NICOLAUS COPERNICUS
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# DOCTORAL DISSERTATION ABSTRACT

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Title of the doctoral dissertation: **Purinergic signaling in glioma – in vitro studies on pathological mechanisms and therapeutic potential**

Doctoral dissertation abstract:


Gliomas, tumors of the nervous system that originate from glial tissue, are considered one of the most aggressive and difficult to treat type of cancer. As they spread throughout the central nervous system and often do not form any solid tumors, they present a challenge to the surgical intervention. In addition, the presence of the blood-brain barrier significantly limits the number of chemotherapeutic agents that can be used, hence the intense search for alternative therapies. One such method is the induction of differentiation towards neural cells, which allows to reduce the proliferative and migratory potential of the cancer cells.

Extracellular ATP (eATP) is the predominant component of the tumor microenvironment, as well as a natural ligand for two families of purinergic receptors: metabotropic P2Y and ionotropic P2X. Among these structures, the P2X7 receptor has received the most attention. Recent studies have identified two distinct isoforms of the P2X7 receptor in the human body: P2X7A and P2X7B. Both of them are involved in the regulation of tumorigenesis. Determination of the success of anti-cancer therapy is dependent on their expression levels in the patient's tumor tissue. Activation of the P2X7A isoform leads to the opening of an ion channel, while with prolonged stimulation it transforms into a non-selective pore, leading to apoptotic cell death. The P2X7B isoform is devoid of one of its intracellular domains - a change that renders such a receptor lacking the pore-opening capacity, whereas its activation has a pro-proliferative effect.

The main research goal was to determine changes in the purinergic microenvironment in cells of three human glioma cell lines, A172, M059K and M059J, subjected to retinoic acid-induced differentiation. Additionally we aimed to access how the alterations in the purinergic signaling system influence cell sensitivity to chemotherapeutics.

Analysis of changes in the expression levels of neurogenesis markers confirmed the differentiation of the cells studied towards the neural lineage. The differentiation procedure resulted in a decrease in cell proliferation and migration rates, as well as a number of modifications in the purinergic signaling system. Among the most important findings were: an increase in the expression level of the P2X7 receptor at the mRNA and protein levels, and a shift in the expression ratio of P2X7A/B isoforms, as well as a decrease in the activity of extracellular adenine nucleotide-degrading enzymes. As a result, we observed an increase in the sensitivity of cells to the cytotoxic effect of eATP and BzATP, a specific P2X7 receptor

agonist, and a decrease in the rate of cell migration - these changes were reversibly modulated by P2X7 receptor activation. Furthermore, we showed that, despite an increase in the expression of the pro-proliferative P2X7B isoform, there was no activation of intracellular signaling pathways that stimulate proliferation, as it was the predominant, pro-apoptotic P2X7A isoform that determined the cytotoxic effect of eATP.

Studies regarding the interactions between elements of purinergic signaling and temozolomide, one of the most commonly used chemotherapeutics in glioma therapy, prove, that concomitant use of a chemotherapeutic agent with P2X7 receptor ligands significantly enhances the cytotoxic effect. At the same time *in silico* studies supported by *in vitro* studies indicate, that temozolomide may act as a P2X7 receptor antagonist.

These results indicate, that retinoic acid differentiation in combination with control of purinergic signaling in the tumor microenvironment, may have potential as a supportive therapy to current anticancer treatments. Additionally, the ability to influence the expression level of P2X7 receptor isoforms and its conditional and site-specific activation may represent a promising therapeutic target in the future.

**Keywords:** glioma, purinergic signaling, eATP, P2X7 receptor, differentiation, chemotherapy

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doctoral student's signature