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ARTICLE

Integrative Multi-Omics Network Analysis Identifies Novel Drivers and Pathways in Glioblastoma Multiforme

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Abstracts
The Cancer Genome Atlas (TCGA) datasets enable integrative analysis of multi-omics alterations in cancer, offering insights into glioblastoma multiforme (GBM) mechanisms. Here, we employ network analysis to identify molecular pathways and candidate drivers in GBM. Functional modules derived from edge-betweenness clustering of a protein interaction network, built from altered genes, revealed enrichment in both established and novel cancer-associated pathways in GBM. Among 72 genes with high-impact deleterious mutations ($\geqslant$ 3 samples), several (for example, fatty acid synthases ACACA and ACACB) represent novel candidates in GBM, though previously implicated in other cancers. Additionally, 89 genes in copy number-altered regions were prioritized for functional relevance based on network interactions. These findings highlight novel genes and pathways with potential roles in GBM pathogenesis, providing candidates for mechanistic studies and targeted therapies.

Keywords: network; drivers; modules; transcription; subnetwork

1.0 Introduction

Cancer arises from genomic and epigenomic alterations, such as mutations, copy number changes, dysregulated gene expression, and methylation, that confer selective advantages enabling uncontrolled proliferation, immune evasion, and metastasis. Identifying the molecular mechanisms driving these processes is critical for developing targeted therapies. Traditionally, driver genes are discovered through frequency-based methods like MutSig (Lawrence et al., 2013) and GISTIC (Beroukhim et al., 2007), which prioritize recurrently altered genes. However, these approaches often overlook infrequent drivers that contribute to tumorigenesis.

An alternative strategy employs network biology, integrating multi-omics data to identify functional modules within protein interaction networks (Wu et al., 2010; Cerami et al., 2010). This approach captures synergistic effects of diverse alterations, offering a systems-level view of cancer pathways. Yet, its efficacy hinges on the quality of interaction data and the criteria for selecting altered genes.

Glioblastoma multiforme (GBM), a WHO grade IV glioma, exemplifies the urgent need for deeper mechanistic insights. Despite standard therapies, GBM remains incurable, with a median survival of 14.5 months (Hegi et al., 2005). While pathways such as PI3K/AKT, RTK signaling, and cell cycle regulation are frequently altered (CGARN, 2008, Wu et al., 2010, Cerami et al., 2010), emerging evidence suggests additional mechanisms contribute to its pathogenesis (Lino et al., 2010; Liang et al., 2010).

Here, we present an integrative network analysis of GBM multi-omics data to achieve three objectives. First, we identified functional modules enriched for known and novel cancer pathways using edge-betweenness clustering of a protein network constructed from mutated and differentially expressed genes. Second, we prioritized putative drivers, including rare, mutated genes and copy number-altered genes interacting with network modules.

By bridging genomic, transcriptomic, and network-level insights, this study uncovers novel therapeutic targets and pathways, advancing precision oncology for GBM.

2.0 Materials and methods

2,1 Generation of gene-centric expression data

Gene expression data for 529 glioblastoma multiforme (GBM) and 10 non-neoplastic brain samples were obtained from the Cancer Genome Atlas (TCGA) data portal. These data were profiled using the Affymetrix HT HG-U133A platform. To generate gene-centric expression data, we followed established methods from TCGA (2008) and Verhaak et al. (2011). The probe sequences from the HT HG-U133A platform were mapped to a database comprising RefSeq version 41 and GenBank 178 complete coding sequences using SpliceMiner. Only perfect matches were considered, and probes mapping to multiple genes were excluded. The resulting output, along with the HT HG-U133A chip definition file (CDF), was processed using the Makealtcdf function from the affyprobeminer package (Liu, 2007). Probe sets with fewer than five probes were removed, and the resulting alternative CDF was converted into an R package using makecdfenv. The generated CDF was then used in Robust Multi-array Average (RMA) for normalization and summarization of the gene expression data, yielding gene-centric expression values for 12,161 genes.

