Uncovering molecular actors of IDO-mediated T cell dysfunction with genome-wide CRISPR/Cas9 knockout screens

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Introduction

Despite tremendous progress in cancer immunotherapy, most patients fail to benefit because of poorly characterized immune resistance mechanisms. Among these, expression of the tryptophan-catabolizing enzyme Indoleamine 2,3-dioxygenase (IDO) has been found in several tumors and associated with local immune suppression, notably by inhibiting T cell functions. However, the exact molecular pathways making T cells sensitive to IDO are still unclear. We propose to exploit the power of whole-genome single-guide RNA (sgRNA) CRISPR screens to uncover novel mechanisms of IDO-mediated T cell dysfunction.

Methods

Cas9-expressing transgenic mice were crossed with mice expressing a transgenic T cell receptor (TCR) specifically recognizing a tumor antigen called P1A. Primary Cas9xTCRP1A $CD8^+$ T cells were isolated from these mice and stimulated with P1A-expressing tumor cells. Cells were then transduced with the Teichmann retroviral genome wide CRISPR knockout library. Seven days after the first stimulation, $CD8^+$ T cells were re-stimulated in a control ($Tryptophan^{high}$, $kynurenine^{low}$) or selective ($Tryptophan^{low}$, $kynurenine^{high}$) medium, mimicking the function of IDO *in vitro*. After four days of screening selection, genomic DNA was extracted from remaining living cells and sequencing libraries were prepared. sgRNA representation was then assessed by next-generation sequencing.

Results

In vitro CRISPR knockout screening pipeline was successfully set up and validated. This includes: (1.) Successful transduction of the CRISPR knockout library, as well as efficient gene knockout in Cas9xTCRP1A primary $CD8^+$ T cells. (2.) Confirming the strong inhibition of T cell proliferation and survival in the selective medium as compared to the control medium, thereby validating the screening selection strategy. (3.) Optimizing genomic DNA extraction procedure, as well as sequencing library preparation. Finally, the proposed *in vitro* CRISPR knockout screening was successfully launched, and analysis of potential candidate genes is ongoing.

Conclusions and perspectives

Our study proposes to perform genome wide CRISPR knockout screens in T cells exposed to conditions mimicking IDO activity *in vitro*. Enriched sgRNA at the end of the selection should reveal genes whose inhibition improved T cell survival in $Tryptophan^{low}$/$kynurenine^{high}$ medium. Resulting potential candidate targets will then be validated by knocking them out in T cells and assessing T cell functions under conditions mimicking IDO-mediated immune suppression *in vitro.* Similar validation of top candidate genes will also be performed *in vivo* in a model of adoptive cell transfer of target-knockout T cells in mice bearing IDO-expressing tumors. The identified genes/pathways involved in T cell sensitivity to Tryptophan shortage / Kynurenine enrichment should reveal new relevant mechanisms of IDO-mediated immune suppression in the tumor microenvironment.