SCIENTIFIC REPORT

-Executive abstract

Project code PN-III-P4-PCE-2021-1572
Contract PCE105/2022
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 1

Metallomic mapping of chemoresistant ovarian cancer cells –part I

June 2022-December 2022

In the first phase of the project, the objectives were implemented through four activities:

Activity 1.1 - Integrative characterization of the SLC membrane transporters and ABC extrusion pumps in platinum-resistant ovarian cancer cells - phase I

To implement the project, the *in vitro* testings were started, by acquiring and characterizing the standard cell lines from authorized cell banks, in the first phase the ovarian cell lines OVCAR-3 and A2780cis were used. From the OVCAR-3 cell line the CD326 lineage was separated with immune-magnetic methods, and than three subsets were isolated based on the surface epitopes of the tumor cells: the ABCG2/CD338^{+}, CD90^{+} and CD133^{+} subcultures, and these populations were cultivated in parallel with their negative analogs. The microscope examination revealed the dissimilar morphology, proliferation rate and bidimensional association of the different subsets(Figure 10), and these finding were confirmed through viability measurements (MTT test) and protein content cuantification.

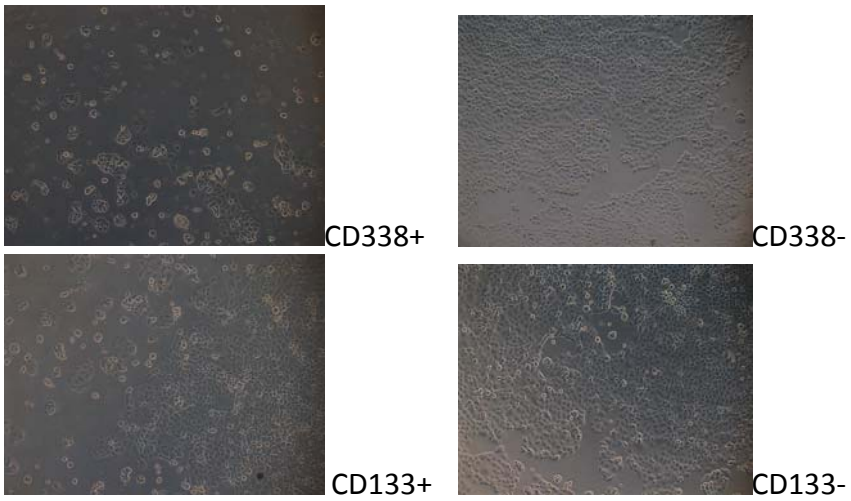

Figure 1. Dissimilar morphology and proliferation rate of CD338+/ABCG2+ versus CD338- cell subcultures (the upper row) and respectively, of the CD133+ vs CD133- (lower row); 10x magnitude images captured with the Observer D1 Zeiss microscope.

To characterize the behavior of the resistant ABCG2/CD338 population, the secreted folate receptor FOLR1, the IL-10 and IL-17 interleukins and the soluble stem cell factor (SCF) were followed through consecutive passages, and different time intervals, using the immune-enzymatic method ELISA (Figure 2).

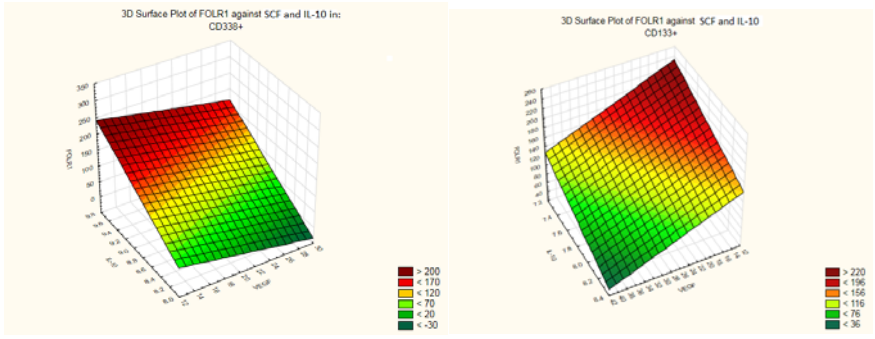

Figure 2. Tridimensional association between the secretome elements: sFOLR1, SCF, IL-10 in ABCG2-positiveand Cd133-positive chemoresistant cell subsets derived from OVCAR-3 parental line.

Several correlations were established by the biostatistic analysis, which proved a distinct secretion of FOLR1 in chemoresistant cells, as well as in SCF production, while IL-10 was relatively constant and less time-dependent. The results of the study resulted a scientific paper submitted to the Cytokine Journal (Elsevier editor), a journal indexed by Web of Science Core Collection, ISSN 1043-4666, IF 3.926.

