# Comprehensive proteomic investigation of high-grade and low-grade gliomas reveals pathways associated with cancer metastasis and candidate protein markers of therapeutic potential

Ayushi Verma[1], Amruth Bhat[2], Epari Sridhar[3], Aliasgar Moiyadi[4], and Sanjeeva Srivastava[1]

[1]Indian Institute of Technology Bombay
[2]Indian Institute of Science
[3]Tata Memorial Hospital
[4]Tata Memorial Centre

July 13, 2023

**Abstract**

High grade gliomas (HGGs), are the most malignant and difficult to treat brain tumors. Despite several studies on glioma pathobiology there is no comparative proteomics study on high-grade and low-grade gliomas which uncovers the mechanism behind the aggressive mesenchymal behaviour of HGGs. In this study, tissue samples of high-grade and low-grade gliomas were processed for label free quantification (LFQ) using HR-LC MS/MS. The analysis identified 140 differentially expressed proteins, GSEA and protein-protein interaction analysis showed over expression of pathways like; ECM remodelling, Focal Adhesion, EMT and Glycan Biosynthesis in HGG. The key proteins were validated using multiple reaction monitoring experiment. ECM glycoproteins including; Fibronectin, Fibrinogens, Collagens, Vitronectin along with mesenchymal markers such as Vimentin and TGF-β came over-expressed in HGGs. The over-expression of oligosaccharyltransferase in HGG indicates its role in enhanced expression of glycoproteins. In-silico molecular docking with catalytic subunits of OST identified two small molecule inhibitors; Irinotecan and Entrectinib as potential candidates to target OST. We propose OST plays a major role in tumor metastasis by promoting EMT and could be used as a potential target to suppress glioma metastasis. Finally, the proteins identified in this study need further clinical research to validate their prognostic values as protein markers.

**Comprehensive proteomic investigation of high-grade and low-grade gliomas reveals pathways associated with cancer metastasis and candidate protein markers of therapeutic potential**

Ayushi Verma[1], Amruth Deepak Bhat[2], Epari Sridhar[3], Aliasgar Moiyadi[3], Sanjeeva Srivastava[1*]

[1]Department of Biosciences and Bioengineering, Indian Institute of Technology Bombay, Powai, Mumbai, India

[2]Centre for BioSystems Science and Engineering, Indian Institute of Science, Bengaluru, India

[3]Advanced Center for Treatment Research and Education in Cancer, Tata Memorial Center, Navi Mumbai, India

*Correspondence: Dr. Sanjeeva Srivastava, E-mail: sanjeeva@iitb.ac.in

Phone: +91-22-2576-7779, Fax: +91-22-2572-3480

**Keywords:** epithelial to mesenchymal transition, extracellular matrix remodelling, glioblastoma, high grade gliomas, proteomics

**Abstract:** High grade gliomas (HGGs), are the most malignant and difficult to treat brain tumors. Despite several studies on glioma pathobiology there is no comparative proteomics study on high-grade and low-grade gliomas which uncovers the mechanism behind the aggressive mesenchymal behaviour of HGGs. In this study, tissue samples of high-grade and low-grade gliomas were processed for label free quantification (LFQ) using HR-LC MS/MS. The analysis identified 140 differentially expressed proteins, gene set enrichment analysis (GSEA) and protein-protein interaction analysis showed over expression of pathways like; ECM remodelling, Focal Adhesion, EMT and Glycan Biosynthesis in HGG. The key proteins were validated using multiple reaction monitoring experiment. ECM glycoproteins including; Fibronectin, Fibrinogens, Collagens, Vitronectin along with mesenchymal markers such as Vimentin and TGF-β came over-expressed in HGGs. The over-expression of oligosaccharyltransferase in HGG indicates its role in enhanced expression of glycoproteins. *In-silico* molecular docking with catalytic subunits of OST identified two small molecule inhibitors; Irinotecan and Entrectinib as potential candidates to target OST. We propose OST plays a major role in tumor metastasis by promoting EMT and could be used as a potential target to suppress glioma metastasis. Finally, the proteins identified in this study need further clinical research to validate their prognostic values as protein markers.

## Introduction

Gliomas are one of the most common brain tumors and account for nearly 30% of primary brain tumors and nearly 80% of all malignant brain tumors (1). The latest 2021 World Health Organization (WHO) classification of tumors of the central nervous system categorizes gliomas into 4 general groups: adult-type diffuse gliomas, pediatric-type diffuse low grade gliomas, pediatric-type diffuse high grade gliomas and circumscribed astrocytic gliomas. The adult-type diffuse gliomas can be further characterized as IDH-mutant astrocytomas; oligodendrogliomas, IDH-mutant and 1p/19q-codeleted gliomas; and Glioblastomas (IDH-wild type gliomas) (2). Within each type of tumor, they are categorized into 4 different grades based on the aggressiveness of the tumor, where Grade I is a mild and low risk glioma, Grade II is slightly more aggressive but has a much better prognosis when compared to Grade III and Grade IV tumors (3). This is particularly important in the case of gliomas which are notorious for their aggressiveness, especially with increasing grade. Gliomas of grade I and II are collectively termed as low-grade gliomas (LGG) whereas grade III and grade IV gliomas are referred to as high grade gliomas (HGG).

The major differences in the biology of LGGs and HGGs lie in the aggressive and highly metastatic nature of these tumors. This is mainly driven by the Epithelial to Mesenchymal transition (EMT) of the tumor cells. This process is characterized by the loss of intercellular connections and is associated with a changed cell morphology which increases the cell motility and metastatic properties (4). To facilitate this transition, there is extensive remodeling of the extracellular matrix (ECM) surrounding the tumor. The biophysical and biochemical properties of the surrounding interstitial ECM are affected by the tumor cells which is required to enable the tumor cells to effectively spread and infiltrate the surrounding region (5). The remodeling of the extracellular matrix during EMT involves alterations in biosynthesis of various glycoproteins. A healthy brain ECM is more flexible and has less abundance of fibrillary glycoproteins such as collagens, fibronectins, fibrinogens and vitronectins. During tumor progression, ECM undergoes several alterations including changes in the formation of ECM components, posttranslational modifications, turnover, and changes in cell–matrix interactions making the ECM more dense and stiffer which supports the aggressive tumor growth (6).

A recent study on ECM remodeling of GBM tissues has also reported several of ECM proteins to be differentially regulated in GBMs when compared with normal brain tissues (7). Another study on proteomic analysis of GBM and healthy brain tissues identified changes in the proteoglycans and glycosaminoglycans in the GBM tissue which favors the aggressive phenotype (8). While proteomic studies have been carried out on Glioblastoma (8) (9), they majorly compare the grade IV tumors with healthy brain tissues and do not provide a complete picture of the proteome differences between high-grade and low grade gliomas (LGGs). While some previous works have characterized the differences in different grade of gliomas (10) (11), the

elucidation of the specific mechanisms driving the EMT in gliomas is yet to be done. Understanding of specific processes by which LGGs transition to HGGs will help in developing treatments and inhibitors of the EMT which will greatly reduce the metastatic properties of HGGs and thereby improved prognosis.

In this study we have performed comprehensive proteomics investigation with the aim to acquire a better understanding of molecular mechanism behind the aggressive behavior of HGG and identification of clinical markers of HGGs that may have therapeutic potential as drug targets.

## 2. Materials and methods:

### 2.1 Tissue sample collection and histopathological characterization

Tissue samples from the radiologically confirmed glioma patients were collected as per the Good Clinical Practice (GCP) guidelines. All patients participated in the study had provided written consent before surgery. The study is approved by Institute Ethics Committee IIT Bombay (IEC number 139) and the IRB (Institute Review Board) Tata Memorial Hospital, Mumbai. Clinical details of glioma patients participated in the study are mentioned in **Table 1.** All tissue samples used in the study were characterized by a histopathologist and categorized in grade I, II, III and IV based on WHO 2016 classification (12). Grade I and II are considered low grade glioma while grade III and IV are grouped under high grade gliomas.

### 2.2 Protein extraction and digestion

Tissue samples which were radiologically and histopathologically confirmed to be glioma were processed for proteomic analysis as reported earlier (13). Briefly, 8M urea buffer was used to lyse the tissue samples followed by sonication (Sonics Materials, Inc; model no. VCX 130) at 40% amplitude for 2.5 min and centrifuged at 6018 x g for 15 min at 4°C to separate the cell debris from the supernatant. For global proteomics analysis, 11 high grade glioma (HGG) and 5 low grade glioma (LGG) tumor samples were included. Further, to validate the significant proteins from global proteomics data, targeted proteomics analysis was performed on 19 samples (HGG = 14, LGG = 5).

