Analysis of selected elements in single cells of brain tissue

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The process of cancerogenesis may influence normal biochemical reactions leading to alterations in the elemental composition of the tissue. Therefore, the element content of neoplastic cells may differ from those in normal specimens as well as it may differentiate various tumor types. Molecular oncology still is in need of the application of structural methods which are capable of monitoring biochemical processes and interactions within the neoplastic tissues. The purpose of our studies was to investigate if the concentrations of minor- and trace elements in the malignant tissues can be used for the differentiation and/or classification (diagnosis) of brain tumors.

Choroid plexus (CP) is a specialized structure within brain which produces cerebrospinal fluid. It is created by meningothelium covered with modified ependymal glia. CP can be found in the whole ventricular system of the brain, except for the so called aqueduct (the narrowest part of the ventricular system of the brain). The cells that cover CP have cubic or cylindrical shape and form the epithelial monolayer resting on the basal lamina. CP consists of numerous small structures called villi. Inside of villi there are numerous vessels: feeding arterioles, draining venules and between them – a tiny capillary network. On the surface of covering cells facing the lumen of ventricles there are numerous microvilli. The main known role of CP is the production and secretion of cerebrospinal fluid (approx. 500 ml/24hrs). The overproduction of cerebrospinal fluid or any hindrance of its outflow to the subarachnoid space, leads to hydrocephalus and even to elevated intracranial pressure.

The synchrotron radiation X-ray fluorescence spectroscopy (SRXRF) was used for the elemental analysis of brain tissues. The samples designed to biochemical micro-imaging were taken intraoperatively from brain gliomas of various grades of malignancy. The samples of choroid plexus were taken during the authopsy. In each case the samples taken for standard histopathological intraoperational examination were cryosectioned at 5 μm thickness for routine hematoxylin-eosin (HE) staining and at 5 μm thickness for the SRXRF spectroscopy experiments. The slices destined for elemental analysis were mounted immediately onto appropriate sample supports and finally freeze-dried. The experiments for this study were carried out at the beamline P06 on PETRA III. The X-ray micro-beam with a diameter of 700 nm and an energy of 17.5 keV was used and the excited characteristic radiation was collected with a Vortex SDD detector. The SRXRF spectroscopy revealed that the elements such as P, S, Cl, K, Ca, Fe, Cu, Zn, Br and Rb were present in all analyzed tissues.

The maps of element distribution illustrate the mass deposits per unit area ($\mu g/cm^2$) of the elements of interest. The correlations between element concentrations were calculated. The various degrees of the correlations between pairs of elements were obtained. Fig. 1 shows the element distribution within CP sample, while Fig. 2 demonstrates the chemical composition of brain glioma tissue. The correlations between Zn and S as well as between Zn and Fe in the CP sample and glioma tissue were presented in Fig. 3. Two maps of cancerous and two of non-cancerous regions were considered. The analysis showed that the correlation between the concentration of selected elements in cancerous tissue was much weaker than that one of non-cancerous tissue.

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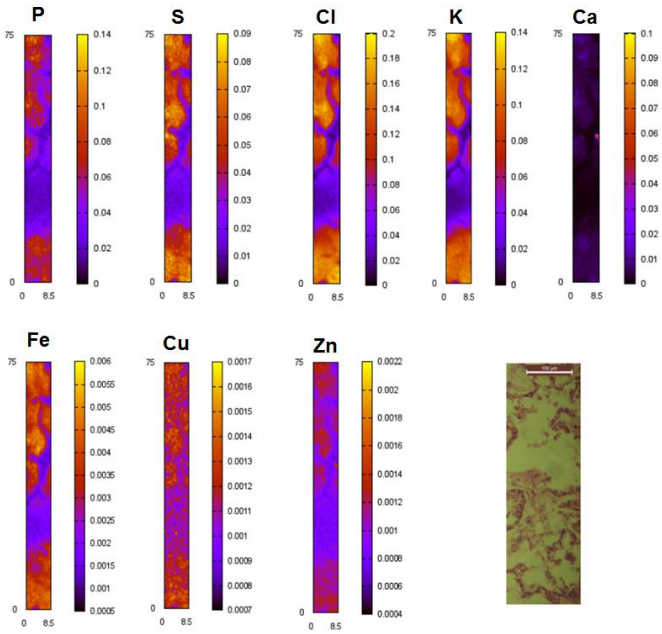

Fig. 1. SRXRF maps of element distribution in brain chorodic plexus tissue and an optical microscope image of the tissue stained with HE. Data presented in µg/cm^2. X-Y coordinates in µm.

