Identification of Potential Therapeutic Targets and Biomarkers for Glioblastomas Through Integrative Analysis of Gene Expression Data

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Abstract

Background: In this study, we conducted a comprehensive analysis of differential gene expression data from studies GSE15824 and GSE4290 to identify up-regulated hub genes with potential as therapeutic targets for glioblastomas.

Results: Ten up-regulated hub genes, TYROBP, C1QA, C1QB, TLR2, C1QC, CD163, VSIG4, ITGB2, VEGFA, and CD14, were identified. Among them, VEGFA was selected for virtual screening, leading to the discovery of six significant hits. Three hits were from the ChemDiv library of kinase inhibitors (D491-4259, J094-0246, D491-4785), and three from the ZINC20 database (ZINC57658, ZINC57652, ZINC57679), showing promising properties for drug discovery. Furthermore, two drugs for repurposing, Sunitinib and Ticlopidine hydrochloride, were identified. All the hits outperformed control drugs Irinotecan, Carboplatin, and Fluorouracil.

Additionally, we identified down-regulated hub genes VAMP2 and ANK3 as potential biomarkers for glioblastomas. Enrichment analysis provided further insights into the molecular pathways involved, shedding light on potential therapeutic strategies for the treatment of glioblastomas.

Conclusion: This computational study sheds light on potential drug candidates for glioblastoma treatment and offers valuable insights into the disease's molecular mechanisms, paving the way for improved therapeutic strategies.

Keywords: Glioblastomas, VEGFA inhibitors, Virtual Screening, Biomarkers.

Introduction

Malignant tumors of the central nervous system (CNS) are characterized by their aggressive nature and poor prognosis. Among these tumors, gliomas are the most common primary CNS tumors, exhibiting the ability to differentiate into various cell types, such as neural stem cells, astrocytes, or oligodendroglia progenitor cells [1].

Glioblastomas, classified as level IV gliomas by the World Health Organization (WHO), are the most aggressive subtype, accounting for 60-70% of malignant gliomas [2,3]. They can be further categorized into three subtypes: proneural, classic, and mesenchymal [4]. The incidence of gliomas increases with age, particularly among individuals over 75 years old, with age also correlating inversely with survival rate [5]. While the exact risk factors for glioblastoma development remain unclear, it has been observed that it is more common in men than in women [6,7].

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In silico analyses enable the identification of compounds that may possess inhibitory effects on overexpressed proteins associated with various diseases and malignancies, such as glioblastomas. They allow for the evaluation of the stability of interactions between candidate compounds and proteins, and offer cost-effective alternatives by studying existing drugs, as compared to the expenses involved in discovering new compounds. Moreover, with the availability of databases containing drugs with inhibitory potential against numerous genes, as well as the integration of Artificial Intelligence, the search for candidate compounds can be expedited [8-10].

It is the purpose of the present work to identify suitable targets and ligands for the treatment of glioblastomas by means of differential expression analysis of relevant studies and virtual screening, involving both molecular docking and ADMET filtering, of small molecules databases. Screening of control drugs of identified targets and enrichment analysis of hub genes are also provided to complement the study.

Methods and Materials

Receptors Search

To identify hub genes associated with glioblastomas, gene expression analyses were performed on two studies from the Gene Expression Omnibus. One study, "Gene expression profiling of human gliomas and human glioblastoma cell lines," [11] with accession number GSE15824, utilized microarray analysis on 30 brain tumor samples (12 primary glioblastomas, 3 secondary glioblastomas, 8 astrocytoma, and 7 oligodendrogliomas) and 5 cell lines (LN018, LN215, LN229, LN319, and BS149) to identify differentially expressed signaling pathways in human gliomas compared to normal brain tissue and normal astrocytes.

The other study, "Expression data of glioma samples from Henry Ford Hospital," [12] with accession number GSE4290, evaluated mRNA expression of 153 samples from patients with brain tumors (77 glioblastomas, 50 oligodendrogliomas, and 26 astrocytoma), with 23 samples from patients with epilepsy without tumors serving as controls.

The analyses were performed using NCBI's online tool GEO2R, with options Benjamini & Hochberg for false discovery rate, log transformation, limma precision weights and forced normalization. Adjusted P value significance cut-off was set to 0.05 and log2 fold change threshold was set to zero.

Analysis of Interaction Network

The up and down regulated genes common to both studies were identified using the Funrich [13-15] software, and the protein – protein interaction (PPI) network among the proteins codified by those genes was obtained using the STRING database [16, 17]. The top 10 up and down regulated hub genes, chosen by their degree of interaction, were subsequently identified using the Cytohubba [18] plugin in the Cytoscape software [19].

Preparation of Receptor

The Vascular Endothelial Growth Factor A (VEGFA) protein, which was identified as a hub gene, was obtained from the Uniprot database. The corresponding protein sequence was selected using the accession number from the Uniprot search results. To obtain receptor proteins, a BLAST search was performed using the amino acid sequence of VEGFA against the Protein Data Bank. The protein with entry 1VPF [20] (Figure1), which had a percentage of identity greater than 80% and an unmutated structure, was selected. Cavity detection was carried out in CASTp [21] and the structure was prepared for molecular docking by removing non-protein chains, solvents, ions, and ligands in Chimera [22]. Energy minimization was performed in Swiss PDB viewer [23], and hydrogens and charges were added to the protein structure in Chimera.

