In Silico Development of EGFRvIII-Targeted Aptamer for Fluorescence Imaging of Glioma

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The most prevalent and deadly type of malignant brain tumor is glioma. It has been established that one of the most significant indicators of overall survival, progression-free survival, and neurological prognosis is the extent of resection. Maximum safe resection therefore becomes crucial. Thus, there is an urgent need for a method that can identify the tumor's border in order to achieve maximal safe excision while safeguarding the healthy brain tissues. Recently, a nanoprobe tagged with quantum dots (QDs) has been designed to specifically bind to tumor cells through aptamers. These aptamers attach to the overexpressed EGFRvIII (epidermal growth factor receptor variation) in glioma cells. Our theory is that these aptamers' strong binding and high activity are caused by their ability to bind to the receptor's active site, which allows them to inhibit the receptor's function. In the present study, we have investigated how these aptamers interact with EGDRvIII receptors using molecular docking simulations. Additionally, we employed artificial intelligence (AI) techniques such as graph neural networks (GNN) and machine learning (ML) to corroborate our findings and elucidate the workings of aptamers. Our findings indicate that the aptamer binds firmly to the receptor's active site. In order to improve the current aptamers, we have also executed changes in the aptamer sequences. As a result, we discovered that E-M4 created binds to the protein's active site. In conclusion, the current study will contribute to the development of aptamers targeted at glioma detection and treatments, as well as to our understanding of the binding mechanism of these aptamers to the EGFRvIII receptor.

1. INTRODUCTION

Glioma can be classified as a type of tumor which originates from glial cells in the brain or spinal cord that vary in terms of location, size, and grade (1, 2). These factors determine the tumor's aggressiveness and prognosis. Depending on the severity of the tumor, it can fall under various subtypes, such as glioblastomas, astrocytomas, and more (3-5). Currently, treatment involves radiation therapy, surgery, or chemotherapy tailoredQ to the tumors grade and patient's overall health (2). Gliomas contribute to the universal number of cancer cases as they account for a significant burden on the USA and on a global scale. Glioblastoma multiforme (GBM) is the most common brain tumor in adults, making up around 27% of brain cancer overall (6). The persistent high mortality rate associated with gliomas emphasizes the urgent need for continued research and their status as a critical public health issue.

EGFRvIII (Epidermal Growth Factor Receptor variant III) is the mutant form of the epidermal growth factor receptor (EGFR), and is particularly associated with glioblastomas, an aggressive brain cancer (7-9), Figure 1. This mutation results in a truncated receptor from the deletion of exons 2-7 in the EGFR gene (9). Consequently, since it lacks a portion of its extracellular ligand-binding domain, this receptor is continually active (9,10). The EGFRvIII mutation is a significant target for cancer therapy because it is not expressed in normal tissues, but in various tumors (10). The perpetual activation of this mutation results in tumor growth and resistance to programmed cell death (apoptosis), as it causes uncontrolled cell proliferation and survival (11). The EGFRvIII mutation is a vital target for novel cancer therapies including vaccines, small molecule inhibitors, and targeted antibodies (12). These are therapeutic approaches to target EGFRvIII by inhibiting its function and stimulating the immune system to attack cells expressing this mutant receptor (13,14). This mutation acts as a biomarker which can help predict disease prognosis and diagnose certain cancers (15-17).

Molecular docking is a sophisticated computational method in molecular biology which predicts the ligand-receptor interactions (7-12). It can be used to identify the binding site on the receptor surface and shares a significant role in pharmaceutical research to identify potential drug candidates with high binding

