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CONNECTIVITY MAP-BASED DRUG REPURPOSING OF SP600125 TO TARGET MESENCHYMAL TRANSITION IN GLIOBLASTOMA*

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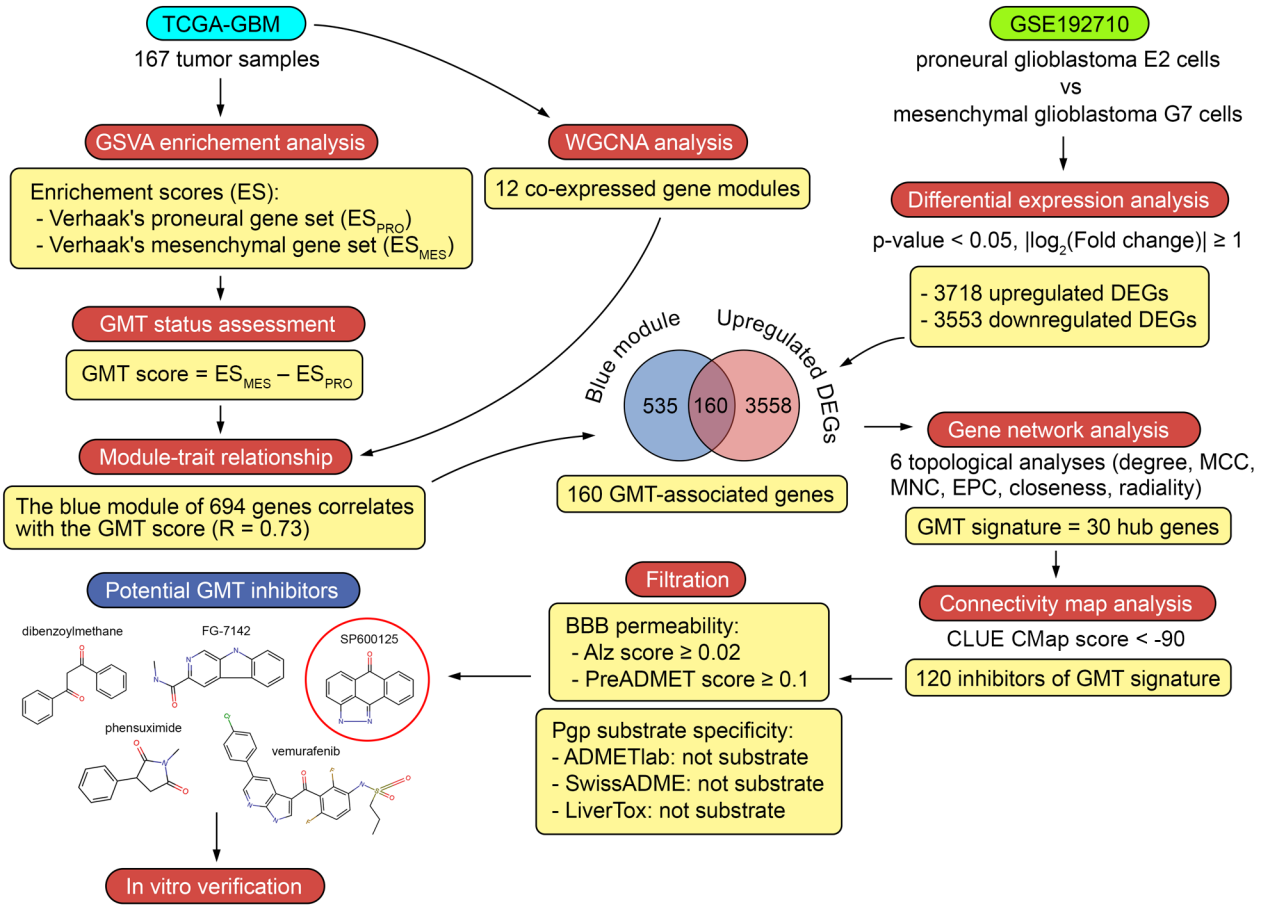
Abstract

Two RNA-seq datasets were reanalyzed using pathway and network analysis. Using connectivity map analysis and chemoinformatics, five small molecules were predicted to cross the blood-brain barrier and inhibit glial-mesenchymal transition (GMT), including SP600125, dibenzoylmethane, FG-7142, vemurafenib, and phensuximide. SP600125 was shown to inhibit GMT in U87 glioblastoma cells *in vitro*.

