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Bioinformatic approach for AS1411 aptamer optimization for the treatment of Glioblastoma

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Abstract

Human glioblastoma (GBM) is one of the most aggressive, infiltrative, and lethal types of brain tumor. In spite of biotechnology progress and the evolution of artificial intelligence tools, the prognosis for GBM remains poor, due to the difficulties associated with targeting anticancer drugs to GBM cells. A major challenge in improving the treatment of GBM is the development of a targeted drug delivery system that is capable of crossing the blood-brain barrier (BBB). On the other hand, aptamers are oligonucleic acids (RNA or ssDNA) molecules able to bind to a molecular target with high affinity and specificity, presenting low immunogenicity, low toxicity, long stability, easy synthesis, chemical modification, and penetration capabilities make aptamers promising therapeutic tools for GBM, as they enhance the efficacy of existing GBM treatments in addition to inhibiting the growth of overexpressed GBM cells, making them promising therapeutic tools for the disease. The main drawback of aptamer therapy is that it can be degraded by nucleases in the body, as well as the fact that aptamers must remain stable in the bloodstream to prevent adverse side effects. The objective of this work is to develop a new bioinformatic approach for optimizing the AS1411 aptamer, a 26-unit guanine-rich DNA oligonucleotide that adopts a G-quadruplex structure. AS1411 was the first oligodeoxynucleotide aptamer to enter phases I and II of clinical trials for several cancer. Our new protocol seeks to identify additional aptamers from the AS1411 aptamer that are capable of increasing their ability to bind to EGFRvIII, a mutant form of the epidermal growth factor receptor (EGFR) that is involved in tumor cell proliferation, angiogenesis, motility, differentiation, and survival.

The crystal structure of protein 1i8i, a mutation of the Epidermal Growth Factor receptor (EGFRVIII) antigen, was downloaded from the Protein Data Bank and completed by homology modeling using the Swiss Model, which reported a GMQE value of 0.85 (see Fig. 1). Using Discovery Studio 2021, the co-crystallized water molecules were removed from the protein structure, the remaining hydrogen atoms were added in Chimera, and the energy minimization was performed in sPDBv with Gromos96 force fields. Verify3D was used to validate the resulting protein structure using ERRAT, which generated an Overall Quality Factor of 99.0654, and PROCHECK reported Ramachandran outlier rate of 88.50% (see Fig. 2).

A primer in the AS1411 aptamer was constructed after preparing the receptor for molecular docking. A sequence for the AS1411 aptamer was obtained from PubChem (sequence: **GGTGGTGGTGTGTGTGGTGGTGGTGGGTGGGTGG**); the secondary structure of the ligand was constructed using RNAstructure (see Fig. 3); and RNAFold WebServer and RNAComposer were used

for the construction of the Vienna format and the 3D structure of the RNA aptamer. Pymol command line was used to replace uracil nucleotides with thymines, and Yasara was used for energy optimization. In order to determine the active contact residues (A:LYS43-B:T12:OP1, A:GLN39-B:G11:O3', A:GLN39-B:A:G11:C4', A:TYR109-B:G14, A:TRP110-B:T12, A:LEU93-B:G10), a rigid-body docking was performed using ZDock and a flexible docking was performed using Haddock 2.4 to refine the molecular docking.

Using the DEAP Python library, hundreds of new DNA aptamers were predicted via genetic algorithms using Google Colab. There were three crucial limitations to the developed script: **1)** it was required to retain the active nucleotides necessary for molecular recognition; **2)** it had a length of 26 nucleotides, similarly to the AS1411 aptamer; and **3)** it allowed the incorporation of new nucleotides such as Adenine and Cytosine to improve the hydrophilicity of the new aptamers, because the clinical efficacy of the AS1411 aptamer is limited by its poor water solubility. The sequences of the newly generated aptamers AptaG1 (**GACTGCTCGGGTCGAGAAAATGCAAT**), AptaG2 (**CACTTACTGGGTCGAGATCATTAAAT**), AptaG3 (**CACTTGCTGGGTGGTGCTCAGTAAAT**), AptaG4 (**CACTTCCTGGGTCGAGATCATGCAAC**) and AptaG5 (**GACTTCCTGGGTGGTGTATATGCAAT**). Using the methodology described previously, 3D structures of DNA aptamers were generated and rigid-body docking with ZDock and flexible docking with Haddock 2.4 were conducted for the best candidates (AptaG1, AptaG2, AptaG3 and AptaG5). Results are presented in Table 1.

