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Anti-tumor efficacy of Tγ9δ2 lymphocytes expressing anti-*O*-acetylated GD2 CAR in pediatric brain tumors

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Objective: Primary brain tumors have the poorest prognosis of all childhood tumors. The current treatments are rarely curative and induce significant toxicity due to the sensitivity of this vital organ. Chimeric antigen receptor (CAR) αβT cell therapy has been recently proposed to improve the outcome of children with brain tumors. Major obstacles involve the complex and costly individualized manufacturing process, the lack of tumor-specific target antigen, immunosuppressive tumor micro-environment and target antigen. A potential solution for these limitations is the use of donor-derived γ9δ2T cells thanks to cells lack allogenicity. In the literature, the O-acetylated GD2 (OAcGD2) ganglioside represents a solution to minimize off-tumor on-target toxicity. Thus, our goal is to demonstrate anti-tumor efficacy in OAcGD2-specific CAR Tγ9δ2 cells against pediatric brain tumors, and to further identify primary/acquired tumor resistance mechanisms using 3D tumor cell models.
Methods: We thus designed Tγ9δ2 lymphocytes expressing a second-generation *O*AcGD2-specific CAR (CD28-CD3ζ), and control Tγ9δ2 lymphocytes expressing *O*AcGD2-specific CAR without transducing domain. Their cytotoxic activity dependent on anti-*O*AcGD2 CAR engagement was evaluated in 2D tumor models expressing the target. In order to understand the mechanisms of primary/acquired tumor resistance, a 3D model was developed using the CHLA-200 high-grade glioma cell line. So, we next studied CAR T cell infiltration, T cell activity and T cell secretion against 3D-model using confocal microscopy, videomicroscopy and flow cytometry.
Results: The cytotoxic activity of CAR T cells showed about fifty percent lysis against CHLA-200 in 2-D model. The confocal microscopy allows us to observe that CAR T cells are able to penetrate the 3-D model and time-lapse videomicroscopy analysis further revealed that these CAR T cells efficiently killed CHLA-200 tumor cells within a time of 96 hours, compared to control CAR T cells. After 48 hours of coculture with the 3-D model, CAR T cells increase their secretion of inflammatory cytokines such as TNFα and IFNγ, and also cytotoxic proteins like granzyme A, B and perforin.
Conclusion: These results suggest that OAcGD2-specific CAR Tγ9δ2 cells hold a great potential for immunotherapy of children with brain tumors. Further phenotyping of CAR γδT cells and CHLA-200 cells will allow us to identify possible escape mechanisms and propose optimized effective strategies.

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