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Ngs Data Analysis And Active Site Identification of Alpha-1-Acid Glycoprotein Bound To Potent Anti Tumor Compound UCN-01 In Malignant Brain Tumor

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***Abstract*:** Malignant brain tumors are the most lethal among all the tumors originating from central nervous system. Alpha-1-acid glycoprotein (PDB ID: 7OUB) is an acute phase transport protein found in blood plasma in abundance. The high affinity of AGP for many molecules shows its effectiveness on the pharmacodynamics and pharmacokinetic properties of several small molecules. In this study, we used AGP bound to a ligand namely, staurosporine, an antitumor compound with its one of the hydroxylated forms UCN-01 to analyse its functionality in malignant brain tumor. In this approach, we used various software and database tools for structural analysis and sequence alignment of the sample, and integrated molecular docking with desired drugs to determine the respective binding affinities with AGP. Tools like PROSITE and STRING were utilized to determine significant information regarding tumor specific, nodular regions, families & domains, and for protein enrichment network analysis respectively. Furthermore, cleft analysis was done for interpreting potential active site and somatic mutation on multiple locations were predicted using COSMIC database which represents unchecked cellular growth due to sequential gaps leading to destruction. In conclusion, this computational study based on NGS provides new biological insights in malignancy research thereby representing alpha-1-acid glycoprotein as a strong candidate for further experimental studies in bioinformatics.

Keywords: **Malignant brain tumor, NGS, Sequence alignment; Structural analysis; Molecular docking; COSMIC**

Introduction

Brain tumors are the most lethal among all the tumors originating from central nervous system. Although their classification can be done on various histophysiological, molecular and genetic factors; brain tumors are majorly classified as benign and malignant tumors based on their ability to metastasize (Campos-Sanchez E. et al 2019; Johnson DR et al., 2017). The malignant brain tumors developing from the embryonal cells such as medulloblastoma are common in children whereas tumors like oligodendroglioma and glioblastoma is prevalent in adults (Ostrom QT et al., 2014; Ostrom QT et al., 2015). According to the World Health Organization, grade 2 and 3 meningiomas have better prognosis and high potential of penetrating into the brain parenchyma than grade 3 meningiomas also known as malignant meninginomas (Schaff L.R. et al., 2023; Rohringer M., et al., 1989; Ostrom Q.T. et al., 2022). For instance, glioblastoma, an intra axial tumor which arises from the brain parenchyma has much poor prognosis with a median overall survival of 14-17 months (Fisher JL et al., 2007; American Cancer Society, 2022) than the intracranial tumors such as meningioma spreading outside (Marosi C. et al., 2008; Schaff L.R. et al., 2023). Low survival, high incidence rate and lack of therapeutic options due to blood brain barrier are few of the important factors which distinguish glioblastoma from other primary tumors (Mitusova K. et al., 2022). Typical symptoms include headache, vomiting, nausea, seizures, excessive drowsiness, fatigue, sleep disturbance, neurocognitive impairment and focal neurological deficits (Schaff L.R. et al., 2023; Megan S Jeon et al., 2021). Conventional technique like magnetic resonance imaging (MRI) has been extensively used approach for the diagnosis and localization of tumor, analyzing its extent, grade, and assessment of treatment response. Moreover, Positron emission tomography (PET) when performed along with advanced MRI is reported to provide further information which in turn, helps in adding to the specificity of tumor diagnosis and better evaluation process (Wynton B Overcast et al., 2021). Radiation treatments including exposure to ionizing radiation and medical imaging technique utilization are the well validated risk factors considered for primary brain and other central nervous system tumors (Braganza MZ et al., 2012). However, patients with some atopic diseases like psoriasis, aczema, asthma or any other allergic history are found to have decreased risk (Amirian ES et al., 2016). Tumors with high potential of malignancies are commonly treated with surgery which howsoever have a poor prognosis and early relapse. Whereas radiation therapy, particularly ionizing radiation which enhanced sensitivity for cancer cells has been routinely employed as an effective option for treatment. Along with this, drug therapy is also considered to be an essential part of cancer treatment. However, very few drugs have been found out to be effective against malignant tumors like meningioma and glioblastoma (Ostrom Q.T. et al., 2022; Wen PY et al., 2020; Karschnia P et al., 2023; Goldbrunner R. et al., 2016;

