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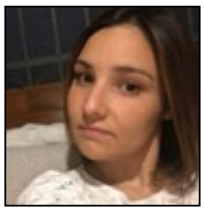
The CDK4/6 inhibitor palbociclib increases the cytotoxicity in the testicular germ tumor cell line NTERA-2



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Biography

Elisa Rossini is a PhD student in Section of Pharmacology, Department of Molecular and Translational Medicine, University of Brescia, Viale Europa 11, Brescia, Italys.

Introduction: 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 in the treatment of TGCTs and about 70–80% of the patients with disseminated testicular cancer can be cured with this treatment. Unfortunately, 20–30% of patients do not respond, or relapse. Because of not satisfying results in the treatment of relapsed TGCTs, evaluation of new treatment strategies and novel drugs with significant antitumor activity, as a single-agent or combination, is a priority.

Methods: Cell culture and treatment. Ntera-2 Clone D1 was purchased from ATCC® and cultured as suggested. Cells (2.0 x 104/well in 24 wells-plate) were treated with increasing concentrations of cisplatin (range: 0.001 - 10 μM) and/or palbociclib (range: 0.5 - 10 μM). Preliminary experiments of time-course have been conducted to establish the optimal duration of cell treatment. To evaluate the duration of cytotoxicity after drug suspension, cells were treated for 2 days with the IC50 value of cisplatin and/or palbociclib, then the medium was replaced with drugfree medium and cells were kept in culture up to 12 days. Cell viability was evaluated by 3- (4,5-Dimethyl-2-thiazol)-2,5-diphenyl-2Htetrazolium bromide (MTT) dye reduction assay and/or by ATP-Lite Luminescence Assay (PerkinElmer, Milan, Italy). Each experiment was performed at least 3 times and run in triplicate. For the evaluation of drug combination, the Chou- Talaly method was applied and the isobologrammultiple drug effect equation was determined using the CompuSyn software.

Gene and protein analysis. Gene and protein expression were evaluated respectively by q-RTPCR (ViiA7, Applied Biosystem, Milan, Italy), using the SYBR Green as fluorochrome; and by western blot, using the 4-12% NuPAGE bis-tris gel system (Life Technologies, Milan, Italy). Densitometric analysis of the immunoblots was performed using ImageJ software.

Data analysis were conducted with the Graph Pad Prism 5 software.Cell cycle analysis. Cells were treated with the palbociclib IC50, cisplatin IC50 and the drug combination for fortyeight hours. After treatment cells were treated with RNase, stained with propidium iodide (Life technologies, Milan, Italy) and analyzed by flow cytometry using a Macs Quant Analyzer (Miltenyi Biotec, GmbH, Germany) for cell cycle status. Data were analyzed using FlowJo (TreeStar).

Results: Ntera-2 cell line exposed to increasing concentrations of cisplatin displayed a concentrationdependent cytotoxicity, that reached its maximum at 48 hours of treatment, with the IC50 of 0.3 μM. Cisplatin exposure induced an increase of both RNA and protein of cdk6, with no modification of cdk4 expression. Based on this results, Ntera-2 cells were exposed to increasing concentrations of the cdk4/6 inhibitor palbociclib, inducing a cytotoxic effect, with the IC50 of 2.3 μM. Interestingly, the combination experiments applying the Chou-Talalay method, indicated that a synergism can be observed when cells are exposed to both drugs. The drug combination exerted a positive effect also for the cell recovery after the toxic insult elicited by these anticancer drugs: indeed, when cells were exposed to both drugs at their IC50, the latency of cell proliferation recovery lasted up to 8 days and cells returned to the untreated cell proliferation rate 10 days after drug withdrawal, while the latency was inferior (up to 5 days) when cells were treated with cisplatin orpalbociclib alone. To evaluate palbociclib activity, gene and protein expression of cdk4/6 and their downstream targets Rb/pRb were measured. Accordingly to the palbociclib mechanism of action, pRb was reduced in Ntera-2 cells after palbociclib treatment, while no modifications were detected in gene expression of cdk4/6, while an increase of protein expression was observed. In order to evaluate cisplatin and palbociclib mechanism of action, cell cycle analysis was conducted. Results indicated an increase in the proportion of cells at the G0/G1 phase after 48 h of palbociclib treatment (untreated cells: 26.99% ± 4.51%; palbociclibtreated cells: 45.84% ± 3.06), and an increase in the proportion of cells at the G2/M phase after cisplatin treatment (untreated cells: 42.41 % ± 19.27%; cisplatintreated cells: 71.35 % ± 3.29%). Cell exposure to the drug combination modified the cell cycle distribution, indeed we measured 14.0% ± 1.91% cells in G0/G1 cell cycle phase and 71.20% ± 7.31% cells in G2/M cell cycle phase.

Conclusions: The cytotoxic effect of cisplatin in Ntera-2 cells was increased when the drug was combined with the cdk4/6 inhibitor palbociclib. Accordingly, palbociclib is currently used as combination therapy in the breast cancer therapy, in association with aromatase inhibitors or fulvestrant. These results could give the background and the rationale to develop further studies in order to improve the pharmacological therapy of testicular cancer.

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