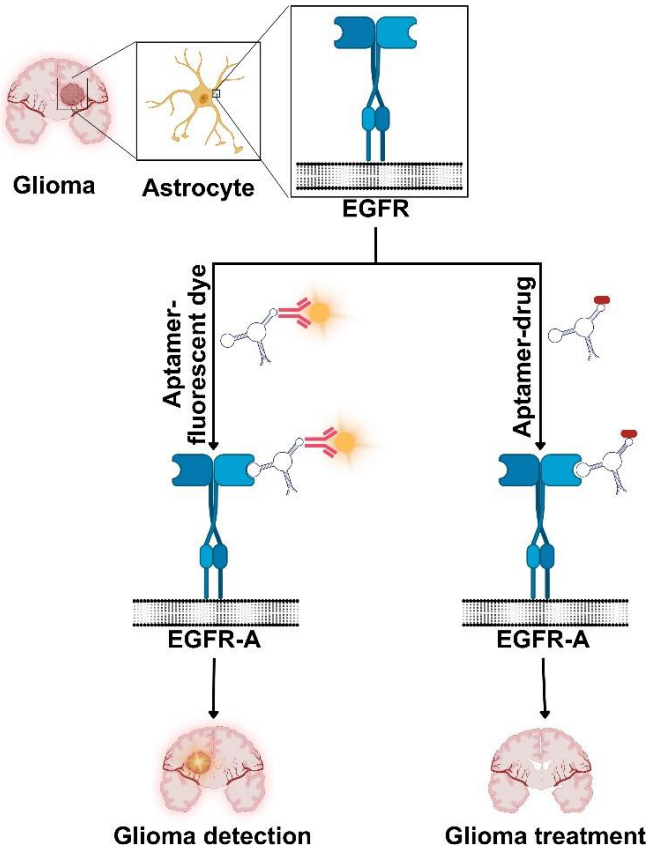

Fig. 1. Scheme of research.EGFRvIII is often overexpressed in gliomas, making it a crucial target for cancer therapies. The aptamers utilized in this research specifically bind to these receptors, serving as both cancer biomarkers and agents for targeted cancer therapy.

affinity. Aptamers are nucleotide that are single stranded, often referred to as "chemical antibodies" that bind to specific target molecules (13). The aptamers are made from a process called as SELEX in which a large pool of random DNA or RNA gets selected based on their interactions to the target protein (14). They are getting popularity due to their target molecules detecting properties, as therapeutic agents to inhibit the activity of target proteins. Write 4-5 lines about alpha Fold 3.

One of the most prevalent genetic variations in glioma is the emergence of EGFRvIII which is highly active, mutant form of EGFR receptor and promotes cell proliferation. The mutant form is predominantly found in glioma and are rarely observed in other types of cancer making them a crucial biomarker in the cancer detection. Recently, aptamer-based fluorescence imaging targeting these receptors has been developed (15). Recently, Tang et al. have developed aptamers bound with quantum dots on its surface (15). EGFRvIII are mutated form of EGFR and are commonly found on the surface of glioma cells (16). The aptamer reported in Tang et al. paper specifically binds to the EGFRvIII proliferated glioma cells, while the QD will fluorophore these cells and help in cancer cell detection (15). Some of the advantages of this approach are as follows: firstly, the aptamer specifically interacts with EGFRvIII receptor that is a mutant and truncated form of wild type (wt) EGFR receptor, Figure 4. We hypothesize that the strong interactions and activity of these aptamers is due to its interaction to the active site of the receptor and hence it can block the activity of the receptor. In the current investigation work we have studies the interactions of these

aptamers to the EGFRvIII receptor and their interactions has been computed. The results demonstrate that upon truncation the binding site of the mutant receptor changes and the aptamer binds to the new binding site. These findings were are validated by using both machine learning suing P2Rank and graph neural network utilizing GrASP analysis. The current investigation will not only help in designing glioma imaging technique but also aid as a potential therapeutic strategy against the disease.

2. RESULTS

In this study, we developed and investigated EGFRvIII-targeted aptamers using molecular docking and artificial intelligence tools to enhance glioma fluorescence imaging. Our results demonstrate that the aptamers bind strongly to the receptor's active site, supporting their potential for glioma detection and treatment. According to our hypotheses, the EGFRvIII receptor has a particular binding location where the aptamer should bind. In order to find this site, we first used graph neural networks (GNN) using the Graph Attention Site Prediction (GrASP) web server, Figure 2: The druggable site is displayed. The term "druggable site" refers to the protein's particular locations that medications can be bound to with great efficiency. Finding the druggable location is essential for creating medications that can attach to these biomolecules.