For MS analysis, approximately 25µg of protein from each sample was digested. The protein was first reduced with 20 mM Tris (2-carboxyethyl) phosphine (TCEP), and 40 mM iodoacetamide (IAA) was used to alkylate the reduced cysteine residues. Further, the samples were digested with trypsin at an enzyme/substrate ratio of 1:50 and incubated at 37°C for 16 hours. Digested peptides were desalted using a C18 stage tip and dried digested sample was resuspended in 0.1% formic acid.

### 2.3 Global proteomic analysis of glioma samples

One microgram of the digested desalted peptide was run in the Q-Exactive Orbitrap Mass Spectrometer (Thermo Fisher Scientific, USA) coupled with an automated Easy-nLC 1200 system with a gradient of solvent A (0.1% formic acid) to solvent B (0.1% FA, 80% Acetonitrile) for 120 min in a positive mode with blanks after every sample.

For MS OT, detector type was set to orbitrap and resolution was set to 60,000. A scan range of 375-1700 m/z with a maximum injection time of 125 ms was set. Monoisotopic peak determination was on peptide mode, charge state was set to 2-6. For dynamic exclusion, mass tolerance was 10 ppm and exclusion duration was set to 40 s. For ddMS2 OT HCD, resolution was set to 30,000, isolation window was 2, HCD collision energy was 30 %, mass range was normal and AGC target was set to standard.

The MS acquired data in .raw format was analysed using Maxquant (Version 1.6.12.0) against the UniProt Human Proteome Database (downloaded on 07092021) for protein identification. Trypsin was used as the enzyme for protein digestion allowing up to two missed cleavages. Carbamidomethylation of cysteine (+57.021464 Da) was set as fixed modification whereas oxidation of methionine (+15.994915) was set as dynamic modifications. The false discovery rate (FDR) for proteins and peptides identification was set to 0.01 to ensure high reliability.

### 2.4 Statistical data analysis

The LFQ intensities of each sample were extracted and statistical analysis was performed using Perseus (Version 2.6.0) (14). Proteins with quantified intensities in less than 70% of the samples were discarded. The data was log2 transformed, median normalized and missing values were imputed separately for each sample group using a kNN based imputation method. A two-sample t-test was then performed on this data and the resultant p-values were corrected using Benjamini-Hochberg FDR correction. Proteins were considered to be significant if they had an FDR corrected p value (q-value) of less than 0.05 and an log 2 fold change value greater than 1.5. Metaboanalyst (Version 5.0) was used for visualization of the heatmap (15); violin plots and volcano plots were created using Python tools. The violin plots were made with Log2 transformed data and the significance level was calculated based on t-test with Bonferroni correction.

### 2.5 Gene set enrichment analysis and protein-protein interaction analysis

The identified protein list was used for pre-ranked Gene set enrichment analysis (GSEA) using Hallmark (16) and KEGG database (17). The number of permutations was set to 1000. Further, Cytoscape (Version 3.9.1) was used to visualize the enriched pathways (18). For protein-protein interaction analysis, significant protein list was used as input for STRING (Version 11.5), keeping the interaction score to 0.4.

### 2.6 Multiple reaction monitoring (MRM) based targeted proteomic analysis

Proteins showing differential expression in HGG and LGG were further validated using MRM experiment. The Transition list of the selected proteins was prepared using Skyline daily version 22.2.1.501 (19) with human database (downloaded on 07092021). The peptide uniqueness was checked using neXtProt (20) and imported to Skyline-daily. Proteins which had 3 or more than 3 unique peptides with 8-20 amino acid length and peptides which had 4 or more than 4 transitions were selected. The missed cleavage criteria was set to 0, precursor charge and product charge was selected as +2, +1 respectively. All y ions from ion 2 to last ion -2 were monitored. Initial optimization of transition list was carried out in sample pools to select the best peptides and their transitions for every protein. For each sample, 1.5 µg peptide was injected in a triple quadrupole mass spectrometer Altis (Thermo Fisher Scientific) coupled with an Ultimate 3000 UHPLC system. A binary buffer system (Buffer A = 0.1% Formic acid in water and Buffer B = 0.1% Formic acid in Acetonitrile) allowed for the separation of peptides. The data was acquired using the same methodology mentioned in the previous study (21). BSA was run to check the instrument's performance on different days. PROSIT library was used as the spectral library (22). Data analysis was performed using MSstats tool (23) in Skyline daily to identify the significant peptides (p value $< 0.05$) and to calculate the fold changes keeping a confidence interval of 95%. Protein abundances were exported from the skyline daily and after log 2 transformation, violin plots showing differential expression were plotted.

### 2.7 *In-silico* molecular docking

*In-silico* molecular docking of OST was performed using catalytic subunits, STT3A and STT3B. The AlphaFold structures of STT3A (AF-P46977-F1) and STT3B (AF-Q8TCJ2-F1) were retrieved from UniProt (24). A library of 89 potential FDA approved drug candidates was prepared by downloading their 3D structure for PubChem database (25). NGI-1, a known OST inhibitor was used as control to set the threshold for the binding energy and to determine the active sites of the proteins. Energy minimization of ligands was performed using open babel and Autodock Vina (Version 1.1.2) (26) of PyRx software was used to perform docking with exhaustiveness value set to 50. The out.pdbqt files generated after the molecular docking were split into individual poses using vina split command and the pose with lowest binding energy was used for post docking analysis. PyMOL (Version 2.5.4) and Discovery Studio Visualizer Software (Version 4.0) was used to visualize the docked structures and binding interactions were identified using protein-ligand interaction profiler (PLIP) server (27).

## 3. Results:

### 3.1 Global proteomic profiling of glioma samples reveals differentially regulated proteins and glycoproteins

We have performed comprehensive proteomic analysis of glioma samples using high resolution LC-MS/MS

followed by MRM based validation**(Figure 1)** . The global proteomic analysis resulted in identification of 1024 unique proteins in at least 70% of the samples.**Figure 2A** represents a heatmap of top 25 differentially expressed proteins in HGG and LGG. The principal component analysis (PCA) plot showed two separate clusters for HGG and LGG with a score of 33% for PC1, 13.3% for PC2 and 10.1% for PC3 **(Figure 2B).**The statistical test provided 140 significant proteins (FDR q value <0.05, absolute Fold change >1.5), of which 72 proteins were downregulated and 68 proteins were upregulated in HGG when compared the protein abundance **(Figure 2C)** . Proteins, namely, Fibronectin (FN1), Collagen alpha-3(VI) chain (COL6A3), Collagen alpha-2(VI) chain (COL6A2), Fibrinogen beta chain (FGB), Plasminogen (PLG), Inter-alpha-trypsin inhibitor heavy chain H2 (ITIH2), Kininogen-1 (KNG1) were found to be significantly upregulated in HGG, whereas proteins like Contactin-1 (CNTN1), Tenascin-R (TNR), Neurofascin (NFASC), Ankyrin-2 (ANK2) and Neurogranin (NRGN) were found downregulated in HGG. A list of key candidate proteins and glycoproteins identified via label-free quantitative proteomics in high grade glioma tumor samples is given in **Table 2.**

### 3.2 Pathway enrichment analysis showed epithelial mesenchymal transition pathway to be highly enriched in high grade gliomas

Gene set enrichment analysis of identified proteins resulted in enriched pathways associated with glioma pathobiology ranked according to their normalized enrichment score (NES) along with the enriched proteins in each pathway **(Figure 2D, Figure S1)** . The top 5 enriched pathways in HGG include, epithelial mesenchymal transition (NES = 1.77), focal adhesion (NES = 1.48), regulation of actin cytoskeleton (NES = 1.41), MTORC 1 signaling (NES = 1.28) and hypoxia (NES = 1.16) (**Figure S2** ). Further GSEA with gene ontology functional database for cellular component using WebGestalt showed maximum number of proteins in HGG were mapped to extracellular matrix, blood microparticle and ER lumen respectively with FDR less than 0.05 (**Figure S3** ).

