Investigation of pathological mechanisms in brain cancers with the use of techniques based on synchrotron radiation

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In recent years literature studies indicate an essential role of minor and trace elements in a number of pathological processes. Selected elements may contribute, directly or indirectly, on the carcinogenic process. The elemental abnormalities, including these related to Zn, Cu and Fe in various forms of cancer were reported over the past decade. However, the exact role of these and many other elements in carcinogenesis remains unknown. Molecular oncology is in need of the application of structural methods which are capable of monitoring biochemical processes and interactions within the neoplastic tissues. The recent development of beam-lines of third generation synchrotron sources enables spatially resolved XRF and XAS biochemical analysis at cellular and subcellular levels.

The methods based on synchrotron radiation i.e. synchrotron radiation x-ray fluorescence (SRXRF), X-ray absorption near edge structure (XANES) spectroscopy, and extended x-ray absorption fine structure spectroscopy (EXAFS) were used for analysis of brain tumor samples. The samples designed to analyses were taken intra-operatively from brain cancers (mainly gliomas) of different types and various grades of malignancy. The investigations included also brain tissue apparently without malignant infiltration. Two types of samples were measured, cryo-preserved thin slices and frozen fragments of resected tumor tissues. The micro-SRXRF and micro-XANES analyses were performed in thin freeze-dried cryo-sections. The elemental chemical micro-imaging was carried out for “homogenous” areas of cancerous tissues as well as for other characteristic structures like blood vessels or areas of calcification. The cryo-EXAFS method was used for studies of tumors samples having mass about 0.5 g. The measurements were performed at the bending magnet beam lines L and C at HASYLAB, Hamburg. The beam on line L was focused to a size of 15 micrometers in diameter. The measurements were carried out in air. The measurements on line C were performed in vacuum at temperature about –168 °C.

The results of micro-SRXRF imaging of areas of calcification in brain tumors showed that high level of Ca was accompanied by increased level of Zn. It may suggest that Zn participate in generation of calcified granules together with Ca. Moreover, it was found that Fe level is significantly lower in the calcified structures in comparison with surrounding tissue. Iron and zinc were also determined in the areas of the tissue containing blood vessel. Significantly higher levels of Fe and Zn were noticed in the closed vicinity of blood vessel as well as in the surrounding tissue structures till about hundreds of micrometers from the vessel. Synergy of the SRXRF and multiple discriminant analysis (MDA) was applied for deconstruction of the samples histopatological structures (neoplastic cells, blood vessels, calcification) based on their elemental content. The MDA of elements commonly found in tumor tissues was also used to differentiate neoplastic samples according to their histopathological classifications. MDA allowed finding the elements of the highest importance for the general discrimination of tumor type. It seems justifiable to suppose that the abnormal reactions related with these elements are a source of the unique elemental fingerprint of different types of brain tumor. It suggests that the created elemental fingerprinting may be a very useful tool assisting the process of histopathological diagnosis of tumors especially in difficult or disputable cases.

The topographic analysis of Fe speciation in the tissues investigated with the use of XANES technique indicated the presence of the microstructures where Fe2+ is dominant as well as these of high abundance of oxidized form of Fe. The quantitative analysis showed that for all cases the content of oxidized form of Fe is significantly higher in comparison to the Fe2+. The highest level of Fe3+ was found in control sample whereas the lowest one for the glioma of the highest grade of malignancy. It was found that position of the edge of Zn in XANES spectra collected for all the tissue structures is the same as for the control material in which zinc is present on +2 oxidation state. The presence of metallic Zn was not found. Distribution of oxidized form of Zn in tissue structures was determined. It was noticed that the areas of calcification reveal high level of Zn 2+. The Fourier transforms of the tumor tissue Fe and Zn EXAFS functions are indicating differences in the paths of scattering obtained for measured samples. These findings, however promised from medical point of view, require verification by investigation of a statistically reliable number of samples relevant for selected pathology.

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