DECIPHERING THE ROLE OF TRANSCRIPTION FACTORS IN HUMAN ANTI-TUMOR CD8 T CELL DYSFUNCTION

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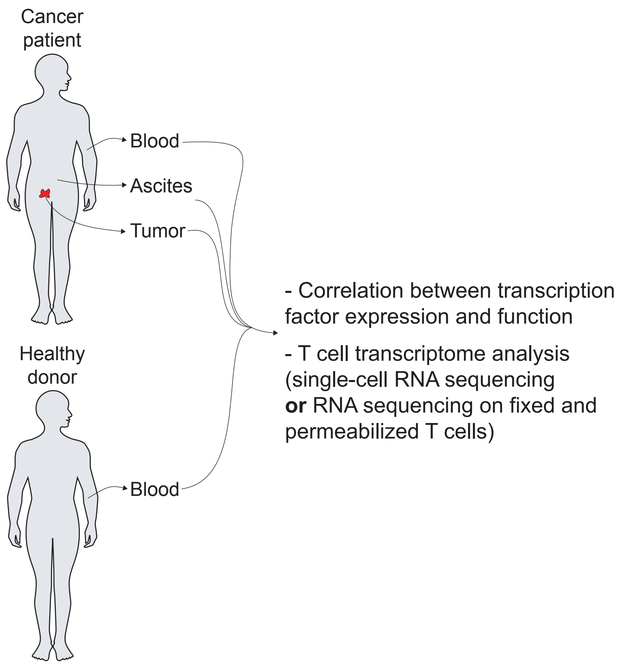
Abstract

Studies in mice, particularly those using chronic LCMV infections, identified expression patterns of transcription factors that characterize dysfunctional or exhausted murine CD8 T cells[1,2]. However, published data about the role of some of these transcription factors implicated in T cell exhaustion are not clear. We intend to approach this question from a different perspective. First, we analyze transcription factor expression patterns in *ex vivo* human CD8 T cells obtained from the blood, ascites and solid tumors of ovarian carcinoma patients by flow cytometry. We correlate observed expression patterns to the loss of functional markers, such as cytokine production or granzyme B expression. We then analyze the impact of selected transcription factors on T cell function *in vitro* by inducing or downregulating their expression in tumor-specific human CD8 T cell clones using lentiviruses. Finally, these modified T cell clones will be compared to their control counterparts for their ability to control tumor growth in a human xenograft model.

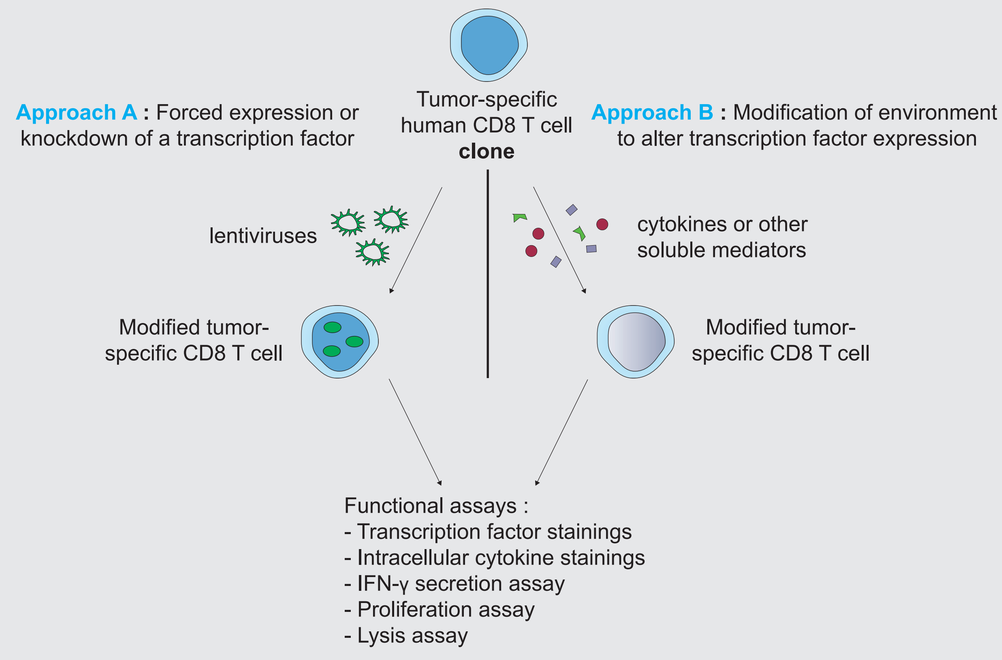
1. Laura M. McLane, Mohamed S. Abdel-Hakeem, and E. John Wherry. (2019) CD8 T Cell Exhaustion During Chronic Viral Infection and Cancer. Annual Review of Immunology 37:457-95
2. Renata M. Pereira, Patrick G. Hogan, Anjana Rao, and Gustavo J. Martinez. (2017) Transcriptional and epigenetic regulation of T cell hyporesponsiveness. Journal of Leukocyte Biology 102(3), 601-615

