Targeted next generation sequencing of cell-free circulating tumor DNA from blood and frozen tumor tissues as a potential diagnostic tool for glioma patients

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Malignant gliomas are the most common primary, diffuse brain tumors in adults. Numerous genetic alterations critical for glioma pathogenesis and responses to therapy have been described and genetic tumor profiling is used in order to assist in the precise classification of disease. Analysis of cell-free circulating tumor DNA (ctDNA) presents a great alternative to biopsy or analysis of cerebrospinal fluid as side effects of lumbar puncture often include central nervous system infection, leptomeningeal tumor spread, or increase of intracranial pressure. Brain surgery involves significant risks for patient and a correct timing of surgery during patient progression would be easier with an application of a non-invasive method. Liquid biopsy provides a great opportunity for fast diagnostics, prognostics, and progression monitoring. In the present study, matching samples of fresh frozen patient's tumor tissues and blood were collected from the cohort of 67 patients with gliomas. Tumor DNA was isolated with a Trizol phenol chloroform extraction method, whole blood DNA was isolated using QIAamp DNA Blood Mini Kit (Qiagen, Germany) and cfDNA was isolated from the corresponding samples using QIAvac 24 Plus pump with QIAamp Circulating Nucleic Acid Kit (Qiagen, Germany). Libraries were prepared from cfDNA using Sure Select library preparation kit (Agilent, USA), size selected and sequenced using targeted NGS sequencing with a custom panel of 50 genes. The custom panel includes genes frequently mutated in gliomas or having diagnostic or therapeutic values. To evaluate a spectrum of mutations occurring in those tumors, libraries were prepared using Kappa Hyper Plus library kit (Roche, Switzerland) from tumors and the whole blood were molecularly characterized by targeted NGS sequencing using a custom panel comprising of 600 cancer related genes and 100 epigenetic genes. Corresponding data was compared to estimate detection levels of the tumor DNA within the circulating blood. The preliminary data of the analysis will be presented.

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