IPD Project Details

Project ID: IPD2954

Project Title: Proteomics analysis of High-grade glioma tissues showing differential fluorescence following 5-Aminolevulinic acid guided surgery

Description: Targeted treatment of high-grade gliomas (HGGs) is challenging due to intra- and inter-tumoral heterogeneity. Prognosis of these tumors relies largely on the extent of resection. Fluorescence guided surgery using 5-ALA as adjunct has been on the rise in the recent years. However, 5-ALA has been ineffective in a small subset of population with similar histological phenotypes but varying metabolic/biochemical properties. Visualized fluorescence can sometimes be subjective and lead to variability in defining fluorescing regions with respect to their biological grade. Objective assessment of fluorescence is possible using spectroscopic techniques and with ex vivo PpIX assessment assays. The biometric study in our previous work revealed that even with objective assessment using PpIX assays, there exists a small subpopulation of tumor cells with similar histological phenotypes but discordant metabolic/biochemical properties w.r.t accumulation of PpIX. In the current study, we extended the investigation further and have carried out proteomic analysis of high-grade glioma tissue samples resected using 5-ALA fluorescence guided surgery to understand molecular differences leading to differential fluorescence in these complex and heterogenous tumors.

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Sample Preparation: a. Protein lysate preparation and quantification - 30 mg of tissue was lysed by sonication at 40% amplitude for 2.5 minutes with 5 seconds pulse cycles in 300 µL of lysis buffer containing 8 M urea, 50 mM Tris pH 8.0, 75 mM NaCl, 1 mM MgCl2, 500 units of Benzonase. Following this, the lysate was clarified by centrifugation at 8000 rpm, 40 C for 15 minutes and clear supernatant was split into multiple aliquots for storage at -800 C until further use. Protein lysates were quantified using the micro-BCA assay. 50 ug of protein lysate was subjected to in-solution digestion. The proteins were first reduced and then alkylated using 20 mM tris (2-carboxyethyl) phosphine (TCEP) and 37.5 mM Iodoacetamide (IAA), respectively.

Peptide Separation: The proteins were then subjected to digestion using trypsin (Pierce) at a ratio of 1:30 at 370 C for 16 hours. Following digestion, the peptides were

desalted using in-house C18 stage-tips, dried and stored for future use. b. Liquid chromatography coupled with Tandem Mass spectrometry (LC-MS/MS) - The desalted and dried peptides were reconstituted in 0.1% FA. Briefly, 1 µg of the peptide was loaded on to the C18 nano viper trap column (Acclaim PepMap 100, 75 µm x 2 cm) and equilibrated at a flow rate of 5 µL/min. Peptides were then resolved on an analytical column (EASY-spray PepMap RSLC C18 reversed phase column, 2 ?m, 75 ?m x 500 mm) at a flow rate of 300 nL/ min over a 120 min gradient in solvent B (80% ACN in 0.1% FA). The spectra were measured using the Orbitrap Fusion tribrid MS platform (Thermo Fisher Scientific) operating in the positive ion mode. Full MS scan was performed at a resolution of 60,000 and full scan range from 350 to 1700 m/z. The MS2 scan was carried out at a resolution of 15000 using the TopN DDA method (N=20). MS2 fragmentation was achieved through Higher Energy Collisional Dissociation (HCD) using a Normalized Collision Energy (NCE) of 27. The mass window was set to be ten ppm with a dynamic exclusion duration of 40 seconds. Mass accuracy during the acquisition was ensured through the lock mass option using the Polysiloxane species (m/z 445.12003). All the sample runs were randomized with each sample injected in triplicates. To account for any run-to-run variation resulting during the experiment, a common pool sample was run after every six sample runs.

Protein Characterization: Primary data analysis on the raw data files generated was performed with MaxQuant (v2.1.3.0) against the Uniprot human database proteome file (proteome ID: UP000005640) searched with the built-in Andromeda Search Engine of MaxQuant. Raw files were processed using Label Free Quantification (LFQ) parameters and setting label-type as ‘standard’ with a multiplicity of 1 on the Orbitrap Fusion mode. Maximum missed cleavages were set to 2, carbamidomethylation of Cysteine (+57.021464 Da) was set as a fixed modification, and oxidation of Methionine (+15.994915 Da) was set as a variable modification. False Discovery Rate (FDR) was set to 1% for protein and peptide levels to ensure high reliability of the protein detection. Decoy mode was set to ‘revert’, and the type of identified peptides was set to ‘unique+razor’. Further downstream analysis was carried out on the ‘proteins groups.txt’ file obtained after MaxQuant analysis. After primary analysis, we identified a few samples with low protein coverage and a few from predominantly necrotic regions of the tumors. These samples were discarded from further analysis. 11 samples (33 MS runs) were discarded from the analysis and the analysis was carried out only on 17 samples (51 MS runs). The metadata contains information of all the samples which were run and considered for final analysis. Proteins containing no unique peptides were filtered out from the dataset along with contaminants and reverse proteins. For each protein in the list, the median value of its abundance was considered, provided the protein was quantified in at least 2 out of 3 runs. Statistical analysis on the samples was performed using ProTIGY (v1.0.1). The resulting dataset was log2 transformed and proteins with greater than 30% of missing values across all samples were filtered out. Based on IDH gene mutation status and fluorescence pattern, the high-grade glioma samples were divided into 4 subgroups – IDH wild type with Fluorescence (IDH wt Flu), IDH wild type without Fluorescence (IDH wt NonFlu), IDH mutant with Fluorescence

(IDH mt Flu) and IDH mutant without Fluorescence (IDH mt NonFlu). To identify proteins with significant differences in the expression between the fluorescing and non-fluorescing regions, two sample t-tests were carried out for comparisons – i) IDH wt Flu vs IDH wt NonFlu and ii) IDH mt Flu vs IDH mt NonFlu.

Experiment Type: Shotgun proteomics

Species: Homo sapiens - 9606

Tissue: Brain (bto:0000142)

Cell Type:

Disease: Glioblastoma (doid:3068)

Instrument Details: Orbitrap Fusion (MS:1002416)

Protein Modifications: No PTMs

PubMed ID: 37358939