Engineering High Affinity and Cleavage Resistant CD16 to Augment ADCC of Placental Hematopoietic Stem Cells-Derived Natural Killer Cells

Xuan Guo1, Srinivas Somanchi1, Shuyang He1, Qian Ye1, Andrea DiFiligia1, Salvatore Rotonda1, Hemlata Rana1, Weifang Ling1, Robert Hariri1 and Xiaokui Zhang1

1Celularity, Inc, Warren, NJ

INTRODUCTION

Celularity, Inc is developing human placental hematopoietic stem cells-derived, cryopreserved, off-the-shelf, ex-vascular and allogeneic natural killer (NK) cells for treatment of various hematological malignancies and solid tumors. NK cells play a central role in antibody dependent cell mediated cytotoxicity (ADCC) through Fc receptor CD16 in monoclonal antibody mediated anti-tumor therapies. Two allelic forms of CD16 have been identified with the 158Val/Val form shown to have higher IgG binding affinity comparing with the 158Phe/Phe form.1 The high IgG binding allelic is found in about 15-20% of the normal population.2,3 In addition, activation of NK cells induces CD16 shedding by metalloproteinase ADAM17 at 197Ser, thus limiting ADCC responses. A single mutation (Ser157Phe) prevents CD16 shedding and increases ADCC activity in NK cells.4 To augment the effector functions of the placental hematopoietic stem cell derived NK cells and to sustain their tumor-killing potential, we expressed a high affinity (158Val) and protenase cleavage resistant (197Phe) CD16 variant (CD16VP). Here we report in vitro and in vivo phenotypic and functional evaluation of CD16VP cells.

MATERIALS AND METHODS

• Cell culture: Human placental CD16+ cells were isolated and cultured in the presence of cytokines including thrombopoietin, SCF, FGF, IL-7, IL-15 and IL-12, for 35 days to generate NK cells.
• Cell expansion and Characterization: Cell expansion was recorded during the culture process. On day 35, CD16+VP cells were evaluated for NK surface markers CD56, CD57, and CD16, using flow cytometry.
• CD16VP Sheding Assay: Expression of CD16VP was evaluated by activating cells with PMA/ionomycin to induce CD16 cleavage followed by immunostaining with CD16 antibody and analyzed using flow cytometry.
• In vitro ADCC Assay: ADCC activity of CD16VP cells was assessed against Daratumumab (anti-CD38) or Rituximab (anti-CD20) opsonized lymphoma cell lines at various effector to target (E:T) ratios. IgG was used as ADCC control. In sustained ADCC assay, CD16VP cells were treated with PMA/ionomycin and then evaluated for ADCC activity as described above.
• Animal Study: In vivo anti-tumor activity was assessed in a Daudi disseminated Xenograft model in NOD mice. Luciferase-expressing Daudi cells (3x105) were intravenously (IV) administered at day 0, followed by CD16VP cells (1x10^7) IV at day 1 and day 3, and Daratumumab at day 3. Tumor burden in mice was monitored by Bioluminescence Imaging (BLI).
• Statistical Analysis: Statistical analysis was performed using Prism/Excel program. Data are presented as mean ± standard deviation. Paired or unpaired two-tailed Student’s t test were used for comparing two groups.

RESULTS

Figure 2. Placental CD34+ Cells Expanded and Differentiated to NK Cells

Figure 3. High Expression of CD16VP Sustained During Culture and Was Resistant to Shedding

Figure 4. CD16VP Cells Demonstrated Augmented ADCC against Tumor Cell Lines in Vitro

Figure 5. CD16VP Cells Demonstrated in Vivo Anti-Tumor Activities

Figure 6. Representative examples of ADCC activities of CD16VP cells compared to NT cells against Daratumumab or Rituximab opsonized lymphoma cell lines. (A) Average ADCC activities of CD16VP cells compared to NT cells against Daratumumab opsonized lymphoma cell lines at 10:1 E:T ratio (n=3 to 5). * indicates significant higher activities compared to NT control (p<0.05). (B) ADCC of CD16VP cells before and after PMA/ionomycin treatment against Daratumumab opsonized Daudi cells.