TARGETING GLUCOSE-6-PHOSPHATE DEHYDROGENASE WITH A COMBINATION OF LIPOSOMAL CISPLATIN AND 6-AMINO NICOTINAMIDE AS NEW STRATEGY TO OVERCOME CISPLATIN-RESISTANCE

Veronica Cocetta[1], Daniela Catanzaro[1], Eugenio Ragazzi[1], Gianfranco Pasut[2], Monica Montopoli[3]

[1]Department of Pharmaceutical and Pharmacological Sciences, University of Padua, Padua - Italy, [2]Department of Pharmaceutical and Pharmacological Sciences, University of Padua, Veneto Institute of Oncology IOV, IRCCS, Padua, Padua - Italy, [3]Department of Pharmaceutical and Pharmacological Sciences, University of Padua / Venetian Institute of Molecular Medicine, Padua - Italy

Ovarian cancer is one of the most aggressive and lethal gynecological cancers with a 5-year survival rate of approximately 30-40%. Currently, the first-line treatment for the advanced stages of ovarian cancer involves surgical resection followed by cycles of chemotherapy. Despite high initial response rates, patients relapse or chemoresistance occur frequently resulting in a therapeutic challenge. Among others, also dysregulated metabolism has been recently associated to cisplatin-resistance. It is known that resistant cells increase their demand for glucose, which is partially redirected toward the pentose phosphate pathway (PPP). G6PDH is the first and rate-limited enzyme of the PPP, and recent studies demonstrated that G6PDH was involved in cell growth modulation and carcinogenesis. In previous studies we demonstrated that the overexpression and increased enzymatic activity of G6PDH were correlated to ovarian cisplatin-resistant phenotype cells, suggesting that up-regulation of G6PDH activity could be a target to counteract cisplatin resistance. In fact, the combined treatment with the G6PDH inhibitor 6-amino nicotinamide (6-AN) and cisplatin (CDDP), exhibited a selective additive effect on cisplatin-resistant cells.Here we aimed to explore which metabolic pathways are exploited by human ovarian cancer cells resistant to cisplatin (IGROV1PT) to escape drug cytotoxicity in order to develop innovative strategies able to limit the onset of cisplatin-resistance, as well to reduce its side effects.

In order to characterize the metabolic profile of the IGROV1PT cell line, cells dependency from glucose to survival was evaluated. By qRT-PCR and Western blot techniques, the expression of the major glucose transporter (GLUT1) was analyzed; moreover, the uptake of glucose was investigated using the glucose analog 6-NBDG.
G6PDH parameters were also evaluated. mRNA and protein levels were analyzed and the G6PDH enzymatic activity was estimated using a G6PDH Activity Assay Kit. Inhibition of G6PDH with 6-AN, showed a restore in drug sensitivity. Then, to reduce cisplatin toxicity and ameliorate its pharmacokinetics, a lyophilized stealth liposomal formulation of cisplatin has been developed.

Interestingly, it has been observed that resistant cells switch their metabolism toward the glycolytic pathway showing a higher sensitivity to glucose deprivation and to glycolysis inhibition. mRNA expression of glycolysis enzymes revealed that IGROV1PT present higher mRNA levels compared to the wild type. In particular GLUT-1mRNA and protein expressions resulted up-regulated. In line with these data, IGROV1PT cells presented increased glucose uptake.
G6PDH mRNA and protein expressions were increased in IGROV1PT cells when compared to the sensitive counterpart. Also, the G6PDH activity was increased in IGROV1PT cells in comparison to IGROV1WT. In order to confirm the specific phenotype, cells were incubated with a competitive G6PDH inhibitor: 6-AN and results showed that resistant clone is significantly more sensitive to 6-AN compared to the sensitive clone.
Then, to reduce the toxicity of cisplatin and prolong its action, a lyophilized stealth liposomal formulation of cisplatin was developed. The combination treatment of liposomal cisplatin and 6-AN showed promising cytotoxic activity in drug-resistant cells.

Recent studies suggest that up-regulation of G6PDH activity could be a target to counteract cisplatin resistance. In fact, the combined treatment with the G6PDH inhibitors (6-AN or DHEA) and cisplatin, exhibit a selective additive effect on cisplatin-resistant cells. Present data support that treatment of liposomal cisplatin, especially in combination with 6-AN, can become a strategy to counteract cisplatin resistance.
This work shows that cisplatin/SSL4formulation has promising cytotoxic activities opening up new pharmacological perspectives against cisplatin-resistant cancers.