Activity 1.2 - Membrane marker identification at single cell level

In the freme of these activity, in vitro membrane markers were identified in the A2780cis cisplatin-resistant cell line, from European Collection of Authenticated Cell Cultures (ECACC, Salisbury, UK), through flow-cytometry and confocal microscopy.
We examined the membrane expression of ABCB1 (ATP binding cassette subfamily B member 1) or CD243, the multidrug resistance marker, simultaneously with the transmembrane receptor CD44/Pgp-1 and Prominin-1 or CD133, the stem-like marker of tumor cells (Figure 3).
As well, the A2780cis chemoresistant cells survival, the modulation of the surface epitopes and the downstream functional changes of secreted ABCB1, CD133 and CD44 were examined when the cells were treated with core-shell magnetite-silica nanostructures coated with aminopropyltriethylsilane (AMPTS and functionalized with folic acid, which target the FOLR falate receptors on the ovarian carcinoma cells surface.

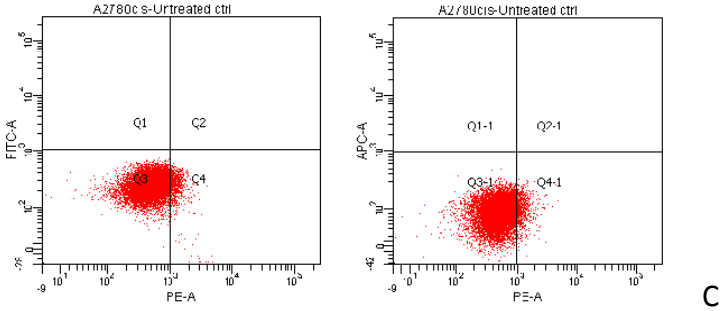

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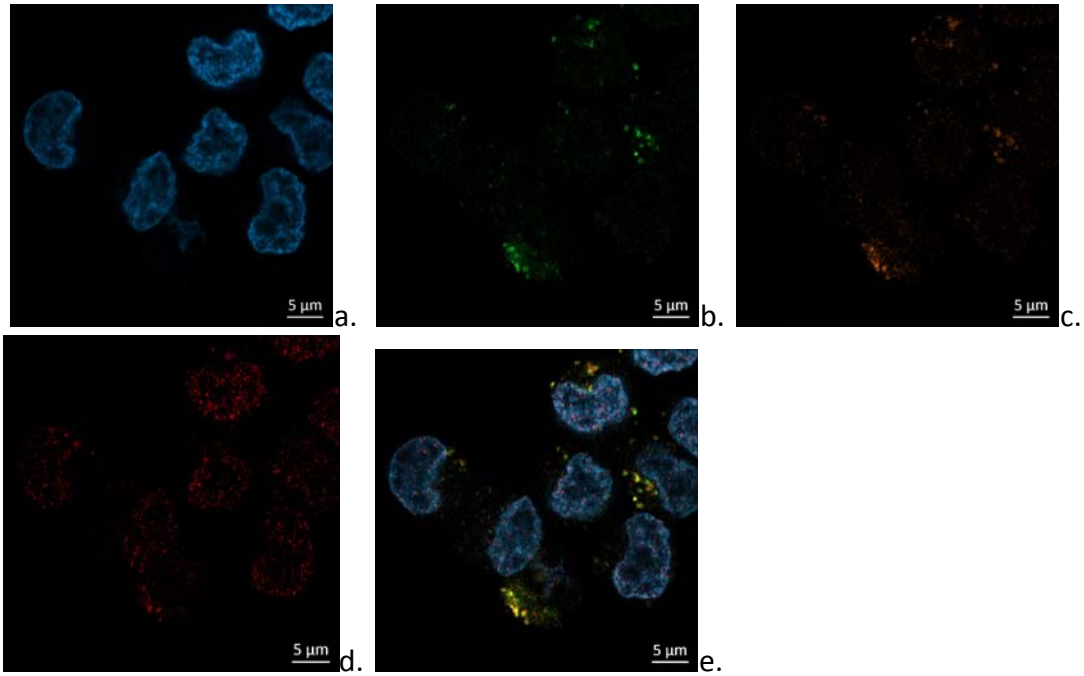
Figure 3. Bidimensional histograms, corresponding to untreated A2780cis chemoresistant cell population, tagged with ABCB1/CD243-PE, CD44-FITC and CD133-APC fluorescent antibodies.

The microscope evaluation was made following the cells staining with ABCB1/CD243-PE, CD44-FITC and CD133-APC antibodies and 4',6-diamidino-2-fenilindol dihodroclorid (DAPI) dye. The images were captured using an inverted phase LSM98 Airyscan 2 microscope with high resolution, in the laboratories of Zeiss Microscopy GmbH, Oberkochen, Baden-Wurttenberg, Germany.



