121 TETRASPANIN MOLECULE CD9 AS A NOVEL MARKER OF TUMOR-REACTIVE T CELLS

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Background Overcoming 'heterogeneity of tumor antigen' by infusion of a variety of T cells that recognize multiple tumor antigens would be beneficial for patients with solid tumor. To this end, two major approaches aiming to comprehensively obtain tumor-reactive T cells (or TCRs) from TIL/PBMC have been examined so far. One approach is to use cell surface molecules such as exhaustion marker PD-1, and the other is to utilize single cell analysis for selecting tumor-reactive T cells with characteristic RNA expression profiles. Although significant progress has been made, it is still required to deepen the understanding of anti-tumor immunity for development of an effective immunotherapy.

Methods We applied the following strategies to dissect human colorectal TIL. (1) Obtain TCRs from single cell sorted $CD8^+$ TIL ex vivo by using cell surface molecules, (2) Analyze tumor reactivity by using autologous tumor organoids, (3) Determine the specificity to neoantigens, and (4) Identify molecules preferentially expressed by neoantigen-specific T cells.

Results We obtained neoantigen-specific 5 TCRs from 4 cases among of 8 tumor-reactive TCRs from 6 cases. Notably, tumor-specific $CD8^+$ TILs were most enriched in the $PD\text{-}1^+$ and $4\text{–}1BB^+$ (DP) population in all cases, and further analysis revealed that the frequency of DP cells in $CD8^+$ TIL tend to correlate with patient survival. Interestingly, scRNA-seq and TCR-seq analysis of TIL in which about 5% of whole $CD8^+$ TILs are neoantigen-specific uncovered that CD9, a member of tetraspanin, was significantly expressed in these T cells. Indeed, addition of CD9 to DP sorting further enriched tumor-specific T cells. To our best knowledge, tetraspanin molecule has not been reported as a marker of tumor-reactive T cells. Therefore, these data prompted us to examine CD9 expression on neoantigen-specific $CD8^+$ T cells in mice. Indeed, not only CD9 but also CD81, another member of tetraspanin, is preferentially expressed on these T cells in a mouse model we previously reported.[1] Furthermore, we successfully identified tumor-reactive TCRs from splenic $CD8^+CD9^+CD81^+$ T cells in two independent tumor bearing mice models (CMS7 and CT26), in which one TCR recognized AH-1 epitope in the case of CT26 tumor bearing mice.

Conclusions We report that tetraspanin molecules would be novel markers of tumor-reactive T cells. Our data strongly indicate that tetraspanin molecules play unexpected roles in the interaction of T cells with tumor, although further analysis is required. Finally, we hope that these findings would be useful for development of an effective personalized cancer immunotherapy in near future.

REFERENCE

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Ethics Approval Written informed consents were obtained from patients and healthy volunteers according to the guidelines of the Declaration of Helsinki. The experimental protocol (ID;3037) was approved by the Institutional Review Board at the Mie University School of Medicine.

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