Project plan

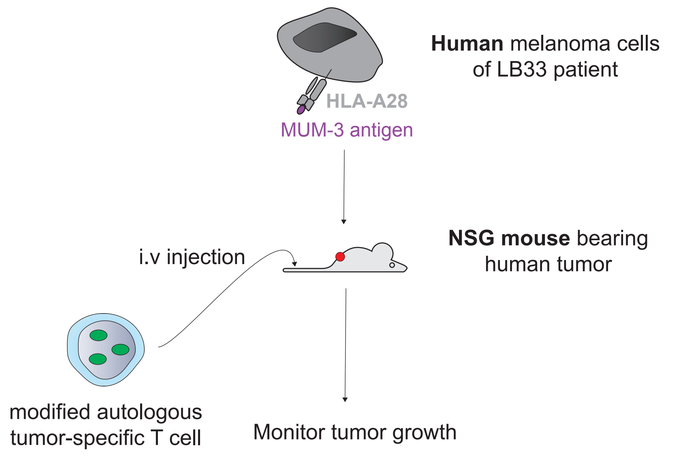
1. *Ex vivo* analysis of transcription factors



2. *In vitro* analysis of transcription factors



3. *In vivo* analysis of transcription factors

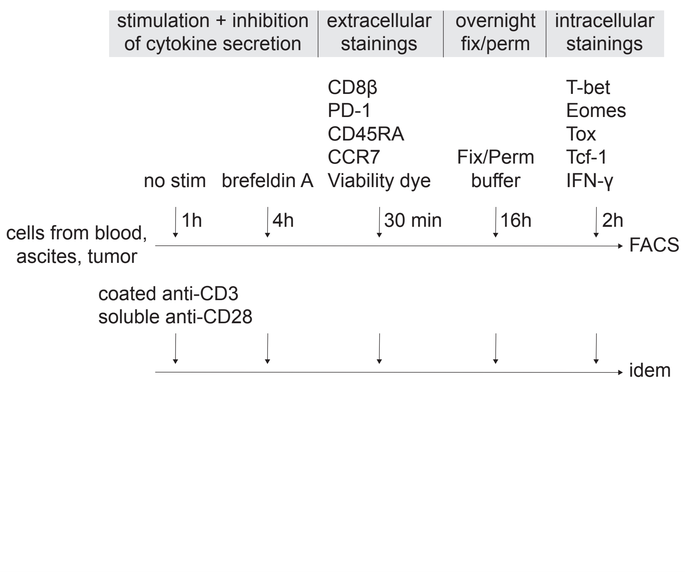


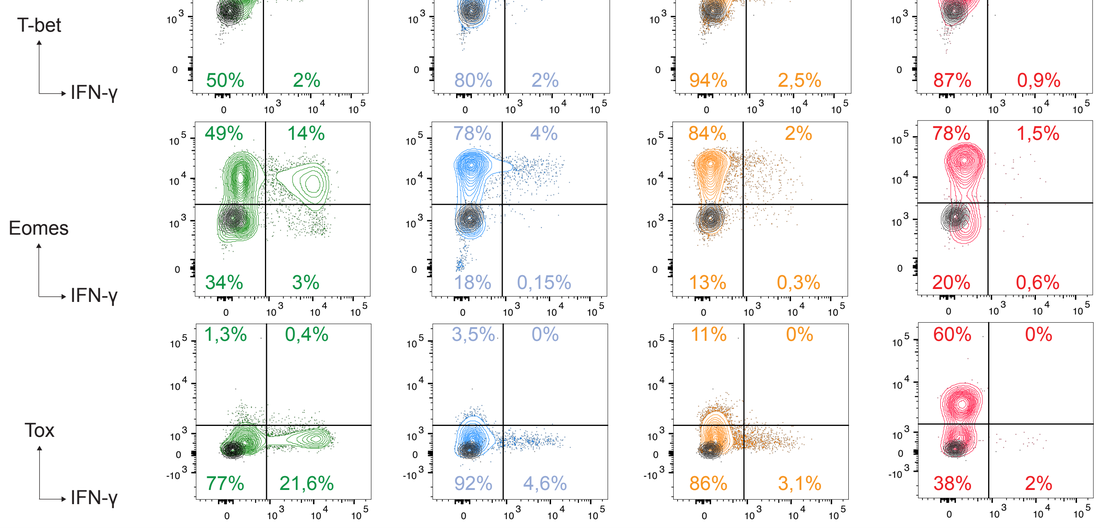
N.B. No data linked to this part of the project have been generated yet. This part is shown here only for perspective.

Results and Perspectives

1. Ex vivo analysis of transcription factors

Protocol - IFN-γ production assay and transcription factor stainings





T bet⁺ CD8 T cells producing IFN-γ are present in healthy donor but not in LB6151 cancer patient.

