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“The spectroscopic methods in the study of the toxicity of the primary tumors after implantation in the rat brain”.

Abstract

Tumors are the second leading cause of death among men and women and about 5-10% of them are primary brain tumors. One of the most aggressive central nervous system tumor, accounting for about 16% of all primary brain tumors, is glioblastoma multiforme (GBM). Despite radical therapies, mortality rate caused by GBM is high and the median survival of patients from diagnosis does not exceed 2 years. Therefore, many interdisciplinary researches are undertaken for better understanding the nature of glioblastoma as well as to get novel knowledge about its pathogenesis. These type of researches are often performed with the use of animal models.

The main purpose of the research performed in the frame of the doctoral dissertation was to identify the elements and biomolecules involved in the development of glioblastoma multiforme in the animal models of the disease. The first stage of the investigation was the implantation of three different human GBM cell lines into rat's brains. The next step was to choose an analytical tool for the determination of the elemental composition of the separate brain hemispheres taken from the rats subjected to implantation. From the various available methods of elemental analysis of biological samples, Total Reflection X-ray Fluorescence (TXRF) spectroscopy was selected. An attempt was made to verify the usefulness of the TXRF in the elemental analysis of biological samples. The investigation included the evaluation of selected validation parameters of performed measurements, i.e. precision, trueness and detection limits, as well as assessment of the inter-laboratory comparison of the results of the mammalian tissue samples analysis. Next, the method of Synchrotron Radiation based X-ray Fluorescence (SR-XRF) was used to examined changes in the distribution of elements in the rat brain tissues. In turn, anomalies in the accumulation and structure of biomolecules in the tissues were analysed using Fourier Transform Infrared Spectroscopy (FTIR) and Raman microscopy.

The obtained results confirmed the high usefulness of the TXRF method in the analysis of biological samples, especially for elements with a higher atomic number. The TXRF analysis of rat brain hemispheres allowed to indicate the elements, which potentially may be involved in the development of GBM, and the number of the observed anomalies correlated with the degree of aggressiveness of the cells used for implantation. Topographic analysis performed using the SR-XRF method, revealed changes in the accumulation of elements in the regions of the tumor and its surroundings. Analysis of the maps of elemental distribution within the examined tissues along with their microscopic evaluation allowed to discuss the potential causes of the observed elemental anomalies in the animal models of GBM. In particular, it was possible to indicate selenium as a possible biomarker of GBM development. Two-dimensional biochemical analysis performed using FTIR and Raman microspectroscopy, showed local changes in the accumulation of the main biological molecules in the rodents brains. The areas

of the tissues affected by the tumor growth were characterized by a reduced content of the lipids, nucleic acids and compounds containing carbonyl groups, as well as changes in the level and structure of the proteins.

To conclude, in the frame of the doctoral dissertation, the various spectroscopic methods were used to examine the toxicity of the glioblastoma multiforme developed in the rodents brains after implantation of human cells of the tumor. The results of elemental and biochemical analysis correlated with the histological changes of the tissues resulting from the GBM development. Furthermore, performed researches allowed to identify potential elemental and biomolecular markers of GBM toxicity.