GENE THERAPY WITH P19ARF AND INTERFERON-BETA SENSITIZES CELLS TO PD-1 CHECKPOINT BLOCKADE AND ENHANCES ANTITUMOR IMMUNE RESPONSES IN RESISTANT MOUSE MODELS

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Background Innate and acquired resistance are major concerns that limit the patient’s response to immune checkpoint therapy. Immune-boosting gene therapies have been shown to improve response in models of checkpoint blockade resistance. Our group has previously shown that a combined gene therapy with p19Arf and interferon-beta promotes immunogenic cell death in melanoma models. However, even responsive mice often relapse, which we hypothesize to be partially due to PD-L1 superexpression. Thus, by combining our approach with anti-PD-1/PD-L1 monoclonal antibodies, we aim to overcome limitations of the gene therapy and increase sensibility to checkpoint blockade in resistant models.

Methods B16F10 cells were treated in vitro with non-replicating adenoviral vectors and were analyzed by flow cytometry. Tumor-bearing mice were treated in situ with the gene therapy and PD-L1 expression was analyzed by flow cytometry, RT-qPCR, and immunohistochemistry. Furthermore, tumor-bearing mice received intraperitoneal injections of the monoclonal antibodies and follow-up continued until reaching 1cm³. Blood serum was then collected for cytokine analysis and tumors were excised for immunophenotyping. Alternatively, B16OVA tumor-bearing animals were euthanatized on treatment day 12 and the splenocytes were pulsed with ovalbumin to assess specific tumor antigen presentation via MHC-I by antigen presenting cells by flow cytometry. Lastly, five different melanoma human cell lines were treated with the vectors and PD-L1 surface expression was analyzed by flow cytometry.

Results The analysis showed upregulation of PD-L1 both in vitro and in vivo, which led us to combine the gene therapy with PD-(L)1 inhibitors. In the in vivo model of B16F10, which is a known resistance model for PD-1 blockade, there was an increase of 10% and 20% responders when associating the gene therapy with anti-PD-L1 and anti-PD-1, respectively, in comparison to the gene therapy alone. Tumor immunophenotyping and cytokine profiling showed increased innate immune cell infiltrates in the conditions treated with the vectors and a high increase in NK cells and IFN gamma sera levels in the combination with both gene therapy and anti-PD-1. The gene therapy also increased tumor antigen presentation via MHC-I by antigen presenting cells, compared to mock group. Nevertheless, the gene therapy increased PD-L1 surface expression in all melanoma human cell lines analyzed.

Conclusions These data show that our immunogenic gene therapy approach increases sensibility to PD-1 and PD-L1 blockade in resistant mouse melanoma models and generates tumor antigen-specific immune responses, with further improved effector immune activity when combining it with checkpoint blockade.

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REFERENCES

Ethics Approval All procedures and conditions have been approved by the Ethics Committee for Animal Use (CEUA, FMUSP) under the protocol number 1300/2019 and by the National Technical Committee on Biosafety (CTNBio) under the process number 01250.034644/2019-31.