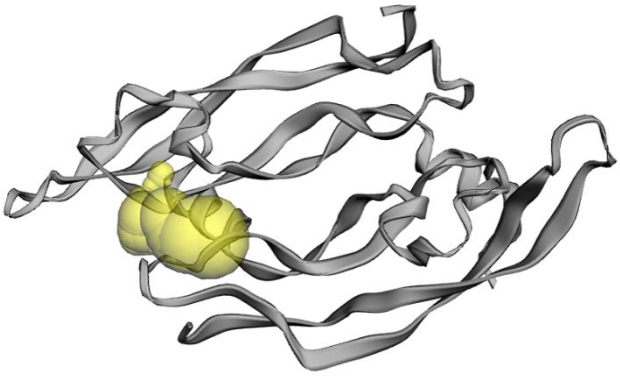


Figure 1. VEGFA Structure PDB 1VPF (chain A), showing the pocket for the active site.

Virtual screening

Ligands were obtained from the ZINC20 database, 100 compounds were downloaded from the subset of inferred compounds targeting the protein VEGFA, and from the Protein Kinases Inhibitors Library from ChemDiv [24], which consists of 36324 compounds. Energy minimization was performed with the Open Babel software [25], using the generalized Amber force field (GAFF) with 5000 steps.

The Drug Gene Interaction Database (https://www.dgidb.org/), with VEGFA as target, was also searched for possible drugs repurposing. Approved drugs Irinotecan, Carboplatin and Fluorouracil were used as controls, based on information about their use in glioblastoma treatment and VEGFA interaction, retrieved from the Drug Bank (https://go.drugbank.com/) and Gene Cards (https://www.genecards.org) databases.

Molecular docking was carried out with Autodock Vina [26, 27], with center coordinates (37, 11, 14) and a symmetric grid size of 25 Å and the results were filtered for druglikeness and toxicity properties using SwissADME [28], OSIRIS Property Explorer and Data Warrior [29]. Finalists' compounds were required to pass all the druglikeness rules of Lipinski [30], Ghose [31], Veber [32], Egan [33], Muegge [34], exhibit none of the structure Brenk [35] and PAINS alerts [36] and permeate the Brain Blood Barrier (BBB). As for toxicity, they were required to show zero alerts for mutagenicity, tumorigenicity, irritancy, and reproductive effects.

Using values provided by the OSIRIS Property Explorer for druglikeness, drug-score, and solubility, as well as the binding energy, in absolute values, obtained from docking, a simple average score was calculated to rank the final hits.

Enrichment Analysis

Enrichment analysis was performed on the hub genes using GeneTrail [37] to obtain the ten most enriched categories according to the KEGG [38], Wikipathways [39], Ractome [40] and Gene Ontology [41, 42] databases. Hub genes expression against data from glioblastoma multiform tumors vs normal samples was also checked using Gepia2 [43] for further validation, with parameters |log2FC|=1 and P-value < 0.05.

Results

We obtained six significant hits, three from the ChemDiv library with identifiers D491-4259, J094-0246, and D491-4785, which presented the highest scores and best overall properties, and three from the ZINC20 database, with identifiers ZINC57658, ZINC57652 and ZINC57679. We also identified two possible drugs for repurposing: Sunitib and Ticlopidine hydrochloride. Tables 1 and 2 provide their properties, as well as their druglikeness and toxicity results, along with the controls.

The results from the differential gene expression analyzes of studies GSE15824 and GSE4290 are presented in Figure 2, while Figure 3 presents the Venn diagrams of the up and down regulated genes from both studies, respectively, showing they share 288 genes for the former case and 664 for the latter. From these, the PPI networks of the associated proteins were obtained from Strings DB (Figures in supplementary material), with significant enrichment P values, showing that the networks constitute biological clusters. Finally, up-, and down- regulated hub genes are given in Figure 4.

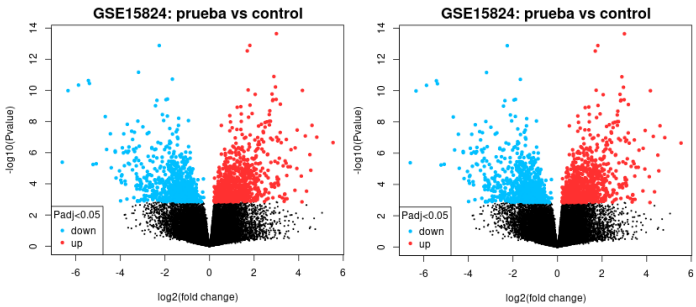


Figure 2. Volcano plot showing the dysregulated genes resulting from differential gene expression analysis, for both studies

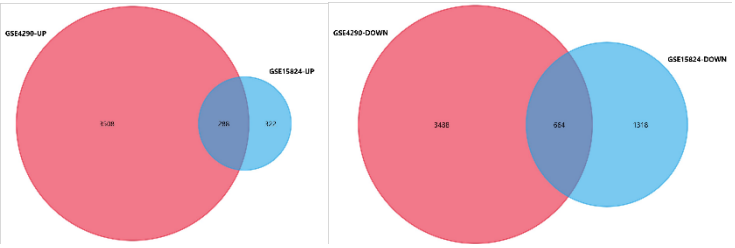


Figure 3. Venn Diagrams showing the number of common genes for up-regulated genes (left) and down-regulated ones (right).

