https://doi.org/10.48047/AFJBS.6.14.2024.1992-2012

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Next Generation Sequencing and Proteomics Analysis of BRD2-BD1 Complexed with MDP5 in Medulloblastoma

Uma Kumari[1] Abishek Ebenezar[1] Motukuri Naveen Kumar[2] Usha Nadikudu[2]

[1]Senior Bioinformatics Scientist, Bioinformatics Project and Research Institute, Noida - 201301, India

[1]Project Trainee at Bioinformatics Project and Research Institute, Noida - 201301, India

[2]Project Trainee at Bioinformatics Project and Research Institute, Noida - 201301, India

[2]Project Trainee at Bioinformatics Project and Research Institute, Noida - 201301, India

Corresponding Author Uma kumari (uma27910@gmail.com)

Article History

Volume 6, Issue 14, Aug 2024

Received: 15 July 2024

Accepted: 7 August 2024

Published: 8 August 2024

doi: 10.48047/AFJBS.6.14.2024.1992-2012

Abstract

Current therapeutic strategies for Medulloblastoma (MB), include surgery, radiation, and chemotherapy, often lead to severe long-term side effects, underscoring the need for more effective and less toxic treatments. Medulloblastoma (MB) is characterized by its origin in the cerebellum or posterior fossa and its heterogeneity, comprising distinct with unique genetic and clinical profiles. Recent research efforts have focused on identifying and targeting the specific molecular pathways involved in these subgroups, aiming to develop tailored therapeutic approaches. The promise of targeted therapies and immunotherapies holds potential for improved patient outcomes, but practical application remains in its early stages. In our study, we performed an extensive analysis of BRD2-BD1 in complex with MDP5 using next-generation sequencing and proteomics. The role of bromodomain-containing proteins, particularly in gene expression regulation through chromatin remodeling, was investigated using advanced molecular visualization tools like Rasmol and PyMOL. Hydrogen bond analysis and the identification of active sites for drug binding were key components of this research, providing insights into the potential for novel drug targets. Furthermore, the use of bioinformatics tools, including Biopython for sequence analysis and principal component analysis (PCA) for structural variability assessment, facilitated a comprehensive evaluation of protein interactions. Validation of protein structures through ERRAT and Ramachandran plots, coupled with functional network analysis using STRING and gene localization via GeneCards, provided a robust framework for understanding the molecular underpinnings of medulloblastoma. Our findings highlight the importance of integrating molecular biology, bioinformatics, and structural biology to advance the development of targeted therapies for medulloblastoma. By focusing on the specific molecular profiles of different subgroups, we aim to improve survival rates , moving closer to the goal of effective and personalized cancer treatment.

Keywords: Medulloblastoma, NGS, Genecard, Molecular Docking, Proteomics, Structure Analysis.

1. Introduction

The most frequent malignant brain tumour in children is medulloblastoma (MB), which was first discovered in 1925 (Ramaswamy and Taylor, 2017; Gajjar and Robinson, 2014; Gibson et al., 2010; Grill et al., 2005). MB is an extremely aggressive embryonal tumour of the central nervous system (CNS) that originates in the cerebellum (Rooper and Brown, 2005). It is brought on by a disruption of normal cerebellar development (Marino, 2005). It accounts for over 40% of tumours in the posterior fossa (PF) and 15% of all paediatric CNS tumours (Park et al., 2016; Withrow et al., 1998). According to Tulla et al. (2015), there are roughly 0.41 instances of MB for every 100,000 patient-years among those 0 to 19 years of age. With a male-to-female ratio of about 1.7:1, it is more frequently diagnosed in men (Sun etal.,2015) At least four subgroups of MB have been identified by molecular studies, which have demonstrated the clinical and biological variability of the disorder (Taylor et al., 2012; Northcott et al., 2011; Cho et al., 2011). These subgroups are wingless (WNT), sonic hedgehog (SHH), group 3, and group 4. 2016 saw a change to the MB classification that included both histologic and molecular criteria. WNT-activated, SHH-activated TP53 wildtype, SHH-activated TP53-mutant, and non-WNT/non-SHH are the currently recognised genetically characterised subtypes of MB (Cotter and Hawkins, 2021). These four categories exhibit distinct clinical, histological, genetic, transcriptional, and prognostic traits that were discovered through integrated genomics research (Northcott et al., 2012; Northcott et al., 2011; Pugh et al., 2012; Ramaswamy et al., 2013) These four subgroups, identified through integrated genomics studies, display well-defined clinical, histopathological, genetic, transcriptional, and prognostic features (Northcott et al., 2012; Northcott et al., 2011; Pugh et al., 2012; Ramaswamy et al., 2013).

Each subgroup has distinct prognoses and therapeutic responses, underscoring the need for personalized treatment (Kool et al., 2012). The standard treatment for MB includes maximal safe resection, chemotherapy, and craniospinal irradiation (CSI). According to Choi et al. (2023), combining multiple treatment strategies and precise risk assessment has improved long-term survival rates to 70%–80% for all patients. However, despite intensive multimodal therapy, about 30% of patients do not survive, and those who do often endure significant long-term side effects that diminish their quality of life.

1.1 Molecular Subtypes of Medulloblastoma

With a 5-year overall survival (OS) rate of over 95%, the WNT subgroup—which accounts for just 10% of all medulloblastomas—has the best prognosis. (Ramaswamy et al., 2016; Kool et al., 2012). This subgroup is characterised by chromosome 6 monosomy, which is present in 80–90% of cases, and mutations in the CTNNB1 gene that accumulate nuclear beta-catenin (Ellison et al., 2011; Goschzik et al., 2015). WNT medulloblastomas frequently have classic histology upon diagnosis and are typically non-metastatic

(Hovestadt et al., 2020). Patients with WNT medulloblastoma still have very good prognoses, with 90% or more of them still alive after five years (Mani S et al., 2024).

SHH-activated medulloblastoma represents about 30% of all cases, predominantly impacting infants, children, adolescents, and adults (Northcott et al., 2012; Kool et al., 2012). These tumors originate primarily from the cerebellar hemispheres, with cerebellar granule neuron precursors identified as their likely cell of origin (Gibson et al., 2010). The defining feature of SHH medulloblastomas is the activation of the Sonic Hedgehog (SHH) signaling pathway, often due to mutations in PTCH1, SMO, and SUFU genes (Lazow et al., 2022). Common genetic alterations include TP53 mutations, particularly in pediatric cases, and MYCN amplifications. Germline mutations in PTCH1 and SUFU can predispose individuals to Gorlin syndrome, associated with SHH medulloblastomas (Lazow et al., 2022).

Group 3 medulloblastoma represents around 25% of cases, mainly affecting infants and young children, with a higher incidence in males (Kool et al., 2012). This subgroup is characterized by a distinct transcriptional profile and genomic instability, showing frequent chromosomal alterations including gains in 1q, 7, and 17q, and deletions in 10q, 11, 16q, and 17p (Shih et al., 2014; Northcott et al., 2012; Cho et al., 2011). Compared to other subgroups, Group 3 tumors often present with metastatic disease at diagnosis (40–45%), large cell/anaplastic histology (40%), and/or MYC amplification, contributing to poorer outcomes (Northcott et al., 2011; Kool et al., 2012; Cho et al., 2011). The prognosis is poor, with a 5-year survival rate of only 50% (Kool et al., 2012).