Eomes⁺ CD8 T cells produce IFN-γ in healthy donor and much less in LB6151 cancer patient.

In the solid tumor, CD8 TILs contain a Tox+ population unable to produce IFN-γ.

PERSPECTIVES

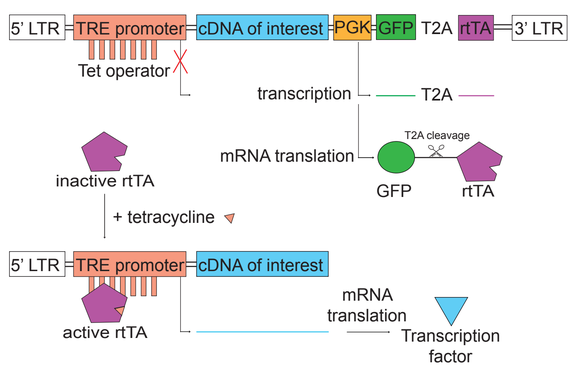
cells from blood, ascites, tumor
↓
activation with coated anti-CD3
↓
FACS stainings for isolation of a population of interest (***e.g*** : Tox⁺ in tumor ***vs*** Tox⁻ population in ascites/blood)
↓
Transcriptome analysis

2. In vitro analysis of transcription factors

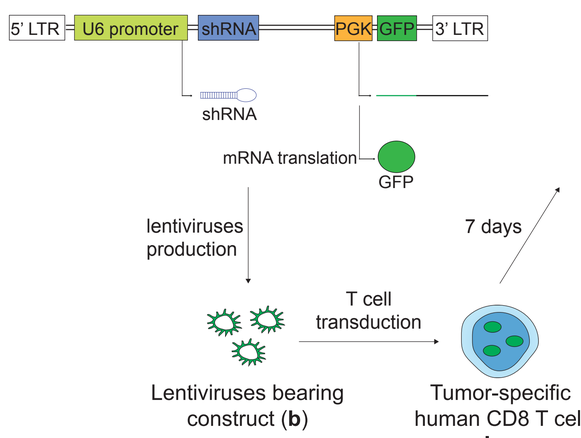
Approach A : Forced expression or knockdown of a transcription factor

DNA construct allowing inducible expression (a) or constitutive knockdown of a transcription factor (b) using lentiviruses

