v7.n9.2026

SCIENTIFIC JOURNAL - RECIMA21 ISSN 2675-6218

IN SILICO STUDY ON PEDIATRIC HIGH-GRADE GLIOMAS

ESTUDO *IN SILICO* SOBRE GLIOMAS PEDIÁTRICOS DE ALTO GRAU

ESTUDIO IN SILICO SOBRE GLIOMAS PEDIÁTRICOS DE ALTO GRADO

Raquel Furlani Rocon Braga[1], Matheus Correia Casotti[2], Lorena Souza Castro Altoé[3], Camilly Victoria Campanharo[4], Maria Eliza Soares Queiroz[5], Iúri Drumond Louro[6] e Débora Dummer Meira[7]

e798918
https://doi.org/10.47820/recima21.v7i9.8918

PUBLISHED: 09/2026

ABSTRACT

Pediatric High-Grade Gliomas (pHGGs) encompass a group of emerging glial cell tumors characterized by their rare and aggressive nature with unpromising therapeutic prospects. Studies on pediatric glial tumors have revealed specific mutations related to histone variants, TP53 (Tumor Protein 53), and growth factors, differing from adult tumors that are widely mutated in IDH (Isocitrate Dehydrogenase gene). Within this framework, it is possible to suggest, given the unique biology of these tumors that sets them apart from High-Grade Gliomas in adults, the need for the development of targeted and specific therapies for pediatric patients. To contribute to the emerging studies on pHGGs, this research aimed to unveil in a more specific manner the protein interactions that characterize these tumors. Computational Biology tools were used for the construction and analysis of a Protein-Protein Interaction Network to elucidate tumor characteristics and analyze important interactions for the development of therapeutic targets. Through the in silico approach, it was possible to conclude the importance of studying central proteins such as VEGFA (Vascular Endothelial Growth Factor A), p53 (Cellular Tumor Antigen p53), EP300 (Histone Acetyltransferase p300), and CREBBP (Histone-lysine Acetyltransferase CREBBP) in the organization of the tumor and to assess, from a general perspective, that these proteins may act as potential therapeutic targets. Thus, the advancement of Computational Biology, Biotechnology, and Systems Biology in partnership with Pharmacogenomics was found to be essential, as these tools provide more refined analyses of tumor characteristics that are difficult to describe in vitro, guiding future studies.

KEYWORDS: *Pediatric High-Grade Glioma. Protein-Protein Interaction Network. Growth Factors. Therapeutic Targets. VEGFA.*

[1] Pharmacist graduated from the Federal University of Espírito Santo (UFES), affiliated with the Center for Human and Molecular Genetics, Vitória, ES, Brazil.
[2] Bachelor's and Teaching Degree in Biological Sciences from the Federal University of Espírito Santo (UFES), affiliated with the Center for Human and Molecular Genetics. Master's and doctoral student in Biotechnology (UFES/RENORBIO), Vitória, ES, Brazil.
[3] Teaching Degree in Biological Sciences and PhD in Veterinary Medicine from the Federal University of Espírito Santo (UFES), affiliated with the Center for Human and Molecular Genetics, Vitória, ES, Brazil.
[4] Pharmacist and Master's student in Biotechnology at the Federal University of Espírito Santo (UFES/RENORBIO), affiliated with the Center for Human and Molecular Genetics, Vitória, ES, Brazil.
[5] Biologist graduated from the Federal University of Espírito Santo (UFES), affiliated with the Center for Human and Molecular Genetics. Master's degree holder and doctoral student in Genetics and Breeding in the Graduate Program in Genetics and Breeding, Vitória, ES, Brazil.
[6] Physician graduated from the Federal University of Espírito Santo (UFES), PhD in Biochemistry and Molecular Genetics from the University of Alabama at Birmingham (UAB), professor at UFES, affiliated with the Center for Human and Molecular Genetics, Vitória, ES, Brazil.
[7] Pharmacist and PhD in Nuclear Biosciences from the State University of Rio de Janeiro (UERJ), professor at the Federal University of Espírito Santo (UFES), affiliated with the Center for Human and Molecular Genetics, Vitória, ES, Brazil.