Table 1. Rigid-body docking results using ZDock and flexible docking results using Haddock 2.4

Aptamer	ZDock score	Haddock Score
AS1411	1589.35	(-60.1 ± -6.7)
AptaG1	1608.19	(-81.9 ± -9.3)
AptaG2	1808.31	(-71.3 ± -0.5)
AptaG3	1687.92	(-69.5 ± -2.8)
AptaG4	1598.01	N/A
AptaG5	1649.57	(-79.1 ± -2.9)

According to the results presented in Table 1, it is observed that the combined use of artificial intelligence tools and bioinformatics treatments increased molecular recognition for all aptamers tested. The flexible docking of AptaG1, AptaG2, AptaG3, and AptaG5 was performed using HADDOCK 2.4. By using this methodology, it was possible to obtain more flexible aptamers, which increased the number of active residues that were in contact with the 1i8i protein. The results indicate that the AptaG1, AptaG2, AptaG3, and AptaG5 ligands may be able to bind to EGFR (epidermal growth factor receptor), a protein commonly overexpressed in GBM cells, thereby inhibiting EGFR signaling and promoting tumor cell death. An example of this is AptaG1 (Haddock score = -81.9 ± -9.3) and AptaG5 (-79.1 ± -2.9), which showed a significant increase in drug-receptor affinity energy. This is primarily due to the increase in non-bonding Van der Waals type interactions (see Fig. 4), offering a promising basis for the discovery of new therapeutic agents for the treatment of glioblastoma.

Referencias:

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Figures

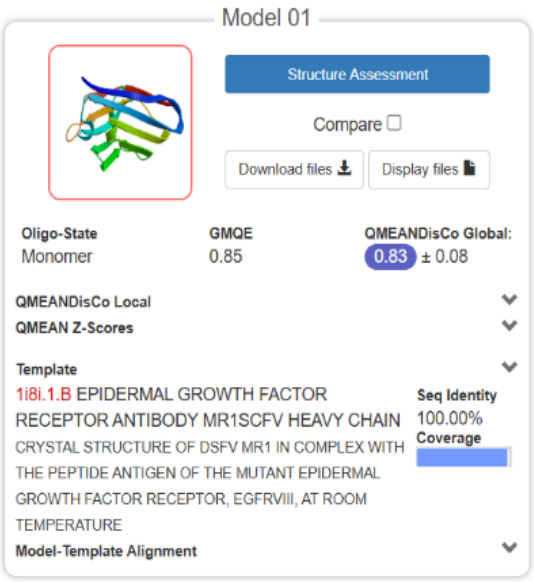

Fig. 1. Homology model of theprotein (1i8i) predicted by SWISS-MODEL.

