Interaction of Tim-3 and Phosphatidylserine in the Tumor Microenvironment

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Introduction

T cell (or transmembrane) immunoglobulin and mucin domain 3 (Tim-3) has been shown to have an immunosuppressive role in the tumor microenvironment (TME).

- 40-60% of Tregs among tumor-infiltrating lymphocytes (TIL) express Tim-3 and produce higher levels of suppressive cytokines such as IL-10
- Tim-3 and PD-1 co-expression on CD8 T-cells is associated with more aggressive tumor growth

The mechanism of Tim-3 mediated immune suppression is still unclear.

- PtdSer is a ligand of Tim-3 and is flipped onto the extracellular surface of apoptotic cells
- Previous studies have shown that tumor burden increased in mice injected with PtdSer-exposing MC38

Question: How does the PtdSer:Tim-3 interaction affect Treg- or CD8 T-cell mediated immunosuppression?

Acknowledgements

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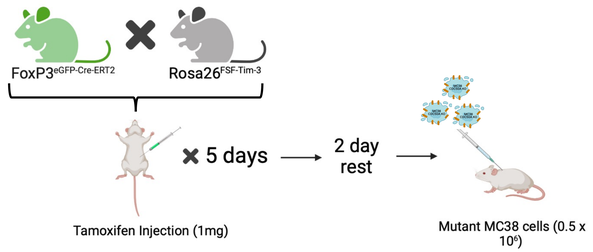
Methods

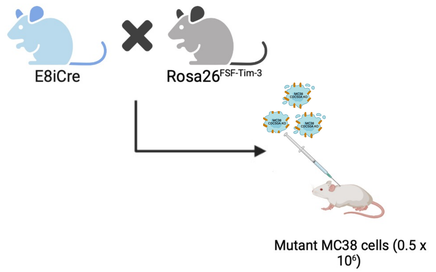
Our collaborators developed a mutant MC38 (colon carcinoma) cell line that constitutively exposes PtdSer on the extracellular surface by knocking out the flippase CDC50A.

Mutant PtdSer-exposing MC38 cells were injected into two different Tim-3 transgenic mouse models: Treg and CD8.

Treg Model: FSF-Tim-3 x Foxp3-eGFP-Cre-ERT2



CD8 Model: E8iCre x FSF-Tim-3



- Tumor growth was monitored for 12 days
- On day 12, tumor infiltrating lymphocytes (TIL) were characterized by flow cytometry

Results

Characterization of TIL from Treg transgenic (Tg), or Cre-only (WT) mice injected with PtdSer-exposing MC38s

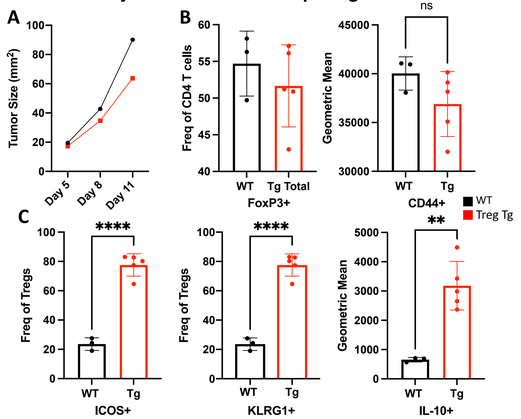


Figure 1: **A** Increased tumor growth in WT (black) compared to Tg (red) mice. **B** Frequency and activation status (indicated my mean fluorescence intensity of CD44+) of Tregs in TIL was similar in WT and Tg mice. **C** Markers of Treg effector phenotype, such as ICOS, KLRG1, and IL-10, were more prevalent in Tregs from Tg mice.

Characterization of TIL from CD8 transgenic (Tg), or Cre-only (WT) mice injected with PtdSer-exposing MC38s

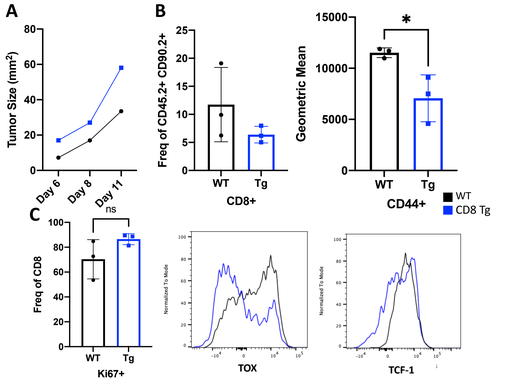


Figure 2: **A** Increased tumor growth in Tg (blue) vs. WT (black) mice. **B** Frequency and activation status (MFI of CD44+) of CD8 T cells in TIL was higher in WT than Tg mice. **C** Markers of CD8 T cell proliferation (Ki67) were similar in both models. TCF-1, a measure of CD8 T-cell naivety was also similar. TOX, a marker of T-cell exhaustion was lower in Tg mice.

Conclusions

Treg Model:

Effects of PtdSer:Tim-3 interaction on Tregs are unclear because:

- Markers of Treg effector function were upregulated in Tim-3+ Tregs from the TME of PtdSer exposing tumors
- Tumor burden is decreased in Treg transgenic mice compared to WT mice

CD8 T-cell Model:

Effects of PtdSer:Tim-3 interaction in CD8 T-cells need to further explored because:

- Tumor burden was increased in Tim-3+ CD8 transgenic mice
- Frequency and activation of CD8 T-cells in the TME of transgenic mice is lower compared to WT mice
  - Indicating either that CD8 T-cells have not yet migrated to the TME in transgenic mice or are dying rapidly

Future Directions:

- Directly compare parental and mutant MC38 tumor burden in both transgenic models
- Explore the timing of Tim-3+ CD8 T-cell migration to TME using parental and mutant MC38 tumor to understand PtdSer:Tim-3 interaction

References:

- Banerjee, H., Nieves-Rosado...Ferris, R. L., & Kane, L. P. (2020). Expression of Tim-3 drives naïve Treg to an effector-like state with enhanced suppressive activity. *Cell Reports* 36(11):109699.
- Banerjee H, Kane LP. (2018) Immune regulation by Tim-3. *F1000Res.* Mar 14;7:316.