SCIENTIFIC REPORT

Executive abstract

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Grant of Ministry of Research Innovation and Digitization, CNCS - UEFISCDI
Host: Institute of Oncology "Prof.Dr. Ion Chiricuta" Cluj-Napoca, Romania

***Metallomic modulation of membrane transporters in chemoresistant ovarian cancer cells through nano-sensitizers* - MetallomeX**

Phase 3: January-December 2024

The objectives of phase 3 were the followings:

Resensitization of chemoresistant cells by core-shell type targeted nanostructures loaded with platinum and novel synthesized metal complexes
Clinical validation of the in vitro 3-D cell culture results

These objectives were achieved through the following activities:

Evaluation of the chemoresistant cells transformations following the sensitization with platinum-functionalized mesoporous nanostructures
In vitro experiments were completed to detect the optimal functionalization of mesoporous nanoparticles in order to modulate the ABCB1 target on ovarian cancer cells. By completing cytotoxicity testing, evaluation of membrane transporters expression, Elisa testing, DNA damage and cellular uptake measurements, we identified novel meso-substituted phenothiazinyl porphyrin complexes of metals Platinum, Indium and Zinc, functionalized with ferrocenylvinyl moiety. These structures are efficient against chemosensitive A2780 and platinum resistant A2780cis cells, by sensitizing the Nrf2-2, TNFα, ABCB1 pathways and triggering apoptotic cell death through NF-kB.

Evaluation of changes in ovarian tumor chemoresistance in a 3-D *in vitro* model obtained by co-cultivation with CD8+ lymphocytes
The OVCAR-3 chemoresistant ovarian cell line was cultivated as hanging drops to obtain an organoid-like tridimensional cell accumulation; the effector CD8-positive T lymphocytes were obtained through gradient density and immune-magnetic separation. The interactions between these structures were evaluated by spectrometry, flow cytometry and immune-enzymatic methods, which evidenced a relationship between ABCB1 overexpression and the CD8+ T cells activation through CD25, CD69 and the soluble form of tumor necrosis factor receptor superfamily member 18 (TNFRSF18), known as GITR.

Modulation of druggable molecular targets of ovarian cancer cells by SLC31A1- and ABCG2-mediated reprogramming of chemoresistant ovarian cancer cells

The SK-OV-3 ovarian cancer cell line was treated with the multidrug transporter ABCG2 inhibitor Ko-143, and the copper transporter SLC31A1 was blocked by its substrate, an anti-SLC31A1 antibody (polyclonal IgG isotype, COPT-1 O15431). The cytotoxicity and oxidative stress triggered by carboplatin and cisplatin in the treated SK-OV-3 cells was inferior to the untreated reference, without evidencing a relationship between the dose and the inhibitory effect, but with an increasing tendency in dynamics, in exposures exceeding 48 hours.

Study of the metal-induced cell death signaling in ovarian cells reprogrammed with antisense oligonucleotides - the chemoresistant A2780cis, OVCAR-3 and OAW-42 cell lines were subjected to custom-made anti-ABCB1/MDR1 antisense oligonucleotide, targeting mRNA, with the deoxyribonucleotide sequence GTCCAGCCCCATGGA; the carboplatin cytotoxicity and the SBA-15 mesoporous platinum-functionalized nanoparticles-induced cytotoxicity was evaluated in the presence or in absence of these modulations. The mesoporous platinum-functionalized structures activity was significant in all cell lines, in the presence or absence of pre-treatment. OWA-42 cell line, with a significant ABCB1 expression evidenced on its cell membrane, was influenced by the oligonucleotide treatment, and a plateau was observed after 24 hours.

Correlations between the residual platinum and the treatment outcome
Blood serum samples were harvested from the patients according to the IOCN Ethical Council approvals, following the written consent of the informed patient. Multielement analysis was performed on these samples, using the ICP-MS method, for quantitative assessment of Pt, Fe, Cu and Zn metals. Regardless of the histological type of the tumors, the distribution of the elements Fe, Zn and Cu was aberrant in the patients treated with carboplatin, the post-therapeutical metal accumulation was inversely correlated with the folate receptor and with the soluble form of SLC7A11, which indicates the occurrence of cell death mechanisms other than apoptosis.

Retrospective study
Paraffin-embedded post-treatment tissue samples of ovarian cancer patients will be selected, following the regulations of the Ethical Council; the samples will be recovered from paraffin, weighted and subjected to multielement analysis using the ICP-MS method. Data were collected from IOCN medical records with respect to confidentiality and the ethical statement; the clinical outcome of the patients was correlated with the standard histological markers such: CK7/ CK(AE1/AE3), PAX-8, p16, p53, WT-1,CA-125, estrogen receptor RE, Ki-67 and also with the expression of AbCB1 extrusion pump and with the SLC3A2 (CD98) solute carrier.

The relationship between the secreted soluble membrane transporter proteins level in blood samples of platinum-treated patients vs tridimensional ovarian cell cultures treated *in vitro*
Aliquotes from the human blood samples (serum) harvested for the multielement analysis were used under the frame of this activity for quantitative Elisa measurements of soluble form ABCB1, ABCG2, SLC31A1 and SLC7A11 proteins. The SK-OV-3 ovarian cancer cell line was cultivated on cylinder-shaped 3D structures, obtained by SolidEdge V20-driven printing from starting materials such Acrylonitrile butadiene styrene, polylactic acid and polypropylene. The cell cultures were treated *in vitro* with carboplatin, the from the cell culture supernates the same proteins were assessed, using the same methods and assay kits; significant correlations were found in soluble ABCB1 expression between the extracellular environment and the serum samples derived from patients having ovarian serous adenocarcinoma histological type.

The results of the research were summarized in the manuscript entitled: “Meso-substituted AB3-type phenothiazinyl porphyrins and their indium and zinc complexes photosensitizing properties, cytotoxicity and phototoxicity on ovarian cancer cells” accepted, Open Access article in RSC Medicinal Chemistry, edited by the Royal Society of Chemistry, UK, available at: https://pubs.rsc.org/en/content/articlelanding/2024/md/d4md00601a.

Part of the experimental results and new methods were comprised in the book chapter entitled: “The effect of ascorbic acid at cellular level: the biological basis of its interferences with chemotherapy in colorectal cancer” (original title in Romanian: Efectul acidului ascorbic la nivel celular si bazele biologice ale interferentelor acestuia cu chimioterapia in cancerul colorectal)
In the book: “Advancement and repurposing in colorectal cancer approach” (original title in Romanian: Progrese si repozitionari in abordarea cancerului colorectal) https://casacartii.ro/editura/carte/progrese-si-repozitionari-in-abordarea-cancerului-colorectal/ , editors: Ioan Cătălin Vlad and Irina Camelia Chiș, Casa Cartii de Stiinta, Cluj-Napoca, Romania, 2024, ISBN 978-606-17-2440-6.

The manuscript entitled: “Novel Schiff base-type platinum(II) complexes: syntheses, physical-chemical characterization and biological activities” was submitted (code JTAC-S-24-03783) and it is under evaluation at the Journal of Thermal Analysis and Calorimetry, Springer Nature.

The results were disseminated as oral or poster presentations at several international and national conferences in 2024, such as: The 9th International Symposium on Metallomics, London, Great Britain; the 11th International Conference on Oxidative Stress in Biology and Medicine”, Andros, Greece; the Days of the Institute of Oncology “Prof. Dr. Ion Chiricuta-95 years of activity”, Cluj-Napoca, Romania; The Annual Congress of the Romanian Society for Radiotherapy and Medical Oncology, Sinaia, Romania.

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