

1108 **SCREENING FOR IMMUNE RESPONSE INDICATORS AFTER P53 GENE TRANSFER AND CABAZITAXEL TREATMENT IN HUMAN PROSTATE CARCINOMA *IN VITRO***

¹Nadine Assis*, ²Bryan Strauss. ¹Universidade de São Paulo, São Paulo, Brazil; ²Instituto do Câncer do Estado de São Paulo, São Paulo, Brazil

Background Prostate cancer is among the most common cancers in men worldwide¹ and despite current treatment options, patients with metastatic disease have a five-year survival rate of only 31% in the United States.² Taxane chemotherapy is the standard of care for metastatic Castration-Resistant Prostate Cancer (mCRPC) with cabazitaxel as second-line treatment, however, it causes grade ≥ 3 adverse effects such as neutropenia, leukopenia and anemia.³ Our group has previously shown that p53 gene transfer sensitized prostate cancer cells to cabazitaxel, permitting the application of lower drug doses, thus avoiding toxicity, while still achieving complete inhibition of tumor progression in a mouse model.⁴ Integrating our encouraging results with recent findings on the relevance of p53 reinstatement in initiating an anti-tumor immune response^{5,6} here we present data related to the validation of experimental conditions, examination of cell death and gene expression analysis of indicators of immune response in human mCRPC cells *in vitro*.

Methods PC3 human carcinoma cells were transduced with AdRGD-PG-p53, AdRGD-PG-eGFP or AdRGD-PG-Luc non-replicative serotype 5 adenoviral vectors and treated with the 25% inhibitory concentration (IC₂₅) of cabazitaxel. Flow cytometry was used for assessment of vector transduction efficiency after transduction with the GFP vector as well as evaluation of hypodiploid cell population after p53 gene transfer and treatment with low-dose cabazitaxel, which was complemented with MTT assay for determination of cell viability. Screening for indicators of immune response was performed by quantitative PCR for genes involved in recognition of tumor cells by immune cells, IFN signaling and antigen processing and presentation in the tumor cells after combined treatment.

Results Combined treatment led to an approximately 50% reduction in cell viability, showing a potentiation of the cytotoxic effects of each treatment alone. Induction of p53-target genes (e.g. CDKN1A, HDM2 and NOXA1) was confirmed together with the modulation of genes of immune activation pathways (e.g. TLR3, IRF1, ISG15 and TAP1). Genes involved in the recently described Viral Mimicry Response (e.g. HERV-E and DNMT1) were also shown to be modulated, mainly as a result of p53 restoration in our model.

Conclusions Both cabazitaxel treatment and p53 gene transfer were shown to display important reduction in viability of PC3 cells, especially when combined. Modulation of important immune response mediators, at least at the mRNA level, encourages us to proceed to protein and immunology assays.

Acknowledgements This work has been supported by the São Paulo Research Foundation (FAPESP). We thank Fernanda Antunes and Nayara Gusmão Tessarollo for technical assistance.

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<http://dx.doi.org/10.1136/jitc-2022-SITC2022.1108>