2.2 Compilation of low-priority gene sets

To exclude genes with low functional impact on tumorigenesis (e.g., high mutation propensity, sex-specificity, or extracellular localization), three gene sets were defined. First, genes encoding extracellular proteins were identified using Gene Ontology "extracellular" cellular component annotations from the Human Protein Reference Database (HPRD) (Keshava et al., 2009). Second, genes encoding proteins longer than 4,000 amino acids were curated from the Consensus Coding Sequence (CCDS) database (Pruitt et al., 2009). Third, sex chromosome-linked genes were obtained from the UCSC Genome Browser (https://genome.ucsc.edu/). These criteria targeted genes less likely to contribute to GBM-driven oncogenic pathways, aligning with the disease's non-sex-specific nature and prioritizing core signaling alterations.

2.3 Differential gene expression analysis

Differential expression analysis was performed on TCGA on ten non-neoplastic and GBM tumour samples. Differentially expressed genes (DEGs) were identified using the Significance Analysis of Microarrays (SAM) method (Tusher et al., 2001) in the R package *siggenes*, with a delta threshold of 6 and FDR-corrected p-values < 0.00001. Low-priority genes from the first

(extracellular proteins) and third (sex chromosome-linked) sets were excluded from the final DEG list.

2.4 Scoring functional effects of somatic mutations

Somatic mutation data for 9505 genes across 282 GBM samples were obtained from TCGA (Level 3). After excluding silent mutations, functional impact scores were assigned to missense mutations using six algorithms (SIFT, Polyphen-2 [HumDiv/HumVar, LRT, MutationTaster, MutationAssessor, FATHMM) from dbNSFP v2.0 (Liu et al., 2001; Ng and Henikoff, 2009; Kumar et al., 2009; Adzhubei et al., 2010; Ramensky et al., 2002; Chun and Fay, 2009; Schwarz et al., 2010; Reve et al., 2011; Schwarz, et al., 2014). A deleteriousness score (0–7) was calculated based on the number of algorithms predicting functional impact. Frameshift, nonsense, splice-site, and translation start-site mutations were assigned the maximum score (7). Genes in low-priority sets (extracellular proteins, sex chromosome-linked) were excluded.

2.5 Identification of Genes in Copy Number-Altered Regions

Copy number data for 470 GBM samples (HG-CGH-244A/HG-CGH-415K platforms) were downloaded from TCGA (Level 3). A custom Python script identified chromosomal segments with log2 signal ratios ≤ -1.2 (homozygous deletion) or ≥ 1.2 (high-level amplification) and mapped these to hg18 gene coordinates from UCSC Genome Browser (https://genome.ucsc.edu/). Genes were included if their coordinates overlapped, enclosed, or partially intersected (≥ 25% of one end) with altered segments. Low-priority genes were filtered out.

2.6 Intracellular Protein Network Construction and Functional Module Detection

A directed protein interaction network was built using Reactome FI version 13 (http://www.reactome.org/pages/download-data/), incorporating experimentally validated functional interactions (FIs) and pathway-derived annotations. To ensure specificity, interactions were filtered to include only pairs annotated with "inhibit" or "activate," exclude terms like "predicted" or "express," and retain a confidence score of 1. This yielded 35,195 unique gene pairs.

The network was constructed using mutated genes and differentially expressed genes (DEGs) that connected at least two mutated genes. Modules were identified via edge-betweenness clustering [109] using the R package *igraph*. Pathway enrichment analysis was performed for each module, and networks were visualized with Gephi (Bastian et al., 2009).

2.7 Identification of Rare Mutated Driver Genes and Prioritization of Copy Number-Altered Genes

A Cytoscape file containing GBM pathway interactions (https://cbio.mskcc.org/cancergenomics/gbm/pathways/) was used to extract genes from frequently altered pathways. Samples lacking deleterious mutations (functional impact score ≥ 3) in these genes were identified, and their network modules were screened for genes with high-impact mutations (score ≥ 5).

To prioritize functionally relevant genes in copy number-altered regions, two criteria were applied:

1. Genes within the intracellular network located in regions altered in ≥ 3 samples.

2. Genes not in the network but interacting with network-resident genes, located in regions altered in ≥ 3 samples.

2.8 Functional Enrichment Analysis of Network and Co-Expressed Modules

Pathway enrichment analysis was performed using a hypergeometric test implemented in R. For each module, the test evaluated the overrepresentation of KEGG pathway genes (from MSigDB) among module genes compared to the full microarray gene set. *p*-values were adjusted for multiple testing using the Benjamini-Hochberg method (Benjamini and Hochberg, 1995).