Khabibov M et al., 2022; Goldbrunner R. et al., 2021; Patel B. et al., 2022; Maier P et al., 2016). In commercially available drugs, lomustine and carmustine are the most extensively used therapeutic agents in the treatment of malignant brain tumors due to their ability to cross blood brain barrier. Carmustine is used in only low-grade glioma therapy due to its highly toxic nature whereas lomustine is utilized in PCV (procarbazine, lomustine, vincristine) for high grade gliomas (Brada M et al., 2010; drug bank; Brandes AA et al., 2016). Temozolomide is an oral chemotherapeutic agent taken in combination with radiation therapy or surgery due to its highest permeability for blood brain barrier and effectiveness in increasing survival period (Wang D et al., 2019). Another chemotherapeutic agent carboplatin, usually prescribed for recurrent disease in patients and irinotecan which causes double strand breakage is known to show less effectiveness and durability for tumor treatment (HB Newton, 2006; Marcucci F et al. 2021; Vredenburgh JJ et al., 2009). Other examples include topotecan, etoposide, cyclophosphamide, and procarbazine (Mitusova K et al., 2022).

Materials and methodology

The three-dimensional high-resolution structure of the proteomic sample of Alpha-1-acid glycoprotein (AGP) bound to potent anti-tumour compound UCN-01 with the PDB ID 7OUB was retrieved from the Protein Data Bank (PDB) which is a worldwide database tool established at Brookhaven National Laboratories (BNL) and managed by Research Collaboratory for Structural Bioinformatics (RCSG) for archiving crystal structures of various biological macromolecules (Helen M. Berman et al., 2000). The sample obtained was already bound to ligand UCN-01. AGP is an acute phase transport protein found in blood plasma in abundance. The ligand namely, staurosporine, found in the protein AGP as an antitumor compound with its one of the hydroxylated forms UCN-01 which is a kinase inhibitor in nature. The two variants of lipocalins found in plasma i.e. AGP1 and AGP2 are present within them (Erik and Williams et al., 2021). Further information including chemical graphs, 3D domains and similar sequences was retrieved from MMDB, an experimentally determined 3D molecular structure database of Entrez which is originally offered by National Center for Biotechnology Information (NCBI) (Hogue, 1996). The visualization of proteomic sample was done using softwares like RasMol2 and PyMol. PyMol is a cross-platform python-based software developed with the aim of fascilitating computational drug designing, molecular modelling including protein-ligand modelling, molecular simulations, and drug screening (Yuan, S. et al., 2017). The software was used for basic molecular graphic representation, crystallographic visualization of molecule, generation of publication quality 3D figures, multiple atom selection syntaxes, molecular editing, animations, and more which are required for molecular visualization (DeLano, W.L. 2002). To validate the structure of protein model, ERRAT server was used to assess atomic interaction based on overall quality and toxicity label. BLAST (Basic Local Alignment Search Tool) was used to compare our sample sequence to the database of sequences stored within, which helped in providing short matches of similarity between the sequences and begin aligning them through these hot spots, thereby giving expect value (Jian Ye et al., 2006). Further, the predicted homologs were used to evaluate the RMSD (root mean square deviation) value in PyMol. Interproscan was used for scanning the sample sequence and to determine different families, domains and important sites present within the protein for further analysis (Evgeni M. et al., 2001). Subsequently, KEGG pathway database was considered for analysing the motifs encoded by positionally coupled genes which helps in predicting the function of genes (Minoru Kanehisa et al., 2000). Another valuable global database STRING was used to predict the functional links between proteins which show similarity in species coverage and compare the significance of individually predicted interactions by providing confidence scores based per predicted association, thereby enhancing the functional enrichment analysis of proteins (Christian von Mering, et al., 2003; Damian Szklarczyk et al., 2023). Information about the families, domains and functional sites present in sample were determined using PROSITE in the form of conserved patterns or profiles (Nicolas Hulo et al., 2006). The investigations including protein secondary structures, ligand-DNA interactions, quality assessments using PROCHECK (Ramachandran plot) and more were done via image-based study in PDBsum (Kumari, Uma and Gupta, Shruti, 2023). Molecular docking was done using CB Dock2, a protein ligand docking tool to determine the binding affinity, active site, size and center calculation of our proteomic sample with desired drugs (Uma Kumari and Gopinath et al., 2024). Another database COSMIC (Catalogue of somatic mutations in cancer) was useful in predicting somatic mutation and other relevant details regarding human cancer at a single location (Bamford S. et al., 2004). This helped interpreting genomic data, understand various somatic alterations and facilitate translational research (Zbyslaw Sondka et al., 2024). Clustal omega is a multiple sequence alignment (MSA) tool used to align three or more sequences was used to analyze sequence similarities among sample and its homologs using seeded guide tree and profile-profile techniques based on Hidden Markov Model (HMM) (Vinita Kukreja and Uma Kumari, 2023). The tool typically displays the output using various symbols like (*) for identical sequences, (:) for conserved substitution, (.) for semiconserved sequences and more to compare and analyse the primary, secondary and tertiary structures as well as for phylogenetic reconstruct and predict functions of sequences (Uma Kumari and Vinita Kukreja, 2023; Uma Kumari and Kartik Tripathi, 2023; Uma Kumari et al., 2023; Gurpreet Kaur et al., 2024).

