IJHSR
ijhighschoolresearch.org
RESEARCH ARTICLE

Structural And Functional Insights into EGFR And EGFRvIII Using Computational Modelling and Docking

Atreya Manaswi

Orlando Science High School, 2600 Technology Dr, Orlando, FL 32804 USA; manaswiatreya@gmail.com

ABSTRACT: Glioblastoma (GBM) is the most aggressive form of adult brain cancer with a five-year survival rate of 6.9%. Epidermal growth factor receptor (EGFR) is a major target for cancer therapeutics, particularly glioblastoma. EGFR mutations are seen in approximately 50-60% of GBM cases. The most frequent EGFR mutation, EGFRvIII, results from a deletion of exons 2-7 and occurs in 34-63% of GBM genetic alterations. In this work, we research the effect of the EGFRvIII mutation by visualizing EGFR and EGFRvIII protein structures and performing molecular docking to 6 ligands. According to our analysis, the EGFR variant III has a generally negative effect on the binding affinity of the selected ligands compared to the wild-type EGFR structure. In addition, visualization of the structures revealed that the mutant is missing several residues of the binding domain. Therefore, we suggest that the EGFRvIII mutation may negatively affect EGFR structure, leading to glioblastoma proliferation.

KEYWORDS: Computational Biology and Bioinformatics, Computational Neuroscience, Glioblastoma, Epidermal growth factor receptor, EGFRvIII.

Introduction

Glioblastoma (GBM) is the most lethal, aggressive, and prevalent primary brain tumor in adults characterized by its malignancy and poor prognosis.[1] The current standard of care for GBM–maximal surgical resection in concert with radiation therapy and chemotherapy–has not resulted in beneficial outcomes for patients with a median survival time of only 14.6 months.[2,3] Epidermal growth factor receptor (EGFR) is a transmembrane receptor tyrosine kinase that is key in regulating cell growth, proliferation, and survival in tissues originating from epithelia.[4] Atypical EGFR signaling is a hallmark of various cancers, particularly glioblastoma.[5] EGFR mutations are expressed in approximately 50-60% of GBM patients, making them attractive targets for therapeutic intervention.[3] The most common EGFR mutation - EGFRvIII (also known as de2-7EGFR and ΔEGFR) - occurs due to the in-frame deletion of exons 2-7, 801 base pairs (bps), from the wild-type (WT) EGFR coding sequence.6 The EGFR variant III mutation is found in ~50% of EGFR-amplified GBM cases and is linked to increased glioma cell proliferation.[7] The EGFRvIII mutation's role as a therapeutic target is controversial but its oncogenic potential has called for recent development in mutant receptor-targeted therapeutics.[8] These EGFR mutations are associated not only with glioblastoma but also with other cancers, including non-small cell lung cancer, head and neck cancers, and colorectal cancer. These mutations result in the constitutive activation of the EGFR signaling pathway, contributing to tumor development and rendering tumors resistant to various therapies. The different ways in which EGFR is targeted by various TKIs influence the different treatment options for these malignancies. Erlotinib and gefitinib, small molecule tyrosine kinase inhibitors (TKIs), have shown early promise in preclinical and clinical studies, but their efficacy is often limited by the emergence of resistance mechanisms.[9,10] The TKIs employed in this analysis were Erlotinib (Tarceva), Gefitinib (Iressa), Afatinib (Gilotrif), Dacomitinib (Vizimpro), and Osimertinib (Tagrisso). Erlotinib and Gefitinib are examples of EGFR TKIs; both drugs are reversible inhibitors generally used in NSCLC with EGFR mutations, although resistance may eventually build up. Afatinib is an irreversible TKI that targets EGFR, HER2, and HER4, administered in specific EGFR mutations. Dacomitinib is also irreversible and provides more potent EGFR inhibition, used as a first-line EGFR-mutated NSCLC treatment. Osimertinib is an irreversible TKI of the third generation, targeting both sensitizing and resistance mutations such as T790M, and is thus indicated in both new diagnoses and resistance.