In Figure 3's 2D pictures, Vfold2D predicted the aptamer secondary structures, and Table 1 displays dot-bracket notation. These secondary structures give rise to base pair interactions, or hydrogen bonds, which are essential for figuring out the aptamer's three-dimensional form. The resulted protein-aptamer complexes are shows in Figure 4 a and b which shows that both the aptamers interact to the binding site (suggested by GrASP(17)) of the mutant receptor. These results were further validated by AlphaFold 3(18) which shows similar binding site. Based on these we have also performed mutations in the aptamer structures and formed four mutant aptamers (E-M1, E-M2, E-M3, and E-M4). Later these mutants were docked on the EGFRvIII receptors among which aptamer E-M4 interact to the surface and binding region of the receptor, Figure 5. The interactions formed between receptor-aptamer complex were calculated by using the PLIP interaction tool (19). PLIP analysis suggested that aptamer E formed 16 hydrogen bonds, one π cation bond, and 3 salt bridges, shown in Table 2. Four salt bridges and eight hydrogen bonds were produced by aptamer G. E-M1 generated two π cation interactions, nine salt bridges, and twenty hydrogen bonds. Four salt bridges, one π cation interaction, and seventeen hydrogen bonds were all produced by aptamer E-M2. Aptamer E-M3 generated three salt bridges, one π cation contact, and 29 hydrogen bonds. Aptamer E-M4 generated two salt bridges, four π cation contacts, and seven hydrogen bonds.

3. DISCUSSION

Surgical resection in glioma is a surgical procedure to remove the glioma tissues and is a critical component in the disease treatment for best possible outcome (20). Unfortunately, this is a complex and challenging process to remove the cancerous tissues without harming normal tissues (4). Therefore, a technique that can more effectively distinguish the normal cells with cancer cells can help in maximum safe resection. Fluorescence-guided surgery have gained attention for its success in targeting glioma cells (20, 21).

The truncated EGFRvIII receptor was modeled using AlphaFold 3 (18) (an AI tool to suggest the 3D structure of proteins). A prime difference between the two proteins is the presence of extracellular ligand binding domain (where the EGF binds) in the wt, while in the mutant form the head region of the protein is truncated and results in the loss of this EGF binding region (16, 22). The truncation and loss of the EGF binding site also results in the receptor dysregulation initiating a signaling cascade and promoting cell proliferation and cancer (16, 23). Therefore, we first predicted the binding site on the surface of this receptor. GrASP(17) web server shows that the EGFRvIII have druggable site as shown in Figure 2a. ESP shows that this site was predominantly positive in nature and should favors the negative aptamer binding. Later molecular docking simulations and AlphaFold 3 protein-aptamer structures prediction further validated the positive protein and negative aptamer interactions as shown in Figure 3. Another advantage using aptamer have over antibody is its lower immunogenicity, heat stability, easy of modification, cost effectiveness, smaller size, and higher receptor specificity (24, 25). The aptamer used in this research have shown higher specificity against the EGFRvIII receptor in glioma (15). In addition, the aptamers can be linked to conventional fluorescent dyes and also with quantum dots making them an effective tool for cancer detection. The work done by Tang et al shows that the QD-Apt complex has decreases toxicity and also increases specificity (15). Finally, since these aptamers specifically target the EGFRvIII they can be used to target drugs against the cancer cell.

Study of aptamers conjugating with various anti cancerous drugs have been done. For instance, pegaptanib was the first FDA approved aptamer used for age-related macular degradation, a disease causes blindness in elderly people (26). Pegaptanib is used as an antagonist of VEGF receptor and is used for age-related macular degradation (26). In addition, the clinical phase II study for As1411 aptamer is currently underway for the therapy of metastatic renal cell carcinoma (27). Similar cancer diagnosing studies have been performed in which DNA based aptamers targeting EGFRvIII receptor in glioblastoma have been developed (3). Another study also depicts the potential of aptamer as a molecular imaging probes against glioblastoma (28). The following are a few of the limitations of this study: The quality of the models and algorithms employed is the primary factor that significantly influences the accuracy of molecular docking. To verify the molecular docking simulations, we have used both graph neural network (GNN) and machine leaning (AlphaFold 3) to confirm the binding site and the docked structures, respectively. In future studies, we would like to include the protein-aptamer behavior in the presence of water surrounding the protein by performing molecular dynamics simulations. In addition, binding energy calculations should be performed to determine the strength of the interaction and whether the aptamer remains bound to the protein for the entire simulation. Furthermore, experimental evaluations like SPR and ITC will help in validating the EGFRvIII-aptamer binding interaction (29).