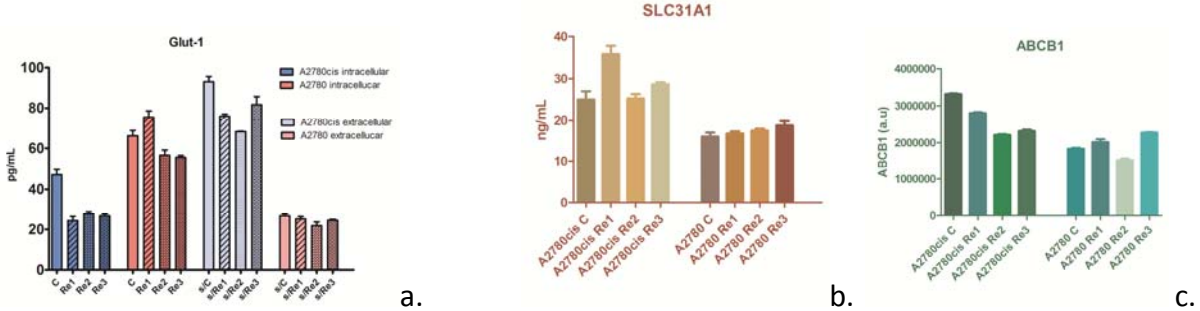
a. b. c. d. e.

Figure 4. Ultraresolution microscopy image of A2780cis cells: a. nuclei evidenced by DAPI; b. CD44 (green); c. CD133 (orange) and d. CD243/ABCB1 positivity e. superposed image depicting the presence of all epitopes.

Activity 1.3 - Evaluation of the soluble secreted SLC and ABC proteins

The solute carrier SLC transporters and the ABC extrusion pumps, implicated in drugs cellular uptake, were evaluated by evaluation the secretome of A2780 and A2780cis ovarian cancer cells in vitro, subjected to the treatment with a class of antitumor compounds, able to reduce the cells proliferation.

The soluble SLC31A1 (copper transporter 1, CTR1), Glut-1 (glucose transporter 1) and ABCB1 (MDR1) were quantified (Figure 5).



a. b. c.

Figure 5. Quantitative evaluation by Elisa testing of the modulation in Glut-1 (a), SLC31A1 (b) and ABCB1 (c) production in platinum-sensitive A2780 vesrus platinum-resistant A2780cis by the nano-denrimers with Rhenium central metal Re1, Re2 si Re3.

The cells were treated with three metallo-dendrimer nanostructures with Rhenium central metal, abbreviated as Re1, Re2 si Re3; their structure, characterization and antitumor potential were published by the team members previousely in the paper: "*Synthesis and antitumour evaluation of mono- and multinuclear [2+1] tricarbonylrhenium(I) complexes*" European Journal of Medicinal Chemistry, 2018, 157, 773-781, doi.org/10.1016/j.ejmech.2018.08.011
In the next phases of the project, the sudy will continue, in order to establish the Re cellular uptake, some molecular features and the mechanisms of tumor cells death, to highlight the involvement of SLC and ABC transporters in the metals cellular uptake.

Act 1.4 - Assessment of the intracellular metal elements in platinum-sensitive vs. platinum-resistant cells -phase I

The activity which aims to elucidate the intracellular metallomic mapping of the ovarian tumor cells was started in this phase, by harvesting the samples of A2780cis and OVCAR-3 cells, after prolonged proliferation and expansion of the cultures in vitro, in order to obtain a critical number of cells.
Ten elements were quantitatively measured from the samples and from their environment: Iron, Copper, Zinc, Lead, Cadmium, Mercury, Selenium, Manganese and Cobalt; three samples were prepared for each, and after appropriate mineralization and dilutions, they were measured using the Inductively coupled plasma mass spectrometry (ICP-MS) method, with a detection limit of: 0,05 µg/l for Hg, 0,1 µg/l for Cd, 0,5 µg/l for Pb, 0,5 µg/l for As, 0,1 µg/l for Mn, 0,1 µg/l for Co, 125 µg/l for Cu, 125 µg/l for Fe, 100 µg/l for Zn and 15 µg/l for Se, and the calibration curves allow the identification of these metals in the intervals: Hg 0 µg/l -1 µg/l; Cd: 0 µg/l - 3 µg/l; Pb: 0 µg/l - 10 µg/l; As: 0 µg/l - 10 µg/l; Se: 0 µg/l - 240 µg/l; Mn: 0 µg/l - 3 µg/l; Co: 0 µg/l - 3 µg/l; Cupru: 0 µg/l - 2000 µg/l; Fier: 0 µg/l - 2000 µg/l and Zinc: 0 µg/l - 1600 µg/l.
The aim of this activity is to identify the aberrations in metal distribution in the tumor cell populations, and the activity will continue with the multi-element evaluation in other two cell lines: SKOV-3 and OAW-42.

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