433 ENHANCED CYTOLYTIC EFFECTOR FUNCTION AND ANTI-TUMOR EFFECTS OF EXHAUSTED CD8 T-CELLS BY THE KLF4 EXPRESSION

[1]Rho H Seong*, [1]Jinwoo Nah, [1]Jieun Kim, Yong Park[2], [1]Sungkyu Lee. *[1]Seoul National University, Seoul, Republic of Korea; [2]MEDGENE Therapeutics, Silver Spring, MD, USA*

Background Antigen-specific CD8 T-cells reach an unresponsive state during chronic inflammatory responses against viral infections and cancer, which is described as exhaustion. Exhausted CD8 T-cells are phenotypically and functionally heterogeneous. It has been reported that the number of progenitor exhausted CD8 T-cells increases in response to anti-PD-1 immunotherapy, which appears to be a critical factor for the successful control of chronic viral infection and cancer. Effective immune responses exerted by the progenitor exhausted CD8 T-cells imply that cells with effector functions may develop from the progenitor cells. Indeed, CD8 T-cells with effector characteristics in the exhaustion context have recently been identified.

Methods Using *in vitro* CD8 T-cell exhaustion model and mouse tumor model, we analyzed the expression of Klf4 and single cell RNA sequence during the exhaustion process. We also combined retroviral transduction of *Klf4* on CD8 T-cells with the models to figure out the role of *Klf4* on anti-tumor immunity. To investigate whether *Klf4* expression could reinvigorate exhausted CD8 T -cells, we used modified *in vitro* exhaustion model in which *Klf4* could be induced after the exhaustion process, not during the activation stage. Lastly, to apply the results to human cancer patients, we used analytical tools to examine the correlation between gene expression and tumor prognosis.

Results We found that *Klf4* is a hallmark of the cytolytic transitory effector CD8 T-cells during the exhaustion process. *Klf4* is required for the differentiation and function of transitory effector CD8 T-cells. In consequence, *Klf4* expression in CD8 T-cells enhances anti-tumor immunity and provides great advantage in controlling tumor growth in mice. Importantly, we demonstrated that *Klf4* expression could reinvigorate the effector function of exhausted CD8 T -cells, in part by restoring epigenetic status of genes related to effector function. In addition, Klf4 expression blocked the exhaustion of human CD8 T cells expressing the GD2 CAR construct, and our TCGA data analysis suggested that *KLF4* expression correlates well with the prognosis of cancer patients. We also found that upregulation of *KLF4* expression by anti-PD-1 therapy increased the survival rates of melanoma patients.

Conclusions *Klf4* promotes the differentiation of CD8 T-cells into cytolytic transitory effector cells and blocks reaching into terminal exhaustion. *Klf4* expression could reinvigorate the effector function of terminally exhausted CD8 T-cells, Thus, the potential effects of *Klf4* on CD8 T-cell exhaustion can be highlighted in terms of anti-tumor immune therapy.

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