Docking results from the ZINC compounds and controls are presented in Table 3, and the docked complexes in Figures 5 to 7. The rest can be found in the supplementary materials. The results from the enrichment analysis and expression from Gepia2 graphs are given in Figures 8 and 9-10, respectively.

Table 1. SMILES strings, molecular weight (MW) in Daltons, topological surface area (TPSA) in $Å^2$, and brain blood barrier permeability (BBB) for the finalists' hits, control and repurpose drugs. The Ligand column shows the name provided by PubChem.

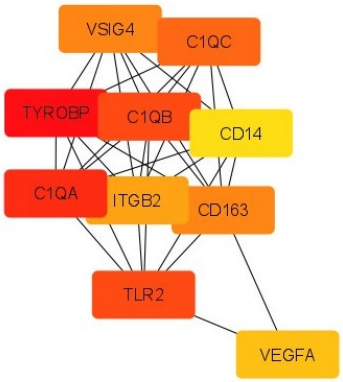
Id	Ligand	SMILES	MW	TPSA	BBB
Sunitinib	Sunitinib	CCN(CCNC(=O)c1c(C)[nH]c(c1C)C=C1C(=O)Nc2c1cc(F)cc2)CC	398.47	77.23	Yes
D491-4785	AKOS002280690	O=C(c1noc(c1)c1ccc2c(c1)CCCC2)N1CCN(CC1)CCc1ccccn1	416.52	62.47	Yes
J094-0246	-	Clc1cccc(c1)[C@H]1CC(=O)Nc2c1c(C)nn2c1nc2c(o1)cccc2	378.81	72.95	Yes
D491-4259	AKOS004929323	O=C(c1noc(c1)c1ccc2c(c1)CCCC2)N1CCN(CC1)CCc1ccncc1	416.52	62.47	Yes
Ticlopidine	Ticlopidine hydroch-loride	Clc1ccccc1CN1CCc2c(C1)ccs2.Cl	300.25	31.48	Yes
ZINC57679	4'-Hydroxyflavanone	Oc1ccc(cc1)c1cc(=O)c2c(o1)cccc2	238.24	50.44	Yes
ZINC57652	5,4'-Dihydroxyflavone	Oc1ccc(cc1)c1cc(=O)c2c(o1)cccc2O	254.24	70.67	Yes
Control	Irinotecan	CCC1(O)C(=O)OCc2c1cc1c3nc4ccc(cc4c(c3Cn1c2=O)CC)OC(=O)N1CCC(CC1)N1CCCCC1	586.68	114.2	No
ZINC57658	Liquiritigenin	Oc1ccc(cc1)c1cc(=O)c2c(o1)cc(cc2)O	254.24	70.67	Yes
Control	Carboplatin	OC(=O)C1(CCC1)C(=O)O.[Pt+2].[NH2-].[NH2-]	371.25	126.64	No
Control	Fluorouracil	Fc1c[nH]c(=O)[nH]c1=O	130.08	65.72	No

Table 2. Druglikenes and toxicity properties. The columns Lipinski (L), Ghose (G), Veber (V), Egan (E) and Muegge (M) show the number of violations to the corresponding rules. The columns PAINS and Brenk show the number of alerts. Toxicity includes information about mutagenicity, tumorigenic, irritant, and reproductive effectiveness, provided by Osiris Property Explorer, while Solubility, Druglikeness, and Drug score are the scores provided by the same software. Energy is the binding energy reported by docking, in kcal/mol, and the final Score is the simple arithmetic mean of the preceding four columns, with Energy in absolute value.