Result and Discussion

1. **Structure validation using ERRAT database**

In save server, proteomic sample analysis detects the incorrect region of protein structure, representing the error residue with red and yellow. Overall quality factor of high-resolution structure of Alpha-1 acid glycoprotein bound to potent anti-tumor compound UCN-01 is **96.296.**

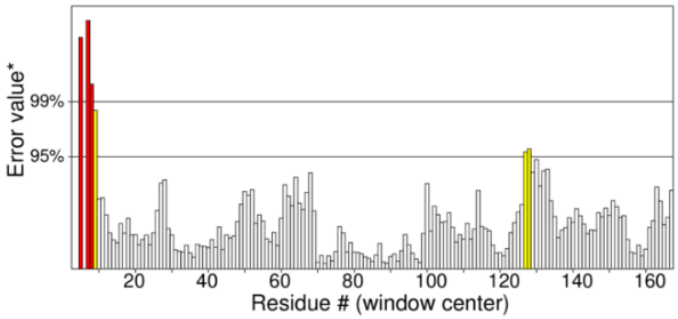

Figure 1: Structure validation analysis (7OUB)

2. **Structural analysis using PyMol**

B factor analysis in PyMol shows **C terminal** (red loop) with **more than 50 Å** which represents **atomic motion or mobile and disordered structure (with high solvent exposure)** comprising of Glutamine residues.

Figure 2: C terminal showing the B factor of more than 50 Å rich in Glutamine residues

Sequence analysis shows the protein position of 7OUB wherein, the amino acid sequence begins at the position of -18, which means that the fasta header could include working with cleavage site or post translational modification.

The active site of both 3APU and 7OUB bound ligand is showing residues within 5 Å distance, thereby making it a good candidate for docking with suitable drug.

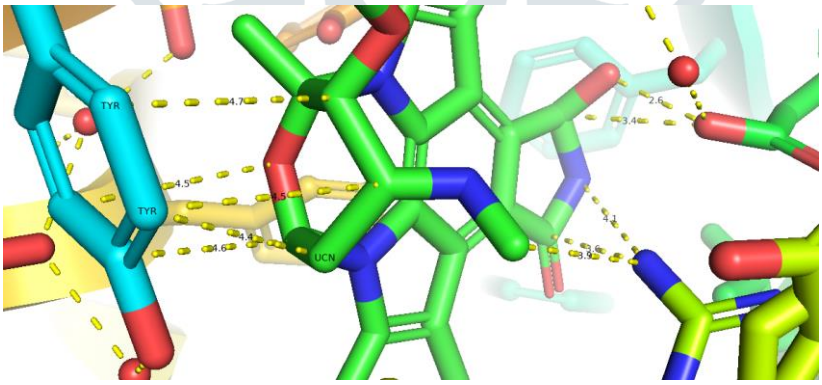

Figure 3: Active site identification in 7OUB bound ligand with the residues within 5 Å

Root Mean Square Deviation (RMSD) Analysis between 7OUB and 3APU

Executive: **RMSD = 0.481** (1097 to 1097 atoms)

The RMSD score of **0.48Å** suggests that the two structures are nearly identical in the regions being compared, representing the same protein in slightly different conformational states or alignments of highly similar homologous proteins.

