DOCTORAL THESIS ABSTRACT

„*Spectroscopic characteristics of in vitro models of selected leukemia subtypes and evaluation of their interaction with exogenous substances*”

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Myeloproliferative neoplasms are a heterogeneous group of cancers of the hematopoietic system, among which leukemias are the most common. They involve the clonal proliferation of malfunctioning blood cell precursors, which can take an abrupt course as in Acute Lymphoblastic Leukemia (ALL) and Acute Myeloid Leukemia (AML), or a chronic as in Chronic Lymphocytic Leukemia (CLL) and Chronic Myeloid Leukemia (CML). A broad spectrum of adverse precursor genetic alterations result in disorders with distinct mechanisms of action affecting the clinical course of the disease and patient prognosis. Diagnosis of molecular subtypes of leukemia is essential for appropriate selection of a medical treatment protocol and subsequent monitoring of the therapy effectiveness. Immunotyping, cytogenetic, or molecular studies are currently being conducted in clinical practise. As one of the alternatives in leukemia diagnosis, Raman spectroscopy, which provides more complete information about the composition of the test sample also in their natural environment, without the need for labeling with exogenous substances is indicated. Supporting the analysis of spectroscopic data with a series of chemometric methods increases the diagnostic potential of this method while providing information on important spectral features related to the state of the cell and the disorders development.

Despite the undoubted advantage of Raman spectroscopy, which is the label-free approach, application of triple bond containing compounds or isotopically substituted draws scientific attention, as it allows the detection of well-isolated narrow Raman bands in the spectral range 2800-1800 cm^{-1}. In biological systems studied by Raman, vibrations important to the sample characteristics are observed beyond this region. So-called Raman reporters composed of a Raman signaling part and groups with affinity for specific biological structure are good candidates to follow the physicochemical conditions of cells. The labelled approach allows high-sensitivity tracking of the distribution of multiple reporters simultaneously, giving an advantage over fluorescence-based methods in which bands of significant width are recorded.

This dissertation is focused on distinguishing molecular subtypes of leukemia as well as monitoring the effects of exogenous substances using *in vitro* models. Raman spectroscopy was used as the leading method. The experimental part was divided into three subsections verifying each research hypothesis.

The first **identifies spectroscopic markers of high-risk molecular subtypes of B Cell Precursor Acute Lymphoblastic Leukemia (BCP-ALL) with present KMT2A rearrangement and *Philadelphia* chromosome** (*BCR-ABL1* gene fusion), which require tailored chemotherapy due to different molecular mechanisms. The next subsection **traces changes in the biochemical state of BCP-ALL cells with the JAK2 kinase point mutation as a result of interaction with the JAK2 kinase inhibitor ruxolitinib**. The most extensive subsection of the results section verifies the hypothesis, assuming **changes in the biochemical state at the level of single acute promyeloblast leukemia cells upon incubation with all-*trans*-retinoic acid** (ATRA) leading to the change in the phenotype of the promyeloblast cells HL-60 cell line to neutrophil-like cells. Moreover, the supplication of the **MitoBADY Raman reporter was shown to improve the classification sensitivity and specificity of promyelocytes and cells with neutrophil phenotype**.

Commercial *in vitro* models popular in a wide range of leukemia research were used for spectroscopic characterization of BCP-ALL subtypes. Cells with *KMT2A-AFF1* rearrangement (t(4;11)(q21;q23)) represented the RS4;11 and SEM lines, while SD-1, SUP-B15 and BV-173 were used as cells with *BCR-ABL1* gene fusion. Employing several *in vitro* models of BCP-ALL subtypes was motivated by the need to find consistent spectroscopic markers for all cell lines, which are a consequence of chromosomal aberrations, rather than resulting from features characteristic only of individual cell lines. With support of unsupervised e.g. Principal Component Analysis (PCA) and supervised Partial Least Squares Discrimination Analysis (PLS-DA), cellular components such as DNA (795 cm^{-1}) hemoproteins (1585, 1130 and 753 cm^{-1}) amino acids phospho-L-tyrosine and phospho-L-serine (825 cm^{-1}) were recognized as prevailing factor in *Philadelphia* positive cells ,while unsaturated lipids (1658 cm^{-1}) and phospholipids (1180, 1076 cm^{-1}) were predominant in BCP-ALL with KMT2A-AFF1 rearrangement. Moreover, this subsection raise an issue of model evaluation metrics in unbalanced groups, pointing to the Matthew's correlation coefficient as the most reliable in comparison with sensitivity and specificity.

In the next section, spectroscopic characterization of BCP-ALL cells with point mutations of the JAK2 kinase clocked with the tyrosine kinase inhibitor ruxolitinib was investigated. Two commercially available JAK2 point mutation models I682F (MHH-CALL-4) and R638G (MUTZ5) were used to follow the interaction with 10 μM of the drug for 0.5 h and 48 h.

With the support of orthogonal PLS-DA, the classification of cell Raman spectra in both *in vitro* models was based on the 788 cm^{-1} band of nucleic acid vibrations despite the incubation time. Differences in the secondary structure of the proteins after 0.5 h of incubation of the cells with the drug were identified based on 1665, 1275, 945 cm^{-1} bands of α-helix, while the 1678, 1243 cm^{-1} bands characteristic of proteins with disordered structure and β-sheet in control cells were also noted.

The most extensive subsection of the experimental part is the spectroscopic characterization of an *in vitro* model of induced differentiation of HL-60 promyelocytes into cells with a neutrophil phenotype in the presence of ATRA. The diagnostic method was developed based on three integral intensities, evaluating the reduced form of cytochrome c (753 cm^{-1}) and lipids (2850 cm^{-1}) in relation to the signal intensity of a Raman reporter such as MitoBADY (2220 cm^{-1}). The application of the reporter improved the classification performance comparing to models based on single bands 2850, 750 and 2220 cm^{-1}. As a result of the presence of a positively charged directing group in the structure of MitoBADY, its accumulation depends on the mitochondrial membrane potential. Despite the decrease in the value of the mitochondrial membrane potential under the influence of ATRA, it was shown that the increased accumulation of MitoBADY in cells with neutrophil phenotype can be associated with an additional factor, the increase in lipid content. In addition, analysis of hyperspectral images by Multivariative Curve Resolution (MCR) provided detailed information about the biochemical state of the cells studied. Trends observed on the mean spectra and integral intensity values were confirmed primarily as changes within the nucleus, an increase in unsaturated lipid content, a decrease in the intensity of the 753 cm^{-1} band or accumulation of ATRA in lipid structures. In addition, it was shown that the selection of several vibrational frequencies of Raman markers is sufficient to describe the biochemical state of the system studied using stimulated Raman scattering.

In conclusion, in the present thesis, markers of *in vitro* models of selected leukemia subtypes were identified and the effects of exogenous substances (chemotherapeutics or Raman reporters) were evaluated using Raman microscopy supported by chemometric methods. All the defined objectives were met. Furthermore, the research presented in the last subsection of the experimental part will be continued within the framework of the Polish National Science Centre Preludium 20 project.