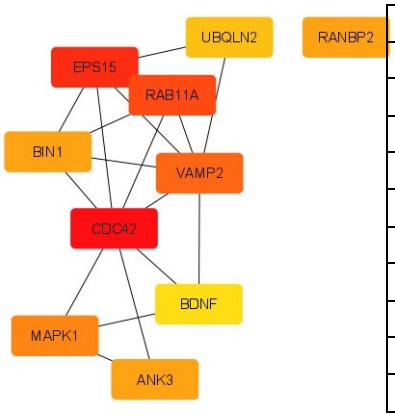
Ligand	L	G	V	E	M	PAINS	Brenk	Toxicity	Energy	Solubility	Druglikeness	Drug score	Score
Sunitinib	0	0	0	0	0	0	1	Irritant	-7.07	-3.47	8.47	0.47	3.13
D491-4785	0	0	0	0	0	0	0	None	-8.76	-3.48	5.59	0.7	2.89
J094-0246	0	0	0	0	0	0	0	None	-8.69	-3.28	5.21	0.77	2.85
D491-4259	0	0	0	0	0	0	0	None	-8.44	-3.46	3.91	0.7	2.4
Ticlopidine hydrochloride	0	0	0	0	0	0	0	None	-6.08	-3.55	4.68	0.81	2.01
ZINC57679	0	0	0	0	0	0	0	None	-7.48	-3.45	2.11	0.79	1.73
ZINC57652	0	0	0	0	0	0	0	None	-7.66	-3.15	1.56	0.79	1.71
Irinotecan	1	3	0	0	0	0	0	None	-8.98	-4.5	0.07	0.35	1.22
ZINC57658	0	0	0	0	0	0	0	None	-7.75	-3.15	-0.36	0.61	1.21
Carboplatin	0	0	0	0	1	0	1	None	-4.93	-0.9	-5.33	0.49	-0.2
Fluorouracil	0	3	0	0	2	0	0	All	-4.46	-1.76	-4.5	0.06	-0.43

Table 3. Docked complexes interactions between flavonoids from ZINC and VEGFA (1VPF)

Compound	Binding energy (Kcal/mol)	Donnor	Distance (Å)	Acceptor	Category	Interaction type
ZINC57652	-7.66	A:GLU64:OE2	4.57	UNL1	Electrostatic	Pi-Anion
		A:GLU64:CA	4	UNL1	Hydrophobic	Pi-Sigma
		A:ASP63:C,O;GLU64:N	4.36	UNL1	Hydrophobic	Amide-Pi Stacked
		A:ASP63:C,O;GLU64:N	4.05	UNL1	Hydrophobic	Amide-Pi Stacked
		A:ASP63:N	3.34	UNL1:O	Hydrogen Bond	Conventional Hydrogen Bond
		A:ASN62:C,O;ASP63:N	3.6	UNL1	Hydrophobic	Amide-Pi Stacked
		UNL1	5.13	B:ILE46	Hydrophobic	Pi-Alkyl
		UNL1	5.03	B:ILE46	Hydrophobic	Pi-Alkyl
		UNL1	5	B:LYS48	Hydrophobic	Pi-Alkyl
		B:LYS48:NZ	3.17	UNL1:O	Hydrogen Bond	Conventional Hydrogen Bond
ZINC57658	-7.75	A:GLU64:OE2	4.63	UNL1	Electrostatic	Pi-Anion
		A:ASP63:C,O;GLU64:N	4.36	UNL1	Hydrophobic	Amide-Pi Stacked
		A:ASP63:C,O;GLU64:N	4.04	UNL1	Hydrophobic	Amide-Pi Stacked
		A:ASP63:N	3.32	UNL1:O	Hydrogen Bond	Conventional Hydrogen Bond
		A:ASN62:C,O;ASP63:N	3.6	UNL1	Hydrophobic	Amide-Pi Stacked
		B:LYS48:NZ	3.27	UNL1:O	Hydrogen Bond	Conventional Hydrogen Bond
		UNL1	5.11	B:ILE46	Hydrophobic	Pi-Alkyl
		UNL1	5.02	B:ILE46	Hydrophobic	Pi-Alkyl
		UNL1	5	B:LYS48	Hydrophobic	Pi-Alkyl
ZINC57679	-7.48	UNL1	5.1	B:ILE46	Hydrophobic	Pi-Alkyl
		UNL1	5.06	B:ILE46	Hydrophobic	Pi-Alkyl
		UNL1	5	B:LYS48	Hydrophobic	Pi-Alkyl
		A:GLU64:OE2	4.6	UNL1	Electrostatic	Pi-Anion
		A:GLU64:CA	4	UNL1	Hydrophobic	Pi-Sigma
		A:ASP63:C,O;GLU64:N	4.35	UNL1	Hydrophobic	Amide-Pi Stacked
		A:ASP63:C,O;GLU64:N	4.05	UNL1	Hydrophobic	Amide-Pi Stacked
		A:ASP63:N	3.32	UNL1:O	Hydrogen Bond	Conventional Hydrogen Bond
		A:ASN62:C,O;ASP63:N	3.6	UNL1	Hydrophobic	Amide-Pi Stacked
		B:LYS48:NZ	3.2	UNL1:O	Hydrogen Bond	Conventional Hydrogen Bond
		A:CYS61:N	3	UNL1:O	Hydrogen Bond	Conventional Hydrogen Bond
		UNL1:H	2.31	A:CYS61:O	Hydrogen Bond	Conventional Hydrogen Bond
		UNL1:H	3.05	B:SER50:OG	Hydrogen Bond	Conventional Hydrogen Bond
		B:SER50:OG	3	UNL1:O	Hydrogen Bond	Conventional Hydrogen Bond
		UNL1:H	2.7	A:LEU66:O	Hydrogen Bond	Conventional Hydrogen Bond
		UNL1:C	3.65	A:GLU64:O	Hydrogen Bond	Conventional Hydrogen Bond
		A:GLU64:CA	3.37	UNL1:F	Hydrogen Bond; Halogen	Carbon Hydrogen Bond;Halogen (Fluorine)
		A:GLU64:OE2	3.34	UNL1:F	Halogen	Halogen (Fluorine)