Group 4 medulloblastoma, the most prevalent subtype accounting for about 35% of cases, primarily affects children aged 5–10 years, with rare occurrences in infants (Cavalli et al., 2017; Juraschka and Taylor, 2019). Cytogenetically, these tumors often show abnormalities involving chromosome 17q; however, unlike Group 3, these anomalies do not independently predict survival outcomes (Northcott et al., 2012). Common genetic alterations in Group 4 include MYCN amplification, mutations in KDM family members, and amplifications of OTX2, SNCAIP, and CDK6, contributing to its distinct molecular profile (Northcott et al., 2011; Shih et al., 2014; Pugh et al., 2012; Robinson et al., 2012). Treatment outcomes generally show an intermediate prognosis, with a 5-year survival rate of 80% under standard therapies. However, approximately 30–40% of patients present with metastases at diagnosis, requiring high-risk treatment strategies (Ramaswamy et al., 2016; Menyhárt et al., 2019).

1.2 Advancements in Treatment Strategies for Medulloblastoma

Treatment for medulloblastoma is extensive and typically involves significant surgical removal of the tumor, followed by craniospinal irradiation and additional chemotherapy (Moxon-Emre et al., 2014; Moxon-Emre et al., 2016; Fossati et al., 2009). Advances in neurosurgical techniques have improved the

effectiveness of tumor removal while reducing surgical complications (Sharma et al., 2009). Radiation therapy, particularly craniospinal irradiation, is essential in treatment but may result in significant long-term side effects such as cognitive decline and hormonal imbalances (Sharma et al., 2009).

Chemotherapy has significantly transformed the treatment approach for medulloblastoma. Previously, standard therapies involving surgery and craniospinal radiotherapy yielded a 10-year survival rate of 45% (Hughes et al., 1988). The incorporation of chemotherapy, administered either before or after radiotherapy, has markedly enhanced treatment outcomes. Risk-adapted craniospinal radiotherapy followed by high-dose chemotherapy and stem-cell rescue is now the norm for children newly diagnosed with medulloblastoma (Gajjar et al., 2006). Currently, over 70% of children aged 3 years or older with average-risk medulloblastoma—defined as no metastasis and residual disease ≤ 1.5 cm^2—can achieve a cure (Gottardo & Gajjar, 2006). Moreover, chemotherapy has enabled a reduction in the necessary dosage of craniospinal radiotherapy for average-risk cases. Nevertheless, challenges persist for patients classified with high-risk disease, where survival rates remain below 55%, primarily due to factors like extensive residual disease or metastasis (Zeltzer et al., 1999; Taylor et al., 2005; Kortmann et al., 2000).

Ongoing research efforts are focused on the development of targeted therapies for medulloblastoma to enhance outcomes while reducing adverse effects. Biomarkers are becoming increasingly significant in risk assessment and individualized treatment, identified through molecular profiling methods such as next-generation sequencing and DNA methylation analysis. Research into the tumor microenvironment and immune cell interactions is revealing new therapeutic targets, including potential immunotherapy approaches. Advances in imaging and surgical tools, such as intraoperative MRI and fluorescence-guided surgery, are improving the precision of tumor resections and minimizing complications. These advancements, along with ongoing clinical trials testing new drug combinations, suggest a shift towards more personalized and effective treatment strategies for medulloblastoma. Genomic characterization is transforming cancer diagnostics by identifying and understanding the genetic events and variants that drive these tumors, aiding the development of better diagnostic, prognostic, and therapeutic approaches. Recent genomic advances have significantly deepened our understanding of medulloblastoma pathogenesis. The detection of variants confirming a predisposition to cancer syndromes may prompt ongoing surveillance and testing of family members. Findings from next-generation sequencing have influenced treatment decisions in 55% of patients, with 24% receiving targeted treatments based on these findings (Barsan et al., 2019). Medulloblastoma, once considered a single disease based solely on pathological features, has been reclassified in recent years through genome-wide sequencing into four distinct molecular subgroups with unique demographics, genetic abnormalities, and clinical outcomes.

1.3 Proteomics in Medulloblastoma

As they carry out the majority of biological functions, proteins are essential in determining the functional state of the cell. Even though gene transcripts are the first step in the production of proteins, studies have demonstrated that for many genes, transcript abundance is not a reliable indicator of the amount of protein produced. This is because of a variety of post-transcriptional regulatory mechanisms, including selective RNA-binding proteins and interfering RNAs, which affect differential translation in response to various cellular conditions (Gry et al., 2009). The categorization of medulloblastoma and its clinical and biological behaviours have been better understood thanks to developments in quantitative mass spectrometry (MS)-based techniques. New research avenues are developing by looking at protein expression and the effects of post-translational modifications on cellular activity. These avenues will help to improve the current subgroup classifications and will aid in the treatment of tumours by identifying novel molecular targets and signalling pathways as well as introducing markers in serum, urine, and CSF for tumor growth and recurrence monitoring (Serra & Mangraviti, 2021).

1.4 BET Proteins: Role in Cancer and Therapeutic Potential

The proteins that make up the mammalian bromodomain and extra-terminal domain (BET) family, which includes BRD2, BRD3, BRD4, and BRDT, control a large number of genes linked to immunity and cancer. By attaching to acetylated histones on chromosomes and enlisting transcriptional regulatory complexes to alter gene expression, these proteins function as epigenetic readers. Because BET proteins interact with several cellular proteins, including transcription factors, chromatin-modifying factors, and histone modification enzymes, they are linked to the advancement of cancer.

The development of small-molecule BET inhibitors highlights the significance of this protein family as targets for anticancer therapy (To et al., 2023). BRD2, BRD3, and BRD4 are involved in regulating the transcription of the MYC oncogene, crucial for the proliferation of many cancer cells. BET proteins maintain MYC expression by binding to its promoter regions (Henssen et al., 2013). Current therapeutic strategies targeting BET proteins use acetylation mimics to prevent bromodomains from binding chromatin, though these agents often face dose-limiting toxicities due to their effects on other bromodomain-containing proteins (To et al., 2023). BRD2 and BRD3 also regulate the transcription of growth-promoting genes like CCND1, suggesting their role in promoting cellular proliferation (LeRoy et al., 2008). Inhibiting BET proteins has been validated as a strategy for cancer chemotherapy. All identified BET proteins contain two bromodomains (BD1 and BD2) that recognize acetylated lysine residues in histones (Slavish et al., 2020).

Many bromodomain-containing proteins exhibit good druggability based on structural and computational analyses (Slavish et al., 2020). Inhibitors targeting BET proteins, such as BRD2, show potential as cancer

treatments by disrupting their binding to acetylated histones, thus downregulating oncogene expression and inhibiting tumor growth (Henssen et al., 2013).

1.5 Drug designing & molecular docking - role in MB

Molecular docking is essential in creating targeted treatments for medulloblastoma (MB), as it helps predict ligand-target interactions at the molecular scale (Pinzi & Rastelli, 2019). Epigenetic proteins are highly sought-after targets in ligand discovery, enabling the identification of inhibitors that can bind to specific molecular targets associated with MB, such as the BRD2 protein.

One of the many genes whose products are commonly activated in human malignancies is the MYC oncogene (Dhanasekaran et al., 2022). As a transcription factor that either directly or indirectly regulates the expression of hundreds of genes, MYC is a crucial regulator of many biological programmes (Kress et al., 2015). According to Juraschka and Taylor (2019), a notable biomarker for high-risk MB patients with worse clinical outcomes is persistent MYC amplification or overexpression.

In 2010, two distinct research groups employing different scaffolds independently found BET bromodomain inhibitors, such as JQ1 and I-BET151 (Filippakopoulos et al., 2010; Nicodeme et al., 2010). These inhibitors target the bromodomain (BD) of BET proteins specifically, competing with histone acetylation sites. Histones dissociate from chromatin as a result of BET proteins' inability to connect with them (Dawson et al., 2011).JQ1, in particular, has been shown to inhibit the transcription and recruitment of c-Myc to chromatin, thereby modifying the transcriptional profile of multiple myeloma cells and hindering their growth both in vitro and in vivo. This selective small molecule inhibitor has demonstrated substantial anti-proliferative effects across various cancer types (Jiang et al., 2020).