3.0 Results and Discussion

This study provides a comprehensive and integrative analysis of high throughput multi-omic data in glioblastoma multiforme. It identified functional network modules, rare, mutated driver genes, functionally relevant genes in copy number altered chromosomal regions, and transcription factors that regulate target genes in co-expression modules. It gives an insight into the underlying mechanism that drives tumorigenic process in GBM.

3.1 The GBM intracellular network reveals cancer-relevant modules

Analysis of 15,137 non-silent mutations (12,985 missense) across 282 GBM samples identified 14 modules via edge-betweenness clustering (Figure 1). Enrichment analysis revealed that 11 of the 14 modules were significantly enriched for cancer-associated pathways ($p \leq 0.05$; Table 1). Notably, modules 2, 5, and 16 were altered in 89 %, 39 %, and 42 % of

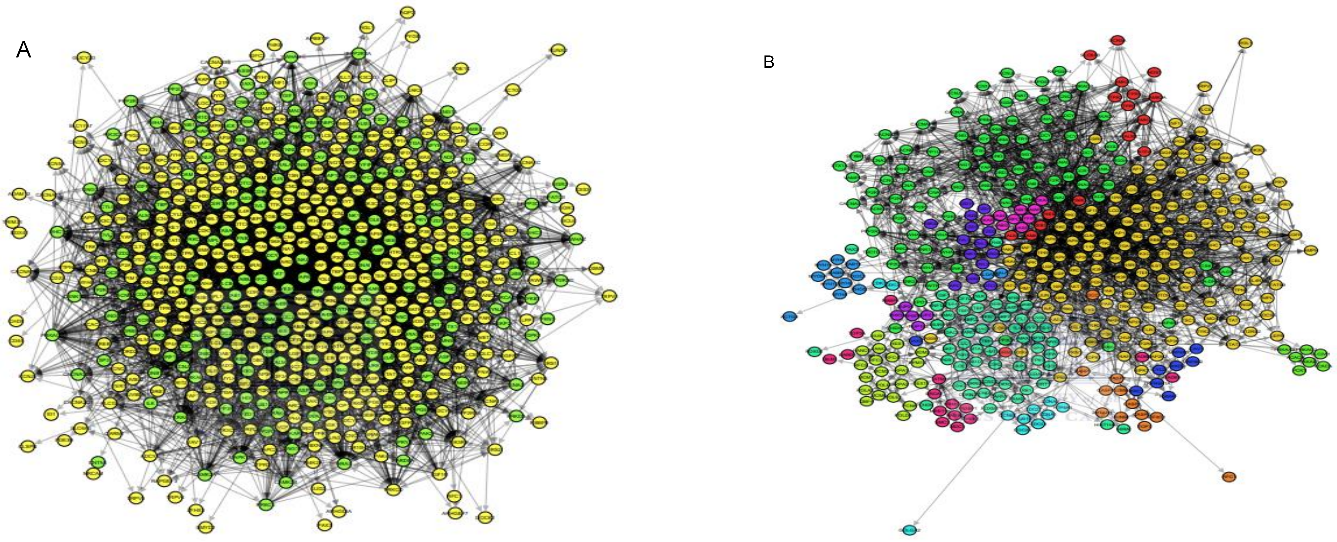

Figure 1: A: The intracellular GBM network. Genes in yellow were mutated genes, and genes in green were non-mutated differentially expressed genes. B: Modules identified in the GBM network. Genes with the same colour are in the same module. Only modules with at least 5

highlighting novel module-level interactions for further exploration.

3.2 Novel signalling pathways were overrepresented in GBM network modules

Novel Signaling Pathways Identified in GBM Network Modules While traditional studies focusing on candidate genes have implicated WNT, NOTCH, TGF-β, and JAK/STAT pathways in GBM, their association with high-throughput omics data has remained underexplored. In this study, modules 2, 5, 16, and 27 were significantly enriched ($p < 0.05$; Table 1) for WNT pathway components, with defects in this pathway identified as causative drivers of GBM. This finding bridges a gap between candidate-driven discoveries and systems-level omics analysis, highlighting the utility of network-based approaches to uncover pathway-level dysregulation in GBM.