Hub genes
TYROBP
C1QA
C1QB
TLR2
C1QC
CD163
VSIG4
ITGB2
VEGFA
CD14



Hub genes
CDC42
EPS15
RAB11A
VAMP2
MAPK1
RANBP2
ANK3
BIN1
UBQLN2
BDNF

Figure 4. Up-regulated (left) and down regulated (right) hub genes obtained from the PPI networks. Colors represent the interaction degree from red (highest) to yellow (lowest).

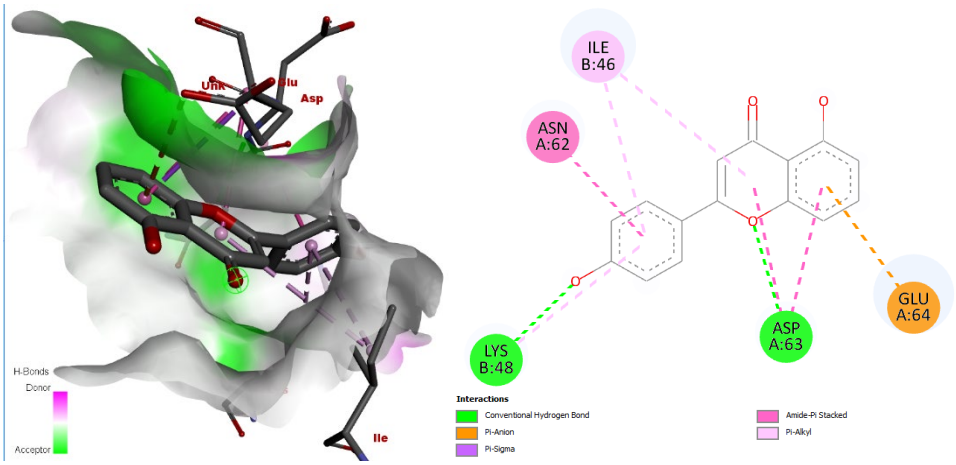


Figure 5. 3D (left) and 2D (right) structure of the docked complex VEGFA and 5,4'-Dihydroxyflavone. The violet and green color shades indicate hydrogen bonds.

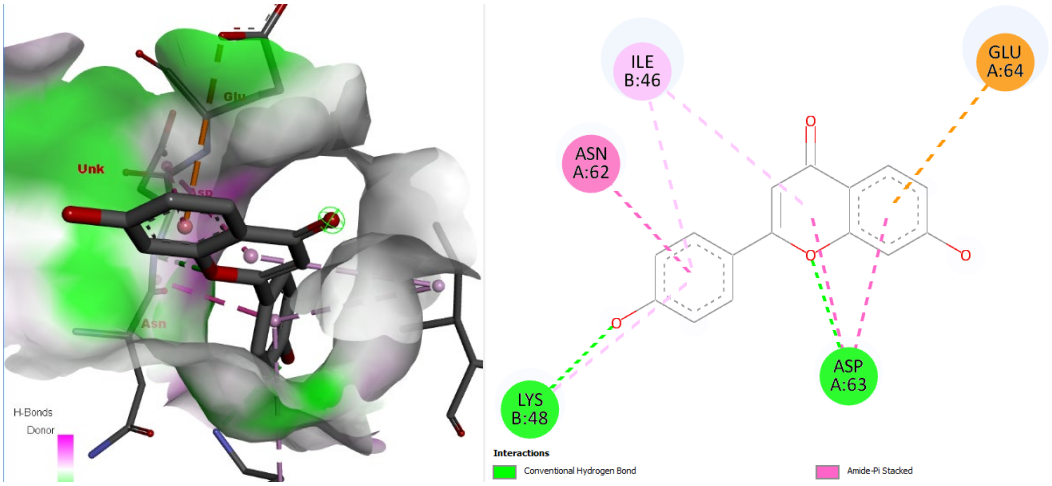


Figure 6. 3D (left) and 2D (right) structure of the docked complex VEGFA and Liquiritigenin. The violet and green color shades indicate hydrogen bonds.