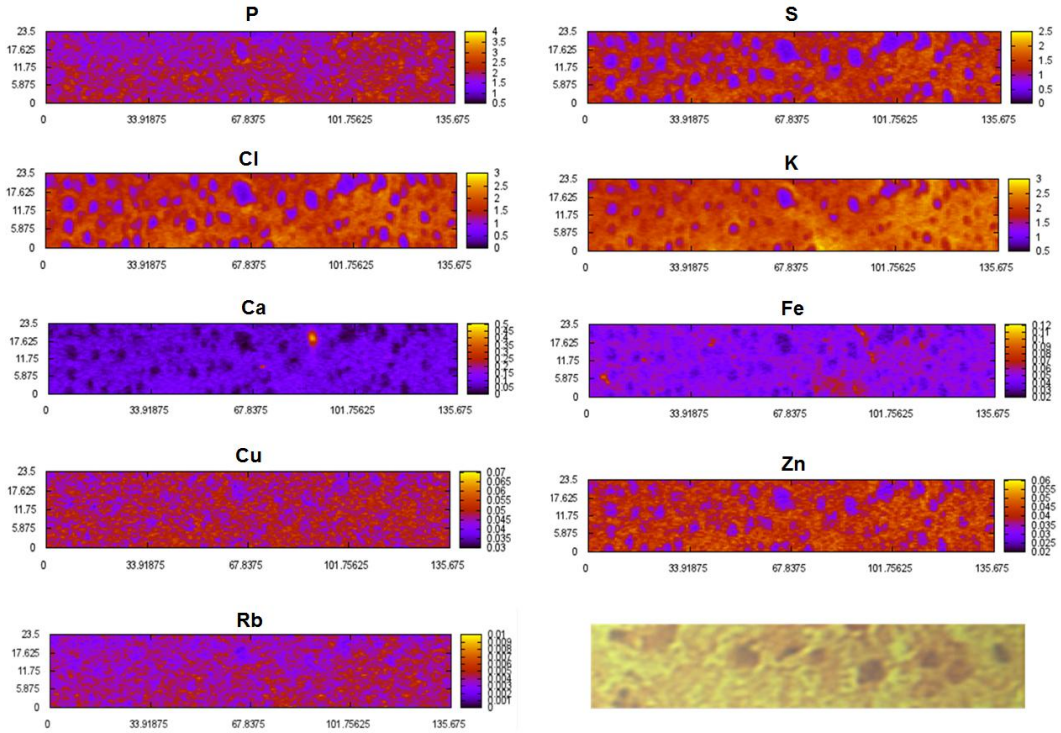

Fig. 2. SRXRF maps of element distribution in brain glioma tissue with the areas of calcification and an optical microscope image of the tissue stained with HE. Data presented in µg/cm^2. X-Y coordinates in µm.

Zn-S correlations

cancerous tissue **non-cancerous tissue (CP sample)**

Zn-Fe correlations

cancerous tissue **non-cancerous tissue (CP sample)**

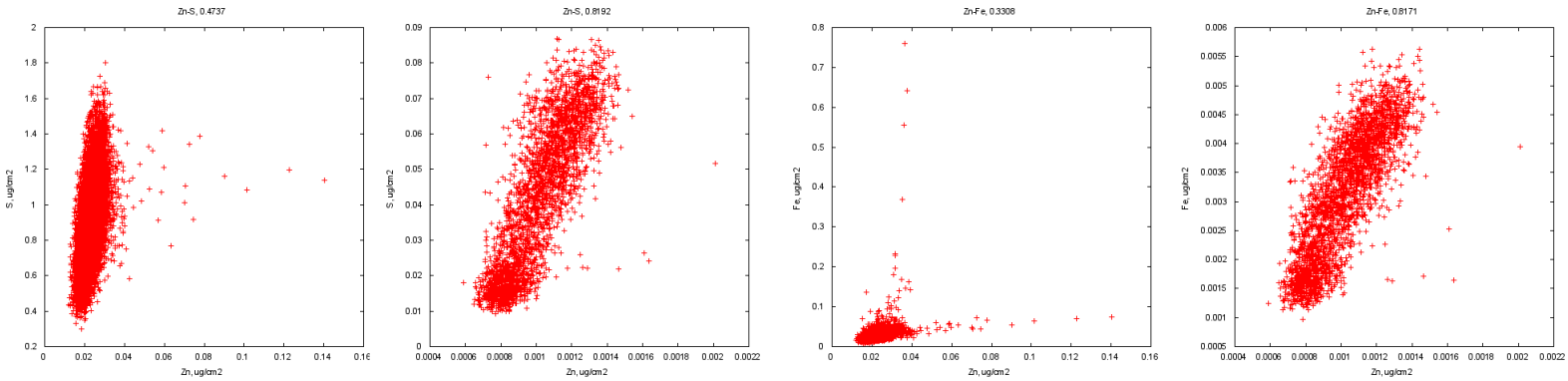

Fig. 3. Correlations between Zn and S as well as between Zn and Fe concentrations in the cancerous tissue and in the chorodic plexus cells taken from the non-cancerous tissue.