Despite improvements in surgical, radiation, and chemotherapy treatments, the median survival time for patients with glioblastoma remains at 15 months. Given the urgent need for novel therapeutic options against glioblastoma, drug repurposing may offer a relatively short timeframe to provide new drug candidates. The use of approved or investigational drugs allows rapid advancement of clinical studies due to the established pharmacokinetic, pharmacodynamic and toxicity profiles of these agents, which also helps reduce development costs.

Here we introduce a drug repurposing study that employs connectivity map analysis, which compares the transcriptomic signature of a disease with the signatures of cells treated with various small molecules (see Figure). First,



The pipeline for bioinformatics analysis

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we established a signature involved in glial-mesenchymal transition (GMT), which drives invasion and drug resistance of glioblastoma cells. Using gene set variation analysis (GSVA), enrichment scores for mesenchymal and proneural gene sets (ES_{MES} and ES_{PRO}, respectively) were calculated and then GMT score was estimated for each tumor sample in TCGA-GBM dataset using the formula: GMT score = ES_{MES} — ES_{PRO}. Subsequently, weighted gene co-expression network analysis (WGCNA) was conducted to identify co-expressed gene modules and a module, named blue, was found to have a positive correlation with GMT score (R = 0.73). Intersection of blue module with genes upregulated during GMT in GSE192710 dataset (p-value < 0.05, $|\log_2 FC| \geqslant 1$) resulted in 160 genes, which were used to construct gene network using STRING plugin in Cytoscape platform. A topological analysis of the gene network was conducted using six criteria of centrality (degree, MCC, MNC, EPC, closeness, and radiality) to identify 30 hub genes. These hub genes were utilized as a GMT signature in a connectivity map analysis on the CLUE platform, which yielded 120 small compounds capable of inhibiting the hub genes. A major challenge in glioblastoma therapy is drug delivery across the blood-brain barrier (BBB). The permeability of the BBB is highly dependent on P-glycoprotein (Pgp), which reverses the transport of various chemotherapeutic agents. We used two chemoinformatics platforms, Alz and PreADMET, to identify 12 small molecules out of 120 that have the potential to cross the BBB. Five compounds, namely SP600125, dibenzoylmethane, FG-7142, vemurafenib, and phensuximide, were then identified as Pgp non-substrates using the ADMETlab, SwissADME, and LiverTox platforms. Previous studies support the potential of these compounds as anti-glioblastoma agents. The antimelanoma drug vemurafenib improves therapy outcomes in $BRAF^{V600}$-mutant gliomas. FG-7142 and phensuximide are drugs that act on the central nervous system, which may facilitate their delivery to the tumor. Dibenzoylmethane has antitumor effects that have not been studied in glioblastoma. SP600125 is a JNK-specific inhibitor used for research purposes, but is also being actively studied as an antitumor candidate in preclinical studies.

Previously, SP600125 was shown to inhibit proliferation, invasiveness, and stemness of glioblastoma cells. However, its effect on GMT is not well understood. In our experiments, SP600125 caused 50% growth inhibition in glioblastoma cell lines U87, U118, and EPNT-5 at concentrations of 7.3 μM, 36.8 μM, and 35.8 μM, respectively. The basal level of invasiveness of U87 cells was also decreased. A trypsin treatment test demonstrated that cell-cell adhesion increased 2-fold after 48-hour incubation with 60 nM and 125 nM SP600125. Scratch assay showed that 30 min incubation with 20 μM SP600125 reduced cell motility by 37% and 47% at 24 h and 48 h, respectively. Finally, SP600125 was shown to suppress GMT. Preincubation with SP600125 blocked morphological changes in U87 cells induced by two GMT inducers, transforming growth factor beta 1 (TGF-β1) and cobalt chloride. SP600125 inhibited transwell migration, vasculogenic mimicry, mRNA levels of Slug and fibronectin, and protein levels of N-cadherin in TGF-β1-induced U87 cells.

In conclusion, our analysis demonstrated that SP600125, dibenzoylmethane, FG-7142, vemurafenib, and phensuximide are promising GMT inhibitors that can be used in combination regimens for the treatment of glioblastoma. GMT inhibitory potential of SP600125 was verified on cell models.