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Natural Killer cells from Umbilical Cord blood stem cells

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Chapter 7:

Summary

The active role of NK cells in controlling cancer has been intensively studied. From the clinical trial summary provided in Table 2 from Chapter 1, it is evident that adoptive transfer of NK cells in a non-transplant setting for hematological malignancies is safe, and has been shown to mediate a Graft versus Tumor (GvT) effect without causing Graft versus Host Disease (GvHD).

It is well known that NK cell functions are tightly regulated by the balance between arrays of NK cell activating and inhibitory receptors. In several cancer types NK cell activating receptors are often down-regulated, thus limiting NK cell target killing. Hence it is essential to have a deeper understanding of the expression levels of relevant NK cell receptors and the functional status of NK cells in tumor conditions. This led us to develop two eight-color NK cell flow cytometry (FACS) panels, one to study the NK cell phenotype and the other to study the NK cell function in PBMC (or single-cell tumor) samples from multiple centers as described in Chapter 2. The NK cell FACS panels were designed, optimized and tested across three different centers using three different flow cytometers with comparable configuration, thus providing a unique platform to generate comparable and reproducible data for multicenter clinical trials. Furthermore, this study also emphasized that cryopreserved NK cells are suitable for studying NK cell phenotypes and functions including NK cell mediated ADCC.

In Chapter 3, to treat anti-EGFR/cetuximab and immunotherapy-resistant cervical cancer cells, allogeneic NK cell-based therapy was explored. The cytotoxic effects of UCB-NK cells and activated PBNK cells were compared either as monotherapy or in combination with cetuximab in an in-vitro set-up. A panel of ten cervical cancer cell lines with different histology and different HPV types were subjected to NK cell killing. All these cell lines expressed low to moderate levels of EGFR (except C33A, which was EGFR negative) and were wild type for the RAS gene, but

failed to respond to cetuximab monotherapy. Upon performing NK cell cytotoxicity assays, it was evident that all cervical cancer cell lines were sensitive to NK-mediated killing, independent of tumor histology and HPV type. Interestingly, UCB-NK-mediated cytolysis rates were significantly higher than those achieved with PBNK alone and equalled those of PBNK + cetuximab. The superior cytotoxicity with UCB-NK cells correlated to their low expression levels of inhibitory KIRs, in keeping with the observed lack of inhibition by HLA-ABC expressed on the cervical tumor cells. These data point to the potential application of UCB-NK cells in the treatment of cervical cancer.

In Chapter 4, we addressed a major hurdle in the treatment of colorectal cancer, i.e. resistance against cetuximab therapy. RAS mutations in the EGFR signalling pathway have left nearly half of the metastatic colorectal cancer (mCRC) population, ineligible for anti-EGFR treatment. In our set-up, using allogeneic and highly activated PBNK cells, we could induce effective killing of colon cancer cells irrespective of EGFR expression levels and RAS status. PBNK induced killing of EGFR⁺ cells was significantly higher when colon cancer cells were coated with cetuximab, mediating ADCC. Importantly, PBNK cells were also highly cytotoxic to EGFR⁻ colon cancer cells, which will obviously not respond to cetuximab therapy. The superior cytolysis observed for both cell lines and primary colon tumors, with variations in EGFR expression levels and RAS mutation status, indicates the potential of combined PBNK and cetuximab application in the treatment of cetuximab-resistant colon cancer.

In Chapter 5, we monitored the frequency and function of NK cells in mCRC patients before and after the first cycle of chemotherapy, and found that mCRC patients not only had a 20% lower frequency of NK cells in peripheral blood before the initiation of chemotherapy, but that this percentage further declined during chemotherapy. In addition to this quantitative defect in NK cells of mCRC patients, their cytotoxic capacity was also impaired. Of interest, though the cytolytic activity of NK cells of mCRC patients could be increased by cetuximab through ADCC,

the level of cytotoxicity was still markedly reduced compared with that mediated by NK cells from healthy adult volunteers. These data suggest that adoptive transfer of fully functional NK cells might be of benefit to restore the NK effector cell pool in mCRC patients. For this purpose, the cytotoxic effects of two allogeneic NK cell products, i.e. activated PBNK and UCB-NK cells, were tested and compared *in vitro* against a panel of colon cancer cell lines. UCB-NK cells were found to exert superior cytotoxicity compared to PBNK cells, their cytotoxicity being comparable to that achieved when PBNK were additionally stimulated using cetuximab. These superior cytotoxic effects of UCB-NK cells were verified *in vivo*, where treatment with UCB-NK cells alone significantly reduced the tumor load in mice inoculated with EGFR⁺ RAS^{mut} colon cancer cells. Of interest, this effect was not increased by the addition of cetuximab *in vivo*, which could be due to a sub-optimal up-regulation of CD16 cell surface expression levels on the adoptively transferred UCB-NK in the immunodeficient mice that was used for these studies. Importantly, as a clinical study in AML patients revealed that CD16a expression levels steadily increased on UCB-NK cells post-infusion, synergy between both approaches may be expected when applied clinically.

To summarize, through the studies described in this thesis we have demonstrated that UCB-NK cells have superior anti-tumor efficacy against epidermoid, colon and cervical cancers as compared to activated PBNK cells, and are equally cytotoxic as the combination of PBNK and cetuximab, demonstrating significant anti-tumor benefit against EGFR⁺ RAS mutant and EGFR⁺ BRAF mutant tumors. This novel UCB-NK expansion and differentiation technique from Glycostem allows the generation of large numbers of cytolytic UCB-NK cells that may overcome the limitations of current NK cell based adoptive transfer strategies and supplement the immune system with sufficient numbers of NK cells to mount an effective anti-tumor immune response in immunosuppressed cancer patients. Soon, UCB-NK cell functions, and thereby antitumor efficacy, may be further improved by genetic modification or combination therapy approaches encompassing novel immune modulatory genes or agents as outlined in chapter 6.