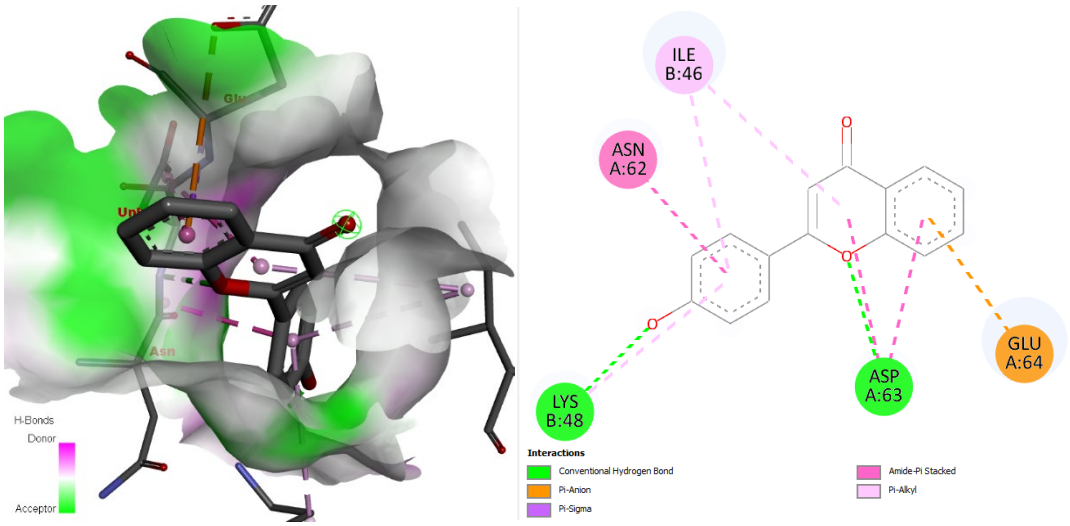


Figure 7. 3D (left) and 2D (right) structure of the docked complex VEGFA and 4'-Hydroxyflavanone. The violet and green color shades indicate hydrogen bonds.

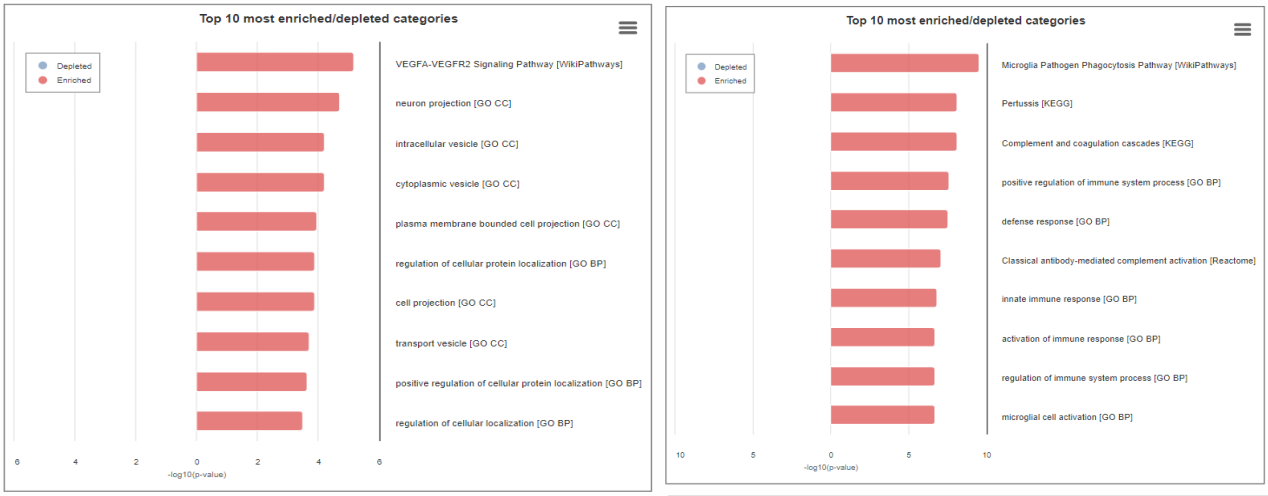


Figure 8. The ten most enriched categories for the up-regulated (left) and down-regulated (right) hub-genes.