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SCIENTIFIC JOURNAL - RECIMA21 ISSN 2675-6218

RESUMO
Gliomas Pediátricos de Alto Grau (pHGGs) abrigam um conjunto de tumores emergentes de células gliais caracterizados por sua natureza rara e agressiva com perspectivas terapêuticas não promissoras. Os estudos a respeito de tumores gliais infantis apresentam mutações específicas voltadas às variantes das histonas, *TP53* (Gene Antígeno tumoral celular p53) e aos fatores de crescimento, divergindo dos tumores adultos, amplamente mutados em *IDH* (Gene Isocitrato desidrogenase). Dentro dessa lógica, é possível sugerir, dada a biologia única desses tumores, a necessidade de elaboração de terapias direcionadas e específicas para pacientes pediátricos. Objetivou-se desvendar de forma mais específica as interações proteicas que caracterizam esses tumores. Foram utilizadas ferramentas da Biologia Computacional para elaboração e análise de uma Rede de Interação Proteína-Proteína, de forma a elucidar características do tumor em questão e analisar interações importantes para elaboração de alvos terapêuticos. Foi possível concluir, por meio da abordagem *in silico*, a importância do estudo de proteínas centrais como *VEGFA* (Fator de crescimento endotelial vascular A), p53 (Antígeno tumoral celular p53), EP300 (Histona acetiltransferase p300) e CREBBP (Histona lisina acetiltransferase CREBBP) na organização do tumor e avaliar, dentro de uma perspectiva geral, que essas proteínas possam atuar como possíveis alvos terapêuticos. Dessa maneira, verificou-se como essencial o avanço da Biologia Computacional, da Biotecnologia e da Biologia de Sistemas em parceria com a Farmacogenômica, uma vez que essas ferramentas proporcionam análises mais apuradas de características tumorais dificilmente descritas *in vitro*, direcionando os estudos futuros.

PALAVRAS-CHAVE: Glioma Pediátrico de Alto Grau. Rede de Interação Proteína-Proteína. Fatores de Crescimento. Alvos Terapêuticos. *VEGFA*.

RESUMEN
Los Gliomas Pediátricos de Alto Grado (pHGGs) albergan un conjunto de tumores emergentes de células gliales caracterizados por su naturaleza rara y agresiva con perspectivas terapéuticas poco prometedoras. Los estudios sobre tumores gliales infantiles presentan mutaciones específicas relacionadas con variantes de histonas, TP53 (Proteína Tumoral 53) y factores de crecimiento, divergiendo de los tumores adultos, ampliamente mutados en IDH (Isocitrato Deshidrogenasa). Dentro de esta lógica, es posible sugerir, dada la biología única de estos tumores, la necesidad de elaborar terapias dirigidas y específicas para pacientes pediátricos. Se objetivó develar de manera más específica las interacciones proteicas que caracterizan estos tumores. Se utilizaron herramientas de Biología Computacional para elaborar y analizar una Red de Interacción Proteína-Proteína, con el fin de elucidar características del tumor y analizar interacciones importantes para la elaboración de dianas terapéuticas. Fue posible concluir, mediante el enfoque in silico, la importancia del estudio de proteínas centrales como VEGFA (Factor de Crecimiento Endotelial Vascular A), p53 (Antígeno Tumoral Celular p53), EP300 (Histona Acetiltransferasa p300) y CREBBP (Histona Lisina Acetiltransferasa CREBBP) en la organización del tumor, y evaluar que estas proteínas pueden actuar como posibles dianas terapéuticas. De esta manera, se verificó como esencial el avance de la Biología Computacional, la Biotecnología y la Biología de Sistemas en asociación con la Farmacogenómica, ya que estas herramientas proporcionan análisis más precisos de características tumorales difícilmente descritas in vitro, orientando los estudios futuros.

PALABRAS CLAVE: *Glioma Pediátrico de Alto Grado. Red de Interacción Proteína-Proteína. Factores de Crecimiento. Dianas Terapéuticas. VEGFA.*

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INTRODUCTION

Childhood cancer refers to a group of diseases characterized by the uncontrolled proliferation of cells with mutated genetic material, similarly to adults. However, childhood cancer presents predominantly embryonic mutations (INCA, 2021). According to Instituto A.C. Camargo (2018), childhood cancer is formed by the disordered multiplication of a cell that did not develop correctly. From this statement, it is possible to infer that pediatric cancer has a molecular profile with significant differences compared to adults, even with histological similarities (Abedalthagafi *et al.*, 2021).

Epidemiological data indicate that, among childhood tumors, Central Nervous System (CNS) tumors show the lowest survival rate from a general tumor-analysis perspective (44.1%) (Leong *et al.*, 2020). Among malignancies, brain tumors exhibit high lethality (Borgenvik; Swartling, 2021), with an incidence of effects over the years exceeding 50% of treated cases (Butler *et al.*, 2021).

Pediatric high-grade gliomas (pHGGs) are considered incurable and intriguing, accounting for 17% of pediatric CNS tumors, with a 5-year survival rate of only 20% (Borgenvik; Swartling, 2021), displaying high aggressiveness, infiltrative capacity, and poor prognosis (Fuchs *et al.*, 2020; Haase *et al.*, 2020). pHGGs comprise a variety of tumor subsets, differing in prognosis, age, and location (Pollack; Agnihotri, 2019), with a median survival prognosis of 9 to 15 months (Haase *et al.*, 2020).

