Targeting cancer drug resistance by modulation of ERCC1-XPF and p53 activity

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Abstract

New disruptors of the ERCC1-XPF interaction interaction have a synergistic effect with traditional NER inhibitors, in p53 positive cells. Furthermore, the synergy can be resumed in p53 negative cells upon reactivation of the TP53 gene.

Introduction

The ERCC1-XPF 5'-3' DNA endonuclease complex is involved in the nucleotide excision repair (NER) pathway, which is one of the key mechanisms responsible for resistance development to chemotherapeutic agents.[1-4] A strategy to improve effects of traditional crosslinking drugs and reduce development of resistance is to target both the endonuclease and p53. These proteins play a key role in the efficacy of DNA damage induced apoptosis. Indeed, a loss of function in p53 allows cells to survive despite great genomic impairment.[3] The overall objectives of the present study are: i) to shed light on the above-mentioned mechanisms, ii) to evaluate, by *in vitro* tests, the efficacy of *in silico* predicted ERCC1-XPF inhibitors[1,4] on both p53 WT and p53 negative cells.

Materials and Methods

Using cytotoxicity studies, synergy between inhibitors and DNA damaging drugs has been assessed. MTT assays were performed and the dose-response curves were fitted with the aid of software CompuSyn, to quantitatively assess the level of synergy for specific drug combinations. Doing so, the key parameter CI (combination index) was obtained, whose value indicates if synergy is present or not. CI95 used in the analysis were mean values from at least seven experiments with various ratios of compounds, and S.E.M. was used to represent errors. Limits of CI95 for synergy determination were set as synergy: (<0.9), additivity (0.9<CI95<1.1), and antagonism (>1.1). PLA was performed on p53 WT cells for further investigation on the nature of the drug synergy, targeting the complex ERCC1-XPF. This allowed to observe the abundance and the location of the complex in the cell trough fluorescence using a ZEISS Axio Scan.Z1 slide scanner (ZEISS, Oberkochen, Germany). Moreover, Annexin-PI apoptosis studies were performed to investigate whether p53 reactivators could restore synergy in p53 negative cells. The apoptosis assay was performed using an AnnexinV-Fluos staining commercial kit (Roche) sample analysis has been performed d by FACS (Fortessa, BD Biosciences, Franklin Lakes, NJ, USA). Percentage of living cells (propidium iodide and AnnexinV negative) was directly linked to the activity of drug combinations. Statistical analysis was performed using one-way ANOVA tests.

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Key words: Drug resistance; ERCC1-XPF; p53; cancer therapy.

Acknowledgments: I wish to acknowledge my group, Dr. Jordheim's and Bruno Chapuis and Denis Ressnikof at CIQLE.

Disclosures: Authors have nothing to disclose.

Conference presentation: This paper was presented at the Third Centro 3R Annual Meeting - L'era delle 3R: modelli *in silico, in vitro* e *in vivo* per promuovere la ricerca traslazionale - 30 September - 1 October 2021, Evento online organizzato dal Politecnico di Torino.

Received for publication: 9 July 2021.
Accepted for publication: 7 September 2021.

Biomedical Science and Engineering 2021; 4(s1):164
doi:10.4081/bse.2021.164

Results

MTT data provided evidence for a possible synergistic effect between compounds, that correlates with affinity values from the in silico predictions.[1] On two p53 WT cell lines, the CI value of new compound combinations seemed to reflect a synergistic effect. Moreover, the synergy itself, was evident in the p53 WT cells, but not in the mut-p53 cells. From the PLA, shown in Figure 1, the new compounds (A4 and B9) were macroscopically observed to reduce the ERCC1-XPF interaction, even in presence of cisplatin, which upregulates this complex. Therefore, experimental results demonstrated that inhibition of the ERCC1-XPF complex is stronger upon usage of the new compounds (A4 and B9), compared to the reference compound (F06).[4] Furthermore, new preliminary data from in vitro cytotoxicity studies and FACS Annexin-PI evidence how the synergistic mechanism can be re-induced in p53 negative cells by using p53 inductors.

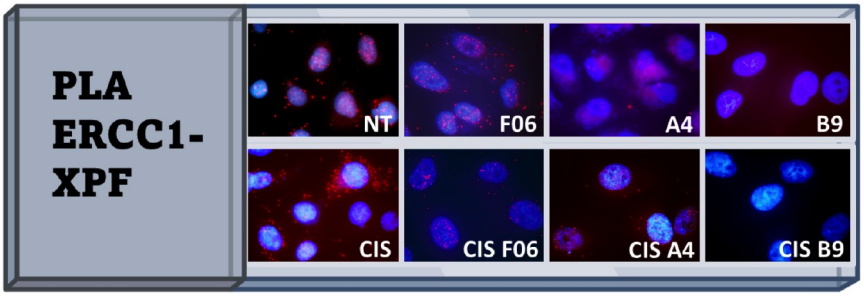

Figure 1. PLA assay on p53 WT cells treated with the inhibitors (F06, A4, B9), also in combination with cisplatin. The ERCC1-XPF interaction complexes are visible as red dots.

Discussion and Conclusions

Cisplatin and MMC are widely used cancer chemotherapy, which are active in several tumor types. However, cisplatin-based treatments are discontinued for many patients because of the associated drug resistance or toxicity. Therefore, to address these issues, the new inhibitors can be employed, tuning the mechanisms described, and adopted as a new route to target cancer and address drug resistance issues, paving the way to development of innovative treatments for clinics. Furthermore, bypassing p53 involvement in the mechanism could finally overcome drug resistance occurrence even in p53 negative tumors, which is nowadays common upon usage of DNA damaging drugs, helping to improve the efficacy of these treatmentsin the clincial setting.

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