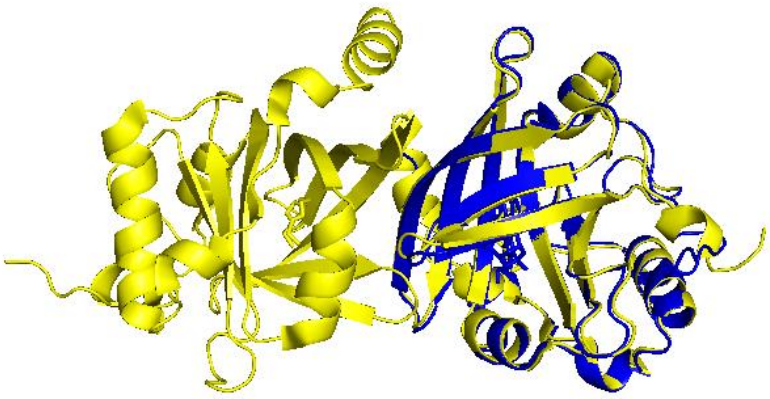

Figure 4: Protein ligand interaction of 7OUB (blue) and 3APU (yellow) showing RMSD score of 0.481

3. InterProscan

Interproscan and NGS for malignancy tumor sequence analysis help to identify functional domain, mutations and pathway disruption. Interpretation of sample 7OUB in interproscan reveals both integrated and unintegrated mean of sequences.

The **integrated sequences include AGP and ALPHA 1 ACID GLYCOPROTEIN** in 7OUB.

Domain (PIRSF PIRSF036899) **AGP** Integrated: IPR001500 family (12 – 191);

Domain (PANTHER PTHR11967) **ALPHA-1-ACID GLYCOPROTEIN** Model: PTHR11967:SF3 (19 – 191);

The **unintegrated sequence include Lipocalin** representing the domain umatched to any functional known sequence.

Cdd cd19451 **lipocalin_AGP-like** (22 – 191)

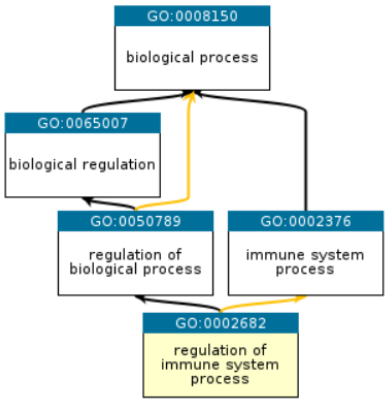

Figure 5: Ancestor chart in InterProScan GO terms (7OUB)

4. STRING database

The computational analysis of predicted functional partners of human malignant brain tumor proteomic sample 7OUB shows **0.99 confidence score** which represents highest protein-protein interaction of 7OUB (ORM2 gene) with other proteins.

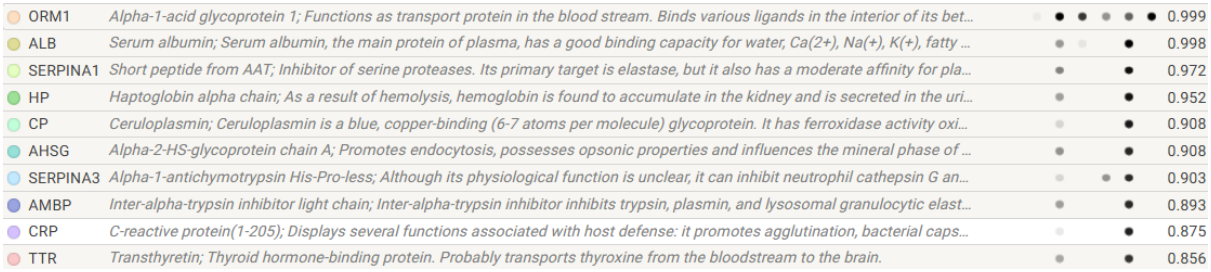

Figure 6: Predicted functional partner analysis of human proteomic sample

Analysis in network stats shows –

number of nodes: 11

number of edges: 55

average node degree: 10

avg. local clustering coefficient: 1

expected number of edges: 13

PPI enrichment p-value: < 1.0e-16
Wherein, P value < 1.0e-16 indicate protein in network are highly connected and functionally related, representing a biologically meaningful network rather than random interaction.