The genetic landscape shaped by the tumor microenvironment appears to be associated with hypoxic and angiogenic conditions (Fuchs *et al.*, 2020). Moreover, the secretion of trophic factors plays an important role in promoting gliomagenesis (Huchedé; Leblond; Castets, 2022). The involvement of factors encoding growth factors — such as vascular endothelial growth factor (VEGF), platelet-derived growth factor receptor A (PDGFRA), the TGFβ superfamily of growth factor receptors, and others — proves essential in the development and aggressiveness of these tumors (Papa *et al.*, 2017; Papale *et al.*, 2018).

Regarding treatment, these tumors have maximal surgical resection as the standard approach, along with chemotherapy and radiotherapy; however, none of these therapies has shown promising responses to date (Cacciotti; Fleming; Ramaswamy, 2020). Among the harms related to these treatments are mental disabilities, organ toxicities, and secondary or recurrent cancers (Gröbner *et al.*, 2018).

Systems Biology plays a prominent role, as it seeks to investigate complex diseases from different perspectives (Casotti *et al.*, 2023a; 2023b). The tools developed by Computational

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Biology offer new strategies for detection, control, and treatment, being essential in the study of complex tumors such as pHGGs. The growing advancement in the field of protein-protein interaction networks (PPINs), central to several biological processes and to the pathogenesis of various diseases, including cancer, complements these perspectives (Cheng *et al.*, 2020; Wahab Khattak *et al.*, 2021).

Therefore, this study intends to add to and contribute to future advances in the characterization and treatment of childhood neoplasms (especially pHGGs) through the analysis of specific protein interactions using Bioinformatics and Computational Biology tools, in order to verify and elucidate new therapeutic targets and points of tumor resistance. The general objective was to elucidate the relationship between the proteins characteristic of pHGGs and Growth Factors through *in silico* analyses, in order to understand the mechanisms of this neoplasm, aiming to determine new targets and potential therapeutic approaches.

1. THEORETICAL FRAMEWORK

1.1. New classification and comparison with adult gliomas

Although pHGGs share histological similarities with adult gliomas, the genomic landscape involving these two tumors differs considerably. Pediatric gliomas mainly present mutations related to platelet-derived growth factor/platelet-derived growth factor receptor (PDGF/PDGFR) and mutations in the histone H3.3 gene. In contrast, the same tumors in adults predominantly present mutations in PTEN and Epidermal Growth Factor Receptor (EGFR).

After a long period in which the classification of pediatric CNS tumors was carried out similarly to that of adults (Varlet *et al.*, 2022), a new configuration was published by the World Health Organization (WHO) in 2021, broadening the need to elucidate the molecular profile of tumors beyond their histological similarities (Louis *et al.*, 2021), such that pHGGs were subdivided into: (1) Diffuse midline glioma, H3 K27-altered; (2) Diffuse hemispheric glioma, H3 G34-mutant; (3) Diffuse pediatric-type high-grade glioma, H3-wildtype and IDH-wildtype; (4) Infant-type hemispheric glioma (Chart 1).

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Chart 1. Comparison between recurrent mutations in adult gliomas and pHGGs.

PEDIATRIC HIGH-GRADE GLIOMAS	
Classification (WHO)	**Recurrent Mutations**
Diffuse midline glioma, H3 K27-altered	H3 K27, TP53, ACVR1, PDGFRA, EGFR, EZHIP
Diffuse hemispheric glioma, H3 G34-mutant	H3 G34, TP53, ATRX
Diffuse pediatric-type high-grade glioma, H3-wildtype and IDH-wildtype	IDH-wildtype, H3-wildtype, PDGFRA, MYCN, EGFR
Infant-type hemispheric glioma	NTRK family, ALK, ROS, MET
ADULT GLIOMAS	
Classification (WHO)	**Recurrent Mutations**
Astrocytoma, IDH-mutant	IDH1, IDH2, ATRX, TP53, CDKN2A/B
Oligodendroglioma, IDH-mutant and 1p/19q-codeleted	IDH1, IDH2, 1p/19q, TERT promoter, CIC, FUBP1, NOTCH1
Glioblastoma, IDH-wildtype	IDH-wildtype, TERT promoter, chromosomes 7/10, EGFR

Source: Adapted from Louis *et al.* (2021).

1.2. Genetic landscape

According to Haase *et al.* (2020), the discovery of recurrent mutations in genes encoding the histone variants H3.3 (H3F3A) and H3.1 (HIST1H3B/C) demonstrated the unique biology of these tumors. This group of mutations revealed recurrent mutations in the amino-terminal tail of histone H3 genes, where, at position 27 of histones 3.1 and 3.3, a lysine is replaced by a methionine (H3K27M), and at position 34 of histone 3.3, a glycine is replaced by an arginine or a valine (H3.3G34R/V).

