Kinome profiling of glioblastoma samples by mass spectrometry

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Introduction

Glioblastoma Multiforme (GBM) is an aggressive type of primary brain tumor. Patients with newly diagnosed glioblastoma have a median survival of approximately one year and no effective treatment is available. Studies on GBM report alterations in pathways in which kinases are involved. However, treatment with individual kinase inhibitors has been shown to be ineffective. Since multiple pathways are involved, administration of a combination of kinase inhibitors might improve treatment. To determine which kinases to target, protein kinase identification, quantification and activity determination in patient samples are needed. This study aims to analyze GBM tissue and primary cell cultures thereof, for kinase expression (genomics and proteomics) and kinase activity; this information can then be used to select kinase inhibitors for treatment.

Methods

From twenty GBM tumor tissues with corresponding primary cell cultures, genomics data (RNA micro-array and DNA sequencing data) and clinical information were obtained. The primary cell cultures were cultured serum-free and cell pellets were harvested and stored at -80˚C. For proteomics analyses the pellet was digested with trypsin and either directly analyzed by nanoLC coupled to Orbitrap Fusion or a phosphopeptide enrichment was realized prior to LC-MS analyses. In addition, a mass spectrometry based kinase activity assay was performed. The cell pellet was lyzed in kinase-buffer, followed by the addition of ATP and kinase substrate peptides and incubation for 90 minutes. Subsequently, the read-out is either done by MALDI-TOF or, when multiple substrate peptides were added, by nanoLC Orbitrap.

Preliminary results

In a preliminary experiment using three of the primary cell cultures, on average 2500 proteins per sample were identified using a shotgun approach on an Orbitrap Fusion mass spectrometer. These identified proteins included 106 protein kinases accounting for about 20% of the ~500 protein kinases that have been described in publically available protein kinase databases (HomoKinase, KinBase, KinWeb etc.). Although the number of primary cell cultures analyzed at the moment is still limited, differences in kinase abundances could already be observed, for instance DNA-dependent protein kinase was highly abundant in one of the three primary cell cultures.

In addition, a phoshopeptide enrichment using TiO_2 spin tips was performed on a single cell culture. This resulted in the identification of 3286 unique phosphopeptides including phosphopeptides of 111 different protein kinases. The presence of these phosphopeptides can be used as indirect evidence that certain kinases or classes of kinases are present in the sample and that in addition, these kinases are active. Also, the identified phosphopeptides can be used to design new substrate peptides for protein kinases. These substrates can eventually be used in the developed mass spectrometry based kinase activity assay. Preliminary experiments for the developed assay with pure kinases showed that our method was successful. Phosphorylation of substrate peptides was evident by a mass increase of 80 Da (phosphate group). Also, phosphorylation of the substrate peptides, designed for PKAc alpha, AKT1, CDK1/CDC2, MAPK1/ERK2 and PRKCA/PKC, could be observed if incubation is performed with a lysate of

the primary cell cultures. Experiments will be further extended to finally include all twenty cell cultures. The combined data will be used to determine the kinome profile of individual tumors. This information can be used in the future for *in vitro* testing of kinase inhibitors that are selected based on the tumor's kinome profile.

Novel aspect

A combined genomics, proteomics and kinase activity approach to determine the kinome profile of individual glioblastomas