DYNAMIC IMMUNE LANDSCAPES DURING MELANOMA PROGRESSION REVEAL A ROLE FOR ENDOGENOUS OPIOIDS IN DRIVING T CELL DYSFUNCTION

1 Davide Mangani*, 2 LingR Hu, 3 Meromit Singer, 4 Ruitong Li, 5 Rocky Barilla, 6 Giulia Escharr, 7 Katherine Tookey, 8 Hanning Cheng, 9 Conor Delaney, 10 Kathleen Newcombe, 11 Jackson Nyman, 12 Nemanja Marjanovic, 13 James Nevin, 14 Orit Rozenblatt-Rosen, 15 Vijay Kuchro, 16 Avi Regev, 17 Ana Anderson. *Evergrande Center for Immunologic Diseases, Ann Romney Center for Neurologic Diseases, Harvard Medical School and Mass General Brigham, Boston, MA, 02115, USA, Boston, MA, United States; 2Department of Biostatistics, Harvard T.H. Chan School of Public Health, Boston, MA, 02115, USA, Boston, MA, United States; 3Dana-Farber Cancer Institute, Boston, MA, 02215, USA, Boston, MA, United States; 4Klarman Cell Observatory, Broad Institute of Harvard and MIT, Cambridge, MA, USA, Cambridge, MA, United States; 5Klarman Cell Observatory, Broad Institute of Harvard and MIT, Cambridge, MA, USA; 6Genentech, South San Francisco, CA, USA, Cambridge, MA, United States

Background Immune checkpoint blockade (ICB) therapies aimed at invigorating the anti-tumor immune response have achieved unprecedented responses in several tumor types, including melanoma.1,2 Despite this great success, approximately 50% of melanoma patients either fail to respond or develop resistance to ICB.3 As the immune response becomes progressively disabled as tumors advance, achieving a better understanding of the immunosuppressive mechanisms that take hold during tumor progression is needed to identify novel therapeutic targets and extend the benefit of immunotherapy to more patients.

Methods We performed an unsupervised examination of the immune infiltrate of B16F10 melanoma tumors over the course of tumor progression using single-cell RNAseq. At each time point we harvested tumors of different sizes to enable parsing of changes associated with tumor size and time from implant separately. We investigated dynamic changes in composition of the tumor infiltrate and in genes and pathways expressed in CD8+ T cells over the course of tumor progression.

Results We uncovered an unexpected role for endogenous opioid signaling in the development of CD8+ T cell dysfunction during melanoma progression. The endogenous opioid-polypeptide hormone pro-enkephalin (Penk) was progressively up-regulated in CD8+ T cells that transitioned from the effector to terminally exhausted T cell state with tumor progression. We applied gain- and loss-of-function approaches to test the role of Penk in tumor antigen-specific responses. Adoptive transfer of OT-I CD8+ T cells transduced with a lentiviral vector to over-express Penk had reduced cytolytic capacity and secretion of effector cytokines (IFNg, TNFa) and failed to control the growth of B16F10-OVA compared to WT OT-I cells. Conversely, CRISPR-Cas9-mediated knock-out of Penk in OT-I cells led to enhanced tumor control. Further, Penk deficiency in CD8+ Pmel T cells that recognize the mouse homologue of the human premelanosome protein, overcame the poor ability of Pmel T cells to exert tumor control. Notably, Penk-deficient CD8+ T cells exhibited increased cytolytic potential and effector function, without acquiring features of terminal dysfunction as determined by TOX, Eomes, and TIM3 expression. Lastly, treatment of established B16F10 melanoma with an inhibitor of opioid signaling significantly improved tumor growth control.

Conclusions Our data reveal an unexpected role for endogenous opioids in driving T cell dysfunction, thereby linking analgesic pathways and the dampening of T cell functionality in cancer. Finally, our findings have high clinical relevance as patients with advanced tumors are often treated with opioids, which may ultimately limit anti-tumor CD8+ T cell responses and ICB efficacy.

REFERENCES