### 3.3 Protein-protein interaction analysis highlights glycoprotein mediated biological pathways in HGGs

A list of significantly upregulated proteins from HGG was used to perform protein-protein interaction analysis. PPI analysis showed the significant upregulated proteins to be mapped to four different biological pathways in HGG. These pathways include; focal adhesion (n = 12), N-glycan biosynthesis (n = 3), ECM receptor interaction (n = 10) and complement and coagulation cascades (n = 9) **(Figure 3)** . Proteins RPN1, RPN2 and DAD1 are the subunits of OST complex which is responsible for glycosylation. Further, FN1, FGA, FGB, FGG, VTN and collagens are the crucial molecules related to ECM and have shown high interaction with each other. The trend of few significant proteins was shown in the form of violin plots **(Figure 3).**

### 3.4 Multiple Reaction Monitoring based validation of proteins identified by global proteomics analysis

Differentially regulated significant proteins were validated using multiple reaction monitoring assay. For Initial optimizations, 20 proteins where 185 peptides and 1508 transitions were monitored to identify the best flying peptides and their transitions using sample pools. Further, data was refined based on peak shape, peak intensity and peak area and final transition list was prepared with 14 proteins comprising of 69 peptides and 447 transitions. For the MRM experiment, the data was acquired for total 19 tissue samples, including 14 high grade glioma samples and 5 low grade glioma samples. To monitor the system suitability, an equal amount of heavy labelled synthetic peptide (ENQTCDIYNGEGR) was spiked in each sample and observed CV was 10%.**(Figure 4).** We observed overexpression of 10 proteins including, FN1, FGA, FGB, TGF-BI, VTN, RPN1, RPN2, FLNA, VIM and DDOST in HGGs while 1 protein TNR showed downregulation during MRM experiment.

Three subunits of OST complex; RPN1, RPN2 and DDOST showed similar trend as discovery data, high abundance in HGG samples compared to LGG. The cumulative log2 fold change (cLog2FC) identified from MSstat analysis was 1.12, 1.56 and 2.64 respectively with a confidence level of 95%. Proteins related to ECM

remodelling such as vitronectin (cLog2FC - 3.27), fibrinogen alpha chain (cLog2FC - 3.25), fibrinogen beta chain (cLog2FC - 4.32), filamin-A (cLog2FC -) also showed over expression in HGG which corelates with the global proteomics data. Mesenchymal markers such as fibronectin (cLog2FC - 2.51), vimentin (cLog2FC - 5.95) and transforming growth factor-β (cLog2FC- 2.59) also showed high expression in high grade gliomas compared to LGG there by validating the results of LFQ experiment. **(Figure 4, Figure S4)** . The final list of the proteins and their corresponding peptides along with p value and Log2 FC was given in **Table S1.**

### 3.5 *In-silico* molecular docking of oligosaccharyltransferase to explore its role as therapeutic target

We performed *in silico* molecular docking of STT3A and STT3B with a library of 89 FDA approved drugs (**Table S2** ). STT3A and STT3B are the catalytic subunits of Oligosaccharyltransferase, STT3A-OST catalyses co-translational N-glycosylation while STT3B-OST catalyses post-translational glycosylation. NGI-1 is a known inhibitor of both oligosaccharyltransferase (OST) isoforms; STT3A-OST and STT3B-OST. Therefore, it was used as a positive control to set the threshold for binding energy and to identify the active site. NGI-1 interacted with STT3A and STT3B with binding energy of -8.3 kcal/mol and -7.7 kcal/mol respectively. In total 44 drugs out of 89 showed lower binding energy than NGI-1 for STT3A while for STT3B, 52 drugs showed lower BE and 46 drugs were common between STT3A and STT3B. Top 5 drugs which showed lower binding energy than the control and share same binding pockets were selected. The list of top 5 drug candidates with their binding energy is given in (**Table S3).** Irinotecan and Entrectinib interacted with both STT3A and STT3B with lowest binding energy**(Figure 5)** . Irinotecan is a camptothecin derivative that has a broad-spectrum antitumor activity against a variety of tumors. It was first approved for the treatment of metastatic colorectal cancer (CRC). Combining irinotecan with other drugs increases the overall survival in CRC patients (39). Irinotecan binds to STT3A with a binding energy of -11 kcal/mol and to STT3B with 10.6 kcal/mol. Entrectinib is another drug which has shown higher affinity, it binds with STT3A with -10.7 kcal/mol and with STT3B with -10.6 kcal/mol. Entrectinib is a multi-target small molecule inhibitor that targets TRK, ROS1 and ALK. It is approved by the FDA for the treatment of locally advanced or metastatic solid tumors with NTRK, ROS1 and ALK gene mutations (40).

## 4. Discussion

High-grade gliomas are particularly challenging tumours to treat due to their invasive nature which requires substantial understanding of molecular mechanism behind their aggressive behaviour compared to low grade gliomas. Since LGGs are less common and exhibit milder symptoms than HGG delayed diagnosis or misdiagnosis are frequent. LGG are also slow-growing and less invasive compared to high-grade gliomas and in some cases do not require immediate surgical intervention. Because of these reasons it is challenging to get the low-grade glioma tissue samples compared to HGG. The current study is the first LC-MS/MS based proteomics study of high-grade and low-grade tumor tissues investigating the protein level differences and identifying the key molecular pathways differentially regulated between the two cohorts. We first performed the label free quantification of glioma samples to identify the differentially regulated proteins in HGGs. Proteins like Fibronectin, Vitronectin Fibrinogens (FGA, FGB, FGG), Collagens (COL1A1, COL1A2, COL4A1, COL4A2, COL6A1, COL6A2, COL6A3), Plasminogen and Transforming growth factor-β came over-expressed in the HGGs, while other proteins like Tenascin R, Contactin-1, Neurofascin, Ankyrin-2 and Neurogranin were found downregulated in HGG.

Fibronectin and vitronectin both are glycoproteins found in the extracellular matrix (ECM), they play a crucial role in cancer cell invasion and metastasis. Fibronectin provides a substrate for cancer cells to adhere to and migrate from the primary tumor site and invade surrounding tissues (41). Fibronectin and vitronectin both are known to interact with integrin receptors on the cancer cell surface, promoting cell adhesion and initiating signaling pathways that regulate migration. Cancer cells secrete enzymes called matrix metalloproteinases (MMPs), which degrade the ECM components facilitating tumor cell invasion (40, 41).

Another class of proteins found overexpressed in HGG were fibrinogens such as FGA, FGB and FGG. In high-grade glioma, the disrupted blood-brain barrier allows fibrinogen from the blood to penetrate the tumor microenvironment where it is converted into insoluble fibrin by the enzyme thrombin, resulting in the formation of fibrin deposits within the tumor. Fibrin deposits in the tumor microenvironment act as a provisional matrix further promoting the migration and invasion of glioma cells (7).

Our data also showed high expression of collagens in HGG, like fibronectin and fibrinogens, collagens are also key components of the extracellular matrix (ECM). Upregulated collagens, particularly collagen types I and IV, play a role in ECM remodeling within HGG. These collagens are deposited around tumor cells and contribute to the formation of a denser and stiffer ECM. This altered ECM composition and structure can facilitate tumor cell invasion and migration through the surrounding brain tissue (43). In HGG, upregulated collagens also contribute to angiogenesis, the formation of new blood vessels that supply nutrients and oxygen to the tumor. They promote endothelial cell adhesion, migration, and tube formation, facilitating the development of an extensive vascular network within the tumor (5).

Gene set enrichment analysis of identified proteins revealed epithelial mesenchymal transition (EMT) pathway to be highly enriched in HGGs followed by enriched focal adhesion and actin cytoskeleton pathway. EMT and the focal adhesion pathway are interconnected and play significant role in the progression of cancer. EMT is a process through which epithelial cells acquire mesenchymal-like characteristics, including enhanced migratory and invasive properties (44). In high-grade gliomas, EMT has been observed and is associated with tumor progression and metastasis. EMT enables glioma cells to detach from the primary tumor mass, invade surrounding brain tissue, and migrate to distant sites. Glioma cells undergoing EMT show phenotypic changes, such as loss of cell-cell adhesion molecules (e.g., E-cadherin) and gain of mesenchymal markers (e.g., N-cadherin, vimentin, fibronectin), facilitating their invasive behavior (5,44). Transforming growth factor-beta-induced protein (TGF-β), a strong inducer of EMT transcription factors, drives the EMT in both SMAD and non-SMAD signalling pathways (45). We have observed high expression of TGF-β in high grade gliomas indicating the over expression of EMT pathway in HGG. EMT has also been linked to resistance to chemotherapy and radiation therapy in gliomas. The altered molecular profile and increased migratory properties conferred by EMT can contribute to therapy resistance and tumor recurrence (44). It has been associated with the acquisition of stem cell-like properties in glioma cells. Cells undergoing EMT exhibit enhanced self-renewal capabilities, increased resistance to apoptosis, and a capacity for multilineage differentiation (44). These stem-like properties contribute to tumor heterogeneity and therapeutic resistance, which is well known phenomenon in HGG.