The H3K27M mutation is characteristic of midline tumors, especially pontine tumors, whereas the H3.3G34R/V mutations are found in at least 16% of tumors located in supratentorial positions of the cerebral hemispheres. Many of these mutations are related to cell-cycle regulation and growth-factor signaling, pointing to a possible therapeutic proposal (Graham; Mellinghoff, 2020). Because of their seemingly predictable nature, histones were not regarded as likely mutation targets; however, this finding, characteristic of pediatric gliomas, with an incidence below

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1% in adult cancers (Kasper; Baker, 2020), prompted the development of research to elucidate their molecular mechanisms.

1.3. Tumor microenvironment

Much of the limitations associated with the treatment of pHGGs involve characteristics intrinsic to the tumor, especially the tumor microenvironment, which confers on this neoplasm a challenging complexity regarding the efficacy of pre-existing treatments, being a determining factor in tumor resistance and progression (Antonucci *et al.*, 2022). The microenvironment may also present heterogeneity, with variations among each glioma subtype, determined by the genetic signature that promotes altered biochemical interactions (Herting *et al.*, 2017).

The tumor microenvironment is composed of cancer cells, glioma stem cells, parenchymal cells, infiltrating immune cells, and a vascular network. Typically, pHGGs are less immunologically active, expressing fewer pro-inflammatory markers and infiltrating immune cells — essentially regarding T lymphocytes — showing minimized responses to immunotherapies (Yao *et al.*, 2023). Studies point to the immunosuppressive trait of the disease and the low T-cell infiltration, with inflammatory capacity corresponding to the presence of macrophages, including microglia and monocytes (Ross *et al.*, 2021).

1.4. Difficulties and resistance to treatment in pHGGs

It is known that there is great difficulty in obtaining promising responses for the treatment of pHGGs, given their characteristic signature and heterogeneity (Cacciotti; Fleming; Ramaswamy, 2020). According to a search on *clinical trials*, current studies have mostly involved biological vaccines, also addressing therapies with selective inhibitors of the serine/threonine kinase mTOR, alkylating agents, and tyrosine kinase inhibitors (TKIs), developed mostly in the North American region.

The alkylating agent Temozolomide (TMZ) was evaluated based on its results in adult gliomas; however, the low response rate confirmed the need for a therapy specific to childhood tumors (Ostrom *et al.*, 2015). Studies involving the IgG1 antibody Nimotuzumab, targeting EGFR, showed promising results, with a survival of 32.66 months and a 2-year survival rate of 54.2% (Cabanas *et al.*, 2013). However, subsequent studies presented heterogeneous profiles regarding EGFR expression.

Another intriguing challenge in the study of pHGGs is permeability across the blood-brain barrier (BBB). As described by Sharma *et al.* (2016), the BBB is responsible for limiting the passage of external immune cells, especially lymphocytes, conferring selectivity and preventing

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the transport of many drugs to the brain, thus requiring a multidisciplinary approach for better drug delivery to the CNS.

2. METHODOLOGY

The development of this research was carried out through *in silico* analysis following the standard protocol/procedure developed by the Computational and Systems Biology Group (SCBG) of the Center for Human and Molecular Genetics (NGHM) (Casotti *et al.*, 2022). Protein interactions were analyzed in order to elucidate certain specific characteristics of the disease; through the PubMed platform, a literature search focused on pHGGs (and their cell lines), mechanisms, and mutations, and their relationships with growth factors, was performed. The method was executed in three stages:

2.1. Manual curation

Performed through searches on PubMed with the search key "*growth factors* AND *high-grade pediatric gliomas*", in an organized manner, using experimental articles linked to the study of pediatric CNS tumors. Up to March 18, 2023, 127 articles were found. From the articles found, 81 proteins from 44 articles were ultimately selected, according to inclusion criteria (focus on pediatric CNS tumors; relationship with growth factors and the brain microenvironment; articles focused on the biological data of the tumor) and exclusion criteria (general articles focused only on histology; articles entirely focused on the analysis of adult gliomas).

From the 81 proteins obtained by curation, only those present in at least 2 experimental articles were selected, totaling 19 proteins. From a search on the STRING platform to select human isoforms, a total of 44 proteins were obtained for the construction of the seed Protein-Protein Interaction Network.

2.2. Construction of the protein-protein interaction networks

After the literature search and protein selection, the data were entered into the STRING platform (https://string-db.org/) using the STRING 11.5 database. To expand the network, the number of interactors was set to no more than 50. From the resulting network, the created network was accessed and formatted through Cytoscape (version 3.8.2).

