ENHANCING IMMUNE RESPONSES TO MELANOMA
WITH THE RIG-I ANTIVIRAL PATHWAY AGONIST SLR14

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Background Despite the transformative impact of immune checkpoint blockade (ICB) in melanoma, only approximately half of patients derive long-term survival benefit. T cells with antiviral signatures have the capacity to secrete inflammatory cytokines/chemokines and deliver potent cytotoxic signals ideal in tumor immunity.1 A promising therapeutic target is agonism of the double-stranded RNA (dsRNA) antiviral sensor RIG-I. The novel RIG-I agonist Stem Loop RNA (SLR)14 enhanced inflammatory cytokine release and improved the control of murine tumors by TILs and other immune cell types.2 However, dsRNA-targeting therapeutic strategies like RIG-I agonism have not yet translated into clinical advances for patients. Further, the effects of SLR14 in human tumors are unknown. We tested the hypothesis that SLR14 transforms T cells to a cyto-toxic antiviral state in immunologically ‘cold’ human tumor specimens via type-I interferon.

Methods We obtained 9 surgical resections from primary melanoma tumors and lymph node metastases and made single-cell suspension replicates of tumor and infiltrating immune cell co-cultures. We stimulated with IFNβ, SLR14, αPD-1, αCD3, CD28+αPD-1 or αCD3/CD28 for 42–48 hours. We Fluorescently Activated Cell Sorted (FACS) live cells and their bar-coded for multiplexed single cell sequencing using 10x scRNAseq. To visualize the transcriptional response and assign differentiation trajectories following stimulation, we applied PHATE (potential of heat diffusion for affinity-based transition embedding), which facilitates visualization of state transitions and Slingshot, which defines lineage relationships.

Results Following SLR14 stimulation, this approach revealed shifts in tumor co-culture cell-type proportions (figure 1A) and alterations in the transcriptional phenotypes of stem-like progenitor and terminally differentiated T cell populations, which have recently been described in single cell RNAseq studies.3,4 RIG-I agonism induced new CD4+ and CD8+ populations not observed in positive or negative control conditions (figure 1B,C). In tumor cells, NK cells and T cells, SLR14 stimulation induced expression of canonical IFN-stimulated genes (figure 1D, bottom). In CD8+ T cells, however, we observed specific programs of activation and survival including CD69 (p=0.00003), and IL2RG (p=0.000060) (figure 1D, top). SLR14-induced stem-like CD8+ T cells maintained high levels of IL7R, similar to unstimulated progenitors, but upregulated CD74 (p=0.00004) and IL2RG—suggesting the potential for inflammatory memory formation.5,6 Similarly, SLR14-induced antiviral CD4+ T cells without any significant increase in Foxp3+ Tregs.

Conclusions RIG-I agonist SLR14 stimulates tumor infiltrating T cells into antiviral states in tumor-immune co-cultures.

REFERENCES

Ethics Approval Tumors were collected with the approval of the Yale University Institutional Review Board, IRB # 0609001869, approval date: 7/19/2022, and expiration Date: 7/18/2023. Participants gave informed consent before taking part.

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