ImmunoTools *special* Award 2023

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Utilizing *Carapichea ipecacuanha* cyclotides as a therapeutic approach for boosting the anti-tumor activity of natural killer cells

Cyclotides are head-to-tail cyclized peptides with a unique cystine-knot motif. Their cystine-knot structure renders those peptides very stable to enzymatic, chemical, or thermal degradation compared to other peptides of similar size. Peptide-based therapeutics have several advantages due to their high specificity and selectivity as well as their low immunogenicity. This makes their use safer compared to small molecules or large biologicals. Cyclotides came into the focus of drug research owing to their anti-cancer properties. Their primary mechanism involves inducing apoptosis in tumor cells, but the impact of cyclotides on cytotoxic immune cells is poorly studied.

Natural Killer (NK) cells are cytotoxic innate lymphoid cells and play an important role in the defense against infected, stressed and transformed cells. Therefore, NK cells depict an important player in cancer immune surveillance: NK cells act against primary tumor cells as well as metastasis by preventing proliferation and metastatic spread to distant tissues. A balance of inhibitory and activating receptors controls NK-cell activity- once they are activated, NK cells can directly lyse target cells by secretion of their effector molecules perforin and granzyme. As NK cells in comparison with other immune cells do not need prior sensitization or have antigen specificity, they have shown promising potential in the field of immunotherapy.

As a first step, we screened various plant extracts on NK-cell functionality and observed that the extract derived from *Carapichea ipecacuanha* augments the killing potential of human NK-cell lines against different tumor targets *in vitro*. Based on these positive findings, we therefore extended this research by isolating different peptides from *C. ipecacuanha* and investigating the effect of those highly purified cyclotides on the function of NK cells. Within short time treatment with a prototypic caripe cyclotide, it was found to boost the cytotoxicity of both primary mouse NK cells as well as human NK-cell lines against various tumor targets.

With this study we hope to be able to offer novel insights into the potential of a peptide-based immunotherapeutic strategy. Our findings regarding the augmentation of NK-cell activity through a plant-derived peptide hold significant promise and could greatly contribute to advancing current immunotherapy approaches.

We therefore now aim to further investigate this phenomenon and gain further insights into the underlying mechanisms leading to the enhanced cytolytic potential of the NK cells. In order to attain those objectives, we intend to utilize both primary human NK cells and human NK-cell lines, where the selected anti-human CD antigens (CD56, CD107a, CD3, CD16) and anti-human antigen (IFN-g) as well as the cytokines (IL-2, IL15, IL-18) from ImmunoTools would be extremely helpful to advance our research, contributing to a better understanding on how to improve NK-cell functionality. For this, we plan several flow cytometry based assays, such as a degranulation assay, conjugation formation assay and intracellular staining, where the antibodies from ImmunoTools would offer great help.

ImmunoTools *special* AWARD for **Sophie Huszarek** includes 7 reagents

FITC - conjugated anti-human CD16, CD56

PE - conjugated anti-human CD3, IFN-gamma

purified anti-CD107a

recombinant human cytokines: rh IL-2, IL-15

DETAILS more AWARDS