Network analysis was performed using the Network Analyzer tool in Cytoscape (version 3.8.2), which calculated the basic topological parameters of the PPINs. Among the parameters analyzed as important for indicating the network as real were the Clustering Coefficient, the

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Characteristic Path Length, the Network Diameter, and the Network Heterogeneity. From this point, MCODE (Molecular Complex Detection), available in Cytoscape, was used for network modularization, applying a score to each module and performing a new analysis with the Network Analyzer to evaluate the modules and clusters found (Casotti *et al.*, 2022).

2.3. Target determination

Targets were determined through the analysis of clusters and bottleneck networks, together with knowledge acquired from the literature. cytoHubba (version 0.1), available in Cytoscape, was used for detailed topological analysis, exploring the important nodes based on degree and betweenness (bottleneck) centrality, with the selection of the 10, 5, and 3 best-scoring proteins.

From this analysis, five tyrosine kinase inhibitors (TKIs) targeting VEGFA were selected and analyzed on the PreADMET platform (https://preadmet.qsarhub.com/adme/) regarding their ease of crossing the BBB and their binding to plasma proteins. The BBB permeability parameters were interpreted as follows: values greater than 2 = drugs that cross freely; values between 0.1 and 2.0 = drugs that cross partially; values below 0.1 = drugs that cross in a reduced manner or do not cross (Sharma *et al.*, 2016). The drugs showing BBB passage were analyzed for toxicity using ProTox-II (https://tox-new.charite.de/protox_II/).

3. RESULTS AND DISCUSSION

3.1. Manual curation

Through the defined search key, 127 articles were found in total (111 experimental and 16 reviews). The most frequent proteins, cited in at least 2 experimental articles, were used to construct the interaction network, highlighting: PDGFR (F=16), EGFR (F=13), TP53 (F=7), PTEN (F=7), Histone H3.3 (F=4), Histone H3.1 (F=3), ACVR1 (F=3), CDK2 (F=3), and VEGFA (F=2), as detailed in Chart 2.

The frequency of proteins found leads to a conclusion evidenced by studies on the particularities of pHGGs relative to adult tumors. In a comparison with the mutation profile described in the literature, similarities were observed in the frequency of proteins such as p53, histone variants, and EGFR (Louis *et al.*, 2021).

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Chart 2. Frequency of proteins selected by curation. “F” = number of experimental articles.

F	UniProt ID	Protein	Gene
16	P09619, P16234	Platelet-derived growth factor receptor	PDGFRB, PDGFR, PDGFR1, PDGFRA, PDGFR2, RHEPDGFRA
13	P00533	Epidermal growth factor receptor	EGFR, ERBB, ERBB1, HER1
7	P04637	Cellular tumor antigen p53	TP53, P53
7	P60484	Phosphatidylinositol 3,4,5-trisphosphate 3-phosphatase and dual-specificity protein phosphatase PTEN	PTEN, MMAC1, TEP1
4	P84243	Histone H3.3	H3-3A; H3-3B
3	P68431	Histone H3.1	H3C1; H3C2; H3C3; H3C4; H3C6; H3C7; H3C8; H3C10; H3C11; H3C12
3	Q04771	Activin receptor type-1	ACVR1
3	P24941	Cyclin-dependent kinase 2	CDK2, CDKN2
2	P09038	Fibroblast growth factor 2	FGF2, FGFB
2	P12643, P12644, P12645, Q7Z5Y6, P22003, O95393	Bone morphogenetic protein	BMP2, BMP3, BMP4, BMP5, BMP8A, BMP10
2	Q15797, P84022, Q99717	Mothers against decapentaplegic homolog	SMAD1, SMAD3, SMAD5

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F	UniProt ID	Protein	Gene
2	P42336	Phosphatidylinositol 4,5-bisphosphate 3-kinase catalytic subunit alpha	PIK3CA
2	P09874, Q9UGN5	Poly (ADP-ribose) polymerase (PARP)	PARP1, PARP2
2	P36222	Chitinase-3-like protein 1	CHI3L1
2	P01106	Myc proto-oncogene protein	MYC
2	P15692	Vascular endothelial growth factor	VEGFA
2	P08069	Insulin-like growth factor 1 receptor	IGF1R
2	P04626	Receptor tyrosine-protein kinase erbB-2	ERBB2
2	P01137, P61812	Transforming growth factor	TGFB1, TGFB2

Source: Prepared by the authors, 2026.