Understanding the structural and functional ramifications of the EGFRvIII mutation remains essential. The primary objective of this study was to analyze the structural differences and explain the potential functional ramifications of the mutated EGFRvIII structure compared to its wild-type EGFR form. We also docked EGFR and EGFRvIII to 6 ligands to compare binding characteristics between the structures. Molecular docking simulations work by predicting the likely orientation of a ligand when bound to a target protein. This aids in identifying binding affinities and simulating interactions to improve therapeutic efficacy. By mapping binding sites, structural similarities, etc. we aimed to gain insights into the structural determinants of drug binding and resistance. Two major parameters have also been analyzed: SwissParam-derived binding affinity scores and Attracting Cavities scores. Binding affinity scores reflect alterations in the receptor-mediator interaction involving EGF and therapeutic agents upon mutation and thus the effects on the efficacy of targeted treatments. AC scores give a detailed analysis of the stability of confirmation and

DOI: 10.36838/v7i3.12

structural changes that may affect receptor function. Collectively, these measurements give a more defined account of how the EGFR mutations confer cancer aggressiveness and therapeutic resistance. The findings of this study may inform the development of novel therapeutic strategies that specifically target EGFRvIII-driven GBM.

Methods

We first determined which protein mutations influenced glioblastoma (GBM) progression through an extensive literature review and data analysis. Genomics repositories such as The Cancer Genome Atlas (TCGA) and cBioPortal were utilized to identify frequently mutated genes associated with GBM. After identifying EGFR as a focal interest, we consulted various repositories to locate appropriate structures for analysis. The WT EGFR structure (ID 1ivo)[11] was retrieved from the Protein Data Bank in Europe (PDBe). The EGFRvIII structure (ID 8UKX)[12] was retrieved from Protein DataBase (PDB).

The EGFR and EGFRvIII PDB files were loaded into PyMOL for visualization. Subsequently, they were aligned using the command [align X, Y] to compare the residues. This highlighted the core regions of EGFR and EGVRvIII having high structural similarity. The root mean square deviation (RMSD) was calculated to quantitatively define alignment quality. The command employed was [rms_cur target, mobile].

Using SwissDock, 12 molecular docking simulations were run. 6 ligand-target simulations were performed for the EGFR and EGFRvII each. The 6 ligands chosen consisted of epidermal growth factor (EGF) and tyrosine kinase inhibitors (TKIs) which are small-molecule EGFR inhibitors designed to block kinase activity and prevent downstream signaling that exacerbates tumor growth. The selected ligands were Epidermal Growth Factor, Erlotinib (Tarceva), Gefitinib (Iressa), Afatinib (Gilotrif), Dacomitinib (Vizimpro), and Osimertinib (Tagrisso). These ligands were selected due to their drug-like size and structural availability. In SwissDock, the ligand and target PDB files were loaded. The search space was automatically defined and Random Initial Conditions was set to 1. Thereafter, the Attracting Cavities (AC) (the ligand binding modes in docking simulation) score and SwissParam scores (approximation of ligand's binding free energy to a target) were interpreted and statistically analyzed to determine differences between the binding of the ligands to the EGFR and EGFRvIII structures.

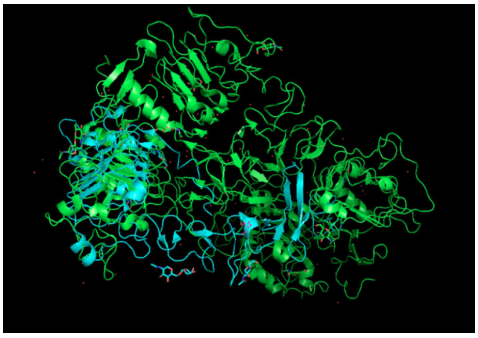

Figure 1: Overlayed structures of EGFR and EGFRvIII in PyMOL visualization. Key conformational differences in the extracellular domain and within the mutation-specific region of EGFRvIII are shown.

