Ocena potencjału terapeutycznego metylotransferazy DNMT2/TRDMT1 w odpowiedzi na liposomalne kompleksy zewnątrzkomórkowego RNA w modelach komórkowych kostniakomięsaka *in vitro*

Summary

Free nucleic acids released from cells as a result of various processes, such as death, senescence, or in response to stress, are known as damage-associated molecular patterns (DAMPs). These molecules mediate intercellular communication, thus regulating many pathways in cells. Free nucleic acids are believed to induce inflammation, which plays a dual role in cancer biology. On the one hand, by activating the immune system, it induces the removal of cancer cells. On the other hand, chronic inflammation can lead to tissue damage and create an environment that promotes cancer development.

Based on previous research conducted by our group, the involvement of the DNMT2/TRDMT1 methyltransferase in the response to free nucleic acids released as DAMPs was hypothesized. Through RNA methylation this protein mediates different pathways such as DNA repair, senescence, and the stress response. Furthermore, DNMT2/TRDMT1 methyltransferase can regulate the immune response, consistently leading to changes in the secretory phenotype of cells.

Taking into account the pleiotropic role of DNMT2/TRDMT1 methyltransferase in the regulation of various cellular pathways, it was decided to determine the role of this protein during the response to extracellular RNA. As a research model, osteosarcoma cell lines were used: U-2 OS, SaOS-2, and MG-63, which were exposed to various concentrations of etoposide. Extracellular RNA was isolated from the cell culture medium from dying or etoposide-induced senescent cells. The obtained extracellular RNA was then used for cell lipofection. The influence of extracellular RNA enclosed in liposomes on the regulation of processes such as proliferation, cell death, redox homeostasis, and the immune response of cells was characterised. The significance of the lack of functional DNMT2/TRDMT1 protein was also determined in cells with chemotherapeutic-induced senescence during response to lipofection with extracellular RNA. In the last stage of the dissertation, an attempt was made to determine whether exogenous RNA can modify the response of cells exposed to chemotherapy drugs and what role DNMT2/TRDMT1 methyltransferase plays in this process.

Extracellular RNA encapsulated in liposomes has been shown to promote redox disturbances of homeostasis in osteosarcoma cells, modulate the pro-inflammatory response, leading to

induction of cell death in the DNMT2/TRDMT1 methyltransferase-dependent manner. DNMT2/TRDMT1 methyltransferase was also observed to be an important factor controlling the innate immune defence of cancer cells with etoposide-induced cell senescence. Moreover, based on the results, it can be concluded that exogenous RNA stimulates the repair of DNA damage in cells treated with etoposide. Due to the fact that loss of DNMT2/TRDMT1 was associated with DNA repair defects despite the presence of exogenous RNA, DNMT2/TRDMT1 methyltransferase represents an important target for cancer therapy. Therefore, the results presented constitute an important addition to the knowledge regarding the involvement of DNMT2/TRDMT1 in the regulation of cancer pathogenesis and indicate a previously uncharacterized mechanism in which the DNMT2/TRDMT1 methyltransferase may regulate the response of cancer cells to extracellular or exogenous RNA.