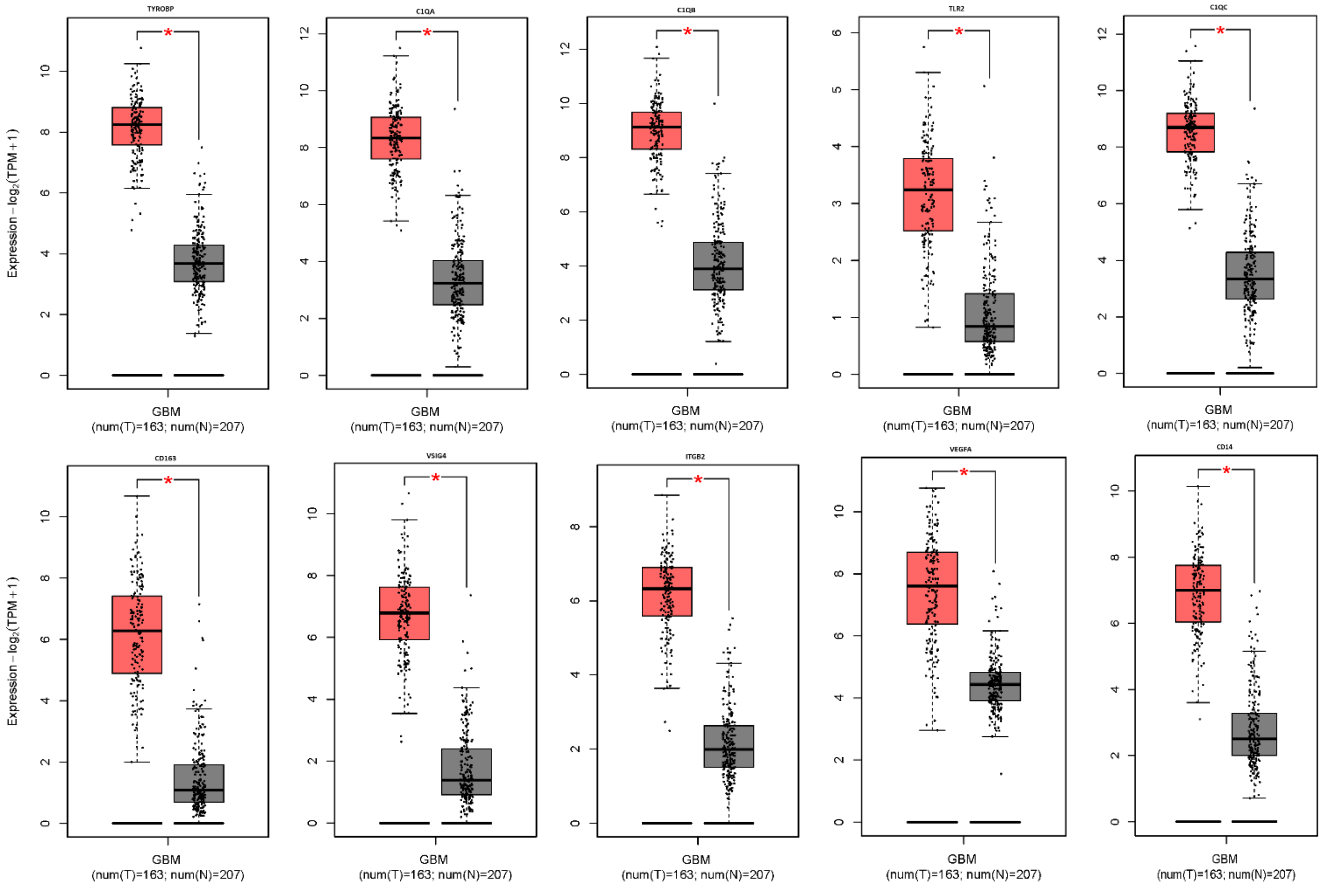


Figure 9. Expression box plots of the ten up-regulated hub genes for tumor (T, in salmon) vs normal (N, in gray) glioblastoma multiforme samples (GBM), obtained from Gepia2. The red asterisk indicates significance (P value < 0.05). Top row: TYROBP, C1QA, C1QB, TLR2 and C1QC. Bottom row: CD163, VSIG4, ITGB2, VEGFA and CD14.

