Differential effects of target ligands and receptor architecture upon NKG2D-based CAR T-cell activation

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BACKGROUND

- The NKG2D receptor is a type II protein that binds to eight stress-induced ligands (NKG2DL). These ligands are absent from most normal tissues, but frequently expressed in various types of tumors, making NKG2DL a promising target for cancer immunotherapy.
- The receptor architecture and its interaction with the different ligands, most likely implies distinct responses, moreover the high polymeric nature of the NKG2DLs implies different lytic consequences. Here we investigated whether different NKG2D receptor-based CAR T-cells architecture has functional consequences to the manufacturing process and functional characteristics of the NKG2D-based CAR T-cells.

METHODS

- We designed different NKG2D-based CAR T-cells, utilizing either a type II or type I based CAR structures. Both CAR T-cells are a second generation CAR T-construct where in the type II only a CD3ζ dimer signaling motif was included (NKG2D expression in this state is mediated by DAP10 that acts as co-stimulatory signal, Figure 1A) and in the type I a hinge, transmembrane-domain and a 4-1BB as a co-stimulatory signal (Figure 1B).
- PBMCs were isolated from whole blood and activated with either CD3/CD28 beads or CD3 antibodies. T-cells were subsequently transduced with retroviral vectors containing the CAR constructs. After expansion cells were harvested and analysed.
- The manufacturing process was assessed and phenotypical analysis was conducted by flow-cytometry at different time-points along the process. CAR T-cell activity was assessed by co-culturing the transduced T-cells with different cancer cell lines expressing different NKG2DLs.
- Cocultures were conducted between NKG2D-based CAR T-cells and different cancer cell lines (K562, HCT116 and RAJI) at different ratios and their lytic activity as well as cytokine secretion was assessed.
- Cell lines expressing a single NKG2D ligand were created by transducing CHO cells with vectors containing different NKG2DLs. These were subsequently purified and co-cultured with the NKG2D-based CAR T-cells.

RESULTS

- NKG2D-based CAR T-cells can be created through different manipulations of the receptor, using either a full-length NKG2D receptor or using only the extracellular domain (Figure 1A and 1B).
- NKG2D-based CAR T-cells were activated in the presence of different cancer cell lines, however a clear distinction could be made between the level of cytokine secretion and target ratios (E:T) had a clear influence on the CAR activity. For instance, if the target cells efficiently could lyse the target cells efficiently when the ratio was increased and similarly the efficiency of several type I CAR T-cells was reduced. Furthermore, the CD8 short hinges showed clear lytic activity while it did not secrete cytokines in response to NKG2DL (Figure 2B and 2C).
- In order to understand the differences observed in the type II or type I CAR T-cells, effector functions of the different CAR T-cells were assessed by flow cytometry. The expression of PD-1 (CD279), TIGIT were increased both as percentage and as the mean fluorescent intensity (MFI), Figure 3A and 3B). Indicating that the type II NKG2D-based CAR T-cells exhibit a more exhausted phenotype in comparison to the type I NKG2D-based CARs. Interestingly, a slight increase was also observed in the type I IgG4h and CD8short NKG2DLs.
- Next, NKG2D-based CAR T-cells capacity to lyse PAN-C1 cells repeatedly was assessed. In the first stimulation although kinetics were different all constructs could lyse the target cells completely, which is similarly observed in the second stimulation. However, from the third stimulation onwards (including stimulation 4 and 5) a clear distinction can be seen between the type I and II CAR Ts, with the type I clearly outperforming the type II NKG2D-based CAR T-cells (Figure 4A).
- The proliferative capacity of the different NKG2D-based CAR T-cells was assessed after the repeated stimulations and the accumulated fold expansion from T0 till T12 days was calculated. The type II NKG2D-based CAR T-cells clearly had a diminished proliferative capacity in comparison to the type I NKG2D-based CAR T-cells (Figure 4B).

CONCLUSIONS

- NKG2D-based CAR T-cells showed distinct functional and phenotypical differences when their receptor architecture was different, with a clear improvement in both cytokytic activity, persistence, exhaustion profile and proliferative capacity when the type I architecture was used.
- A clear preference was further seen when short hinges were used rather than the long hinge, with a slight preference for the CD8short hinge compared to the IgG based hinges.

REFERENCES


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