2. Methodology

The Protein Data Bank (PDB) serves as a standardized repository for atomic coordinates and bond connections derived from crystallographic studies of macromolecular structures. Each entry includes details such as the organism's origin, data resolution, bibliographic references, and relevant structural information. Scientists and biochemists worldwide contribute to the PDB, which is universally accessible online through various member entities like PDBe, PDBj, RCSB PDB, and BMRB, ensuring wide availability for researchers globally.Molecular visualization tools, such as the raster software, are integrated with servers to visualize superimposed 3D structures, including water molecules. The Molecular Modeling Database (MMDB) allows detailed examination of individual molecular architectures and

facilitates comparative analyses across different biological entities. BLAST identifies regions of similarity between the query sequence and database sequences, aiding in elucidating functional connections, inferring evolutionary links, and conducting analyses.

PyMOL, with its integrated Python interpreter, offers features and expandability unmatched by traditional software. Released under an unrestricted open-source license, PyMOL allows scientists and developers to freely adopt and distribute derivative works without cost or limitation. ERRAT is a novel method for detecting incorrect regions in protein structures by identifying errors that cause random distributions of atoms, which are distinguishable from correct distributions.

An multinational group of volunteer developers is working together to create Python modules for a range of bioinformatics issues under the Biopython project. It contains modules for handling 3D macromolecular structures, reading and writing various sequence file formats, executing multiple sequence alignments, interacting with common tools like BLAST, ClustalW, and EMBOSS, accessing large online databases, and providing numerical techniques for statistical learning.
While the fundamentals of protein interactions are becoming more known, new interactions are still being found, and the information about them is dispersed among a variety of database sources, experimental techniques, and levels of mechanistic depth. Data originates from databases of interaction experiments, curated complexes/pathways, automated text mining, and computational interaction predictions. Using information from hierarchical orthology, these interactions are evaluated critically, scored, and automatically transmitted to less-studied organisms.
Researchers may browse and relate human genes, diseases, variations, proteins, cells, and biological pathways with the help of the human gene compendium. Improved user experience, more rapid data updates, and more focused queries are all included in version 4. It gives the GeneCards suite—which includes pharmacological information, proteome expression, unified biological pathways via PathCards, gene-disease linkages via MalaCards—a firmer base. VarElect is a next-generation sequencing phenotypic prioritizer that makes use of the GeneCards and MalaCards knowledge base.
A web-based application called the UCSC genomic Browser allows you to view genomic segments of any size along with aligned annotation tracks. Gene predictions, SNPs, expression and regulatory data, mRNA and expressed sequence tag alignments, phenotypic and variation data, and comparative genomics data are among the annotations. The database tables beneath can be seen, downloaded, and worked with with the

The majority of analyses are image-based and include PROCHECK structural quality evaluations, protein-ligand and protein-DNA interactions, and secondary structure of proteins. Three programmes that allow for interactive viewing of 3D structures include RasMol, PyMOL, and 3Dmol.js, a JavaScript viewer. Users who wish to perform password-protected PDBsum analyses can submit their own PDB files.The

examination of BRD2-BD1 interaction with MDP5 in reference samples revealed significant variations, highlighting potential mechanisms in medulloblastoma pathogenesis, as shown through next-generation sequencing and proteomics analysis.

3. RESULTS

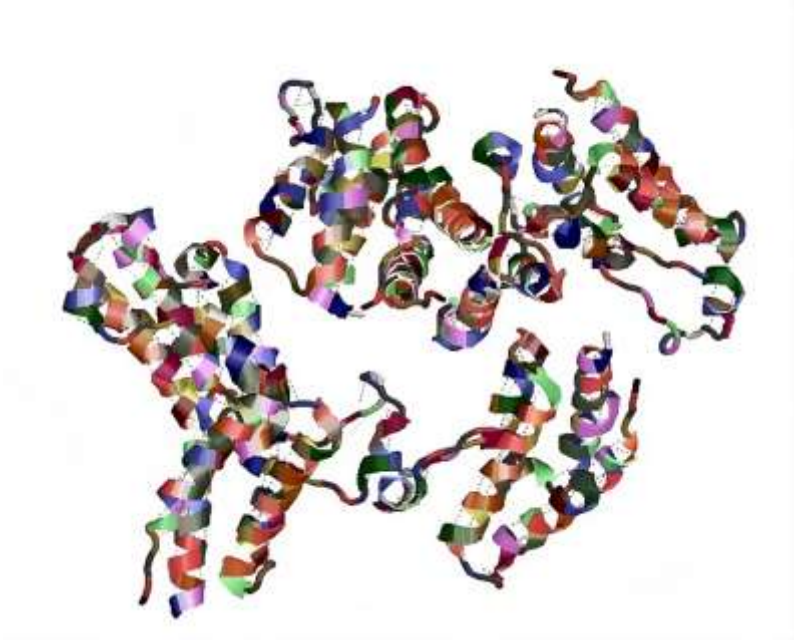


Fig 2. Analysis of Hbond on in Medulloblastoma sample (Homosapiens) in Rasmol

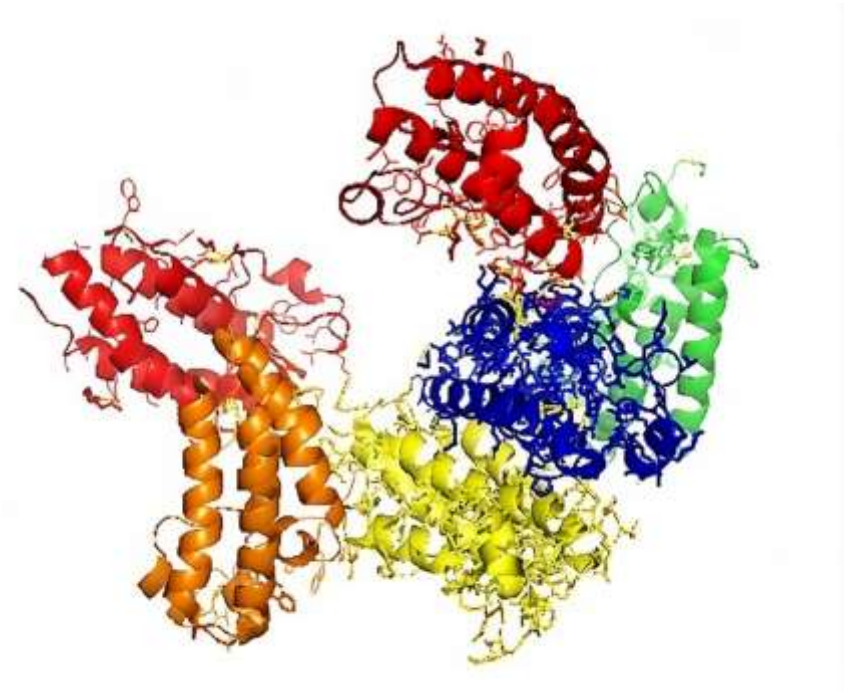


Fig 3. Active site identification on chain A (YELLOW) B (BLUE)

