

Effective treatment of cancer is currently one of the major challenges of modern medicine. Epidemiological and clinical research has confirmed that the development of cancer is largely affected by prolonged infection with simultaneous inflammation. Moreover, studies revealed that pro-inflammatory factors secreted at the site of infection promote tumor growth and reduce the sensitivity of cancer cells to treatment, leading to the development of multidrug resistance (MDR). This phenomenon is associated with reduced susceptibility of tumor cells to anti-cancer drugs with unrelated mechanism of action or chemical structure. It is estimated that up to 40% of currently diagnosed tumors may be resistant to conventional chemotherapy, which greatly increases the risk of recurrence and mortality. Given the above, the search for new drugs with significant anti-cancer activity, effective in the treatment of tumors resistant to conventional treatment with cytostatics, is an important priority for modern science.

In order to sensitize cancer cells to anticancer drugs, immunomodulatory compounds affecting the immune system might be employed. One such agent is cathelicidin LL-37, present in the human body and belonging to the family of natural antimicrobial peptides (AMPs). It has been shown that the antimicrobial effect of LL-37 is due to the membrane activity of this compound - the peptide interacts with negatively charged surface membranes, creates pores within it and ultimately leads to its disruption resulting in cell death. Importantly, the LL-37 peptide inhibits the secretion of pro-inflammatory cytokines, which are responsible for initiating the inflammatory process in healthy cells. This is particularly important considering studies that report the possibility of cancer induction through pro-inflammatory factor-mediated drug resistance. It was also shown that LL-37 peptide, its fragments, and synthetic analogs (ceragenins) possess significant anti-tumor potential, which is associated with the ability to interact with the cancer cell membrane. Additionally, these peptides may be employed in the treatment of colon and gastric cancers, and hematological malignancies due to the induction of apoptosis and cell cycle arrest. The biological activity of these compounds can be intensified by the engagement of nanoparticles as drug carriers, which significantly improve the activity of anti-tumor substances and facilitate their penetration into cancer cells. Importantly, nanoparticles (structures with size up to one billionth of the meter in at least one dimension) reduce the viability of cancer cells, affecting their metabolism and leading to the breakdown of their genetic material, which creates the possibility of combined interaction of these structures with tested compounds.

The aim of the project is to evaluate the immunomodulatory and anti-tumor activity of nanosystems composed of magnetic nanoparticles and membrane-active agents (LL-37 peptide, its fragment FK-16, ceragenins) or an anthracycline antibiotic. The studies will be conducted on anthracycline-resistant breast, ovarian and colon cancer cell lines. The main hypothesis guiding this project is that the designed nanosystems will possess significant anti-tumor activity and immunomodulatory properties that will enable them to overcome the drug resistance of the cells. In the project, a number of methods assessing the detailed biological activity and mechanism of action of studied nanosystems will be employed. First of all, synthesized nanosystems will be thoroughly tested for physicochemical and morphological properties. The potential of nanosystems to reduce secretion of pro-inflammatory cytokines initiating inflammation in healthy cells will also be investigated. The biocompatibility of tested agents, number of cancer cells entering the apoptosis process and level of DNA fragmentation after treatment with analyzed nanosystems will be analyzed. In the next stage of the research, the stiffness of the treated cancer cells will be assessed. Recent studies indicate that cancer cells are less stiff (hard) than normal cells. Accordingly, the increase in stiffness of the treated cells may indicate the anti-tumor activity of studied agents. The next stage of this project will examine the detailed mechanism of action of the tested nanosystems. First, we will investigate whether the nanoparticles achieve a sufficiently high concentration inside the cancer cells. Next, the activity of enzymes necessary for the proper action of ABC transporters (determining the resistance of tumor cells to the drugs) and the activity of the proteins that control the process of apoptosis will be explored. Moreover, it will be assessed to what extent test compounds affect the metabolism of cancer cell. In the last stage of the project, information obtained from the *in vitro* research will be tested in a mouse model of resistant cancer.

Realization of the project will allow for accurate and comprehensive examination of the potential of the proposed nanosystems to reduce the resistance of tumors. This will create an opportunity to develop new therapeutic methods applicable in cancer chemotherapy, especially against cancer cells insensitive to the conventional therapeutic methods. Achieving all the goals will not only increase patients' quality of life, but also will improve the efficiency and safety of currently used therapeutic methods.