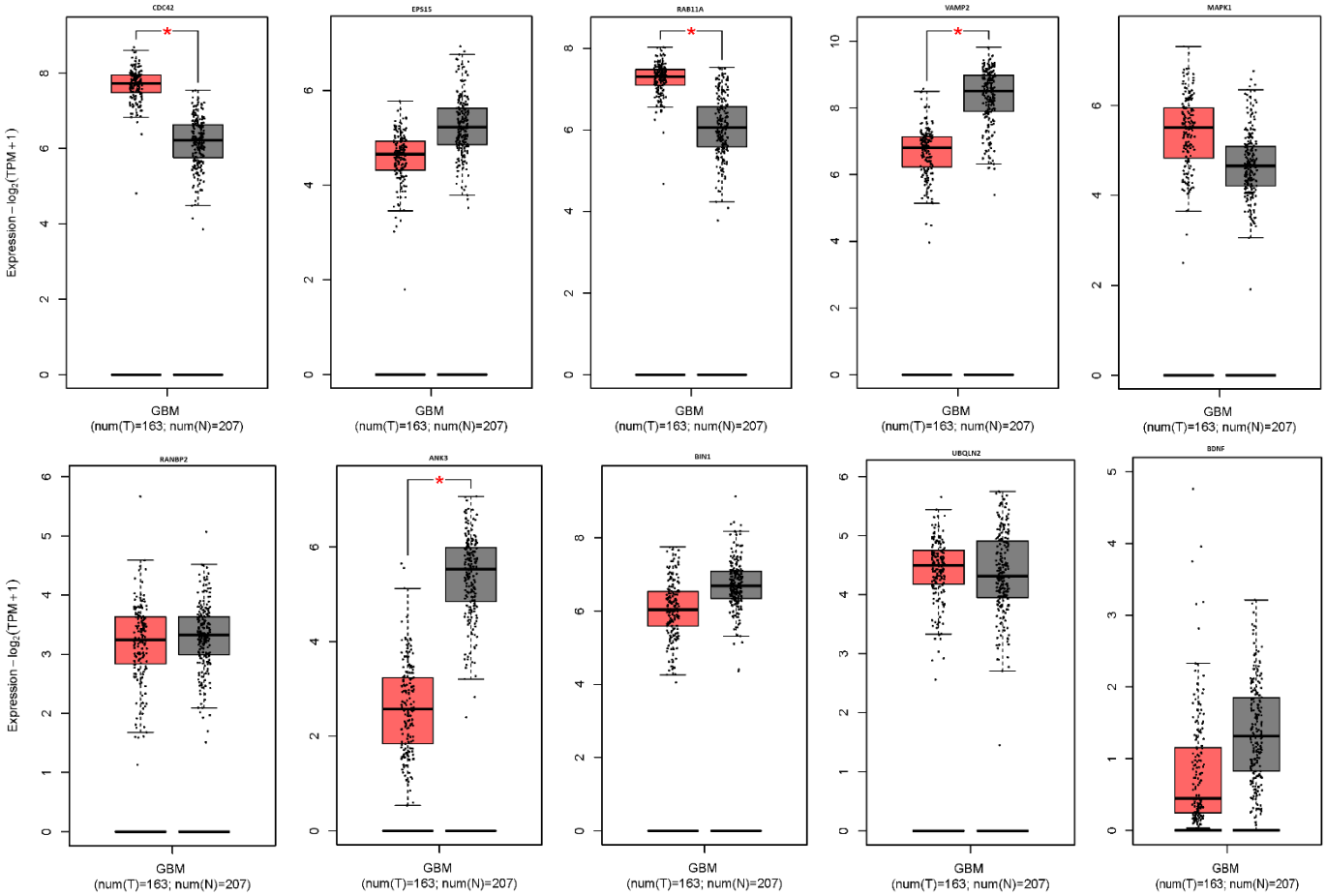


Figure 10. Expression box plots of the ten down-regulated hub genes for tumor (T, in salmon) vs normal (N, in gray) glioblastoma multiforme samples (GBM), obtained from Gepia2. The red asterisk indicates significance (P value < 0.05). Top row: CDC42, EPS15, RAB11A, VAMP2, and MAPK1. Bottom row: RANBP2, ANK3, BIN1, UBQLN2, and BDNF.

Discussion

The hits obtained from the ZINC database consist of flavonoids, with 5,4'-Dihydroxyflavone and Liquiritigenin classified as flavones, and 4'-Hydroxyflavanone identified as a flavanone. Despite being outperformed by other hits in various metrics, the analysis of the docked complexes revealed a substantial number of Pi interactions, suggesting improved structural stability and specificity [44]. As a result, these compounds were selected for further emphasis. Previous studies have reported these compounds as inhibitors of tankyrase [45] and monoamine oxidase [46] (4'-Hydroxyflavanone), androgen receptor [47] (5,4'-Dihydroxyflavone), and aromatase [48] (Liquiritigenin), although their potential as VEGFA inhibitors remains unexplored.

Conversely, hits from the ChemDiv library exhibited superior scores and represent novel compounds not documented in existing literature, indicating their potential for lead development. Despite VEGFA not being a kinase, the screening of kinase inhibitors was based on an in silico study demonstrating binding affinity between VEGFA and tyrosine kinase inhibitors [49], a hypothesis supported by the current findings.

Among the two drugs identified for repurposing, sunitinib demonstrated the highest score, surpassing the hits, but was flagged for a Brenk alert and identified as an irritant in toxicity screenings, diminishing its value. In contrast, Ticlopidine hydrochloride exhibited no drawbacks, ranking between the hits from ChemDiv and ZINC, positioning it as a promising candidate for repurposing. While Irinotecan was the highest-ranked control drug, it exhibited Lipinski and Ghose violations, with none passing the blood-brain barrier (BBB) test.

In selecting the target, all ten up-regulated hub genes displayed significant overexpression in tumor vs. normal data, with VEGFA being the sole validated target for cancer treatment, particularly in glioblastomas [51-53], justifying our choice. Conversely, no curated association between the other nine hub genes and glioblastomas was found in the Comparative Toxicogenomics Database (CTD) [54], indicating novel findings.

Among the down-regulated hub genes, only VAMP2 and ANK3 exhibited significant underexpression in tumor vs. normal data, suggesting their potential utility as biomarkers. Once again, no curated association with glioblastomas was identified for these genes in the CTD.

Enrichment analysis of the up-regulated genes supported their involvement in the VEGFA signaling pathway, neuron projection, and cellular processes. In contrast, the down-regulated hub genes were enriched for processes related to immune system regulation, aligning with expectations.

Conclusions

This computational study identified nine up-regulated hub genes as potential targets for glioblastoma treatment and four down-regulated hub genes as candidate biomarkers for the disease. Leveraging the validated target VEGFA, six hits with favorable binding affinity, pharmacokinetic properties, and toxicological profiles were identified for further development as leads, along with two potential candidates for glioblastoma treatment, outperforming control drugs. These findings not only contribute to the development of improved drug candidates for glioblastoma treatment but also offer insights into the molecular underpinnings of the disease.

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