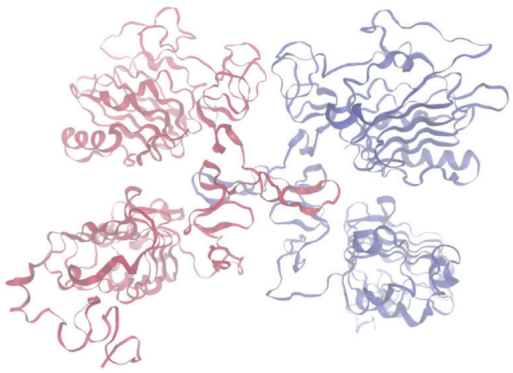

Figure 2: Epidermal growth factor and epidermal growth factor receptor dimerization arm in SwissDock. Binding occurs within the amino-terminal 622 amino acids extracellular region.

Results and Discussion

Structural Comparison of EGFR and EGFRvIII:

Visualization confirmed the EGFRvIII mutation leads to the loss of nearly the entire domain I and a portion of domain II in the EGFR extracellular region. The EGFRvIII structure was significantly smaller than EGFR due to the truncation of exons 2-7. Despite this notable size difference, the structures showed similar alignment with several regions corresponding (Figure 1). The MatchAlign score of 1183.500 generated by PyMOL indicated high alignment but the RMSD score of 15.415 suggests significant structural differences, being, on average, the corresponding atoms in the structures are 15 Å apart after alignment. Visualization of the protein structures confirmed these differences in conformation and visibility of the ligand binding site between the structural variants (Figure 2). Additionally, the absence of a part of domain II in EGFRvIII disrupted the dimerization arm, a key structural element involved in ligand-induced EGFR dimerization and subsequent activation.

Molecular Docking Simulations

Molecular docking simulations were performed to characterize the interactions between EGFR, EGFRvIII, and six ligands in SwissDock. The results, summarized in Table 1 show that EGFRvIII has consistently higher AC scores compared to EGFR across all ligands. However, the SwissParam scores, which estimate binding free energy, generally favored EGFR over EGFRvIII indicating a stronger predicted binding affinity for the wild-type receptor.

Statistical Analysis:

All data were analyzed using R version 4.2.0 (R Core Team, 2021). Paired t-tests were performed to compare the AC scores and SwissParam scores between EGFR and EGFRvIII for each ligand. The resulting p-values were adjusted using Bonferroni correction to account for multiple comparisons. Statistical analysis revealed significant differences for both AC scores ($t = -9.03$, $p < 0.001$) and SwissParam scores ($t = -3.86$, $p = 0.024$) between EGFR and EGFRvIII across all six ligands, even after Bonferroni correction. These results confirm that EGFRvIII leads to a significant reduction in ligand binding affinity compared to the wild-type receptor.

DOI: 10.36838/v7i3.12

Table 1: Molecular Docking Results for EGFR and EGFRvIII to 6 Ligands.

Ligand	EGFR AC	EGFRvIII AC	EGFR SwissParam	EGFRvIII SwissParam
EGF	436.500	5306.751	-7.5629	-6.6026
Erlotinib	145.410	5098.166	-7.0965	-6.2711
Gefitinib	159.543	4430.670	-7.0214	-6.4773
Afatinib	170.150	3655.460	-6.6018	-6.5527
Dacomitinib	71.971	7488.680	-7.6954	-6.0985
Osimertinib	233.906	6477.591	-7.6124	-6.7766

Discussion

This research investigated the structural and functional implications of a major oncogenic driver in glioblastoma: the EGFRvIII mutation. We used *in-silico* modeling and molecular docking simulations to compare EGFRvIII structure with that of wild-type EGFR and found critical differences in the ligand-binding region and dimerization arm of the receptor. Our structural data indicate that EGFRvIII adopts a tethered-like conformation, as has been observed in the unliganded state of wild-type EGFR.[13] This conformation may prevent ligand-induced dimerization with wild-type EGFR and may affect its signaling and interaction in GBM.