3.3 Deleterious Mutations in GBM Network Modules Reveal Novel and Known Drivers

Analysis of deleterious mutations in network modules identified 15 genes previously reported by both frequency-based (CGARN, 2008; Brennan et al., 2013) and network-based (Cerami et al., 2010) studies, including *NF1* (frequency-based only) and *IL18RAP* (Table 2). Module 2 contained all major known drivers (*PTEN*, *TP53*, *EGFR*, *NF1*, *PIK3CA*, *PIK3R1*, *RB1*, *PDGFRA*), with ≥ 90 % of their mutations classified as deleterious. Notably, this module also harboured genes such as *TEK* and *KDR,* encoding angiogenesis regulators angiopoietin-1 and VEGF receptors, previously linked to copy number alterations (Cerami et al., 2010) but now shown to carry functionally impactful mutations. TEK activation is associated with astrocytoma progression (Zadeh et al., 2004), while KDR variants correlate with rectal cancer survival (Slattery et al., 2014).

Additional cancer-associated genes in Module 2 included *MTOR* (Guertin and Sabatini, 2007; Matsubara et al., 2013), *PAK4* (Wong et al., 2013; Minden, 2012; Tabusa et al., 2013), *TYK2* (Zhang et al., 2011; Bel et al., 2013; Sanda et al., 2013), and *PTPN11* (Chan et al., 2008). Other modules contained established cancer genes: checkpoint kinases *CHEK1/2* (Nevanlinna and Bartek, 2006; Bartek et al., 2003), tumour suppressors *TP63* (Flores, 2007; Costanzo et al., 2014; Wei et al., 2011) and *TERT* (Heidenreich et al., 2014; Vinagre et al., 2013), and chromatin remodeler *EP300* (Gayther et al., 2000; Kim et al., 2013; Wallberg et al., 2003; Spin et al., 2010). Nitric oxide synthases *NOS2* and *NOS1,* implicated in pro-tumorigenic processes like angiogenesis (Fukumura et al., 2006; Williams and Djamgoz, 2005), were also mutated, aligning with studies linking nitric oxide to tumour progression.

3.4 Non-Muscle Myosin and Metabolic Genes Harbor Deleterious Mutations in GBM

Module 10 contained five non-muscle myosin genes (*MYH4*, *MYH6*, *MYH8*, *MYH13*, *MYH15*) with deleterious mutations in ≥ 3 samples (Table 2). Non-muscle myosins regulate critical cancer processes, including proliferation, immune evasion, angiogenesis, and metastasis (Ouderkirk and Krendel, 2014).

Module 9 was enriched for metabolic genes, including *ACACA* and *ACACB* (acetyl-CoA carboxylases mediating fatty acid synthesis), *PCK1* (a gluconeogenesis regulator), and *SLC2A1*

Table 1: Alteration types and KEGG pathway enrichment of GBM network modules

Module	# genes	# samples with mutation in modules n=282	# sample with CNV in modules n=470	Enriched pathway	# genes mapped in the pathways
1	9	7	4	Regulation of acting cytoskeleton	7
2	149	251	299	ErbB signalling pathway	35
				Neurotrophin signalling pathway	37
				Chemokine signalling pathway	44
				Focal adhesion	38
				Glioma	25
				Insulin signalling pathway	27
				Regulation of actin cytoskeleton	28
				VEGF signaling pathway	20
				JAK/STAT signaling pathway	23
				Adipocytokine signaling pathway	17
				MTOR signalling pathway	15
				Tight junction	18
				Gap junction	14
				Apoptosis	13
				NOD-like receptor signalling pathway	11
				Phosphatidylinositol signalling system	12
				Inositol phosphate metabolism	9
				WNT signaling pathway	11
3	15	38	3	Homologous recombination	4
4	11	13	1	Cell cycle	6
5	60	111	303	Cell cycle	17
				WNT signalling pathway	13
				NOTCH signaling pathway	7
				TGF- signalling pathway	8
				p53 signalling pathway	5
				Ubiquitin mediated proteolysis	5
9	7	21	3	Adipocytokine signalling pathway	6
				Insulin signalling pathway	6
				Pyruvate metabolism	3
10	13	33	19	Tight junction	6
15	17	43	6	Calcium signalling pathway	11
				GnRH signalling pathway	9
				Neurotropin signalling pathway	8
				Glioma	5
				Phosphatidylinositol signalling system	5
				Gap junction	4
16	89	11	29	Chemokine signalling pathway	21
				Calcium signalling pathway	16
				Gap junction	12
				MAPK signalling pathway	16
				GnRH signalling pathway	10
				WNT signalling pathway	10
				Tight junction	9
23	21	34	45	DNA replication	13
				Mismatch repair	7
				Cell cycle	6
				Base excision repair	4
27	14	18	6	WNT signalling pathway	10
				Pathways in cancer	9
				NOTCH signalling pathway	4