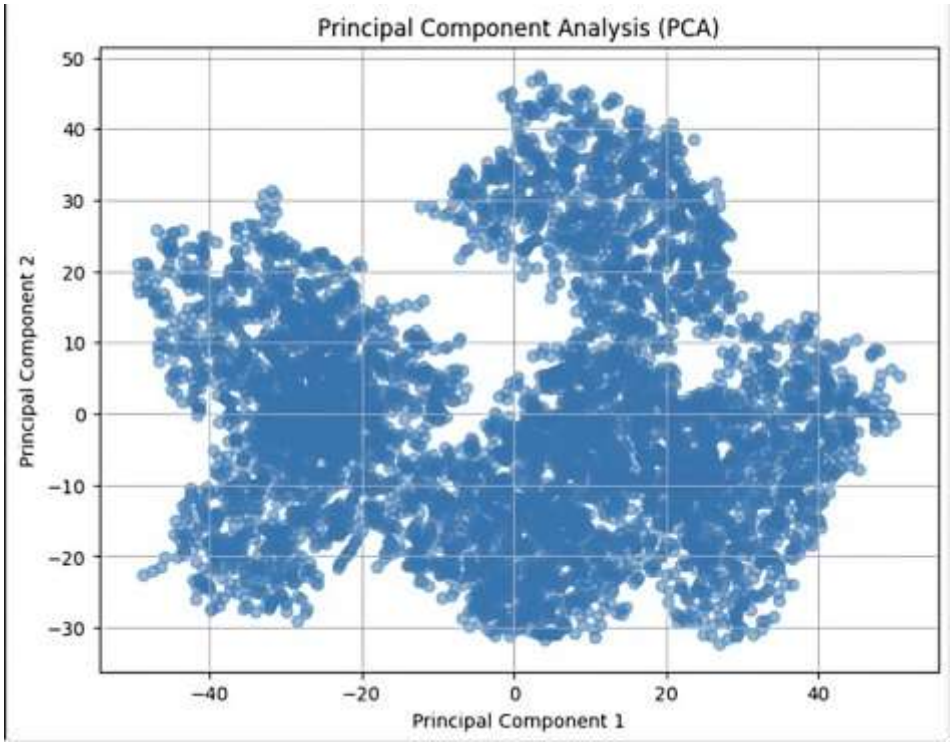


Fig 4. Principal component analysis using Biopython

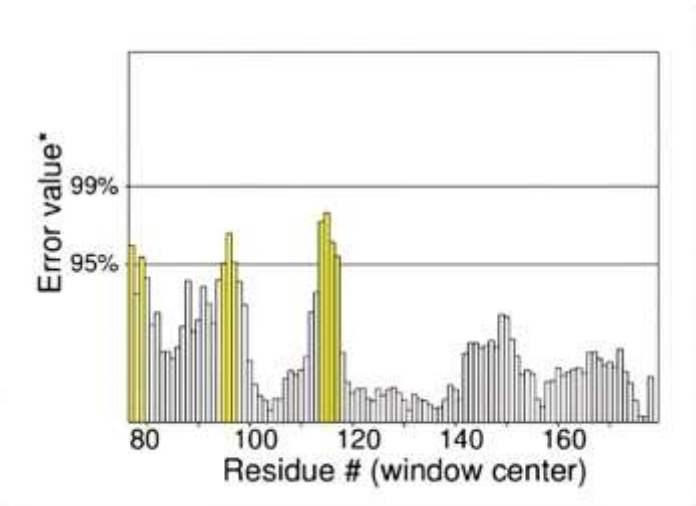


Fig 5. Errat plot for structure validation (7USI) Initial model

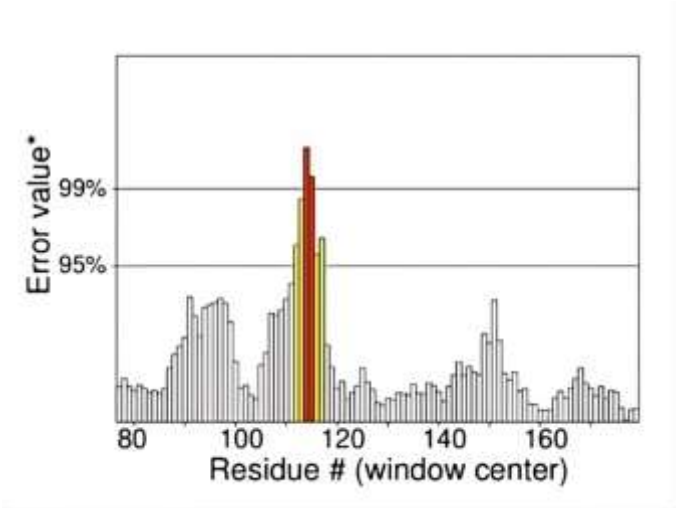


Fig 6. Final model for structure validation of protein sample

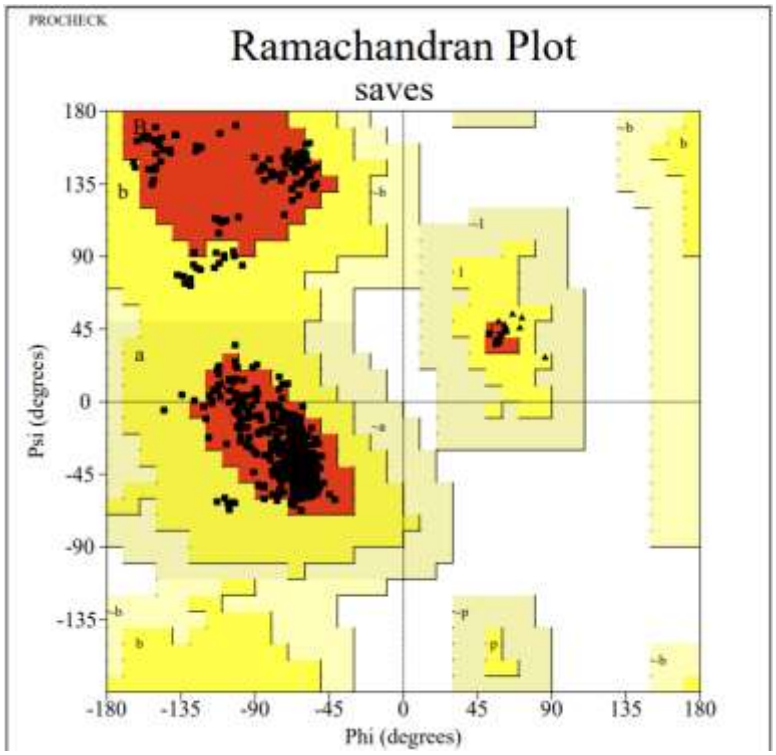


Fig 7. Validation of modeled protein using Ramachandran plot of PROCHECK analysis.

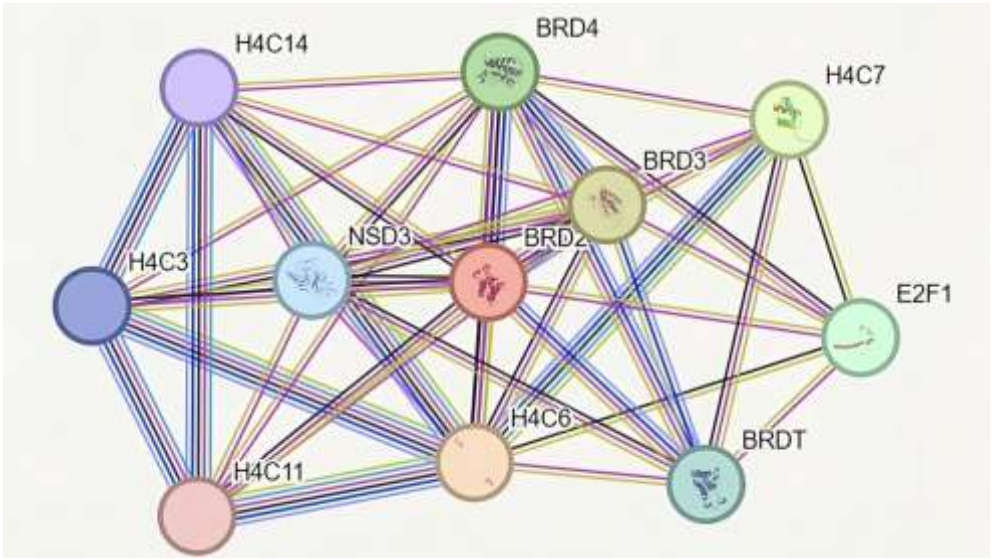
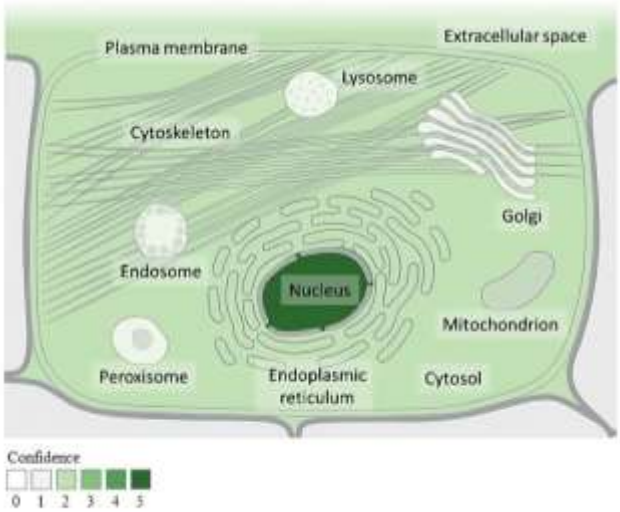


