ImmunoTools *special* Award 2024

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Uncovering new NK-cell-specific immune checkpoints in the context of triple-negative breast cancer

Triple-negative breast cancer (TNBC) is a subtype of breast cancer, which lacks the expression of oestrogen (ER) and progesterone receptors (PR) and has normal expression of the human epidermal growth factor receptor type 2 (HER2). It accounts for approximately 15-20% of all breast tumours and typically is a very aggressive disease with an increased mortality rate, a high risk of relapse and increased metastasis rates. Moreover, metastasis is the leading cause of death in TNBC patients and remains the biggest challenge for the therapy. Chemotherapy is the current standard-of-care and although TNBC is highly sensitive to chemotherapy, relapse is frequent, and a large effort is done to develop novel therapeutic approaches. Immune checkpoint inhibitors represent a breakthrough novel therapy in cancer treatment. The so-called immune checkpoints are essential regulators of immune cells, such as NK cells, guaranteeing controlled actions and preventing autoimmunity. In cancer, malignant cells often upregulate ligands for inhibitory receptors of cytotoxic cells – thereby disabling their cytotoxic capacity.

NK cells are innate lymphoid cells, which are able to recognize and kill virally infected and transformed cells. They discriminate between healthy and stressed cells by screening the ligands on the surface of surrounding cells. NK cell-mediated actions are strictly controlled by the balance of inhibitory and activating receptors, which either prevent or trigger the lysis of target cells. In the context of cancer NK cells are especially important to limit metastasising cells in breast cancer patients and can eliminate up to 80% of circulating tumour cells in the blood. Thus, high numbers of blood NK cells, high expression of activating receptors and improved NK-cell cytotoxicity correlate with patient survival. However, cancer growth and progression are associated with immune suppression and evasion and breast cancer patients typically show normal levels of circulating NK cells, but decreased NK cell

functionality. Apart from reduced cytotoxicity, NK cells in these cases show lower sensitivity to stimulation, downregulate activating receptors and upregulate inhibitory receptors.

Immune checkpoints are often overexpressed during cancer progression and thereby impair the cytotoxic functions of NK cells, which makes immune checkpoint blockade a promising new treatment option. Therefore, we hypothesize that targeting of NK cell-specific immune checkpoints will limit the metastasis of TNBC.

To investigate how blood NK cells and potential NK cell-specific immune checkpoints change during the progression of TNBC we made use of a TNBC mouse model, where we injected the TNBC cell line EO771 orthotopically in the mammary fat pad of female Rag2$^{-/-}$ mice. When the primary tumour reached approximately 70-75 mm^2 blood NK cells of tumour-bearing mice and healthy controls were isolated and used for 10x single-cell RNA sequencing. RNA sequencing showed that in the blood of tumour-bearing mice an NK cell population with an activated phenotype is strongly enriched and compared to healthy controls the NK cells in this population show downregulation of activating receptors such as NKG2D and other molecules important for NK cell function such as Septin1. We will validate these findings *in vitro* – using murine and human NK cells – and *ex vivo* by flow cytometry and Western blot.

The rh IL-2 and rh IL-15 from ImmunoTools would help us to do so, as IL-2 and IL-15 are essential cytokines to cultivate NK cells *in vitro*. We would use the rh IFNβ 1a and mouse anti NK1.1 to stimulate NK cells for functional assays. The antibodies against human CD56, IFN gamma, CD16, CD52 and CD314 and against murine CD25 we would use to validate targets we found in our RNA sequencing experiment.

ImmunoTools *special* AWARD for **Theresa Harm**

includes 10 reagents

FITC - conjugated anti-human CD52

PE - conjugated anti-human CD56, IFN-gamma

PerCP - conjugated anti-human CD

APC - conjugated anti-human CD16, CD314

anti-human NK1.1 func application

FITC - conjugated anti-mouse CD25

recombinant human cytokines: rh IL-2, rh IL-15, rh IFN-beta1

DETAILS more AWARDS