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# Badanie profilu migracji komórek NK i poszukiwanie metody poprawiającej ich infiltrację w terapii nowotworów litych

**Rozprawa na stopień doktora nauk medycznych i nauk o zdrowiu**

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# Mapping the chemotactic landscape of NK cells and seeking methods to enhance their infiltration in solid tumor therapy

## Abstract (Streszczenie w języku angielskim)

Natural killer (NK) cells hold promise as a highly functional template for genetic engineering in the development of next generation of anticancer cell therapies. The hostile conditions of tumor microenvironment (TME) in solid tumors often preclude NK cells from effectively performing their function, necessitating for modifications improving their resistance to suppressive factors in the TME. Another often overlooked prerequisite for extending the success of the cell therapies against solid tumors is that the cells must reach the intended target organ. The aim of this dissertation, based on three publications, is an in-depth characterization of NK cell migratory patterns and identification of a strategy improving tumor homing of NK cell-based therapies.

The first publication, „*Prospects for NK Cell Therapy of Sarcoma*", is a review article published in *Cancers*, MDPI´s open access journal focused on cancer biology and immunology. In the article, we review the role of NK cells in sarcomas immune surveillance, as well as the immunoevasion mechanisms of sarcomas that affect NK cell function. These include MHC class I upregulation, shedding of NK activating ligands, altered metabolism, and increased secretion of inhibitory cytokines. All of the above-mentioned factors contribute to creating a hostile tumor microenvironment, leading to impaired NK cell homing, cytotoxicity suppression, and ultimately, progression of the disease. We discuss various priming strategies and genetic modifications enhancing cancer cell recognition, tumor homing, and resistance to suppressive factors in the TME. We also discuss the possibility of sensitizing sarcoma cells to NK cell-mediated cytotoxicity by monoclonal antibodies, radiotherapy, hyperthermia, and other modalities.

The second publication, „*The Tumor Microenvironment—A Metabolic Obstacle to NK Cells' Activity*", is also a review article published in *Cancers*. It summarizes how the tumor immune evasion mechanisms employed by different solid tumors, including hypoxia, acidosis, oxidative stress, immunosuppressive cytokines, amino acid deprivation, immunosuppressive lipid, and adenosine metabolites, alter the effector functions of NK cells such as NK cell recruitment, lytic synapse formation and cytokine production. We also discuss strategies

aiming to combat the hostile TME conditions, restoring NK cell-mediated immune surveillance, that are evaluated in the *in vitro* and *in vivo* studies as well as clinical trials.

The third publication is an original research article entitled „*Mapping the Chemotactic Landscape in NK Cells Reveals Subset-specific Synergistic Migratory Responses to Dual Chemokine Receptor Ligation*" published in *eBioMedicine*, the *Lancet´s* open access journal for discovery science. This work bases on an observation that in contrast to other cell types, highly differentiated NK cells are rarely found in solid tumors, with their trafficking patterns remaining poorly understood. Therefore, we investigated the trafficking patterns of human NK cells utilizing high-dimensional flow cytometry, mass cytometry by time-of-flight (CyTOF), and single-cell RNA-sequencing combined with functional assays. We found that the chemokine receptor repertoire of peripheral blood NK cells changes in a coordinated fashion becoming gradually more diversified during the differentiation process. The chemokine receptor expression correlated tightly with the migratory response of the distinct NK cell subsets. We also found that simultaneous ligation of CXCR1/2 and CX3CR1 receptors led to a synergistically enhanced migratory response. Investigation of 9471 solid cancer cases in the TCGA/TARGET databases revealed nine predominant chemokine profiles that varied among tumor types, but none of them had ligands for more than one chemokine receptor expressed on mature NK cells. Our results show that the sparsity of naturally occurring pairs of chemokines-chemokine receptors may explain the systematic exclusion of NK cells from the tumor microenvironment and represent an untapped potential for engineering next-generation NK-cell based therapies in oncology.

In summary, through literature review we identified the issue of impaired infiltration of NK cells into solid tumors as well as some of the underlying mechanisms. Then, we performed systematic characterization of NK cell migratory profile in subset resolution, that combined with bioinformatic analysis of over nine thousand solid tumors provided evidence for systematic exclusion of NK cells from the TME by limiting their homing through restrictive chemokine profile. Most importantly, the results presented in this dissertation indicate that arming NK cells with at least two chemokine receptors matching the chemokine profile of the tumor, represents a novel approach for engineering of NK cell-based therapies, based upon the discovered chemokine receptor synergy phenomenon.