Oponentský posudek diplomové práce

Jméno a příjmení uchazeče: **Bc. Vojtěch Graman**

Název práce: **Regulation of intracellular calcium levels as a tool to control NK cell cytotoxicity**

Oponent: **doc. RNDr. Michal Šimíček, Ph.D.**

The presented diploma thesis on the topic "*Regulation of the level of intracellular calcium as a tool for controlling the cytotoxicity of NK cells*" is conceived as a study dealing with the key mechanisms of innate immunological defence mediated by natural killer (NK) cells. The main aim of this work is to investigate the effect of Toll-like receptors (TLR) stimulation on intracellular calcium levels (Ca2+) and NK cell function, and thereby better understand the potential possibilities of improving NK cell-based immunotherapies, especially in the context of acute myeloid leukemia (AML).

In the beginning, the author provides an extensive theoretical introduction to the topic, including a detailed description of NK cells, their functions, mechanism of NK cell-mediated cytotoxicity, the role of TLRs in the immune system and the specifics of AML. It provides the reader with a solid foundation for understanding the following experimental section. The thesis is well written, according to general professional standards and is almost free of stylistic and grammatic mistakes. Only in certain sections the text is unnecessarily complicated and simpler sentence constructions could be used for better clarity.

To achieve the suggested goals, multiple methods were used. Namely, isolation of primary NK cells from peripheral blood mononuclear cells, fluorescent-based measurement of Ca2+ influx, cytotoxicity assays, degranulation tests, qPCR analysis and others. All methodologies and used reagents are well described and correspond to modern standards in immunological research. The results of the study are clearly presented and logically interpreted. The author demonstrates the ability to perform complex experimental procedures and analytical techniques.

Although the results are presented clearly, the thesis could reflect more on the limitations of the used methodology and experimental design. For example, it would be appropriate to discuss in more detail possible sources of variability in Ca2+ influx measurements or cytotoxicity assays and how these variability factors were controlled. Additionally, the gene expression analysis revealed an increase in NFAT and Orai1 expression in NK cells stimulated with selected TLR ligands. The results are critically discussed in the context of current knowledge in the field. Nevertheless, this section could include a more detailed discussion of certain mechanistic details such as how upregulation of NFAT and Orai1 genes might contribute to the observed changes in cytotoxicity and degranulation.

The conclusion of the thesis summarizes the main results and indicates the direction for further research. It is well worded and coherently concludes the entire study that provide potential benefits for clinical practice, especially for the improvement of NK cell-based immunotherapies. Specifically, the results suggest that chemotherapy-induced expression of

DAMP molecules can disrupt Ca2+ homeostasis and thereby impair the cytotoxic activity of adoptively transferred NK cells. These findings may lead to the development of new strategies to optimize NK cell therapies in AML patients.

In summary, the diploma thesis of Vojtěch Graman represents a significant contribution to the understanding of the mechanisms of NK cell cytotoxicity regulation through intracellular Ca2+ signaling. The work is carefully prepared, methodologically sound, and as already stated its results have the potential to contribute to the optimization of NK cell immunotherapies. Overall, this is a high-quality thesis that reflects the author's deep knowledge of the subject and determination for scientific work.

I have following questions related to the presented diploma thesis:

1. What is the potential effect of long-term exposure to TLR ligands on NK cell function and cytotoxicity?

2. Are there any specific inhibitory mechanisms or molecules that could be targeted to increase the efficacy of NK cell therapy in the context of altered Ca2+ signalling?

3. Could you speculate, which other genes or molecular pathways besides NFAT and Orai1 could be affected by changes in intracellular Ca2+ levels?

in Ostrava, 27. 5. 2024

doc. RNDr. Michal Šimíček, Ph.D.