ALTERATIONS IN 3D CHROMATIN CONFORMATION CONTRIBUTE TO T CELL EXHAUSTION IN MURINE MELANOMA

Aaron Yang*, 1Rhodes Ford, 2Amanda Poholek, 1University of Pittsburgh, Pittsburgh, PA, USA; 2Children’s Hospital of Pittsburgh, Pittsburgh, PA, USA

Background Cancer immunotherapy has revolutionized treatment options for patients. However, many patients do not respond to immunotherapy, requiring a better understanding of the T cell response to cancer.1 Immunotherapy functions in part by improving CD8 TIL (Tumor Infiltrating Lymphocytes) functionality. CD8 TIL progress from a progenitor state into a more terminal state of dysfunction termed exhaustion, with diminished cytolytic capacity and an inability to clear tumors.2 Understanding the mechanisms regulating the development of exhausted T cells has clinical implications for improving current and development of new immunotherapies.

Terminally exhausted CD8 TIL are transcriptionally distinct from progenitor exhausted CD8 TIL and our previous study identified epigenetic mechanisms that contributed to T cell dysfunction.3 We found that terminally exhausted CD8 TIL had an increased proportion of histone modifications corresponding to active enhancers and active promoters that did not correspond with expected gene transcription. Intriguingly, we also found decreased expression of AP-1 family members in exhausted CD8 TIL, which are key transcription factors that play roles in transcriptional regulation by mediating enhancer-promoter looping. Intriguingly, enhancers in terminally exhausted CD8 TIL were enriched for AP-1 (bZIP) binding motifs. The physical interaction of enhancers to promoters are essential to their function to promote optimal transcription and recruitment of key transcription factors to these genes. Recent work has shown the importance of chromatin structure for T cell activation and differentiation.4 5 However, the role of 3D chromatin structure in the development of T cell exhaustion has not been explored.

Methods We hypothesized that exhausted CD8 T cells may have alterations in 3D chromatin looping due to a loss of AP-1 factor expression that regulates their transcriptional potential and decreases their functionality. To investigate changes in chromatin interactions, we performed low-input Hi-C on terminally exhausted and progenitor CD8 T cells from B16 melanoma. We performed CUT&Tag for CCCTC-binding factor (CTCF) which regulates chromatin looping.

Results We observed a loss of long-range chromatin interactions in terminally exhausted CD8 TIL compared to progenitor CD8 TIL and differential topological associating domains (TADs). We found loss of CTCF binding between precursor and terminally exhausted CD8 T cells at TAD boundaries.

Conclusions Our findings indicate that chromatin conformation changes may be associated with T cell exhaustion and poor tumor control. Immunotherapies that aim to restore enhancer-promoter looping via AP-1 family binding may promote antitumor immunity.

REFERENCES

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