5. PROTEIN SITE AND PATTERNS

Prosite server as a valuable resource to annotate protein sequence and predict function to explore evolutionary relationship within protein sequence. The analysis in PROSITE scanning shows **Condition S**, **Condition T** and **Condition N** representing the protein sequence which ensures proper function but indirectly, the tumor specific and nodular regions respectively.

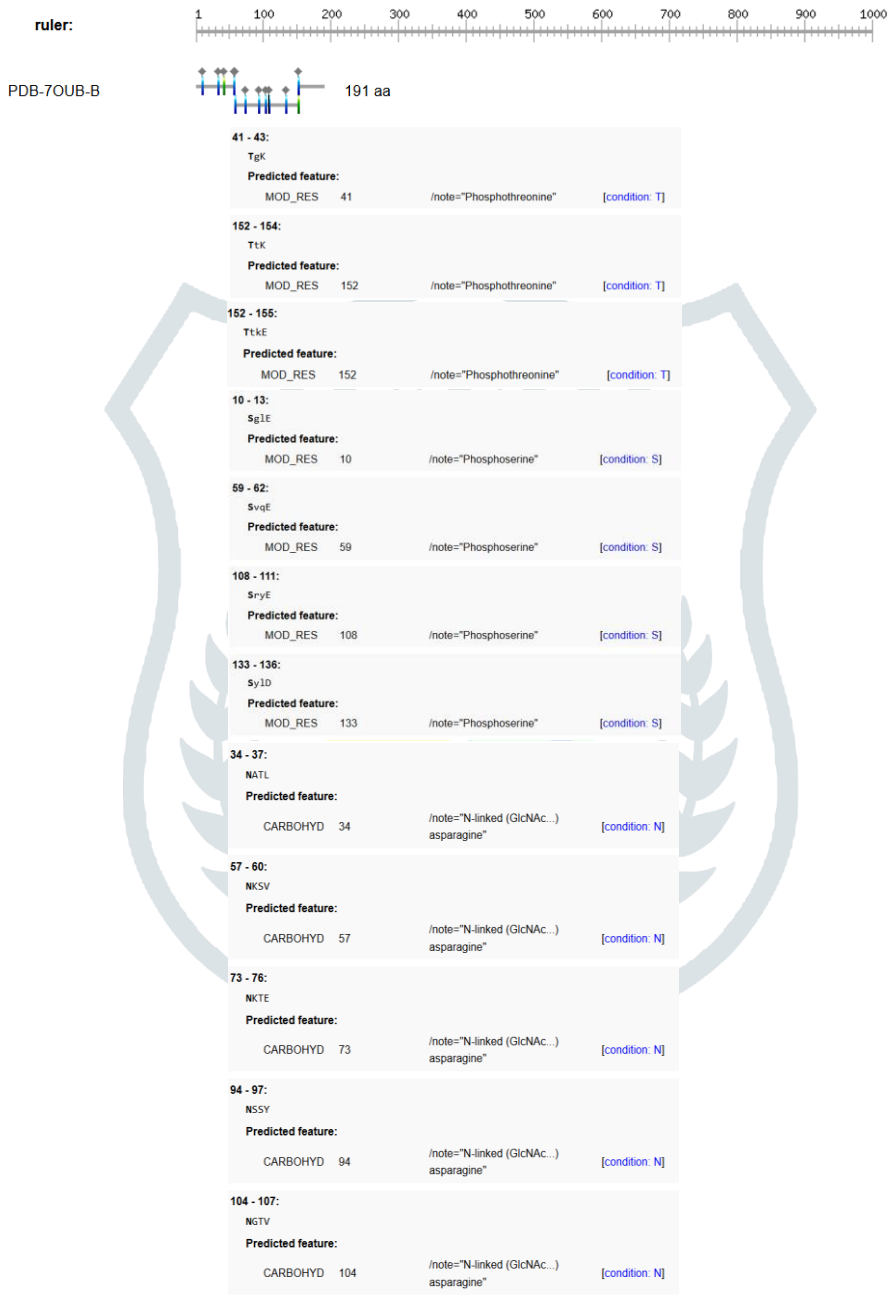

Figure 7: ScanProsite result analysis (Detection of Prosite signature matches of protein families and domains)

6. Cleft analysis in PDBsum

Cleft analysis was done which represents the potential binding site of 7OUB for ligand making which is crucial for drug designing purpose.

