P70 Natural killer cells in combination with mAb cG250: a potential new therapy approach for RCC

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Introduction & Objectives: Natural killer (NK) cells are major effector cells of the innate immune system. Clinically applicable NK cell products with high purity can be generated from $CD34^+$ hematopoietic progenitor cells (HPC). Functional analysis demonstrated that these HPC-NK cells induce natural cytolysis of cancer cells. Monoclonal antibody cG250 recognizes carbonic anhydrase IX (CAIX), highly expressed in clear cell renal cell cancer (ccRCC). Clinical studies suggest that cG250 administration can influence the clinical course of the disease, possibly by NK cell-mediated antibody-dependent cellular-cytotoxicity (ADCC), but the anti-tumor effects need to be improved. Combining cG250 with highly activated HPC-NK cells may be an effective therapy approach for patients with metastasized RCC or at high risk of recurrence. The aim of this study was to examine the lytic potential of HPC-NK cells on RCC cells and to investigate whether increased CD16 expression leads to enhanced cell death through ADCC with cG250.

Materials & Methods: HPC-NK cells were expanded and differentiated from CD34+ cells in 35 days. Non transduced NK cells (NK-NT) with low CD16 expression, CD16 transduced NK-cells (NK-CD16) or PBMC were cocultured with RCC target cells SK-RC-52 ($CAIX^+$) and SK-RC-17 ($CAIX^-$) at various E:T ratios, with mAb cG250. Cell death and confluence was monitored with live cell imaging and the number of viable cells was quantified by flow cytometry. NK cell degranulation (CD107a) and IFNγ production were determined by flow cytometry after 4 hr stimulation of NK-NT and NK-CD16 cells with SKRC52 cells, with or without antibody. To increase CD16 expression (in vivo maturation), NK cells ($14*10^6$ /mouse) were injected i.v. in NOD/SCID/IL2Rg(null) mice (n=15). Mice received 2.5 µg rhIL-15, i.p. at day 0, 1 and 3 to support NK cell survival. At day 7, spleens were harvested, PBMC were isolated and expression of CD16, KIR, NGK2a was analyzed with flow cytometry.

Results: Coculture of SK-RC-52 and SK-RC-17 cells with PBMC resulted in significant cell death. Addition of cG250 increased cytotoxicity of SK-RC-52 but not of SK-RC-17. Cytotoxicity was not increased in cocultures of SK-RC-52 cells with NK-NT, or NK-CD16 cells in the presence of cG250. However, stimulation of NK-CD16 but not of NK-NT cells with SKRC52 cells showed elevated levels of IFNγ production and degranulation when antibody was added suggesting increased lytic potential of NK cells in combination with cG250. Recovered NK cells from the NSG mice demonstrated upregulation of CD16 from <1% before injection up to 46% $CD56^+CD16^+$ NK cells after harvesting. KIR and NKG2a expression increased from 8% to 37 % and from 32% to 89% respectively, showing improved functionality of NKs.

Conclusions: Our results suggest that HPC-NK cells expressing high levels of CD16 combined with mAb cG250 may enhance specific RCC cell death and may provide a new treatment option for patients with RCC.