In conclusion, in the current research work molecular docking simulations and different artificial intelligence tools has validated the strong binding affinity of these aptamers to the active site of EGFRvIII. This demonstrates their potential to block receptor activity and aid in glioma detection and therapeutics. The study has first explored the binding interactions of EGFR mutant and based on this knowledge mutant form of aptamers were obtained. This study adds to our understanding of EGFRvIII-

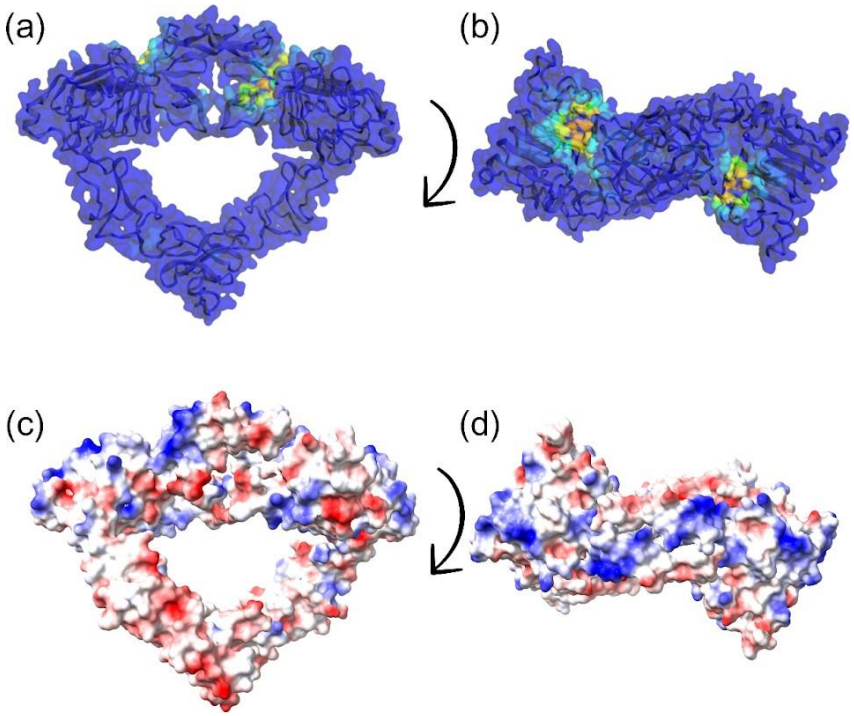

Fig. 2. Binding site of EGFRvIII. (a) shows the side view of the receptor; (b) shows the top view of the receptor. These two pictures show the binding site (in yellowish green) of the receptors; and (c) and (d) shows the electrostatic surface potential (ESP) of the receptor which shows that the top part of the protein is positive/blue in nature.

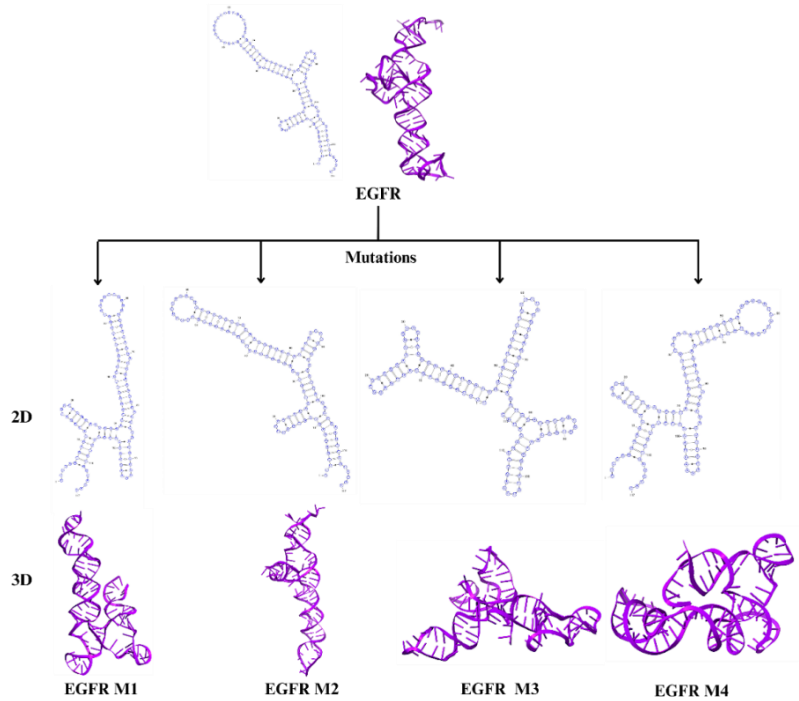

Fig. 3. Aptamer secondary (2D) and tertiary (3D) structure. The 2D and 3D structure shows that they are of different shapes which can affect their binding. The 2D structures are crucial to get the nucleotides binging. This information was used to model the 3D structure of aptamers.