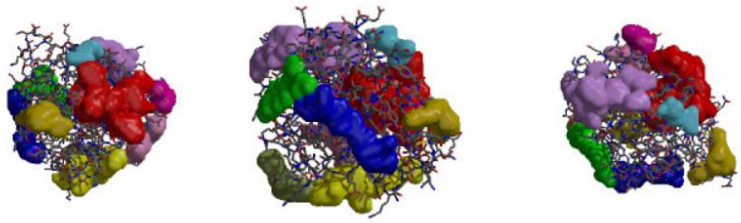

Figure 8: Cleft analysis of 7OUB

Residue-type colouring						
Positive	Negative	Neutral	Aliphatic	Aromatic	Pro & Gly	Cysteine
H,K,R	D,E	S,T,N,Q	A,V,L,I,M	F,Y,W	P,G	C

Figure 9: Cleft residue-type coloring

7. CB Dock

Molecular docking simulations revealed that 7OUB has multiple active sites for binding with the selected ligands i.e. Topotecan (Pubchem CID: 60700) and Irinotecan (Pubchem CID: 60838), making it a potential candidate for drug designing.

TOPOTECAN
Pocket: C1 and **Score: -10.1**

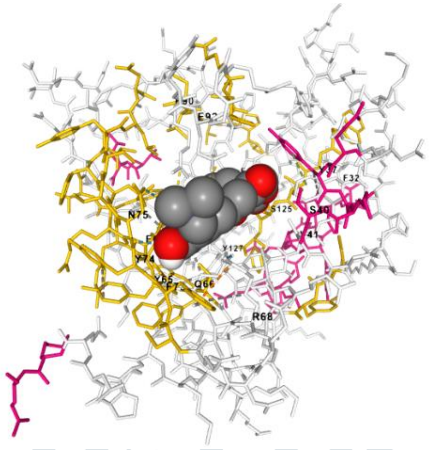

Figure 10: Molecular docking with protein – ligand interaction

Table 1: Molecular docking table score calculation

CurPocket ID	Vina Score	Cavity volume (Å^3)	Center (x, y, z)	Docking size (x, y, z)
C1	-10.1	1778	-6, 29, 0	24, 24, 24
C2	-8.0	157	-1. 23, -3	24, 24, 24
C4	-6.7	88	-13, 34, -16	24, 24, 24
C5	-6.7	76	-14, 39, 11	24, 24, 24
C3	-6.4	110	-10, 47, -4	24, 24, 24

Molecular docking indicates **stable binding affinity** between the ligand topotecan and 7OUB, with the **score -10.1 Kcal/ mol** representing relatively strong binding between the ligand and the protein.

IRINOTECAN
Pocket: C1 and **Score -9.2**

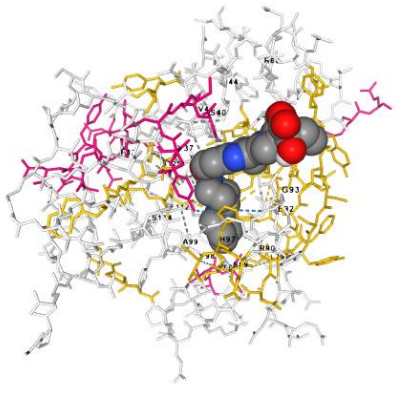

Figure 11: Molecular docking with protein – ligand interaction

Table 2. Molecular docking table score calculation

CurPocket ID	Vina Score	Cavity volume ($Å^3$)	Center (x, y, z)	Docking size (x, y, z)
C1	-9.2	1778	-6, 29, 0	30, 30, 30
C2	-8.7	157	-1. 23, -3	30, 30, 30
C4	-8.3	88	-13, 34, -16	30, 30, 30
C3	-7.5	110	-10, 47, -4	30, 30, 30
C5	-7.2	76	-14, 39, 11	30, 30, 30

Cb dock molecular docking indicates **stronger binding affinity** between the ligand Irinotecan and 7OUB with the **score -9.2 Kcal/ mol** representing favorable and strong binding between the ligand and the protein.