Another enriched pathway observed in HGG was focal adhesion pathway which is critical for cell adhesion, migration, and mechano-transduction. In HGG, the focal adhesion pathway is upregulated and contributes to invasive and migratory behaviors. Focal adhesion proteins also participate in ECM remodeling by mediating the interaction between cells and the surrounding matrix (46). Dysregulated focal adhesions in gliomas contribute to ECM degradation, facilitating tumor invasion and angiogenesis. Focal adhesion proteins, including FAK (focal adhesion kinase), activate downstream signaling pathways involved in tumor progression, such as MAPK and PI3K/AKT pathway (46) . These signaling cascades regulate cell survival, proliferation, and migration, promoting glioma progression and resistance to therapy. Understanding the interplay between EMT and the focal adhesion pathway in high-grade glioma progression is crucial for developing targeted therapies. Strategies aimed at inhibiting EMT, disrupting focal adhesion signaling, or targeting key molecules within these pathways hold promise for limiting glioma invasiveness and improving treatment outcomes.

Hypoxia is the other pathway which was found enriched in HGG, characterized by low oxygen levels. Tumors often develop regions of inadequate oxygen supply due to their rapid growth and aberrant vasculature. Hypoxia triggers the release of various pro-angiogenic factors, such as vascular endothelial growth factor (VEGF), to stimulate the formation of new blood vessels (47) . This process, known as angiogenesis, helps supply oxygen and nutrients to the tumor, promoting its growth and survival. Increased angiogenesis also contributes to tumor aggressiveness and metastasis. Hypoxic conditions force cancer cells to adapt

their metabolism to survive and grow. They undergo metabolic reprogramming, shifting towards anaerobic glycolysis, known as the Warburg effect, which allows them to generate energy even in the absence of oxygen. This metabolic switch not only provides energy for cancer cell growth but also leads to the accumulation of metabolic byproducts that can promote tumor progression (47). Hypoxia can also induce genetic and epigenetic changes in cancer cells that promote their growth and survival. Hypoxia-inducible factors (HIFs) are key transcription factors that are stabilized under low oxygen conditions and regulate the expression of various genes involved in angiogenesis, metabolism, cell survival, and invasion. HIFs can promote the expression of pro-growth and pro-survival genes while inhibiting genes involved in cell death (47) . Hypoxia has been implicated in promoting EMT. Understanding the role of hypoxia in cancer cell growth is crucial for developing effective therapeutic strategies. Targeting hypoxia-related pathways, such as angiogenesis and HIF signaling has emerged as a promising approach to inhibit tumor growth and improve treatment outcomes in cancer.

The results of GSEA analysis were also in concurrence of protein-protein interaction analysis which resulted focal adhesion, ECM receptor interaction as top pathways. Two additional pathways identified were complement coagulation cascade and N-glycan biosynthesis. Fibrinogens (FGA, FGB, FGG), plasminogen, kininogen and antithrombin-III were mapped to complement and coagulation pathways. Three subunits of oligosaccharyltransferase enzyme RPN1, RPN2 and DAD1 were mapped to N-glycan biosynthesis pathway. Whereas Multiple reaction monitoring based targeted proteomics experiment identified another subunit DDOST to be upregulated in HGGs.

Oligosaccharyltransferase (OST) is an enzyme complex with 11 subunits, it is responsible for protein glycosylation. RPN1 and RPN2 subunits are considered as receptors of membrane bound ribosomes hence called ribophorins (48). DAD1 helps in maintaining the structural integrity of the OST complex by forming a heterotetrameric complex with RPN1, RPN2 and DDOST while OST48 or DDOST keeps the OST complex in the ER with its cytosolic domain (49). Higher OST expression is corelated with the aberrant glycoprotein expression. Abnormal expression of glycoproteins, can impact the adhesive properties of cancer cells, affecting cell-cell and cell-ECM interactions. These alterations may contribute to increased invasiveness and metastatic potential of high-grade glioma cells.**Figure 6** summarizes the changes in the extra cellular matrix during the epithelial to mesenchymal transition and role of OST in cancer cell migration. In several tumour cells, higher expression of glycosyltransferase has been linked to enhanced aggressiveness. In a study by Wu et al. (50), immunohistochemistry identified N-Acetylgalactosaminyltransferase-14 as a potential biomarker for breast cancer. Further, higher expression of sialyltransferases and fucosyltransferases is associated with enhanced malignant behaviour (51). Similar findings have been observed in various other tumour cells, including liver, ovary, and melanomas, demonstrating the significance of glycosylation in tumour aggressiveness. Similarly, OST can also be explored as potential biomarker for HGGs and it can act as a potential therapeutic target to control mesenchymal behaviour of HGGs.

We have also performed *in-silico* molecular docking to identify OST inhibitors which can suppress its expression. Molecular docking results identified Irinotecan and Entrectinib to be potential inhibitors of catalytic subunits of OST. Both Irinotecan and Entrectinib are FDA approved therefore safe for human use and showed good binding affinity towards OST isomers STT3A and STT3B. These inhibitors can be explored further for their anti-cancer efficacy in cell culture or animal model.

High-grade gliomas, including glioblastoma, are highly heterogeneous tumors. This heterogeneity poses challenges in identifying consistent and reliable biomarkers that accurately represent the complex molecular alterations within gliomas. In this study we have performed comprehensive proteomic investigation of high-grade and low-grade gliomas with the aim to identify potential clinical marker/therapeutic targets. We have found oligosaccharyltransferase (OST) complex to be highly upregulated in high grade gliomas. Four subunits of OST complex; RPN1, RPN2, DAD1 and DDOST have shown higher expression in HGG compared to LGG. While the specific role of oligosaccharyltransferase in high-grade glioma metastasis remains to be fully elucidated, it is likely that alterations in glycoprotein expression, potentially influenced by oligosaccharyltransferase activity, can impact various aspects of metastasis, including cell adhesion, invasion, immune

interactions, and ECM remodeling. Further research is needed to uncover the specific mechanisms and implications of glycosylation in glioma metastasis and the potential involvement of oligosaccharyltransferase in this process.

## 5. Conclusions

Several studies have been performed towards understanding the aggressive behavior of high-grade gliomas. However, the significance of oligosaccharyltransferase mediated tumor progression and invasiveness is still unclear. High grade gliomas are extremely mesenchymal in nature which further contributes towards the aggressive nature of gliomas. This mesenchymal transition is accompanied by the over expression of several glycoproteins and in turn responsible for extra cellular matrix remodelling. Numerous studies have suggested that OST-mediated glycosylation contributes to the development of various malignancies. In the current study, we have found a connecting link between the over expression of OST and tumor invasiveness of high-grade gliomas. Since the study involves small set of samples particularly for the LGG cohort, therefore the findings should be validated on the large cohort of samples. In addition, glycoprotein enriched samples could provide better insight in the role of OST mediated glycosylation. This study identifies the key proteins involved in ECM remodelling and in turn responsible for EMT transition, these can be used as novel clinical markers or therapeutic targets for tailored management of high-grade gliomas. Finally, the study reveals the importance of oligosaccharyltransferase in aggressive behaviour of high-grade gliomas, highlighting OST as a potential target for cancer therapy.

## Ethics approval

The study was approved by the Institute Ethics Committee, IIT Bombay (IEC Number: 139) and institutional review board at Tata Memorial Centre (TMC-ACTREC) wherein all patients were recruited as per the Institute Review Board guidelines with prior informed consents.

