Analyzing AKT1 Mutants Towards the Progression of Meningioma

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ABSTRACT

Meningioma, a common primary brain tumor, is frequently driven by recurrent somatic mutations in the AKT1 gene, particularly the E17K mutation, which leads to constitutive kinase activation and unchecked cell proliferation. This study investigates the structural basis of AKT1 activation and the potential for targeted therapeutic intervention using high-resolution crystal structures (PDB: 8UW7 and 8UW9). Notably, the E17K mutation enables a unique neo-zinc chelation mechanism that allows for mutation-specific inhibitor selectivity. Residue scanning analysis identified ASP 274 as a critical residue for protein stability, where specific substitutions (e.g., to MET, GLN, or GLU) significantly increased stability compared to other mutations. These findings elucidate the interaction landscape of AKT1 inhibitors and provide a foundation for the rational design of more precise, mutation-specific treatments to improve therapeutic outcomes for meningioma patients.

INTRODUCTION

Meningioma is a predominantly benign, slow-growing tumor that originates from the meninges, the protective layers surrounding the brain and spinal cord. Although most meningiomas are classified as non-malignant, their location within the central nervous system often leads to significant neurological complications. Meningiomas account for approximately one-third of all primary brain tumors, with their rate increasing with age and occurring more commonly in women.

Among the molecular drivers of meningioma, AKT1 mutations play a critical role in tumorigenesis. The AKT1 gene encodes serine/threonine kinase that is part of the AKT pathway, which regulates cell survival, growth, and proliferation. In normal cells, AKT1 activation is tightly controlled through phosphorylation and upstream growth factor signaling. However, recurrent somatic mutations in AKT1, specifically E17K mutation results in constitutive activation of the kinase. This signaling bypasses normal regulatory checkpoints, allowing meningeal cells to proliferate unchecked and resist apoptosis, thereby contributing to meningioma initiation and progression.

To investigate the structural basis of AKT1 activation in meningioma, we examined two high-resolution crystal structures of the mutant protein. Specifically, we used PDB: 8UW7 (1.97 resolution) and PDB: 8UW9 (1.90 Å resolution), which provide detailed insights into the conformational states of AKT1. PDB 8UW7 represents the wild-type AKT1 protein bound to a small-molecule inhibitor, while PDB 8UW9 represents the clinically relevant E17K mutant bound to an inhibitor. These structures are essential for structure-based drug discovery, as they reveal key domains and binding pockets that could be targeted to inhibit aberrant AKT1 signaling.

Several AKT inhibitors have already been developed, including capivasertib, ipatasertib, and MK-2206, which act as ATP-competitive or allosteric inhibitors AKT signaling. Capivasertiv has received FDA approval for cancers harboring AKT1, PIK3CA, or PTEN alterations, demonstrating that AKT1 is clinically actionable target. However, most current inhibitors target all AKT isoforms rather than being mutation-specific. This lack of selectivity underscores the need to design small-molecule inhibitors that specifically recognize the AKT1 mutation to minimize toxicity and improve therapeutic precision in meningioma treatment.

Currently, treatment options for meningioma include surgical resection and radiotherapy, though these approaches carry risks of recurrence and damage to surrounding neural tissue. Through our study we hope to find a more viable alternative through targeting small-molecular inhibitors present in AKT1. To achieve this we are particularly interested in performing site-directed mutagenesis to study key interactions in the inhibitor-binding region. By introducing targeted amino acid substitutions, particularly at residues involved in structural stabilization. We can identify which interactions are essential for binding and activity. These mutagenesis studies will provide a greater understanding of how inhibitors engage the AKT1 mutant and will guide the rational design of more specific and effective inhibitors for therapeutic development.

MATERIALS AND METHODS

All computational studies, including protein preparation, ligand optimization, molecular docking, and binding affinity calculations, were performed using the Schrödinger Suite 2024-3 (Schrödinger, LLC, New York, NY).

The crystal structure(s) of the target proteins were retrieved from the Protein Data Bank (PDB). Before docking studies, the protein was prepared using the Protein Preparation Wizard (Schrödinger Suite 2024-3) to ensure a chemically and sterically sound structure. This process involved several steps:

1. Bond Order Assignment: Covalent bonds, bond orders, and formal charges were assigned.
2. Hydrogen Addition: Hydrogen atoms were added to the protein structure, and their positions were optimized.
3. Metal Ion State Correction: The protonation and oxidation states of metal cofactors (e.g., zinc, magnesium) were corrected.
4. Water Removal: All water molecules outside a 5 Å radius of the active site were removed. The remaining water molecules were retained.

5. Side Chain Correction: The protonation states of histidine residues were determined based on the local hydrogen-bonding network.

6. Energy Minimization: A final energy minimization was performed using the Optimized Potentials for Liquid Simulations (OPLS4) force field to relieve steric clashes and optimize the geometry of the protein, while maintaining the positions of heavy atoms.

The nature of the interactions between the ligands and the target protein was analyzed to identify key binding motifs and residues.

