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Expansion and genetic modification of human natural killer cells for adoptive immunotherapy of cancer

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ABSTRACT

A century after the initial proposition that the immune system has the capacity to fight against tumors, evading destruction by immune cells is now well recognized as a hallmark of cancer. Recent decades have witnessed extraordinary improvements in the use of immunotherapy against malignancies and adoptive transfer of Natural Killer (NK) cells stands among promising tools in the fight against cancer. Clinical studies have demonstrated the anti-tumor responses generated by NK cells both in the autologous and allogeneic settings in various cancers. Direct adoptive transfer, *ex vivo* activation and/or expansion, as well as genetic modification of NK cells aspire novel improvements to current immunotherapy strategies. As such interventions develop, the quest for better preparation of NK cell based therapies continues.

This thesis, primarily investigates the feasibility and potential of *ex vivo* expanded NK cells for cancer immunotherapy. Our results produced a system that has the capacity to expand polyclonal and highly cytotoxic NK cells showing selective anti-tumor activity. Protocols for expansion of these cells from healthy donors and patients with Multiple Myeloma (MM) using current Good Manufacturing Practice (cGMP)-compliant methods have been optimized in conventional cell culture systems as well as automated bioreactors. The elevated cytotoxic activity of expanded NK cells against autologous tumor cells, along with detailed analysis of phenotypic changes during the expansion process has subsequently shifted attention to the interaction between NK and tumor cells.

Both as a basic method to identify these interactions, and as part of further plans to use genetically retargeted NK cells in cancer immunotherapy, we have investigated methods for efficient lentiviral genetic modification of NK cells. This study has resulted in an optimized stimulation and genetic modification process for NK cells that greatly enhances viral gene delivery. Along with NK cell stimulating cytokines, an inhibitor of innate immune receptor signaling that blocks the intracellular detection of viral RNA introduced by the vector was successfully utilized to enhance gene transfer efficiency, also constituting a proof-of-concept for various other gene therapy approaches.

Taken together, the work presented in this thesis aims to bring us closer to optimal *ex vivo* manipulation of NK cells for immunotherapy. Clinical trials with the long-term expanded NK cells as well as further preclinical development of NK cell genetic modification processes are warranted.

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