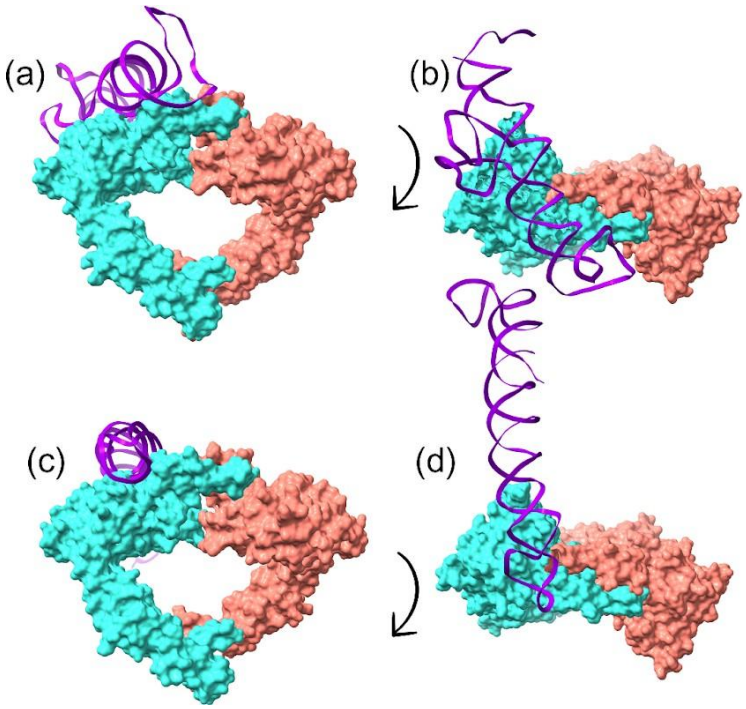

Fig. 4. EGFRvIII-aptamer interactions obtained from molecular docking and AlphaFold 3. (a) and (b) EGFRvIII and aptamer binds to the positive head region of the protein obtained from molecular docking; and (c) and (d) shows the aptamer binds to the similar region obtained from AlphaFold 3.

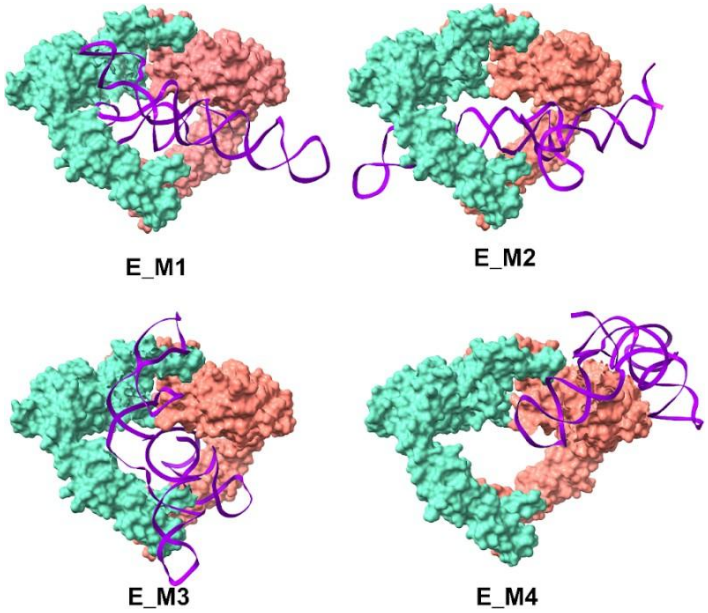

Fig. 5. EGFRvIII and mutant aptamer interactions. Based on molecular docking simulations aptamers were mutated to obtain aptamers with modified binding properties. Out of these four mutant aptamers, E_M4 shows promising results since the binding site is occupied by the aptamer.