## Author Contributions

The study was conceptualised and designed by A.V. and S.S.; A.V. performed the mass spectrometry-based shotgun and targeted proteomic analysis, A.D.B. performed the statistical data analysis. Data visualization and molecular docking was performed by A.V., A.M. performed the surgery and E.S. conducted the pathological examination of tumor samples. Review and writing part of the manuscript was done by S.S., A.V., A.D.B., E.S., A.M.

## Funding

The study was supported by Indian Council of Medical Research (RD/0120-ICMR000-001) and MASSFIIT (Mass Spectrometry Facility, IIT Bombay) (BT/PR13114/ INF/22/206/2015) Department of Biotechnology (DBT) for MS-based proteomics work. AV and ADB was supported by DBT-JRF and MHRD (Ministry of Human Resource Development) respectively.

## Conflict of interest statement

The authors declare no conflicts of interest.

## Data availability statement

**1.** The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE [1] partner repository with the dataset identifier PXD042646.

Submission details:

**Project Name:** Proteomics investigation of high-grade gliomas to understand their aggressive mesenchymal behaviour

**Project accession:** PXD042646

**Project DOI:** Not applicable

Reviewer account details:

**Username:** reviewer_pxd042646@ebi.ac.uk

**Password:** z0z8KNoS

**2.** Multiple reaction monitoring based targeted proteomics data have been deposited in Panorama public (https://panoramaweb.org/HGGvLGGtissue.url).

The reviewer account details to access the data is mentioned below:

Email: panorama+reviewer185@proteinms.net Password: pFddcliX

**References**

1. Weller M, Wick W, Aldape K, Brada M, Berger M, Pfister SM, et al. Glioma. Nat Rev Dis Primer. 2015 Jul 16;1:15017.

2. Osborn AG, Louis DN, Poussaint TY, Linscott LL, Salzman KL. The 2021 World Health Organization Classification of Tumors of the Central Nervous System: What Neuroradiologists Need to Know. AJNR Am J Neuroradiol. 2022 Jul;43(7):928–37.

3. Maher EA, Furnari FB, Bachoo RM, Rowitch DH, Louis DN, Cavenee WK, et al. Malignant glioma: genetics and biology of a grave matter. Genes Dev. 2001 Jun 1;15(11):1311–33.

4. Pucci M, Malagolini N, Dall'Olio F. Glycobiology of the Epithelial to Mesenchymal Transition. Biomedicines. 2021 Jul 2;9(7):770.

5. Winkler J, Abisoye-Ogunniyan A, Metcalf KJ, Werb Z. Concepts of extracellular matrix remodelling in tumour progression and metastasis. Nat Commun. 2020 Oct 9;11(1):5120.

6. Dong Y, Zheng Q, Wang Z, Lin X, You Y, Wu S, et al. Higher matrix stiffness as an independent initiator triggers epithelial-mesenchymal transition and facilitates HCC metastasis. J Hematol OncolJ Hematol Oncol. 2019 Nov 8;12(1):112.

7. Trombetta-Lima M, Rosa-Fernandes L, Angeli CB, Moretti IF, Franco YM, Mousessian AS, et al. Extracellular Matrix Proteome Remodeling in Human Glioblastoma and Medulloblastoma. J Proteome Res. 2021 Oct 1;20(10):4693–707.

8. Sethi MK, Downs M, Shao C, Hackett WE, Phillips JJ, Zaia J. In-Depth Matrisome and Glycoproteomic Analysis of Human Brain Glioblastoma Versus Control Tissue. Mol Cell Proteomics MCP. 2022 Apr;21(4):100216.

9. Wang LB, Karpova A, Gritsenko MA, Kyle JE, Cao S, Li Y, et al. Proteogenomic and metabolomic characterization of human glioblastoma. Cancer Cell. 2021 Apr 12;39(4):509-528.e20.

10. Ghantasala S, Gollapalli K, Epari S, Moiyadi A, Srivastava S. Glioma tumor proteomics: clinically useful protein biomarkers and future perspectives. Expert Rev Proteomics. 2020 Mar;17(3):221–32.

11. Gollapalli K, Ghantasala S, Atak A, Rapole S, Moiyadi A, Epari S, et al. Tissue Proteome Analysis of Different Grades of Human Gliomas Provides Major Cues for Glioma Pathogenesis. Omics J Integr Biol. 2017 May;21(5):275–84.

12. Wesseling P, Capper D. WHO 2016 Classification of gliomas. Neuropathol Appl Neurobiol. 2018 Feb;44(2):139–50.

13. Verma A, Kumar V, Ghantasala S, Mukherjee S, Srivastava S. Comprehensive Workflow of Mass Spectrometry-based Shotgun Proteomics of Tissue Samples. J Vis Exp JoVE. 2021 Nov 13;(177).

14. Tyanova S, Temu T, Sinitcyn P, Carlson A, Hein MY, Geiger T, et al. The Perseus computational platform for comprehensive analysis of (prote)omics data. Nat Methods. 2016 Sep;13(9):731–40.

15. Chong J, Yamamoto M, Xia J. MetaboAnalystR 2.0: From Raw Spectra to Biological Insights. Metabolites. 2019 Mar 22;9(3):57.

16. Liberzon A, Birger C, Thorvaldsdóttir H, Ghandi M, Mesirov JP, Tamayo P. The Molecular Signatures Database (MSigDB) hallmark gene set collection. Cell Syst. 2015 Dec 23;1(6):417–25.

17. Kanehisa M, Furumichi M, Tanabe M, Sato Y, Morishima K. KEGG: new perspectives on genomes, pathways, diseases and drugs. Nucleic Acids Res. 2017 Jan 4;45(D1):D353–61.

18. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003 Nov;13(11):2498–504.

19. MacLean B, Tomazela DM, Shulman N, Chambers M, Finney GL, Frewen B, et al. Skyline: an open source document editor for creating and analyzing targeted proteomics experiments. Bioinforma Oxf Engl. 2010 Apr 1;26(7):966–8.

20. Lane L, Argoud-Puy G, Britan A, Cusin I, Duek PD, Evalet O, et al. neXtProt: a knowledge platform for human proteins. Nucleic Acids Res. 2012 Jan;40(Database issue):D76-83.

21. Ghantasala S, Pai MGJ, Srivastava S. Quantitative Proteomics Workflow using Multiple Reaction Monitoring Based Detection of Proteins from Human Brain Tissue. J Vis Exp JoVE. 2021 Aug 28;(174).

22. Gessulat S, Schmidt T, Zolg DP, Samaras P, Schnatbaum K, Zerweck J, et al. Prosit: proteome-wide prediction of peptide tandem mass spectra by deep learning. Nat Methods. 2019 Jun;16(6):509–18.

23. Choi M, Chang CY, Clough T, Broudy D, Killeen T, MacLean B, et al. MSstats: an R package for statistical analysis of quantitative mass spectrometry-based proteomic experiments. Bioinforma Oxf Engl. 2014 Sep 1;30(17):2524–6.

24. UniProt Consortium. UniProt: the universal protein knowledgebase in 2021. Nucleic Acids Res. 2021 Jan 8;49(D1):D480–9.

25. Kim S, Chen J, Cheng T, Gindulyte A, He J, He S, et al. PubChem in 2021: new data content and improved web interfaces. Nucleic Acids Res. 2021 Jan 8;49(D1):D1388–95.

26. Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem. 2010 Jan 30;31(2):455–61.

27. Salentin S, Schreiber S, Haupt VJ, Adasme MF, Schroeder M. PLIP: fully automated protein-ligand interaction profiler. Nucleic Acids Res. 2015 Jul 1;43(W1):W443-447.

28. Schor SL, Schor AM. Phenotypic and genetic alterations in mammary stroma: implications for tumour progression. Breast Cancer Res BCR. 2001;3(6):373–9.

29. Fu Y, Weissbach L, Plant PW, Oddoux C, Cao Y, Liang TJ, et al. Carboxy-terminal-extended variant of the human fibrinogen alpha subunit: a novel exon conferring marked homology to beta and gamma subunits. Biochemistry. 1992 Dec 8;31(48):11968–72.

30. Elgundi Z, Papanicolaou M, Major G, Cox TR, Melrose J, Whitelock JM, et al. Cancer Metastasis: The Role of the Extracellular Matrix and the Heparan Sulfate Proteoglycan Perlecan. Front Oncol. 2019;9:1482.