Fig 8. Protein Enrichment network analysis showing first cell of interaction (String)



Compartment	Confidence
nucleus	5
cytosol	2
endoplasmic reticulum	2
mitochondrion	2
cytoskeleton	2
extracellular	2
plasma membrane	2
golgi apparatus	1
lysosome	1
endosome	1
peroxisome	1

Fig 9. Compartment analysis showing Localization of BRD2 gene from human sample 7USI

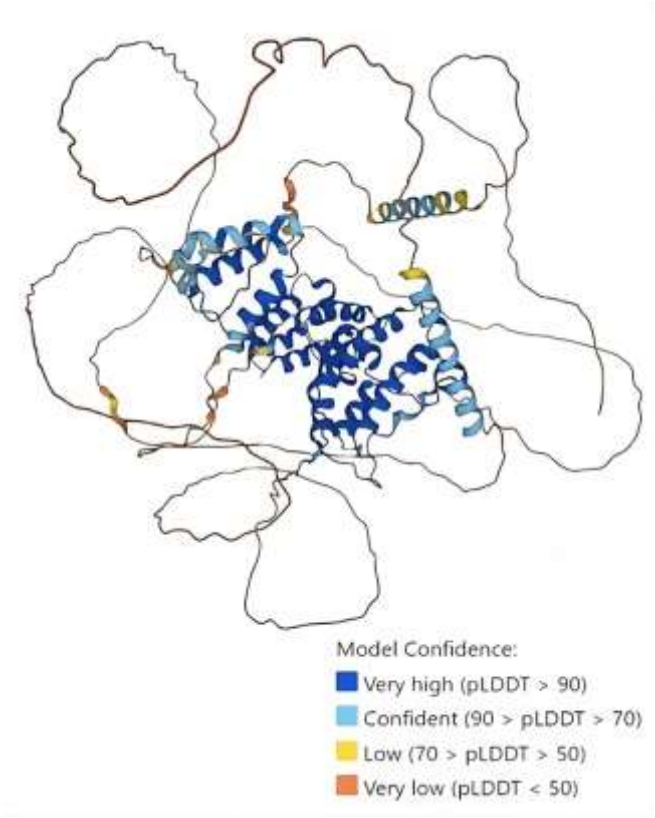


Fig 10. 3D structure of BRD2 gene

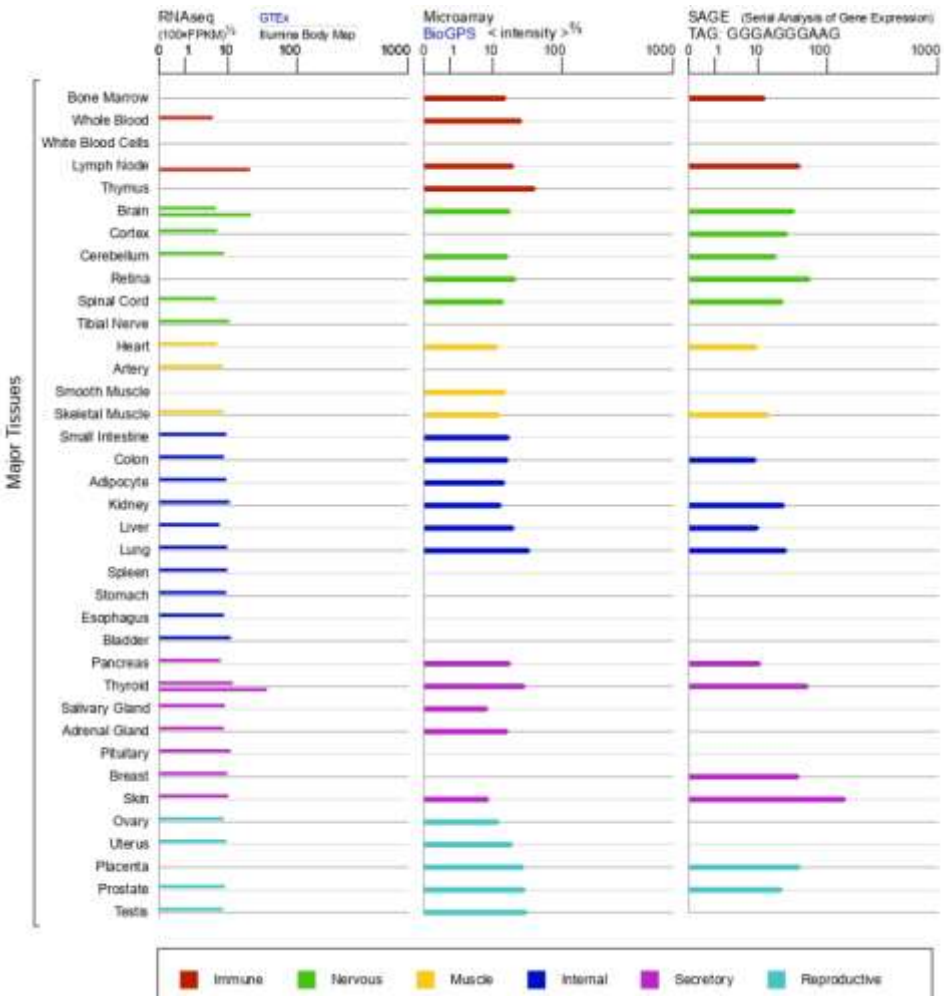


Fig 11. In genetic variant database analysis showing Expression of BRD2 gene in Genecards

Chain	No. of interface residues	Interface area (Å^2)	No. of salt bridges	No. of disulphide bonds	No. of hydrogen bonds	No. of non-bonded contacts
A	18	998	-	-	2	71
B	19	999				

