MAKING THEIR OWN OFF SWITCH: FGL2 PRODUCED BY ANTIGEN-SPECIFIC CD8+ T CELLS TEMPSERS THE CD8+ T-CELL RESPONSE VIA APOPTOSIS OF FCRIIB+ CD8+ T CELLS

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Background Effector CD8+ T-cell tumor infiltration is a critical factor to immunotherapeutic success. Our lab recently discovered that FcyRIIB, the sole inhibitory IgG-Fc receptor, is upregulated on effector-like memory CD8+ T cells at the tumor in mice and humans1-3 and signaling through FcyRIIB regulates CD8+ T cells in a cell-intrinsic manner. We discovered that fibrinogen-like protein 2 (Fgl2) binds FcγRIIB and induces FcγRIIB-mediated cell death, but the source of Fgl2 was unknown. Here, we investigate the possibility that CD8+ T cells produce Fgl2 in an auto-regulatory feedback loop.

Methods In vivo mouse studies utilized the B16-OVA and B16-F10 melanoma mouse models. For melanoma tumor-infiltrating lymphocyte (TIL) studies, melanoma tumor tissues were collected (IRB #00095411), deidentified, and distributed by the Cancer Tissue and Pathology shared resource of Winship Cancer Institute of Emory University. Publicly available single-cell RNA-sequencing human data was analyzed using the BBrowser2 platform.4

Results Here, we show that CD8+ T cells can express and produce Fgl2 upon activation in mice and humans compared to unstimulated controls (p<0.01). To determine the clinical relevance of Fgl2 on CD8+ T cells, we analyzed two publicly available datasets of melanoma patient TILs.6,7 We found that Fgl2 is expressed on patient CD8+ TIL (p<0.0001), upregulated on activated vs. naive CD8+ TIL (p<0.0001), and that this expression is correlated with decreased patient survival (p<0.05). To determine the isolated impact of Fgl2 from CD8+ T cells, a conditional knockout model was generated wherein only the tumor-specific CD8+ T cells lack Fgl2. B16-challenged mice given Fgl2-deficient tumor-specific CD8+ T cells exhibited enhanced antitumor response as measured by increased persistence (p<0.01), decreased exhaustion (p<0.01), and decreased tumor size (p<0.05) compared to mice given WT tumor-specific CD8+ T cells. Increased persistence of Fgl2-/- tumor-specific CD8+ T cells was underpinned by a decrease in apoptosis of FcγRIIB+ CD8+ T cells, as Fgl2-/- CD8+ T cells possessed a higher frequency of FcγRIIB+ CD8+ T cells compared to WT CD8+ T cells (p<0.05).

Conclusions These data support the immunosuppressive role of Fgl2 in dysregulating the T-cell antitumor response and highlight the clinical importance of the FcγRIIB-Fgl2 pathway on CD8+ T cells. Furthermore, these data demonstrate a regulatory signaling axis whereby effector-like memory CD8+ T cells produce their own off-switch. The discovery of this pathway opens several potential avenues to manipulate the expression and signaling of this axis to increase patient treatment options.

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REFERENCES

Ethics Approval This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals. The protocol (PROTO201700538) was approved by the Institutional Animal Care and Use Committee of Emory University. All surgery was performed under general anesthesia with maximum efforts made to minimize suffering.

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