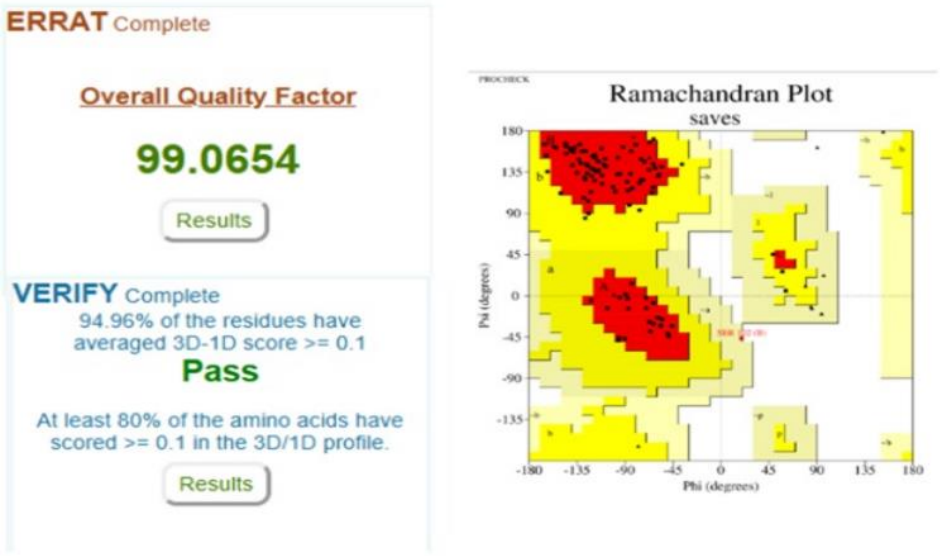

Fig.2. Validation parameters of protein (1i8i) predicted by verify3D.

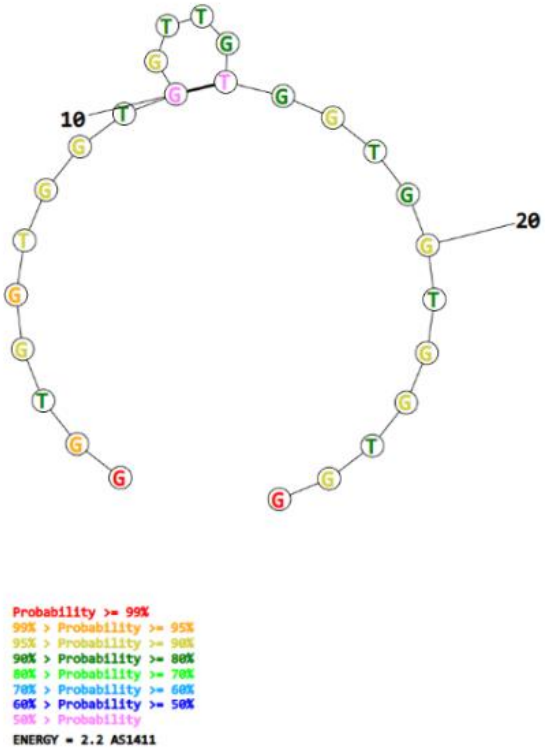

Fig. 3. Secundary structure of the aptamer AS1411 predicted by RNAStructure.

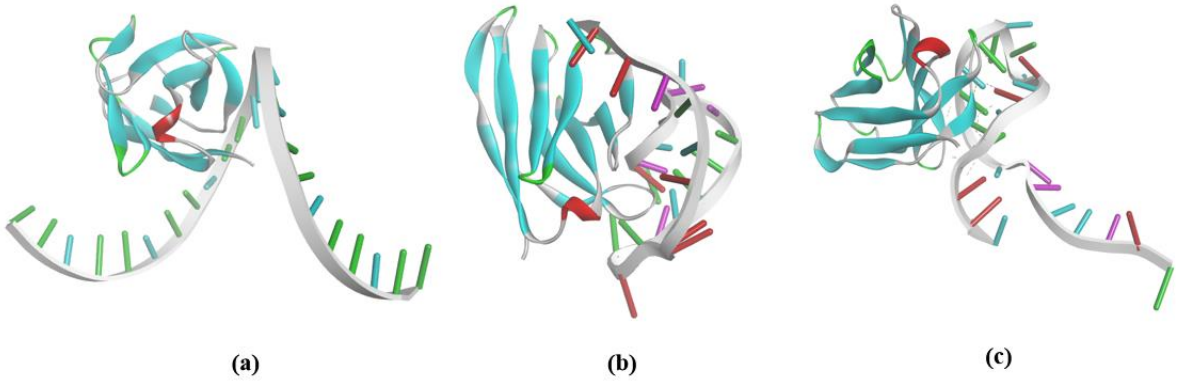

Fig. 4. Molecular docking of aptamers and protein (1i8i) obtained using flexible docking with Haddock 2.4. AS1411 **(a)**, AptaG1 **(b)**, AptaG5 **(c)**.