31. Rossignol P, Ho-Tin-Noé B, Vranckx R, Bouton MC, Meilhac O, Lijnen HR, et al. Protease nexin-1 inhibits plasminogen activation-induced apoptosis of adherent cells. J Biol Chem. 2004 Mar 12;279(11):10346–56.

32. Crimaudo C, Hortsch M, Gausepohl H, Meyer DI. Human ribophorins I and II: the primary structure and membrane topology of two highly conserved rough endoplasmic reticulum-specific glycoproteins. EMBO J. 1987 Jan;6(1):75–82.

33. Millake DB, Blanchard AD, Patel B, Critchley DR. The cDNA sequence of a human placental alpha-actinin. Nucleic Acids Res. 1989 Aug 25;17(16):6725.

34. Derynck R, Rhee L, Chen EY, Van Tilburg A. Intron-exon structure of the human transforming growth factor-beta precursor gene. Nucleic Acids Res. 1987 Apr 10;15(7):3188–9.

35. Suzuki S, Oldberg A, Hayman EG, Pierschbacher MD, Ruoslahti E. Complete amino acid sequence of human vitronectin deduced from cDNA. Similarity of cell attachment sites in vitronectin and fibronectin. EMBO J. 1985 Oct;4(10):2519–24.

36. Ramírez AS, Kowal J, Locher KP. Cryo-electron microscopy structures of human oligosaccharyltransferase complexes OST-A and OST-B. Science. 2019 Dec 13;366(6471):1372–5.

37. Nakashima T, Sekiguchi T, Kuraoka A, Fukushima K, Shibata Y, Komiyama S, et al. Molecular cloning of a human cDNA encoding a novel protein, DAD1, whose defect causes apoptotic cell death in hamster BHK21 cells. Mol Cell Biol. 1993 Oct;13(10):6367–74.

38. Yamagata T, Tsuru T, Momoi MY, Suwa K, Nozaki Y, Mukasa T, et al. Genome organization of human 48-kDa oligosaccharyltransferase (DDOST). Genomics. 1997 Nov 1;45(3):535–40.

39. Fujita K ichi, Kubota Y, Ishida H, Sasaki Y. Irinotecan, a key chemotherapeutic drug for metastatic colorectal cancer. World J Gastroenterol. 2015 Nov 21;21(43):12234–48.

40. Jiang Q, Li M, Li H, Chen L. Entrectinib, a new multi-target inhibitor for cancer therapy. Biomed Pharmacother Biomedecine Pharmacother. 2022 Jun;150:112974.

41. Lin TC, Yang CH, Cheng LH, Chang WT, Lin YR, Cheng HC. Fibronectin in Cancer: Friend or Foe. Cells. 2019 Dec 20;9(1):27.

42. Schvartz I, Seger D, Shaltiel S. Vitronectin. Int J Biochem Cell Biol. 1999 May;31(5):539–44.

43. Zhang J, Liu J, Zhang H, Wang J, Hua H, Jiang Y. The role of network-forming collagens in cancer progression. Int J Cancer. 2022 Sep 15;151(6):833–42.

44. Ribatti D, Tamma R, Annese T. Epithelial-Mesenchymal Transition in Cancer: A Historical Overview. Transl Oncol. 2020 Jun;13(6):100773.

45. Miyazono K. Transforming growth factor-beta signaling in epithelial-mesenchymal transition and progression of cancer. Proc Jpn Acad Ser B Phys Biol Sci. 2009;85(8):314–23.

46. Provenzano PP, Keely PJ. The role of focal adhesion kinase in tumor initiation and progression. Cell Adhes Migr. 2009;3(4):347–50.

47. Kaur B, Khwaja FW, Severson EA, Matheny SL, Brat DJ, Van Meir EG. Hypoxia and the hypoxia-inducible-factor pathway in glioma growth and angiogenesis. Neuro-Oncol. 2005 Apr;7(2):134–53.

48. Kreibich G, Czakó-Graham M, Grebenau R, Mok W, Rodriguez-Boulan E, Sabatini DD. Characterization of the ribosomal binding site in rat liver rough microsomes: ribophorins I and II, two integral membrane proteins related to ribosome binding. J Supramol Struct. 1978;8(3):279–302.

49. Kelleher DJ, Gilmore R. DAD1, the defender against apoptotic cell death, is a subunit of the mammalian oligosaccharyltransferase. Proc Natl Acad Sci U S A. 1997 May 13;94(10):4994–9.

50. Wu C, Guo X, Wang W, Wang Y, Shan Y, Zhang B, et al. N-Acetylgalactosaminyltransferase-14 as a potential biomarker for breast cancer by immunohistochemistry. BMC Cancer. 2010 Apr 1;10:123.

51. Arriagada C, Silva P, Torres VA. Role of glycosylation in hypoxia-driven cell migration and invasion. Cell Adhes Migr. 2019 Dec;13(1):13–22.

**Figure legends:**

**Figure 1. An overview of the study and schematic of methodological workflow.** A) Glioma tissue samples were collected from 11 high grade glioma patients and 5 low grade glioma patients. After the lysate preparation and protein digestion, label free quantification was performed. B) The raw files generated from the mass spectrometer was analysed using MaxQuant followed by statistical data analysis. C) Significant proteins were selected from the global proteomics data and multiple reaction monitoring assay was performed for validation. D)*In-silico* molecular docking of target proteins was performed to identify the potential drug candidates.

**Figure 2. Quantitative proteomic profiling and pathway enrichment analysis of glioma samples.** A) Heatmap of top 25 differentially expressed proteins in HGG and LGG. B) PCA plot showing two separate clusters for HGG and LGG with a score of 33% for PC1, 13.3% for PC2 and 10.1% for PC3. C) Graphical representation of the significance in the differential expression was quantitatively performed using volcano plot: $\log_{10}$ (p-value) vs. $\log_2$ (fold change). D) Gene set enrichment analysis revealed positively and negatively enriched pathways. E) Epithelial Mesenchymal Transition pathway is found to be highly enriched pathways in HGG with normalized enrichment score 1.77. F) Oxidative phosphorylation process is found to be highly enriched pathways in LGG with normalized enrichment score -1.87.

**Figure 3. Protein-protein interaction analysis of significant proteins in high grade gliomas and their mapped pathways.** Focal adhesion, ECM receptor interaction, N-Glycan biosynthesis and Complement coagulation cascade pathways are found to be highly upregulated in HGGs. Violin plots showing differentially expressed proteins in HGG and LGG (**1.00e-03 < p [?] 1.00e-02; ***1.00e-04 < p [?] 1.00e-03; ****p [?] 1.00e-04).

**Figure 4. Validation of significant proteins by multiple reaction monitoring assay.** A) each sample was spiked with fixed amount of heavy labelled peptide and the Coefficient of variance is 10%. RPN1 and RPN2 are the subunits of oligosaccharyltransferase (OST). Box plots showing differentially expressed proteins in HGG and LGG (**1.00e-03 < p [?] 1.00e-02; ***1.00e-04 < p [?] 1.00e-03).

**Figure 5. *In-silico* molecular docking of catalytic subunits of oligosaccharyltransferase complex with FDA approved drugs.**A) The 3D representation of predicted binding pocket of Entrectinib. The binding affinity of Entrectinib with STT3A is -10.7 kcal/mol and with STT3B is -10.6 kcal/mol. B) The 3D representation of predicted binding pocket of Irinotecan. The binding affinity of Irinotecan with STT3A is -11 kcal/mol and with STT3B is -10.6 kcal/mol. C) The predicted 2D interaction map of Entrectinib with STT3A. D) The predicted 2D interaction map of Irinotecan with STT3A.

**Figure 6. Molecular mechanism associated with OST mediated aggressive behaviour of high-grade gliomas.** Malignant transformation of glioma cells involves extra-cellular matrix remodelling which is accompanied by OST mediated aberrant glycosylation. Glycosylation also results in activation of TGF-β signalling pathways. TGF- β is a strong inducer of EMT transcription factors, drives the EMT in both SMAD and non-SMAD signalling pathways.

