SUMMARY

Malignant neoplasms are a serious health, social and economic problem in Poland and in the world. Over the past thirty years the incidence of malignant neoplasms in Poland has doubled. One of the most common haematological neoplasms in adults is acute myeloid leukemia. Acute myeloid leukemia is characterized by uncontrolled proliferation and lack of differentiation of immature hematopoietic stem cells and progenitor cells. This leads to the accumulation of immature and functionally impaired blast cells in the body. Infiltration of bone marrow by blast cells leads to hematopoiesis disorder, which results in anemia, thrombocytopenia and neutropenia. The treatment of recurrent and primary refractory acute myeloid leukemia is the most serious problem. Despite continuous attempts to improve the effectiveness of acute myeloid leukemia therapy, the outcome of treatment has remained almost unchanged for decades. The most effective therapy is intensive chemotherapy and hematopoietic cell transplantation, which are accessible for a limited number of patients. Because of high phenotypic and genotypic heterogeneity of acute myeloid leukemia it is necessary to develop a personalized therapy based on determining the individual sensitivity of blast cells to chemotherapeutics.

The aim of dissertation is to develop diagnostic and therapeutic methods for chemoresistance in acute myeloid leukemia. The dissertation presents interdisciplinary research closely related to the field of Biomedical Engineering with use of the latest optical measuring techniques. The proposed method of diagnosis of chemoresistant acute myeloid leukemia clones is based on the changes in biomechanical properties of cells following the incubation with drug. Cells obtained from the bone marrow of patients with acute myeloid leukemia were incubated with topoisomerase II inhibitors (epirubicin and daunorubicin). Stiffness of cells was studied with holographic optical tweezers. The results of the experiments were correlated with nuclear localization of drugs, which was determined by confocal fluorescence microscopy. It was found that changes in the biomechanical properties of blast cells occur only when the cytotoxic substance accumulates within the cell nucleus. No changes in blast cell stiffness were observed when the drug was localized in the cytoplasm. It was concluded that changes in the biomechanical properties of cancer cells after incubation with drug are observed only in chemosensitive cells. It was also found that the nuclear localization of drugs may indicate the sensitivity of cells to chemotherapeutics. Clinic of Hematology, Blood Neoplasms and Bone Marrow Transplantation at the University Teaching Hospital in Wroclaw provided the results of cytogenetic and molecular studies of patients with acute myeloid leukemia. The results of genetic and immunophenotypic studies of patients with acute myeloid leukemia were correlated with blast cell stiffness measurements and with the intracellular localization of topoisomerase II inhibitors. It has been found that the presence of genetic mutations (FLT3-ITD, NPM1) or surface antigens (CD33, CD34, CD123), which are an unfavourable prognostic factors, cause that topoisomerase II inhibitors do not localize within the nucleus, and no changes in cell stiffness due to incubation with these compounds are observed. The effect of daunorubicin on cell nuclei stiffness was also studied. Daunorubicin is an anthracycline antibiotic used in every treatment regimen for patients with acute myeloid leukemia. It was found that with increasing drug concentration, the stiffness of cell nuclei also increases. Increased stiffness of cell nuclei

was also observed as a result of low concentrations of DNA methylation inhibitors – azacitidine and decitabine. DNA methylation inhibitors trigger the activation of suppressor and cell cycle regulating genes. Low dose azacitidine and decitabine affect nucleic acid methylation by inhibiting DNA methyltransferase.

A method has been developed to determine the intracellular location of daunorubicin by flow cytometry. It has been found that by flow cytometry it is possible to study the presence of daunorubicin in cells using more than 100 times lower drug concentration than in confocal microscopy studies. A method to study the release of daunorubicin from leukemia cells using flow cytometry has also been developed. The developed method enables studies of changes in the amount of drug in cells. Active removal of the drug from cells was demonstrated by this method. Daunorubicin does not passively diffuse across the cell membrane outside the cell. Drug removal from the cells is an active process.

The proposed method of the therapy of refractory acute myeloid leukemia is based on sensitizing chemoresistant cells using DNA methyltrasferase inhibitors. Decitabine has been found to increase the mortality of leukemia cells treated with low concentrations of cyclophosphamide. Decitabine affects cell morphology, number and length of cellular protrusions and reduces the proliferative potential of leukemic clones in the long term. Decitabine inhibits cellular protrusions formation, which may reduce the proliferative potential of leukemia cells.

The results of experiments confirm the possibility of developing a fast and effective test that detects chemoresistant cells based on their biomechanical properties and intracellular drugs location using the equipment available in the hospital. Sensitizing of chemoresistant cells to chemotherapeutic agents would increase the therapeutic options for patients with acute myeloid leukemia and reduce the side effects of chemotherapy.

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