3.2. Protein-protein interaction network

From the proteins and their respective isoforms selected through STRING, the Protein-Protein Interaction Network was constructed, totaling a network with 139 nodes and 2,163 edges/interactions. The topological parameters obtained are summarized in Table 1, showing a Clustering Coefficient = 0.601; Characteristic Path Length = 2.441; Network Diameter = 5; and Network Heterogeneity = 0.604. From these parameters, the resulting network can be analyzed as real (Casotti *et al.*, 2022), and its structure of biological connections can be visually observed in Figure 1.

Table 1. Topological parameters of the network obtained by Network Analyzer (Cytoscape v3.8.2).

Parameters	Result
Clustering Coefficient	0.601
Characteristic Path Length	2.441
Network Diameter	5
Network Heterogeneity	0.604

Source: Prepared by the authors, 2026.

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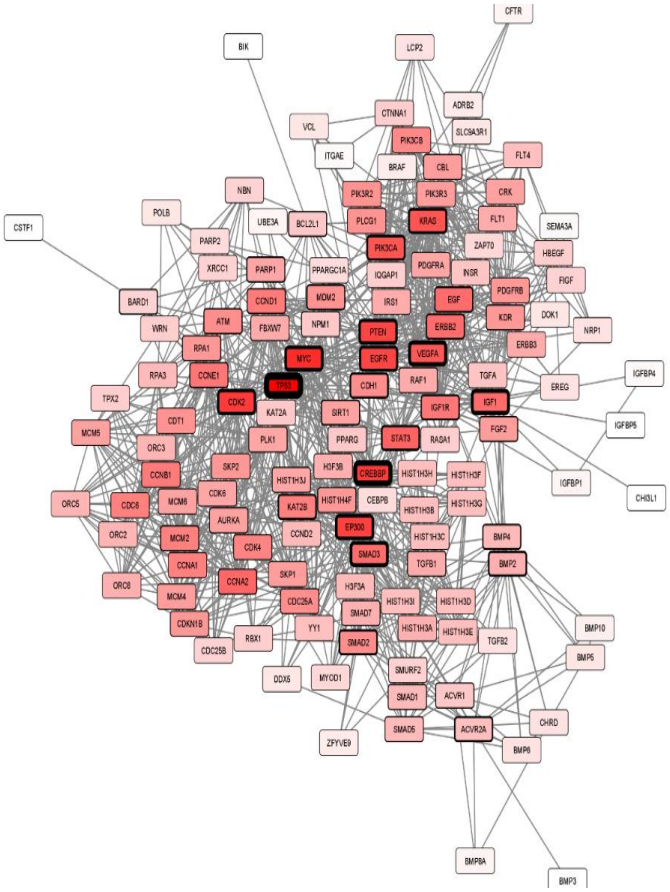
Figure 1. Main interaction network obtained by STRING, in the configuration of up to 50 interactors.



Source: Prepared by the authors, 2026.

The degree centrality of the proteins, organized in descending order by number of interactions, highlights p53 (degree 101), the Myc proto-oncogene protein/MYC (degree 79), EGFR (degree 75), and PTEN (degree 74), all with 74 or more interactions, indicating their important centrality. Network enrichment mainly involved Gene Ontology (GO) pathways, with the following standing out: Cellular response to organic substance (FDR: 8.15E-48), Cellular response to chemical stimulus (FDR: 9.87E-45), Enzyme-linked receptor protein signaling pathway (FDR: 2.29E-43), and Cell surface receptor signaling pathway (FDR: 2E-42).

Studies indicate that the downstream pathway of MYC is closely involved in tumor suppression, playing a fundamental role in VEGFA-induced angiogenesis (Zhang *et al.*, 2022). The TP53 gene, in turn, undergoes the most recurrent mutation in cancers in general

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(Kastenhuber; Lowe, 2017), having a critical function as a tumor suppressor through its response to DNA damage (Haase *et al.*, 2021). Regarding mutations in PTEN and EGFR, these are among the most frequently found mutations in pHGGs (Abedalthagafi *et al.*, 2021).

3.3. Cluster analysis

Using the ClusterMaker application, 4 clusters were obtained from the central network. Cluster 1 presented the interrelationship among histone variants, with centrality for the CREBBP protein (Histone-lysine acetyltransferase CREBBP), containing 32 nodes and 2,144 edges, and a Clustering Coefficient of 0.854. The main enrichment was related to the Cell Cycle (hsa04110, FDR: 1.42E-26), Protein-DNA complex assembly (GO: 0065004, FDR: 4.55E-25), and Mitotic G1 phase and G1/S transition (FDR: 2.28E-24). The dynamic regulation of chromatin configurations is essential for controlled gene expression (Valencia; Kadoch, 2019), and the functional diversity of histones depends on a concerted functioning (Audia; Campbell, 2016).