Enriched pathways with FDR-corrected *p-values < 0.05* are shown

Table 2 Putative driver genes* identified in GBM network modules

Module	Drivers identified previously	Putative driver genes
2	PTEN, TP53, EGFR, NF1, PIK3CA, PIK3R1, PDGFRA, BRAF, PIK3R2, PIK3CB, IRS1, IL18RAP, IGF1R, EPHA3	KDR, MAP3K1, PTPN11, PLCG1, IL4R, MTOR, FGFR3, AMPH, ZAP70, VAV1, TSC2, TLR2, TEK, PRKCD, PRKCB, PLCG2, PDGFRB, PAK4, MLLT4, MAP3K7, LPAR3, EPHB2, RXRA, PTK2B, PGR, MAP2K3, ITGB4, ITGB2, INSRR, ESR2, EPHB1, TYK2, PTPRR, PTPN6, NTRK1, MPL, IFNGR2, DNM2, CTTN
3		SMC3, CHEK2, MDC1, RBBP8, BRCA1
4		CDC27
5	RB1	CHD9, TP63, CREBBP, CLOCK, ZFHX3, NFIB, NCOA2, FOXG1, CDKN2C, TLE4, PAX3, HIRA, HDAC2, CARM1, TERT, RBPJL, EP300, EHMT2, CUL1
9		ACACB, ACACA, SLC2A1, PCK1
10		MYH8, MYH13, MYH15, MYH6, MYH4
15		NOS1, ITPR3, ITPR1, SCN5A, SLC6A3, ITPR2, GRM5,
16		GRM3, CACNA1S, GRM8, SCN1A, CHRM2, CACNA2D1, CACNA1D, CACNA1C, ADCY9, RGS9, NOS3, MAP4K1, GNAT3, GABBR2, CACNG3, ADCY2, ADCY1, CHEK1, CACNB3, CACNA2D2, CACNA1A, ADCY6
23		TEX15 MCM7 PPM1D MCM6
27		FZD10 DVL2 AXIN1
39		DCC SPTAN1 GZMB
41		MAP4K3 MAP4K2 CYLD
63		CPSF1

*Genes in which over 50 % of the mutations were of high impact and found in at least 3 samples are shown.
[a]Driver genes previously identified by frequency-based and/or network-based approaches.

(glucose transporter GLUT1). These findings align with the Warburg effect, a hallmark of cancer metabolism, where tumours reprogram glucose and lipid pathways to support growth (Cairns et al., 2011; Warburg, 1956). Notably, *ACACA* and *ACACB* mutations suggest dysregulated lipid synthesis as a potential therapeutic vulnerability in GBM.

3.5 Infrequently Mutated Genes in Key Pathways

While GBM is driven by frequent mutations in RTK, PI3K/AKT, and cell cycle pathways, we identified 49 rarely mutated genes (≤3 samples) in network modules that may contribute to tumorigenesis when canonical pathways are intact. Notable candidates include *ABL1*, previously reported in gliomas, with one deleterious mutation. Other genes such as *DNM2* (a regulator of endocytosis), *EPHB1* (an Eph receptor tyrosine kinase), and *LPAR3* (a GPCR promoting metastasis) were identified as potential novel drivers. *STAT3/5B* (JAK/STAT signaling), *BARD1* (a BRCA1-interacting tumour suppressor), and *MYC* (an oncogenic transcription factor) further exemplify cancer-associated genes with rare but functionally impactful mutations. *ACACB*, encoding a fatty acid synthesis enzyme, was mutated in seven samples (five deleterious), aligning with metabolic reprogramming observed in cancer. These findings underscore the importance of rare mutations in pathways like JAK/STAT, Eph signaling, and lipid metabolism, which may complement or bypass frequent driver alterations.

