Differential effects of target ligands upon NKG2D CAR T cell activation

Breman E, Fontaine M, Ramelot N, Agaugué S, Sotiropoulou PA, Gilham DE.

**Background**

The Natural Killer group 2D (NKG2D) receptor binds to eight stress-induced ligands (NKG2D-L), the major histocompatibility complex class I chain-related A and B (MICA, MICB) and the UL16 binding protein family (ULBP1-6) (Figure 1A). These ligands are absent from most normal tissues, but frequently expressed in various types of tumors, making NKG2D a promising tool for cancer immunotherapy.

We created a chimeric antigen receptor (CAR) T cell containing the full length human NKG2D fused to the CD3zeta signaling domain (Figure 1B). The binding affinity of NKG2D to all its ligands is not completely known, and it is likely that different ligands elicit distinct responses. Here we assessed the ability of each ligand (alone or in combination) to activate NKG2D CAR T cells.

**Results**

NKG2D CAR T cells target cancer cells expressing heterogenous target ligand profiles

The 8 distinct NKG2D-L are expressed in different combinations and levels on cancer cell lines. To understand the potential differential role of ligands in NKG2D CAR T cell mediated killing, cancer cells were incubated with or without NKG2D CAR T cells or control CD19 T cells. Ligand expression profile was analyzed on the remaining cancer cells (Figure 2). The following cancer cell lines were used: PANC-1 (epithelial carcinoma), OVCAR3 (ovarian cancer), RAJI (Burkitt’s lymphoma), HL60 (acute promyeloblastic leukemia), MCF7 (breast adenocarcinoma), K562 (chronic myelogenous leukemia), HCT116 (colorectal carcinoma).

Expression of MICA/B, MICB, ULBP2/5/6 and ULBP1 on cancer cells was decreased after the co-culture with NKG2D CAR T cells, indicating that these cells were more susceptible to target specific killing. CYAD-01 cells secrete IFN-γ in response to coated MICA and MICB and to a lesser extent to ULBP1, ULBP2 and ULBP3. To assess the role of each individual ligand, plate-coated recombinant NKG2D-L were used. All NKG2D-L tested could bind to NKG2D CAR T cells when present at adequate concentrations (30µg/mL), and this interaction could be completely inhibited by anti-NKG2D Ab (5µg/mL, Figure 3A). Interaction at lower concentrations using wild type NKG2D uncovered differences in the kinetics of binding between the ligands, with ULBP6 showing the highest binding affinity followed by ULBP2 (Figure 3B).

**Conclusions**

Our data indicate that in a NKG2D CAR T cell context, MICA and MICB are potent activators while ULBP1, 2 and 3 can also induce NKG2D CAR T cell function. Recombinant ULBP6 was the weakest activator of NKG2D CAR T cell activity of those tested.

Importantly, MICA and MICB are the predominant NKG2D-L present in human tumors along with ULBP1 and 3. For instance, nearly 100% of AML blasts express one or more of these specific ligands. Notably, the soluble NKG2D ligands often detected in patient sera do not appear to inhibit NKG2D CAR T cell function.

We continue to explore the role of other ligands including ULBP4 and ULBP5 for which specific reagents are more limited. However, these data demonstrate that NKG2D CAR T cells are attractive for their ability to target multiple ligands that are highly expressed on a broad range of cancers.

**References**

7. Celyad, et al. unpublished results