Fig 12. Protein protein interaction statistical analysis

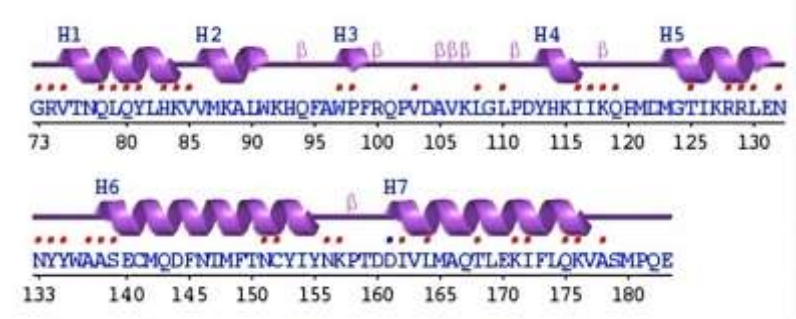


Fig 13. Secondary structure prediction of Human protein sample (7usi) in PDBsum

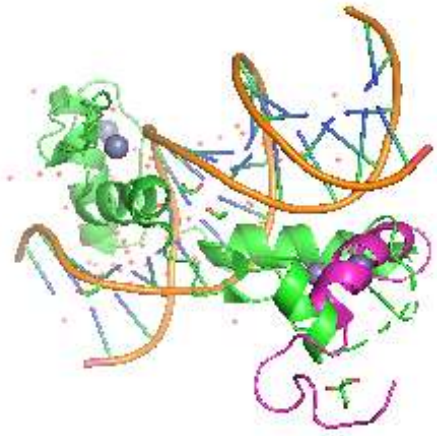


Fig 14. Low similarity RMSD VALUE above 4A indicate significant Structural Differences

Fig 15. Phylogenetic Tree analysis using sequence similarity Search (BLAST TREE)

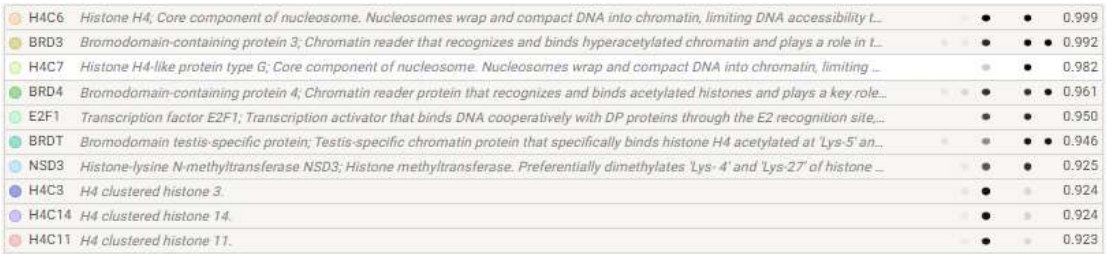


Fig 16. Predicted function partners analysis observe 0.9 score shows highest confidence score

Conclusion

Medulloblastoma, a malignant pediatric brain tumor, has seen advancements in understanding and treatment through the integration of next-generation sequencing (NGS) and proteomics analysis. BRD2, particularly its BD1 domain (bromodomain 1), has emerged as a potential therapeutic target due to its role in gene expression regulation. NGS enables genetic profiling of medulloblastoma tumors, while proteomic analysis elucidates protein interactions involving BRD2-BD1. Proteomic signatures, pathway analyses, and personalized treatment strategies derived from these analyses aim to improve outcomes for patients with medulloblastoma.

References

1. Ramaswamy V, Taylor MD. Medulloblastoma: from myth to molecular. J Clin Oncol. 2017;35(21):2355-2363. doi:10.1200/JCO.2017.72.7842.

2. Gajjar AJ, Robinson GW. Medulloblastoma-translating discoveries from the bench to the bedside. Nat Rev Clin Oncol. 2014;11:714-722.

3. Gibson P, Tong Y, Robinson G, Thompson MC, Currle DS, Eden C, et al. Subtypes of medulloblastoma have distinct developmental origins. Nature. 2010;468:1095-1099.

4. Grill J, Sainte-Rose C, Jouvet A, Gentet JC, Lejars O, Frappaz D, et al. Treatment of medulloblastoma with postoperative chemotherapy alone: an SFOP prospective trial in young children. Lancet Oncol. 2005;6:573-580.

5. Park HJ, Moon EK, Yoon JY, Oh CM, Jung KW, Park BK, et al. Incidence and survival of childhood cancer in Korea. Cancer Res Treat. 2016;48:869-882.

6. Withrow DR, Berrington de Gonzalez A, Lam CJK, Warren KE, Shiels MS. Trends in pediatric central nervous system tumor incidence in the United States, 1998-2013. Cancer Epidemiol Biomarkers Prev. 2019;28:522-530.

7. Tulla M, Berthold F, Graf N, Rutkowski S, von Schweinitz D, Spix C, et al. Incidence, trends, and survival of children with embryonal tumors. Pediatrics. 2015;136.

8. Sun T, Plutynski A, Ward S, Rubin JB. An integrative view on sex differences in brain tumors. Cell Mol Life Sci. 2015;72:3323-3342.

9. Taillandier L, Blonski M, Carrie C, et al. Les médulloblastomes: revue générale [Medulloblastomas: review]. Rev Neurol. 2011;167(5):431-448.

10. Rooper AH, Brown RH, editors. Intracranial neoplasms and paraneoplastic disorders. In: Adams and Victor's Principles of Neurology. New York: McGraw-Hill; 2005.

11. Marino S. Medulloblastoma: developmental mechanisms out of control. Trends Mol Med. 2005;11:17-22.

12. Taylor MD, Northcott PA, Korshunov A, Witt H, Cho YJ, Tsherniak A, et al. Molecular subgroups of medulloblastoma: the current consensus. Acta Neuropathol. 2012;123(4):465-472. doi:10.1007/s00401-011-0922-z.

13. Northcott PA, Korshunov A, Witt H, et al. Medulloblastoma comprises four distinct molecular variants. J Clin Oncol. 2011;29:1408-1414.

14. Cho YJ, Tsherniak A, Tamayo P, et al. Integrative genomic analysis of medulloblastoma identifies a molecular subgroup that drives poor clinical outcome. J Clin Oncol. 2011;29:1424-1430.

15. Kool M, Korshunov A, Remke M, et al. Molecular subgroups of medulloblastoma: an international meta-analysis of transcriptome, genetic aberrations, and clinical data of WNT, SHH, Group 3, and Group 4 medulloblastomas. Acta Neuropathol. 2012;123(4):473-484. doi:10.1007/s00401-012-0958-8.

16. Choi JY. Medulloblastoma: Current Perspectives and Recent Advances. Brain Tumor Res Treat. 2023;11(1):28-38. doi:10.14791/btrt.2022.0046.

17. Northcott PA, Korshunov A, Pfister SM, Taylor MD. The clinical implications of medulloblastoma subgroups. Nat Rev Neurol. 2012;8:340-351.

18. Pugh TJ, Weeraratne SD, Archer TC, et al. Medulloblastoma exome sequencing uncovers subtype-specific somatic mutations. Nature. 2012;488:106-110.

19. Ramaswamy V, Remke M, Bouffet E, Faria CC, Perreault S, Cho YJ, et al. Recurrence patterns across medulloblastoma subgroups: an integrated clinical and molecular analysis. Lancet Oncol. 2013;14:1200-1207.

20. Ramaswamy V, Remke M, Adamski J, et al. Medulloblastoma subgroup-specific outcomes in irradiated children: who are the true high-risk patients? Neuro Oncol. 2016;18(2):291-297. doi:10.1093/neuonc/nov210.

21. Kool M, Korshunov A, Remke M, et al. Molecular subgroups of medulloblastoma: an international meta-analysis of transcriptome, genetic aberrations, and clinical data of WNT, SHH, group 3, and group 4 medulloblastomas. Acta Neuropathol. 2012;123(4):473-484. doi:10.1007/s00401-012-0958-8.

22. Clifford SC, Lusher ME, Lindsey JC, et al. Wnt/Wingless pathway activation and chromosome 6 loss characterize a distinct molecular sub-group of medulloblastomas associated with a favorable prognosis. Cell Cycle. 2006;5(22):2666-2670. doi:10.4161/cc.5.22.3446.