8. Catalogue of somatic mutation in cancer analysis

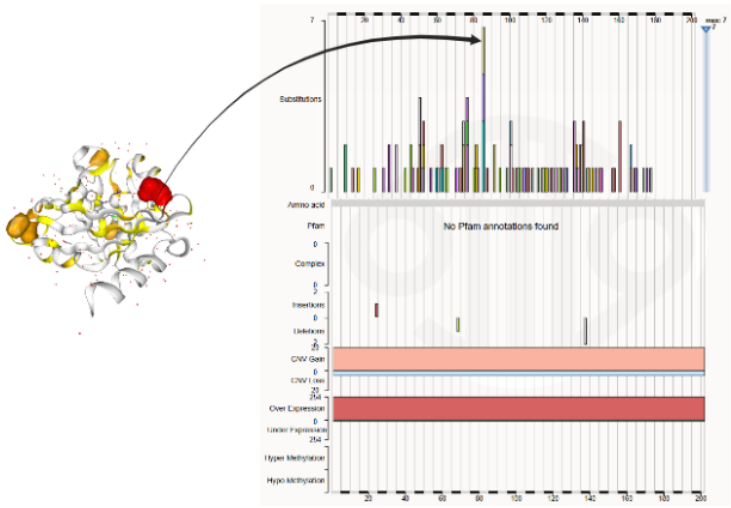

Figure 12: Gene histogram representation of ORM2 gene

The overexpression of gene from position 1-202 and deletion is observed at position 69 and 138 of amino acid residues. The most frequent two mutations observed across all cancers in ORM2 gene at position 86 (p.R86C (c.256C>T)) which is relevantly at peak in protein structure.

9. Multiple Sequence Alignment Analysis

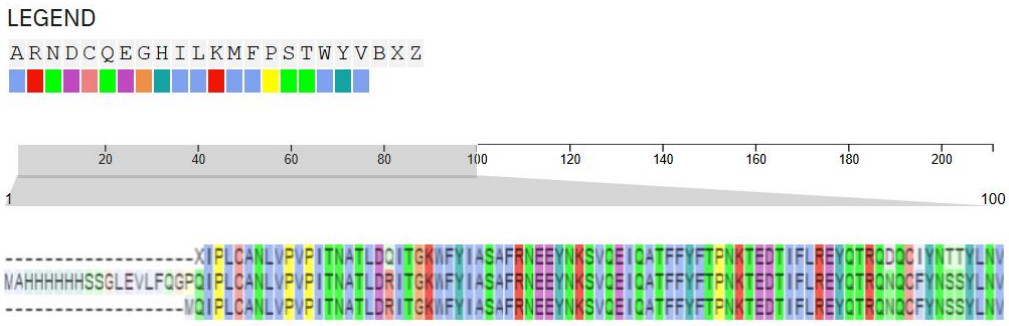

Figure 13: Multiple sequence alignment analysis (7OUB, 3APU and 3KQ0)

When performing a Multiple Sequence Alignment (MSA) using Clustal Omega for the protein sequences from PDB files 70UB, 3APU, and 3KQ0, the color coding in the alignment shows 151bp highly conserved amino acid residues with similarity among sequences.

Conclusion

Computational analysis based NGS pipeline server validation analysis detects the incorrect region of protein structure with high quality score which is accurate and reliable, representing a strong candidate for further computational or experimental studies in bioinformatic research for further analysis. In B factor region of structure, it will indicate destabilization. BIT score of 406 was observed which shows excellent alignment of sequence of sample, root mean square analysis two structures are nearly identical in the regions being compared, representing the same protein in slightly different conformational states or alignments of highly similar homologous proteins. The pathway analysis of malignancy reveals that cancer arise from complex, interconnected molecular network rather than simple mutation. STRING network helps to understand the interaction of AGP2 functional association, which represents insight into biological role and potential implication in cancer research. PROSITE scanning shows Condition S which represent specific criteria require for amino acid in protein sequence to ensure proper function and this term is not directly connect to malignant tumor but rather to functional character of protein which are involved in cancer development, alongwith Condition T which represents tumor specific region and Condition N which represents nodular regions. In computational analysis, PDBsum is a fundamental tool in structural biology and computational drug discovery that provides crucial insights into protein function and ligand interaction. In molecular docking, the score represents favorable and strong binding between the ligand and the protein. Cosmic database in malignancy research showing biological perspective to unchecked cellular growth which leads to destruction but also drives adaptation and survival. In MSA enables researchers to uncover critical biological insights from sequence data and the gap represents mutation in the form of insertion and deletion over evolutionary time.

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