oxidative stress at the transcriptional level and to enhance cytotoxic activity of Dox as a highly potent and widely used antineoplastic drug.

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