23. Ellison DW, Onilude OE, Lindsey JC, et al. beta-Catenin status predicts a favorable outcome in childhood medulloblastoma: the United Kingdom Children's Cancer Study Group Brain Tumour Committee. J Clin Oncol. 2005;23(31):7951-7957. doi:10.1200/JCO.2005.01.5479.

24. Ellison DW, Dalton J, Kocak M, et al. Medulloblastoma: clinicopathological correlates of SHH, WNT, and non-SHH/WNT molecular subgroups. Acta Neuropathol. 2011;121(3):381-396. doi:10.1007/s00401-011-0800-8.

25. Goschzik T, Zur Mühlen A, Kristiansen G, et al. Molecular stratification of medulloblastoma: comparison of histological and genetic methods to detect Wnt activated tumours. Neuropathol Appl Neurobiol. 2015;41(2):135-144. doi:10.1111/nan.12161.

26. Northcott PA, Buchhalter I, Morrissy AS, et al. The whole-genome landscape of medulloblastoma subtypes. Nature. 2017;547(7663):311-317. doi:10.1038/nature22973.

27. Taylor MD, Northcott PA, Korshunov A, Remke M, Cho YJ, Clifford SC, et al. Molecular subgroups of medulloblastoma: the current consensus. Acta Neuropathol. 2012;123(4):465-472. doi:10.1007/s00401-011-0922-z.

28. Orr BA. Pathology, diagnostics, and classification of medulloblastoma. Brain Pathol. 2020;30(3):664-678. doi:10.1111/bpa.12837.

29. Hovestadt V, Ayrault O, Swartling FJ, Robinson GW, Pfister SM, Northcott PA. Medulloblastomics revisited: biological and clinical insights from thousands of patients. Nat Rev Cancer. 2020;20(1):42-56. doi:10.1038/s41568-019-0223-8.

30. Mani S, Chatterjee A, Dasgupta A, et al. Clinico-Radiological Outcomes in WNT-Subgroup Medulloblastoma. Diagnostics. 2024;14(4):358. doi:10.3390/diagnostics14040358.

31. Hughes EN, Shilito J, Sallan SE, et al. Medulloblastoma at the Joint Center for Radiation Therapy between 1968 and 1984: the influence of radiation dose and pattern of failure. Cancer. 1988;61:1992-1998.

32. Gajjar A, Chintagumpala M, Ashley D, et al. St Jude Medulloblastoma-96: long-term results from a prospective, multicentre trial. Lancet Oncol. 2006.

33. Choi, Jung Yoon. “Medulloblastoma: Current Perspectives and Recent Advances.” Brain tumor research and treatment vol. 11,1 (2023): 28-38. doi:10.14791/btrt.2022.0046

34.Kool, Marcel et al. “Molecular subgroups of medulloblastoma: an international meta-analysis of transcriptome, genetic aberrations, and clinical data of WNT, SHH, Group 3, and Group 4 medulloblastomas.” Acta neuropathologica vol. 123,4 (2012): 473-84. doi:10.1007/s00401-012-0958-8

35.Martirosian, Vahan, and Josh Neman. “Medulloblastoma: Challenges and advances in treatment and research.” Cancer Reports vol. 2,3 e1146. 18 Nov. 2018, doi:10.1002/cnr2.1146

36. Cooney, Tab et al. “Current studies and future directions for medulloblastoma: A review from the pacific pediatric neuro-oncology consortium (PNOC) disease working group.” Neoplasia (New York, N.Y.) vol. 35 (2023): 100861. doi:10.1016/j.neo.2022.100861

37.Laskowski RA, Swindells MB. LigPlot+: multiple ligand-protein interaction diagrams for drug discovery. J Chem Inf Model. 2011 Oct 24;51(10):2778-86. doi: 10.1021/ci200227u. Epub 2011 Oct 5. PMID: 21919503.

38.Laskowski RA, Jabłońska J, Pravda L, Vařeková RS, Thornton JM. PDBsum: Structural summaries of PDB entries. Protein Sci. 2018 Jan;27(1):129-134. doi: 10.1002/pro.3289. Epub 2017 Oct 27. PMID: 28875543; PMCID: PMC5734310.

39.Karolchik D, Hinrichs AS, Kent WJ. The UCSC Genome Browser. Curr Protoc Hum Genet. 2011 Oct;Chapter 18:18.6.1-18.6.33. doi: 10.1002/0471142905.hg1806s71. PMID: 21975940; PMCID: PMC3222792.

40.Szklarczyk D, Kirsch R, Koutrouli M, Nastou K, Mehryary F, Hachilif R, Gable AL, Fang T, Doncheva NT, Pyysalo S, Bork P, Jensen LJ, von Mering C. The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. Nucleic Acids Res. 2023 Jan 6;51(D1):D638-D646. doi: 10.1093/nar/gkac1000. PMID: 36370105;PMCID: PMC9825434.

41.Cock PJ, Antao T, Chang JT, Chapman BA, Cox CJ, Dalke A, Friedberg I, Hamelryck T, Kauff F, Wilczynski B, de Hoon MJ. Biopython: freely available Python tools for computational molecular biology and bioinformatics. Bioinformatics. 2009 Jun 1;25(11):1422-3. doi: 10.1093/bioinformatics/btp163. Epub 2009 Mar 20. PMID: 19304878; PMCID: PMC2682512.

42.The GeneCards Suite: From Gene Data Mining to Disease Genome Sequence Analyses Stelzer G, Rosen R, Plaschkes I, Zimmerman S, Twik M, Fishilevich S, Iny Stein T, Nudel R, Lieder I, Mazor Y, Kaplan S, Dahary, D, Warshawsky D, Guan - Golan Y, Kohn A, Rappaport N, Safran M, and Lancet DCurrent Protocols in Bioinformatics(2016), 54:1.30.1 - 1.30.33.doi: 10.1002 / cpbi.5

43.Varma SN, Ye S, Ferlin S, Comer C, Cotton K, Niklison-Chirou MV. The Proteasome Inhibitor CEP-18770 Induces Cell Death in Medulloblastoma. Pharmaceutics. 2024 May 16;16(5):672. doi: 10.3390/pharmaceutics16050672. PMID: 38794334; PMCID: PMC11124782.

44.Leary SES, Packer RJ, Li Y, Billups CA, Smith KS, Jaju A, Heier L, Burger P, Walsh K, Han Y, Embry L, Hadley J, Kumar R, Michalski J, Hwang E, Gajjar A, Pollack IF, Fouladi M, Northcott PA,

Olson JM. Efficacy of Carboplatin and Isotretinoin in Children With High-risk Medulloblastoma: A Randomized Clinical Trial From the Children's Oncology Group. JAMA Oncol. 2021 Sep;7(9):1313-1321. doi: 10.1001/jamaoncol.2021.2224. PMID: 34292305; PMCID: PMC8299367.

45. Choi JY. Medulloblastoma: Current Perspectives and Recent Advances. Brain Tumor Res Treat. 2023 Jan;11(1):28-38. doi: 10.14791/btrt.2022.0046. PMID: 36762806; PMCID: PMC9911713.

46. Henssen A, Thor T, Odersky A, Heukamp L, El-Hindy N, Beckers A, Speleman F, Althoff K, Schäfers S, Schramm A, Sure U, Fleischhack G, Eggert A, Schulte JH. BET bromodomain protein inhibition is a therapeutic option for medulloblastoma. Oncotarget. 2013 Nov;4(11):2080-2095. doi: 10.18632/oncotarget.1534. PMID: 24231268; PMCID: PMC3875771.

47.Vinchon M, Leblond P. Medulloblastoma: Clinical presentation. Neurochirurgie. 2021 Feb;67(1):23-27. doi: 10.1016/j.neuchi.2019.04.006. PMID: 31494131.