Our docking simulations with different ligands, including the natural ligand EGF and different types of TKIs, emphasize that EGFRvIII, in general, has lower binding affinity compared to the wild-type EGFR. This observation of our structural analysis where we identified that the EGFRvIII mutation led to the loss of residues crucial in ligand binding. The lower ligand binding affinity of EGFRvIII could interfere with normal signaling by EGFR and this could partly explain the uncontrolled cell division associated with glioblastoma. Moreover, the modified ligand binding pocket might account for the poor response of the existing EGFR-targeted therapies in treating glioblastoma with EGFRvIII.

Interestingly, docking results also revealed that EGFRvIII can still interact with certain ligands, including EGF, despite its impaired binding affinity. This observation is consistent with previous studies suggesting weak ligand binding to EGFRvIII.[2,5] The ability of EGFRvIII to bind ligands, even with reduced affinity, raises questions about its potential to activate downstream signaling pathways and contribute to tumorigenesis.[6] The structural analysis from the reference paper further supports this notion, showing that domain III of EGFRvIII remains accessible for ligand binding. The EGFRvIII is a mutant form of the epidermal growth factor receptor that contains ligand-independent, constitutive activation. Whereas its wild-type counterpart requires EGF binding to attain an active state, the EGFRvIII is, by contrast, constitutively active without this requirement. This leads to persistent activation of downstream signaling pathways, such as PI3K/AKT and RAS/RAF/MEK pathways, implicated in tumor growth and survival. Moreover, defective endocytic degradation of EGFRvIII results in prolonged residence of the receptor at the cell surface and an additional contribution to chronic oncogenic signaling. These properties explain why diminished binding of EGF does not diminish the tumorigenic competence of EGFRvIII and instead renders it more aggressive and challenging for therapeutic interventions.

Due to the heterogeneity of EGFR mutations in glioblastoma, targeted approaches should focus on specific molecular anomalies driving tumor growth. Future work should address characterizing structural and functional implications of other common EGFR mutations for glioblastoma. While this computational analysis provides promising insights, these results should be experimentally validated by future research studies. Additionally, further research should investigate combination therapies that target numerous pathways.

Conclusion

In summary, our computational study of EGFR and EGFRvIII suggests that the EGFRvIII mutation drastically changes the conformation of the receptor's ligand-binding domain and therefore decreases the binding of different ligands to the receptor. Such structural changes may be related to the malignant behavior of glioblastoma and may be the reason for the poor sensitivity of current EGFR-targeted therapies in treating tumors with the EGFRvIII mutation.[14] The lowered ligand binding affinity of EGFRvIII may further lead to uncontrolled cell division and tumor formation – two characteristics of glioblastoma. Because EGFRvIII is such a significant factor in GBM progression, the creation of targeted drugs that block the receptor continues to be an active line of investigation.[3]

These results highlight the importance of individualized treatment approaches targeting molecular subtypes of GBM, especially EGFR.[2] Further research should concern the possibility of targeting EGFR along with EGFRvIII or other signaling pathways related to glioblastoma development.

Acknowledgments

The author sincerely thanks Taylor Hailstock from Emory University School of Medicine and Dr. Hamidreza Shaye from Stanford University Department of Molecular and Cellular Physiology for providing guidance and technical support to this research.

References

1. Cloughesy, T. F.; Cavenee, W. K.; Mischel, P. S. Glioblastoma: Fro m Genetic Disease to Precision Medicine. *Cell* **2014**, *156* (3), 715 –29.
2. Nathanson, D. A.; Mischel, P. S. EGFR Inhibitors in the Treatme nt of Glioblastoma: When One Target Is Not Enough. *Cancer Cel l* **2010**, *17* (3), 221–222. https://doi.org/10.1016/j.ccr.2010.02.017.
3. Brennan, C. W.; Verhaak, R. G. W.; McKenna, A.; Campos, B.; N oushmehr, H.; Salama, S. R.; Verhaak, R. G. W. The Somatic Gen omic Landscape of Glioblastoma. *Cell* **2013**, *155* (2), 462–477.htt ps://doi.org/10.1016/j.cell.2013.09.034.
4. An, Z.; Aksoy, O.; Zheng, T.; Fan, Q.-W.; Weiss, W. A. Epidermal Growth Factor Receptor and EGFRvIII in Glioblastoma: Signal ing Pathways and Targeted Therapies. *Oncogene* **2018**, *37* (12), 15 61–1575.
5. Huang, P. H.; Xu, A. M.; White, F. M. Oncogenic EGFR Signali ng Networks in Glioma. *Sci. Signal.* **2009**, *2* (87), re6. https://doi. org/10.1126/scisignal.287re6.
6. Sugawa, N.; Ekstrand, A. J.; James, C. D.; Collins, V. P. Identical S

