In vitro identification of molecular determinants that may enhance drug cytotoxicity in the testicular tumor germ cell line NTERA-2.

1)Rossini E.. 2)Fragni M.. 3)Vezzoli S.. 4)Berruti A.. 5)Memo M.. 6)Sigala S..

Università degli studi di Brescia

Testicular germ cell tumors (TGCTs) are the most common type of cancer in men between 20 and 40 years of age. Cisplatin-based chemotherapy is the mainstay drug therapy, although 20%–30% of the patients do not respond or relapse (Cierna et al., 2016). The mechanisms underlying cisplatin resistance seemed to involve the increase of glycoprotein P (PgP) efflux pump expression on tumor cell surface. Thus, evaluation of new cisplatin-based combination therapy with other drugs may help to identify new pharmacological approach to treat patients with TGCTs. In this study, using the in vitro experimental model of TGCTs, the Ntera-2 cell line, we evaluated the effect of cisplatin and we identify molecular markers that may represent new drug target.

Ntera-2 Clone D1 was purchased from ATCC® and cultured as suggested. Cells were treated for different times with increasing concentrations of cisplatin (0.1 - 20 μM) and/or palbociclib (0,001 – 10 μM). Cell viability was evaluated by MTT dye reduction assay. Total RNA was ex-tracted using the RNAeasy kit (Qiagen, Milano, Italia) and transcribed into cDNA. Gene expres-sion was evaluated by Q-RT-PCR (ViiA7, Applied Biosystem, Milano, Italy), using the SYBR Green as fluorochrome. Differences of the threshold cycle Ct values between the β-actin housekeeping gene and MDR1, PD-L1, CDK4 and CDK6 gene (ΔCt) were then calculated. Data analysis were performed using Graph Pad Prism 4 software. The synergism/antagonism assay was performed using the constant ratio method (cisplatin: palbociclib = 2:1); the Combination Index to evaluate synergism/antagonism and isobologram-multiple drug effect equation were determined using CompuSyn. (Chou et al., 2006).

Ntera-2 cell line was exposed to increasing concentrations of cisplatin for different times: our results indicated that cisplatin induced cytotoxicity on Ntera-2 cells with an IC50 of 2,9 μM (95% CI: 1.3-6.2 μM); this effect reached its maximum after 24h of treatment and it is maintained up to 48h. We then studied the gene expression of markers, namely CDK4/6, that is the target of CDK4/6 inhibitors such as palbociclib, PD-L1, that is the target of check-point inhibitor immune therapy and MDR-1, that is the gene encoding the PgP. Untreated Ntera-2 cells express high level of mRNa encoding CDK4/6, with a ΔCt of, respectively, 4,1 ± 0.3 and 8.1 ± 0.5, while MDR1 and PD-L1 mRNA are expressed at low level. Exposure of Ntera-2 cells to cisplatin at its IC50 for up to 48h induced an increase of mRNA expression of PD-L1 and MDR1, with no modification of CDK4/6 expression. Based on the high level of CDK4/6 expression, we investigated the effect of palbociclib on Ntera-2 cell viability. Our results demonstrated that palbociclib has it's maximum cytotoxicity at a concentration of 10 μM (percentage of mortality: 60 ± 2.9) To investigate the effect of cisplatin and palbociclib combination treatment, we ap-plied the Chou-Talalay method and we demonstrated that palbociclib was synergistic with cisplatin at high concentrations.

These results indicated that exposure of the Ntera-2 cells to cisplatin induced an increase of mRNA encoding MDR1 and PD-L1, thus suggesting that cisplatin-treated cells could become sensitive to drugs targeting (or insensitive) PgP efflux pump or the check-point inhibitor path-ways. Further, we demonstrated that combination of palbociclib and cisplatin exerted a syner-gistic effect. This result agree with the recently published comment on CDK4–CDK6 inhibitors, that suggest that these drugs may have limited value as monotherapy, but may offer greater promise when combined with other therapies (Sherr et al., 2017).

Cierna et al. (2016). Annals of Oncology 27: 300 – 305.

Chou et al. (2006). Pharmacological Review 58(3): 621-681.

Sherr et al. (2017). NEJM 375 (20): 1922-1923

This work was supported by a donation from Vanoli Basket "Memorial Michelangelo Barcella". M.F. was supported by a grant from the Italian Society of Pharmacology.