1. Ligand Interaction Analysis: Visual analysis of the docked poses was performed using Maestro to identify crucial hydrogen bonds, π-π stacking, cation-π, and hydrophobic interactions between the ligand and the active site residues.

2. Protein Interaction Analysis: The Protein Interaction Analysis tool was used to quantify and characterize the network of interactions, including hydrogen bonds, salt bridges, and steric clashes, within the prepared protein structure and its complex with the ligand. This provided a detailed view of the interaction landscape beyond simple visual inspection.

To predict the effect of mutations on ligand binding, Residue Scanning Analysis was performed. This tool computationally simulates alanine mutations at key active site residues and calculates the change in binding energy (ΔΔG) for each mutation. The results were used to identify key residues that are critical for binding and to inform potential strategies for overcoming drug resistance or designing more potent ligands.

RESULTS AND DISCUSSION

PDB entries 8UW7 and 8UW9 both represent crystal structures of the kinase AKT1 bound to small-molecule inhibitors, but they both differ in the mutation status and design. The key difference in the two structures lies in the protein variant and the ligand. 8UW7 contains wild-type AKT1 complexed with compound 3, while 8UW9 contains the E17K mutant form of AKT1 bound to compound 4. This E17K mutation, which substitutes glutamate for lysine, is oncogenic and found in several human cancers. The 8UW9 structure reveals how this mutation enables a neo-zinc chelation mechanism, where the inhibitor interacts with the introduced lysine and recruits a zinc ion, resulting in selective inhibition of the mutant over the wild-type. In contrast, 8UW7 serves as a baseline comparison showing inhibitor binding in the normal protein without this zinc-dependent interaction.

Many mutations and research studies have been done on the protein AKT1. In one study, AKT1 mutations were specifically analyzed to determine their role in Moroccan bladder cancer patients. In another study, AKT1 mutations were analyzed in prostate cancer tissues from men from Jordan, focusing on exons 3 and 4 which include the PH domain important for protein signaling and localization. While the common E17K hotspot mutation was not found, several other alterations were identified, particularly in exon 4, including nine missense mutations (Phe27Tyr, Ser56PHe, Arg41Trp, etc.) and two novel variants (N53Y and Q59K). These mutations are predicted to potentially affect AKT1's structure and function. In another study, the

AKT1 mutation, E17K (a point substitution in the pleckstrin homology domain that constitutively activates the kinase), was included alongside ten ESR1 mutations in a 12-plex digital PCR assay designed to detect tumor-derived mutations in plasma cell-free DNA from breast cancer patients.

We conducted protein interaction analysis which allowed us to find the residues in the protein protein interface. We then mutated the residues in the active site to every other amino acid. This was done to study the stability of these mutants over the wild-type, to study the effect of the mutants on the protein.

Table 1: Mutated amino acids and their stabilities

Original	Mutated	d Stability
ASP 274	MET	-19.09
ASP 274	GLN	-17.7
ASP 274	GLU	-13.16
ASP 274	ILE	-11.34
ASP 274	VAL	-10.14
ASP 274	ARG	-8.92
THR 211	MET	-8.9
ASP 274	LEU	-8.11
ASP 274	TYR	-6.96
ASP 274	TRP	-6.07
ASP 274	PHE	-5.65
ASP 274	THR	-5.29

Table 1 summarizes the effects of specific amino acid substitutions on protein stability, indicating that most mutations result in a decrease in stability. In particular, mutations on ASP 274 consistently produce large increases in stability, with the most stabilizing ones being ASP 274 mutated with MET, GLN, and GLU. Substitutions to chemically similar or different residues cause substantial destabilization indicating that ASP 274 plays a critical role in maintaining the protein's structural form. Overall ASP 274 is a key spot for stability within the protein.

Our future plans involve focusing on the most stable residue mutants. We will use MMGBSA calculations to study small molecules binding to mutants. This method will allow us to measure how changes in energy affect binding. By mutating specific residues, we can see how each change directly impacts how the ligand interacts with the protein.

Comparing the results from different mutants will help us figure out which residues are important for strong binding and which ones weaken the interaction. This allows us to choose the best mutants for further study. Overall, this process will help us better understand the changes in the protein structure that affect ligand binding and stability.

CONCLUSION

In this study, we examined the role of the AKT1 protein and its E17K mutation in meningioma and why it is an important target for treatment. Our mutation analysis also showed that specific residues, especially ASP 274, are critical for maintaining protein stability, as mutation at this position caused large differences in stability. Overall, these findings help explain how AKT1 inhibitors interact with the protein and highlight the importance of studying specific mutations to support the design of more effective and targeted treatments for meningioma.

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ABBREVIATIONS

AKT1: AKT Serine/Threonine Kinase 1; ASP 274: Aspartic Acid at position 274; ATP: Adenosine Triphosphate; ESR1: Estrogen Receptor 1; OPLS4: Optimized Potentials for Liquid Simulations 4; PIK3CA: Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha; PTEN: Phosphatase and Tensin Homolog

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