Cluster 2 demonstrated relevant protein relationships in cell signaling (24 nodes, 74 edges), with important centrality for EGF, PTEN, and KRAS. Enrichment presented the Enzyme-linked receptor signaling pathway (GO:0007167, FDR: 3.3E-19), Protein phosphorylation (GO:0006468, FDR: 2.94E-17), and Cellular response to growth factor stimulus (GO:0071363, FDR: 9.18E-17). PTEN is a tumor suppressor that negatively regulates the AKT pathway, and its mutation is associated with reduced survival in pediatric gliomas (Thorarinsdottir *et al.*, 2008).

Cluster 3 indicated an important relationship with growth factors and signaling pathways relevant to cancer (23 nodes, 97 edges), with emphasis on EGFR, PIK3CA, and VEGFA. Enrichment evidenced MicroRNAs in cancer (hsa05206, FDR: 2.86E-21), EGFR tyrosine kinase inhibitor resistance (hsa01521, FDR: 1.95E-18), and Glioblastoma signaling pathways (WP2261, FDR: 1.31E-17).

Cluster 4 presented important interactions among EP300 (Histone acetyltransferase p300), MYC, and p53 (15 nodes, 29 edges). Enrichment showed Pathways in cancer (hsa05200, FDR: 4.64E-7), Deubiquitination (HSA-5688426, FDR: 1.71E-6), and Generic transcription pathway (HSA-212436, FDR: 1.7E-6).

3.4. Target determination and drug analysis

Once the clustered interactions were verified, using cytoHubba it was possible to observe the top 10, 5, and 3 proteins (Top 10, Top 5, and Top 3), based on degree and betweenness (bottleneck) centrality criteria, in the study of pHGGs (Figure 2). The central proteins obtained

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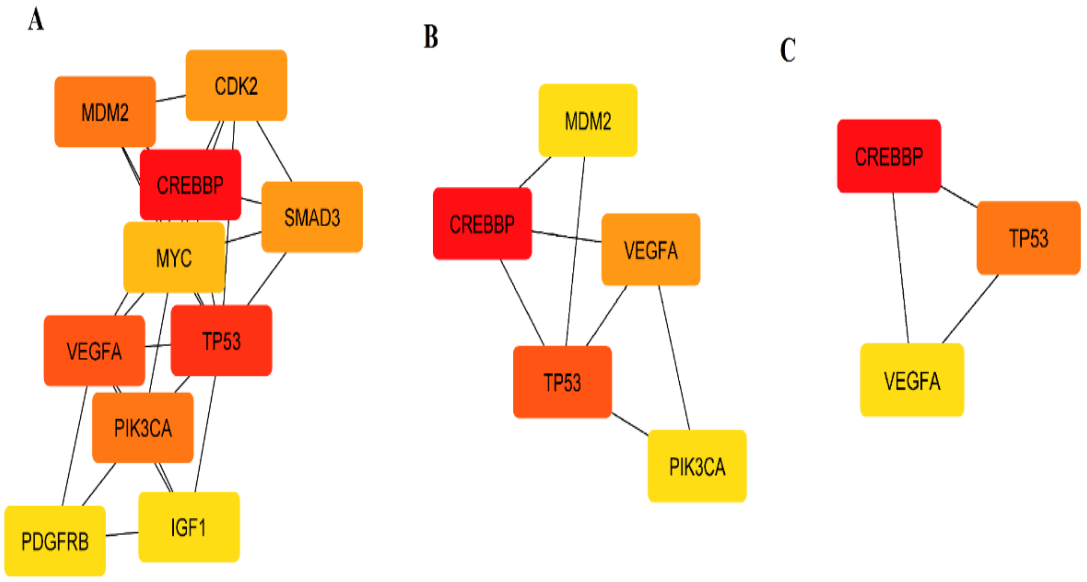
were CREBBP, p53, and VEGFA. Based on the results of Cluster 1, the EP300 protein was also selected, since it is directly related to the histone variants, being involved in the acetylation of histone and non-histone proteins (Luo *et al.*, 2023).

Figure 2. Networks clustered by cytoHubba based on degree and betweenness (bottleneck) centrality criteria. A: Top 10 bottleneck proteins; B: Top 5 bottleneck proteins; C: Top 3 bottleneck proteins.



Source: Prepared by the authors, 2026.

In general, both EP300 and CREBBP may act as tumor suppressors, regulating important physiological functions such as apoptosis, differentiation, and DNA repair (Zhu *et al.*, 2023), and are significantly related to the activation of p53 via acetylation. Since three of the four selected proteins (CREBBP, p53, and EP300) have suppressor functions in tumor development, they constitute more challenging therapeutic targets, as drugging them would require restoring their function — an approach still under investigation through compounds such as COTI-2, PRIMA-1, APR-246, and PK11007 for p53.