EGFRvIII-aptamer bonds . The bonds formed between the aptamer base pairs and protein amino acids are depicted in Angstrom aptamer interactions and advances the development of accurate glioma diagnostic methods and treatment approaches.

4. METHOD

THE PDB ID of the EGFR receptor was 3njp and was downloaded from the protein data bank (30). The mutant form of EGFRvIII is a truncated EGFR and was derived from the AlphaFold3 software (18). The starting point for structural predictions was the primary nucleotide sequence obtained from prior work(15), and was first subjected to secondary structure prediction using Vfold2D (31). The server returns the nucleotide sequence in dot-bracket notation to represent their three-dimensional conformation. Unpaired nucleotides are shown by dots, while paired nucleotides that form base pairs are indicated by matching opening and closing parenthesis to show the coupling. The tertiary structure was modeled using the molecular visualization tool, Vfold3D (32). The first aptamer with the lowest energy state was utilized for molecular docking if it had more than one cluster. HDock docking software was used for docking of aptamer to the epidermal growth factor receptor (EGFRvIII) (33). The receptor and aptamer structures were uploaded to the HDock server, to predict the aptamer-EGFRvIII interaction modes. PyMOL(34) and ChimeraX(35) software were used to visualize and further refine the final binding modes.

Table 1: Aptamer sequence. The 2D is represented through dot and bracket notation to help visualize the base pairing binding and overall 3D structure without requiring a 3D model. The paired nucleotides are indicated by parenthesis ( ), whereas the non-pairing nucleotides are shown in (.) dots. When a pairing ends, a closing parenthesis (')' is used to signify it, while an opening parenthesis ('(')' indicates it begins.

Aptamer ID	Aptamer sequences and dot and bracket notations
E	CUUCGGGGAGCAGCGAUGCGACGCAAUGGUACGGUACUUCCUGAAUGUUGUUUUUUCUCUUUUCUAUAG UACAAAAGUGCACGCUACUUUGCUAAACCAAUACCUAUUCCGUUACAC ..(((((((((((((....)))))((((((.((((((.((((((.....)))))))))))))(((((....)))).))))))..))).))))).....
E-M1	CUUCGGGGAGCAGCGAUGCGACGCAAUGGUACGGUACUUCCUGAAUGUUGUUUUUUCUCUUUUCGAUAC UACAAAAGUGCACGCUACUUUGCUAAACGAAUACCUAUUCCGUUACAC .....((((((((((....)))))).(((((((((((((.((((((((((.....)))))))).)))))))).)))..((((((...))))))))))))).....
E-M2	CUUCGGGGAGCAGCGAUGCGACGCAAUGGUACGGUACUUCCUGAAUGUUGUUUUUUCUCUUUUCGAUAC UACAAAAGUGCACGCUACUUUGCUAAACCAAUACCUAUUCCGUUACAC ..(((((((((((((....)))))((((((.((((((.((((((((((.....)))))))).)))))))))).(((....)))).))))))..))).))))).....
EI-M3	CUUCGGGGAGCAGCGAUGCGACGCAAUGGUACGGUACUUCCUGAAUGUUGUUUUUUCUCUUUUCGAUAG UACAAAAGUGCACGCUACUUUGCUAAACGAAUACCUAUUCCGUUACAC (((((((((((((((....)))))(((....)))))))))))))(((((((((((.....)))))))))))).((((.((((....)))).(((((((....)))).)))))))
E-M4	CUUCGGGGAGCAGCGAUGCGACGCAAUGGUACGGUACUUCCUGAAUGUUGUUUUUUCUCUUUUCUAUAC UACAAAAGUGCACGCUACUUUGCUAAACGAAUACCUAUUCCGUUACAC .....((((((((((....)))))).((((((((((((.....(((((((.....)))))))..)))))))))))).)))..((((((...)))))))))))).....
G	CUUUGGUAUCGUGGAAGGACUCGCAAUGGUACGGUACUUCCUGAAUGUUGUUUUUUCUCUUUUCUAUAG UACAAAAGUGCACGCUACUUUGCUAAGUAGAGGCAGGGAUGAUGUUCU ...(((((((((.....(((....)))))))))))..(((((((((((((((((..(((.(((((...((((((...))))))))))))))))))).)))))))))))))

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