plicing of Aberrant Epidermal Growth Factor Receptor Transcripts from Amplified Rearranged Genes in Human Glioblastomas. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87* (21), 8602–8606.
7. Nishikawa, R.; Ji, X. D.; Harmon, R. C.; Lazar, C. S.; Gill, G. N.; Cavenee, W. K.; Huang, H. J. A Mutant Epidermal Growth Factor Receptor Common in Human Glioma Confers Enhanced Tumorigenicity. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91* (16), 7727–7731.
8. Bagchi, A.; Haidar, S.; Richards, C. W.; Halmos, B. Targeting EGFR Mutations in Glioblastoma: Challenges and New Therapeutic Strategies. *J. Cancer Ther.* **2024**, *13* (5), 234–242.
9. Tanaka, K.; Uchida, H.; Yokogami, K.; Wakabayashi, T.; Yamashita, K. EGFR-Dependent and - Independent Activation of Akt/mTOR Signaling in Glioblastomas. *Neuro-Oncol.* **2011**, *13* (6), 622-630. https://doi.org/10.1093/neuonc/nor025.
10. Vivanco, I.; Rohle, D.; Verhaak, R. G. W.; Mellinghoff, I. K. Cancer Biology: Resistance to EGFR Inhibitors in Glioblastoma. *Nature* **2012**, *487* (7408), 396–397.
11. Ogiso, H.; Ishitani, R.; Nureki, O.; Fukai, S.; Yamanaka, M.; Kim, J. H.; Saito, K.; Sakamoto, A.; Inoue, M.; Shirouzu, M.; Yokoyama, S. Crystal Structure of the Complex of Human Epidermal Growth Factor and Receptor Extracellular Domains. *Cell* **2002**, *110* (6), 775–787. https://doi.org/10.1016/s0092-8674(02)00963-7.
12. Bagchi, A.; Stayrook, S. E.; Xenaki, K. T.; Starbird, C. A.; Doulkeridou, S.; El Khoulati, R.; Roovers, R. C.; Schmitz, K. R.; van Bergen En Henegouwen, P. M. P.; Ferguson, K. M. Structural Insights into the Role and Targeting of EGFRvIII. *Structure* **2024**, *32* (9), 1367–1380.e6. https://doi.org/10.1016/j.str.2024.05.018
13. Lopez-Gines, C.; Cerda-Nicolas, M.; Gil-Benso, R.; Lluch-Bernal, M. Mutant EGFRvIII in Glioblastomas: Pathological and Clinical Implications. *J. Clin. Oncol.* **2010**, *28* (15), e19375.
14. Inda, M. M.; Bonavia, R.; Mukasa, A.; Narita, Y.; Sah, D. W. Y.; Vandenberg, S.; Cavenee, W. K. Tumor Heterogeneity Is an Active Process Maintained by a Mutant EGFR-Induced Cytokine Circuit in Glioblastoma. *Genes Dev.* **2010**, *24* (16), 1731–1745. https://doi.org/10.1101/gad.1890510.

Author

Atreya Manaswi is a senior at Orlando Science High School. He is passionate about finding solutions to real-world problems using biotechnology. He hopes to major in the biological sciences and continue pursuing his love of research.

Data Availability Statement

The data supporting the results are available in a public repository at: https://zenodo.org/records/13731484.

DOI: 10.36838/v7i3.12