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703.CELLULAR IMMUNOTHERAPIES: BASIC AND TRANSLATIONAL

Development of a New CAR-T-Cell Therapy Targeting an Immune Checkpoint for Effective Treatment of Solid and Liquid Tumors

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Development of a new CAR-T-cell therapy against relapsed/refractory malignancies including solid tumors is an interest and a struggling issue. Immune-checkpoint blockade therapy is a successful modality for the treatment of solid tumors. We hypothesized that CAR-T-cell therapy targeting immune-checkpoint protein expressed by tumor cells results in involving T cells suppressed in tumor microenvironment as well as inducing direct cytotoxicity against tumor cells. Programmed cell death ligand 1 (PD-L1) is a major immune-checkpoint expressed by tumor cells. However, PD-L1 is also expressed by activated T cells, therefore it might not be straightforward as a target for CAR-T cells. Sialic-acid-binding immunoglobulin-like lectin 15 (SIGLEC15) is expressed by a panel of tumor cells including hematological malignancies, such as leukemia and lymphoma cells, and its expression in normal cells is limited to macrophages including myeloid-derived suppressor cells (MDSCs). Interestingly, it was reported that SIGLEC15 can suppress T-cell function in tumor microenvironment. Blocking therapy of SIGLEC15 using monoclonal antibody enhanced antitumor effects induced by T cells, thereby CAR-T-cell therapy for SIGLEC15 can be a new treatment modality targeting both an immune-checkpoint and tumor cells while evading cytokine release syndrome by targeting macrophages. Recently, we have developed a new strategy which is focusing on the optimization of a single chain fragment variable (scFv) important for the functions of CAR-T cells *in vivo*, therefore in this study, we have generated CAR-T cells expressing an optimized scFv for SIGLEC15 to achieve enhanced antitumor responses. First, SIGLEC15 mRNA expression was observed in a series of solid tumors using cDNA arrays. Particularly, ovarian cancer cells with moderate/poor characteristics had a trend of high expression of SIGLEC15 mRNA. Immunohistochemistry revealed that those ovarian cancer tissues were likely to express SIGLEC15 when compared to normal ovarian tissue. Next, variable regions of a monoclonal antibody specific for SIGLEC15 (SIGLEC15-VL, SIGLEC15-VH) were employed, and an scFv was integrated into a 2 nd generation CAR gene (CD28/CD3z). SIGLEC15 CAR-T cells recognized SK-OV-3, U87-MG cells from solid tumor cells, and Reh cells from liquid tumor cells, which was confirmed by ELISPOT assay, and were stained with in house soluble SIGLEC15 dimers. Then, using our technology, an scFv library which possessed variable regions of human immunoglobulin (hVK, hVL, or hVH) fused with SIGLEC15-VH or SIGLEC15-VL was generated, and re-integrated into the CAR gene. CAR-library T cells expressing an scFv library were stimulated with SIGLEC15 $^{+}$ antigen presenting cells, and SIGLEC15-specific population was isolated using soluble SIGLEC15 dimers after expansion. As the results, we have identified new clones of hVKs with SIGLEC15-VH. Among them, K145 CAR-T cells recognized and killed U87-MG and SK-OV-3 cell lines which express SIGLEC15, and were likely to proliferate well when compared to original CAR-T cells. Further analyses are needed, but these results suggest that SIGLEC15 might be an attractive target for new CAR-T-cell therapy, resulting in advancement of the treatment for solid and liquid tumors.

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