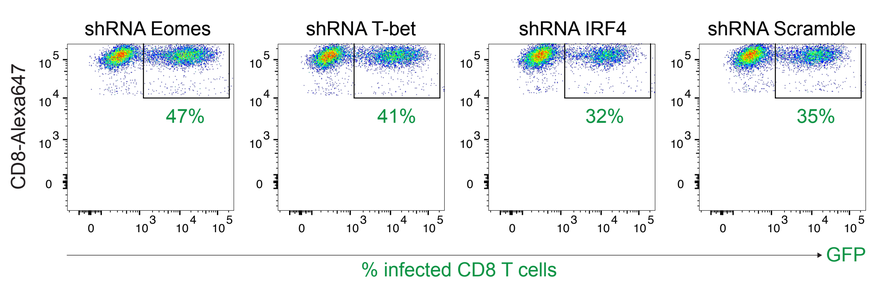
(a) inducible expression



(b) knockdown with shRNAs



(c) FACS plots showing the percentage of infected human CD8 T cells

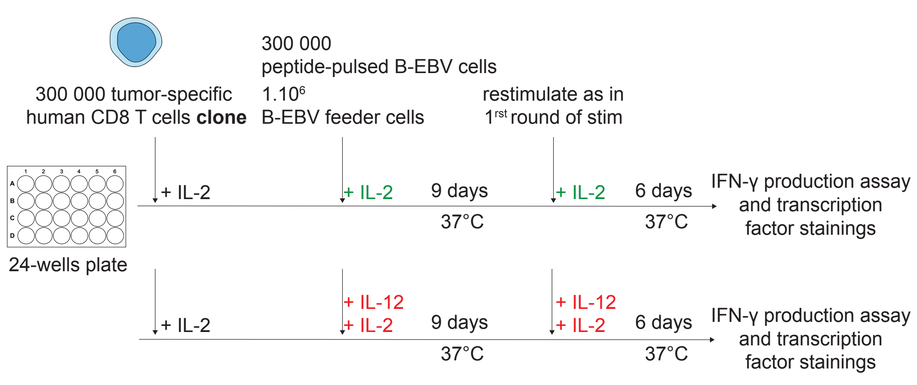


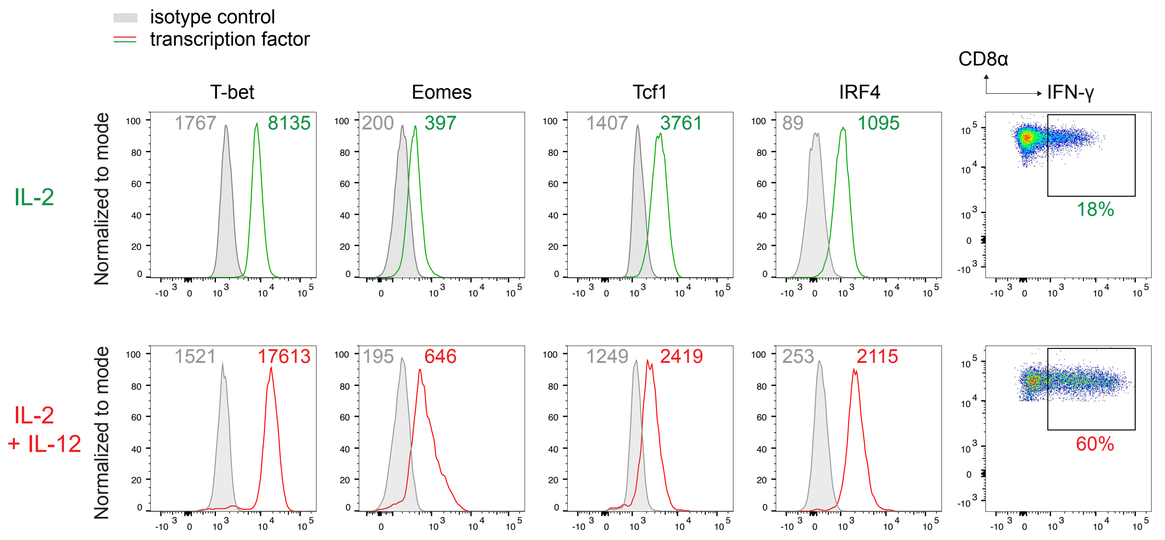
PERSPECTIVES

- transcription factor stainings to validate shRNAs
- CD8 T cell transduction with lentiviruses bearing inducible constructs (see (a)) and tetracycline titration
- FACS sorting & stimulation of infected CD8 T cells
↓
In vitro functional assays + *In vivo* ability to control tumor growth

Approach B : Modification of environment to alter transcription factor expression

Protocol





PERSPECTIVES

- which transcription factor is responsible for increased IFN-γ production ?
- injection *in vivo* in NSG mice to monitor ability to control tumor growth
- RNA sequencing to define the transcriptional landscape of T cells displaying differential anti-tumor activity *in vivo*

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