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927/10: “Deep Proteomic Profiling of CD4+ and CD8+ T Cell-depleted Tumor Models in PD-1 Treatment”

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Background

Immunotherapies targeting the PD-1/PD-L1 axis have been shown to be effective in only around 20% of cancer patients and the mechanism of action (MOA) underlying the differences between responders and non-responders remains poorly understood. It is therefore critical to understand the roles of different lineages of immune cells play mediating PD-1 response, while gaining an overall understanding of the interplay between tumor microenvironment (TME) and immune cell populations. To address this, tumor-bearing syngeneic mice were treated with an anti-PD-1 antibody in combination with targeted CD4+ or CD8+ T cell depletion. Tumor tissues were subsequently investigated with an unbiased proteomics workflow based on data-independent acquisition (DIA) mass spectrometry to provide insights into TME in different treatment contexts.

Methods

T cell subpopulations were depleted in two subcutaneous murine syngeneic models (MC38, and Hepa 1-6) followed by anti-PD-1 treatment (10mg/kg). The depletion of CD4+ and CD8+ T cells were accomplished with anti-CD4 (GK1.5) and anti-CD8 (2.43). The effectiveness of each depletion was assessed by tumor growth, followed by flow cytometry analysis of tumor infiltrating lymphocytes. FFPE tumor tissue samples, taken at the end of study (17-20 days), were digested using standard procedures and analyzed using 4h gradients on a C18 column coupled to a Thermo Scientific Q Exactive HF-X mass spectrometer. Data were extracted using Spectronaut™ (Biognosys) with a sample specific library.

Results

Consistent with expectation, depletion of CD8+ T cells significantly attenuated the antitumor effects of anti-PD-1 treatment in both tumor models, confirming the crucial roles of CD8+ T cells in tumor cell killing and inhibition of growth. However, depletion of CD4+ T cells showed opposing effects in the two models tested with a significant enhancement of anti-PD-1 efficacy in MC38 tumors and a reduction of anti-PD-1 efficacy in Hepa1-6 tumors. These effects were further studied by a deep proteome analysis (>9’000 proteins quantified. Statistical analysis

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showed large differences on proteome level between the two models (>3,000 proteins differentially expressed). We observed a strong upregulation of IFNγ inducible proteins upon anti-PD-1 therapy in the MC38 model, which is boosted upon CD4+ depletion, while many IFNγ pathway related markers were downregulated in the combo group of anti-PD-1 and anti-CD4 in Hepa 1-6. The data of the proteome analysis will be correlated to additional data from RNAseq analysis.

Conclusions

This study shows an unequivocal role of CD8+ T cells in anti-PD-1 induced tumor growth inhibition whereas the role of CD4+ T cells depends on the specific tumor type and TME. In our study we demonstrate how a deep proteome analysis improves the understanding of the interdependency of immune cells and the TME.

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