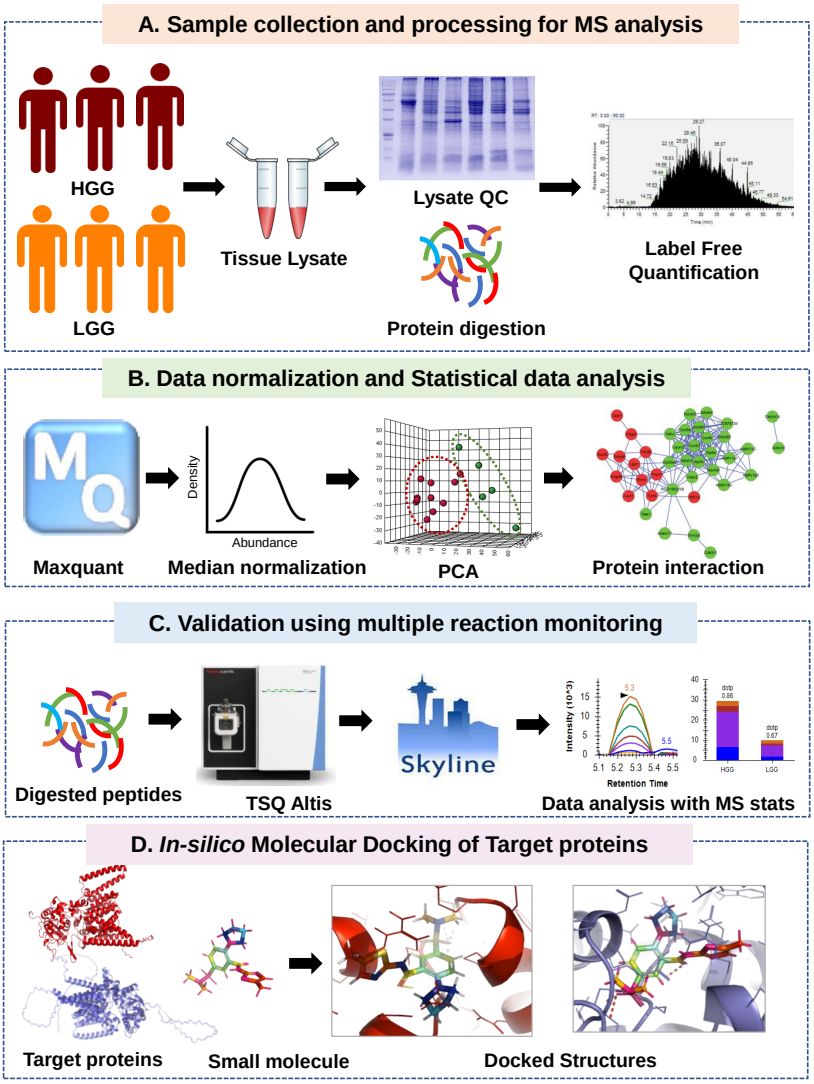


**Figure 1**

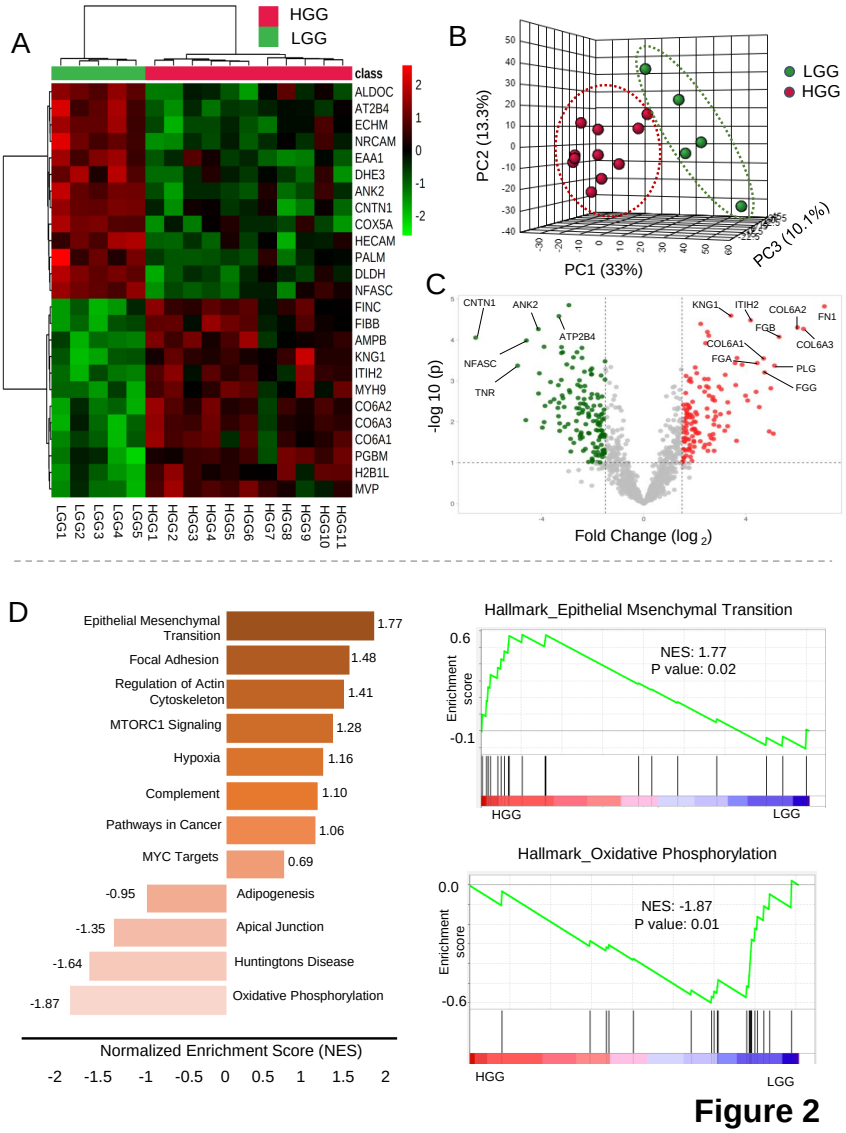


**Figure 2**

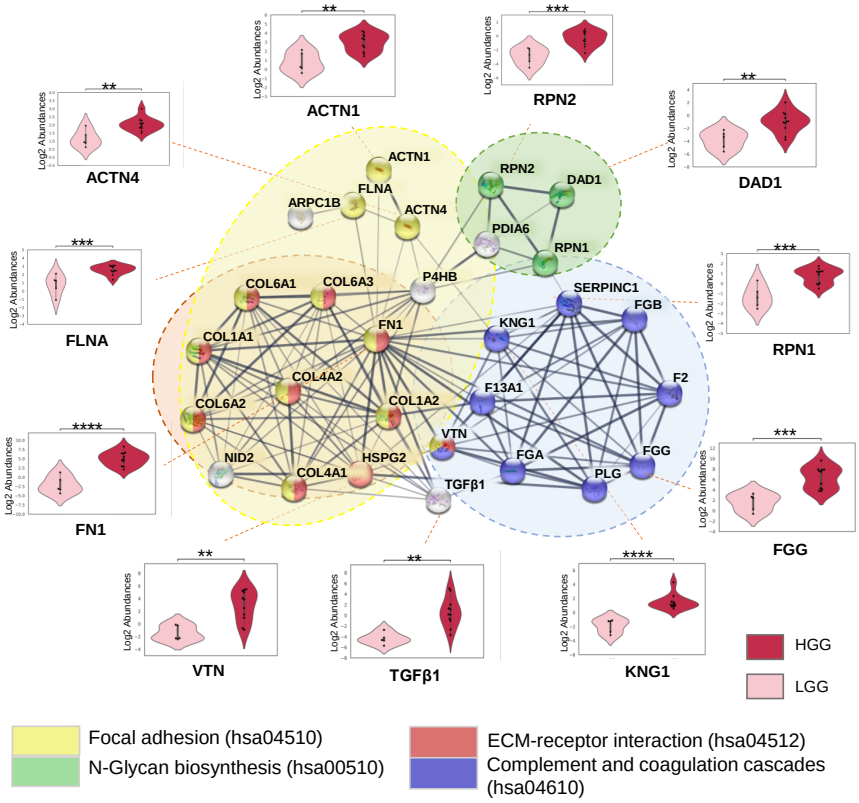


**Figure 3**

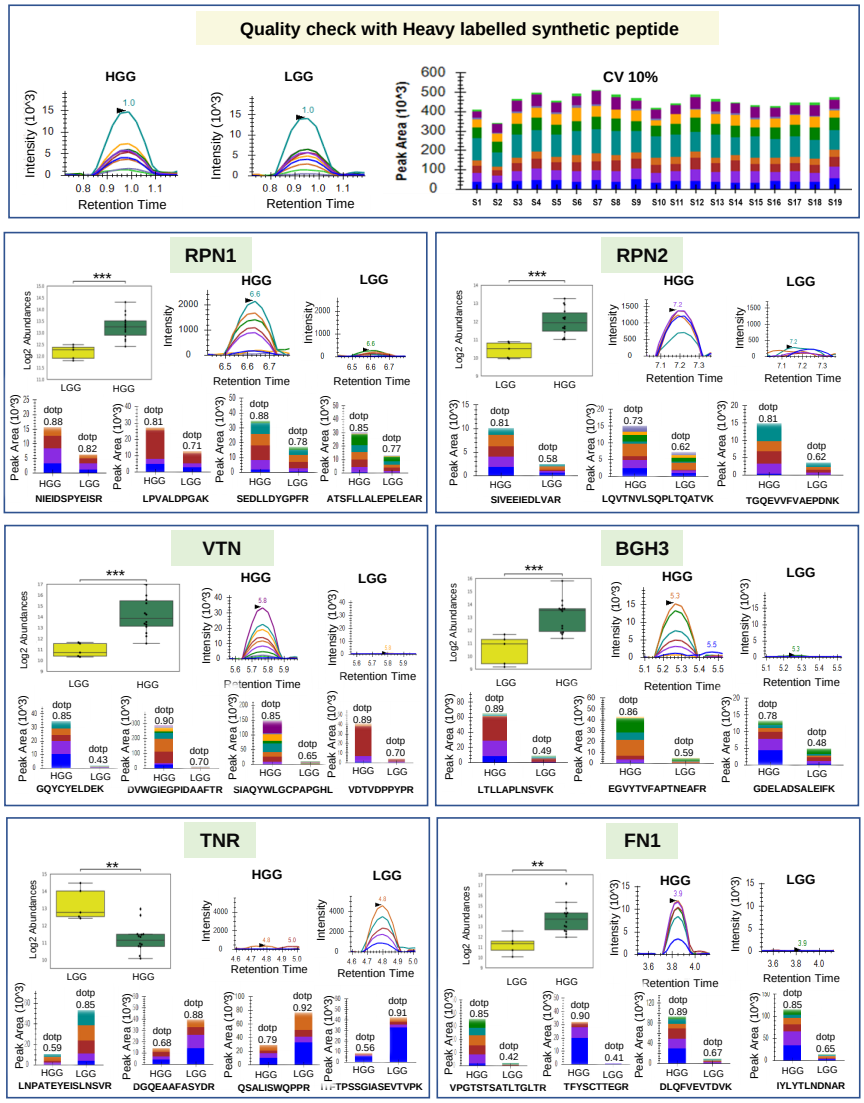


**Figure 4**

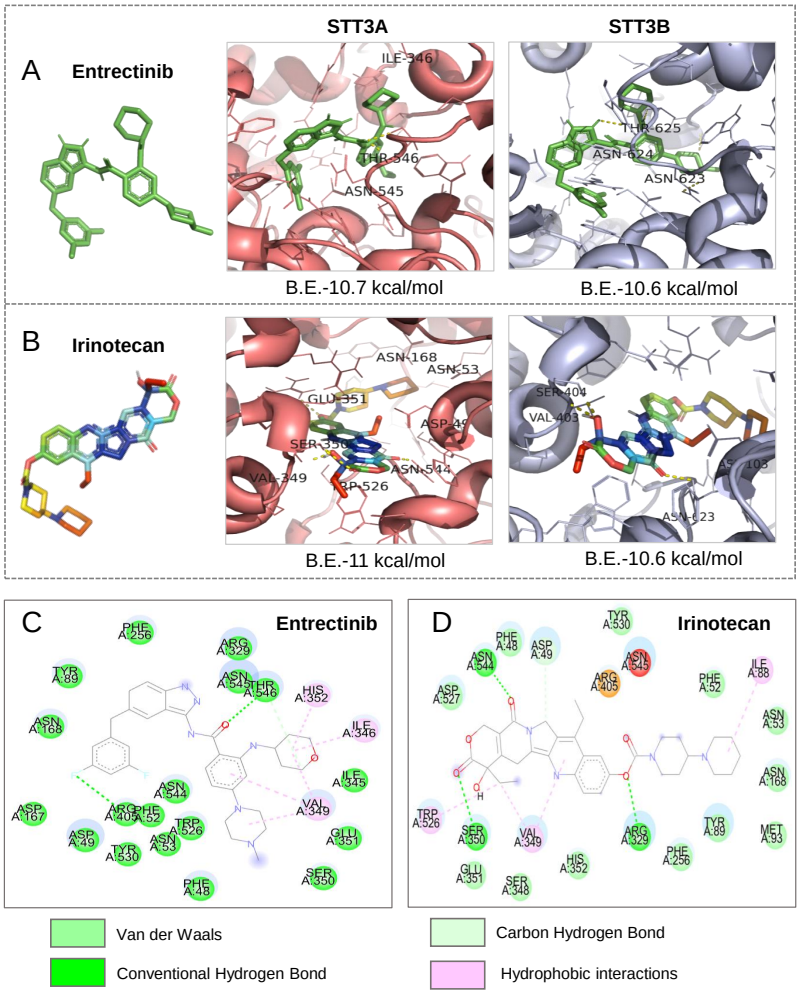


**Figure 5**

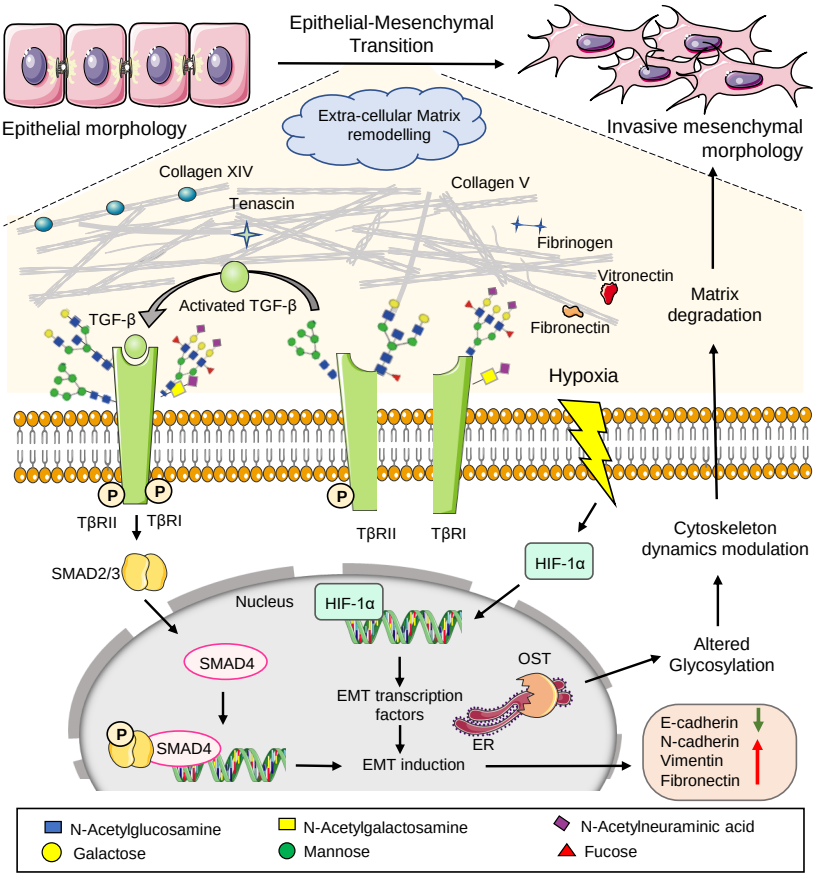


**Figure 6**

### Hosted file

Table 1.docx available at https://authorea.com/users/638983/articles/654538-comprehensive-proteomic-investigation-of-high-grade-and-low-grade-gliomas-reveals-pathways-associated-with-cancer-metastasis-and-candidate-protein-markers-of-therapeutic-potential

### Hosted file

Table 2.docx available at https://authorea.com/users/638983/articles/654538-comprehensive-proteomic-investigation-of-high-grade-and-low-grade-gliomas-reveals-pathways-associated-with-cancer-metastasis-and-candidate-protein-markers-of-therapeutic-potential