48. Albright AL, Wisoff JH, Zeltzer PM, Boyett JM, Rorke LB, Stanley P. Effects of medulloblastoma resections on outcome in children: a report from the Children's Cancer Group. Neurosurgery. 1996 Feb;38(2):265-271. doi: 10.1097/00006123-199602000-00007. PMID: 8869053.

49. Cotter JA, Hawkins C. Medulloblastoma: WHO 2021 and Beyond. Neurosurg Clin N Am. 2022 Jan-Feb;25(1):23-33. doi: 10.1177/10935266211018931. PMID: 35168417.

50. Barsan V, Paul M, Gorsi H, Malicki D, Elster J, Kuo DJ, Crawford J. Clinical Impact of Next-generation Sequencing in Pediatric Neuro-Oncology Patients: A Single-institutional Experience. Cureus. 2019 Dec;11(12), doi: 10.7759/cureus.6281. PMID: 31827999; PMCID: PMC6892579.

51. Funakoshi Y, Sugihara Y, Uneda A, Nakashima T, Suzuki H. Recent advances in the molecular understanding of medulloblastoma. Cancer Sci. 2023 Mar;114(3):741-749. doi: 10.1111/cas.15691. PMID: 36520034; PMCID: PMC9986075.

52.Gry M, Rimini R, Strömberg S, Asplund A, Pontén F, Uhlén M, Nilsson P. Correlations between RNA and protein expression profiles in 23 human cell lines. BMC Genomics. 2009 Aug 7;10:365. doi: 10.1186/1471-2164-10-365. PMID: 19660143; PMCID: PMC2728742.

53. Serra R, Mangraviti A. A systematic view of pediatric medulloblastoma proteomics-current state of the field and future directions. Childs Nerv Syst. 2021 Mar;37(3):779-788. doi: 10.1007/s00381-020-04988-7. PMID: 33409616.

54. To KKW, Xing E, Larue RC, Li PK. BET Bromodomain Inhibitors: Novel Design Strategies and Therapeutic Applications. Molecules. 2023 Mar 29;28(7):3043. doi: 10.3390/molecules28073043. PMID: 37049806; PMCID: PMC10096006.

55. LeRoy G, Rickards B, Flint SJ. The double bromodomain proteins Brd2 and Brd3 couple histone acetylation to transcription. Mol Cell. 2008 Apr 11;30(1):51-60. doi: 10.1016/j.molcel.2008.01.018. PMID: 18406326; PMCID: PMC2387119.

56.Slavish PJ, Chi L, Yun MK, Tsurkan L, Martinez NE, Jonchere B, Chai SC, Connelly M, Waddell MB, Das S, Neale G, Li Z, Shadrick WR, Olsen RR, Freeman KW, Low JA, Price JE, Young BM, Bharatham N, Boyd VA, Yang J, Lee RE, Morfouace M, Roussel MF, Chen T, Savic D, Guy RK, White SW, Shelat AA, Potter PM. Bromodomain-selective BET inhibitors are potent antitumor agents against MYC-driven pediatric cancer. Cancer Res. 2020 Sep 1;80(17):3507-3518. doi: 10.1158/0008-5472.CAN-19-3934. PMID: 32651255; PMCID: PMC7483993.

57. Pinzi L, Rastelli G. Molecular Docking: Shifting Paradigms in Drug Discovery. Int J Mol Sci. 2019 Sep;20(18):4331. doi: 10.3390/ijms20184331. PMID: 31487867; PMCID: PMC6769923.

58.Dhanasekaran R, Deutzmann A, Mahauad-Fernandez WD, Hansen AS, Gouw AM, Felsher DW. The MYC oncogene - the grand orchestrator of cancer growth and immune evasion. Nat Rev Clin Oncol. 2022 Jan;19(1):23-36. doi: 10.1038/s41571-021-00549-2. Epub 2021 Sep 10. PMID: 34508258; PMCID: PMC9083341.

59.Kress TR, Sabò A, Amati B. MYC: connecting selective transcriptional control to global RNA production. Nat Rev Cancer. 2015 Oct;15(10):593-607. doi: 10.1038/nrc3984. Epub 2015 Sep 18. PMID: 26383138.

60.Juraschka K, Taylor MD. Medulloblastoma in the age of molecular subgroups: a review. J Neurosurg Pediatr. 2019 Oct 1;24(4):353-363. doi: 10.3171/2019.5.PEDS18381. PMID: 31574483.

61.Filippakopoulos P, Qi J, Picaud S, Shen Y, Smith WB, Fedorov O, Morse EM, Keates T, Hickman TT, Felletar I, Philpott M, Munro S, McKeown MR, Wang Y, Christie AL, West N, Cameron MJ, Schwartz B, Heightman TD, La Thangue N, French CA, Wiest O, Kung AL, Knapp S, Bradner JE. Selective inhibition of BET bromodomains. Nature. 2010 Dec 23;468(7327):1067-1073. doi: 10.1038/nature09504. Epub 2010 Sep 24. PMID: 20871596; PMCID: PMC3010259.

62. Nicodeme E, Jeffrey KL, Schaefer U, Beinke S, Dewell S, Chung CW, Chandwani R, Marazzi I, Wilson P, Coste H, White J, Kirilovsky J, Rice CM, Lora JM, Prinjha RK, Lee K, Tarakhovsky A. Suppression of inflammation by a synthetic histone mimic. Nature. 2010 Dec 23;468(7327):1119-1123. doi: 10.1038/nature09589. Epub 2010 Nov 10. PMID: 21068722; PMCID: PMC5415086.

63. Dawson MA, Prinjha RK, Dittman A, Giotopoulos G, Bantscheff M, Chan WI, Robson SC, Chung CW, Hopf C, Savitski MM, Huthmacher C, Gudgin E, Lugo D, Beinke S, Chapman TD, Roberts EJ, Soden PE, Auger KR, Mirguet O, Doehner K, Delwel R, Burnett AK, Jeffrey P, Drewes G, Lee K, Huntly BJP, Kouzarides T. Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia. Nature. 2011 Oct 2;478(7370):529–533. doi: 10.1038/nature10509. PMID: 21964340; PMCID: PMC3679520.

64. Jiang G, Deng W, Liu Y, Wang C. General mechanism of JQ1 in inhibiting various types of cancer. Mol Med Rep. 2020 Mar;21(3):1021–1034. doi: 10.3892/mmr.2020.10927. PMID: 31922235; PMCID: PMC7003028.

65.Uma Kumari, K. S. (2023). CADD Approaches For The Early Diagnosis Of Lung Cancer. *Journal of Clinical Otorhinolaryngology, Head, and Neck Surgery, 27*(1), 5190-5199.

66.Uma Kumari, N. B. (2023). Computer Aided Drug Designing Approach for Prospective Human Metastatic Cancer. *International Journal for Research in Applied Science and Engineering Technology, 11*, 1874-1879. doi:10.22214/ijraset.2023.550014

67.Vinita Kukreja, U. K. (2023). Data Analysis of Brain Cancer with Biopython. *International Journal of Innovative Science and Research Technology, 8*(3), 2147-2154

68. Uma Kumari,Gurpreet Kaur *et al*,"Biopython/Network Of Protein Identification And NGS Analysis Of Glioma Cancer ATP Competitive Type III C-MET Inhibitor,Volume 11, Issue 2 : 27-Jun-2024 :pp 41-51 : http://doi.one/10.1729/Journal.40229

69.Uma Kumari,Sharvari Santosh Kulkarini etal "Structure Analysis And Glioma Targeted Therapeutics Cancer In ATP Competitive Type III C-MET Inhibitor", International Journal of Emerging Technologies and Innovative Research, Vol.11, Issue 6, page no. ppi271-i282, June-2024