3.6 Network-Prioritized Copy Number-Altered Genes

From 6,107 genes in copy number-altered regions, 89 (altered in ≥ 3 samples) were prioritized based on network interactions. Known drivers such as *EGFR* and *CDKN2A* were confirmed, while novel candidates included amplified genes like *NAIP* (an anti-apoptotic protein linked to aggressive breast and prostate cancers), *DVL3* (a WNT signaling mediator), and *SKP1* (a component of the SCF ubiquitin ligase complex; Table 3). Deletions in *PRKG1* (a cGMP-dependent kinase) and *CTNNA3* (a β-catenin-related tumour suppressor) were also identified. These results highlight genes whose copy number changes likely contribute to GBM pathogenesis through apoptosis evasion, WNT activation, or cell cycle dysregulation.

3.7 Altered Subnetwork Integrates Oncogenic Pathways

A subnetwork of 238 genes (1,085 interactions) integrated major cancer pathways, including MAPK, p53, WNT, RB1, and GPCR signaling. Key hubs such as *AKT1/3* segregated the network, reflecting their central role in promoting proliferation, survival, and aggressive progression. This systems-level view underscores how genomic alterations converge on critical

Table 3 Genes with copy number alterations in at least three samples.

Gene	# samples with alterations	Gene	# samples with alterations
Genes in the network		Genes not in the network but have interaction targets in the network	
High-level amplification			
EGFR	210	KIT	35
CDK4	61	PHKG1	33
AGAP2	61	DDIT3	33
PDGFRA	48	NUP107	27
MDM2	39	MDM4	27
PIK3C2B	27	FRS2	12
KDR	21	NAIP	10
RAP1B	17	CDK6	10
CLOCK	17	GRB10	8
EXOC1	15	DYRK2	6
SKP1	10	CREB5	6
MET	10	AKT3	6
PTPRR	9	EIF2B5	5
CCND2	7	WNK1	5
PTPRB	7	EPHB3	5
IRAK3	7	AP2M1	5
PIK3CA	6	TIMELESS	4
EIF4G1	5	TFDP1	3
DVL3	5	SENP2	3
ACTL6A	5	MAG12	3
STAT3	3	GNGT1	3
IRS2	3	GNG11	3
CACNA1C	3	GCK	3
AKT1	3	CTNNA2	3
ADCYAP1R1	3	CCNE1	3
Homologous Deletion			
CDKN2A	228	PRKG1	12
CDKN2B	224	FAS	10
PTEN	38	CTNNA3	6
TEK	17	PIK3CD	3
RB1	12	ITPKB	3
CDKN2C	11	DUSP22	3
FAF1	11	CREB3L1	3
HLA-DRB1	8	CHRM4	3
TP53	5	BLNK	3
PER3	4		
VAMP3	4		
NFIB	4		
EPHA3	4		
NF1	3		

oncogenic nodes, reinforcing the utility of network analysis in identifying therapeutic targets.

4.0 Conclusion

Systems-level integration of GBM multi-omics data via network analysis identified novel drivers (for example, *ACACA*, *ACACB*) and pathways (WNT, metabolic reprogramming), extending beyond canonical alterations like RTK/PI3K/AKT. Rare mutations in genes such as *STAT3/5B* and copy number alterations in *NAIP* (anti-apoptotic) and *DVL3* (WNT signaling) reveal underappreciated tumorigenic mechanisms. Central oncogenic hubs, including *AKT1/3*, integrate signaling networks and highlight actionable targets for molecularly stratified therapies. This work demonstrates the power of network biology to decode GBM heterogeneity and prioritize candidates for mechanistic studies and therapeutic development.

Competing interests

The authors declare that they have no competing interests.

Author's contributions

AAF conceived the research idea, designed and performed the experiments, and wrote the draft manuscript. JG supervised all aspects of the work and read, corrected and approved the final manuscript.

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