For this reason, VEGFA was selected as the potential therapeutic target for the treatment of pHGGs. Given its central role in the bottleneck network interactions, it can be inferred that acting specifically on this factor would likely produce changes in the expression of adjacent

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proteins, in a “cascade” or “domino effect”, thereby theoretically reaching the histone variants and p53 indirectly. VEGFA is characterized by mechanisms of expansion of the tumor vascular bed and is detected at least 50 times more in tumor cells than in healthy brain tissue (Sobol-Milejska *et al.*, 2017).

Five TKIs targeting VEGFA were selected: Orantinib, Sunitinib, Apatinib, Sorafenib, and BDBM102337. In the BBB permeability analysis via PreADMET, Sorafenib (BBB: 1.00027; PPB: 89.87%), Sunitinib (BBB: 0.909; PPB: 64.22%), and Orantinib (BBB: 0.445; PPB: 83.46%) showed partial passage. Apatinib (BBB: 0.051) and BDBM102337 (BBB: 0.014) showed low permeability (Table 2).

Table 2. Blood-brain barrier penetration (BBB) and plasma protein binding (PPB) results obtained by PreADMET.

Drug	BBB	PPB
Sorafenib	1.00027	89.865177
BDBM102337	0.0136264	38.92474
Sunitinib	0.908823	64.221341
Apatinib	0.0507448	92.685207
Orantinib	0.445026	83.458926

Source: Prepared by the authors, 2026.

Two of the permeability results are directly related to lipophilicity. Sorafenib showed the highest BBB value, which can be explained, in general terms, by its LogP of 4.32 (obtained from the ChemAxon platform for comparison purposes); conversely, BDBM102337 has a LogP of -0.16, accounting for its low lipophilicity and, consequently, its low permeability, reflected in the lowest BBB value among the analyzed drugs. Another observed parameter is plasma protein binding (PPB), whose intensity determines the level of drug diffusion into tissue and also affects drug elimination (Bastos *et al.*, 2020).

The three drugs selected by membrane permeability showed PPB values above 60%, indicating a strong interaction with plasma proteins; nonetheless, it is worth assessing whether the high binding between sorafenib and plasma proteins is related to the degree of toxicity predicted by ProTox-II.

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In the toxicity analysis by ProTox-II, Sorafenib showed 67.38% prediction accuracy and toxicity class 4, with toxicity for TP53, mitochondrial membrane potential, hepatotoxicity, cytotoxicity, and immunotoxicity. Sunitinib showed 54.26% accuracy and toxicity class 4, with immunotoxicity and cytotoxicity. Orantinib showed 54.56% accuracy and toxicity class 4, showing no specific toxicity for any of the ProTox-II classifications.

Sorafenib, an orally active inhibitor targeting BRAF, c-Kit, RET, and VEGFR, showed partial BBB permeability but exhibited several points of toxicity, including for the tumor suppressor p53, silencing it and promoting carcinogenesis (Ghosh *et al.*, 2019). Sunitinib showed partial permeability and some points of toxicity; completed phase II studies demonstrated good tolerability but insufficient efficacy (Wetmore *et al.*, 2016). Orantinib, a small molecule that competitively inhibits the tyrosine kinase of the VEGF, bFGF, and PDGF receptors (Chen *et al.*, 2017), showed the value furthest from favorable regarding membrane permeability but without specific toxicity points; phase II studies indicated inefficacy for recurrent gastric cancer, suggesting that the drug is probably not suitable for future analyses in pHGGs.

4. FINAL CONSIDERATIONS

The contribution of *in silico* analyses to the development of targeted therapies is indisputable, since understanding the particularities of complex diseases is essential for the design of effective pharmacotherapy. Within the study of pHGGs, this methodology was observed to contribute to the analysis of protein interactions and, consequently, to the determination of possible therapeutic targets.

Through the analyses and contextualization with the available literature, the need for a greater understanding of the particularities of pHGGs becomes evident, assessing their inter- and intratumoral heterogeneity. The results pointed to the presence of interactions among proteins that justify certain characteristics and involvements in recurrent cancer pathways, such as EP300, p53, VEGFA, and CREBBP, highlighting the recurrent mutation in histone variants, present in most of these tumors.

The development of studies that more closely investigate the functions of VEGFA in pediatric gliomas, in order to advance *in silico* and *in vitro* studies to verify efficacy, is extremely necessary. The present study demonstrated uncertain efficacy patterns, since the selected drugs showed either only partial BBB permeability or signs of relevant toxicity. Furthermore, there is also a need to deepen studies on the non-seed proteins, retrieved by the network through the required interactors, since they may present targets of the disease not yet verified. In this way, it

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is believed that it will be possible to add knowledge to existing studies in order to contribute to future therapeutic development and to the treatment of Pediatric High-Grade Gliomas.

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