Anti-EGFR Aptamers as Targeted Therapeutic Agents for Non-Small Cell Lung Cancer (NSCLC)

MEGHA SUDHIR NAMBISAN[1,2] AND GAURAV SHARMA[2]

[1]Downers Grove North High School, Downers Grove, IL, [2]Eigen Sciences, Apex, NC, Compiled January 27, 2025

Non-Small Cell Lung Cancer (NSCLC) is the most common type of lung cancer and is characterized by the rapid and uncontrolled reproduction of abnormal cells. Based on the receptor found on the cancer cell's surface, NSCLC can be classified as either (a) wild-type Epidermal Growth Factor Receptor (EGFR) or (b) mutant EGFR (EGFRvIII). Aptamers are single-stranded nucleic acids that bind to specific targets, such as proteins like EGFR. Recently, aptamers targeting the EGFR cancer cells have been developed. We hypothesize that the aptamers used in this research should specifically bind to the EGFR receptor and bind minimally to its mutant EGFRvIII receptor. Therefore, a targeted therapy for NSCLC that focuses on EGFR can be developed. First, we employed the Vfold2D and Vfold3D webservers to construct 2-dimensional and 3-dimensional models of aptamers. Subsequently, we conducted molecular docking analysis to view the interactions between aptamers and the EGFR binding site. Finally, we used the PLIP software to collect data on receptor-aptamer interactions, including hydrophobic interactions, hydrogen bonds, cation interactions, and salt bridges. The results show that out of eight aptamers used in this research, five binds to the EGFR receptor, while only one binds to the EGFRvIII receptor. This shows the specificity of these aptamers to the EGFR receptor and that they could be used in the EGFR NSCLC. This research work will help us design novel aptamers that can be used in the EGFR NSCLC targeted therapy.

1. INTRODUCTION

The most common type of lung cancer, Non-small cell lung cancer (NSCLC), comprises approximately 85% of all given lung cancer cases (1). NSLC consists of multiple subtypes that are classified based on the appearance of the cell under a microscope, such as adenocarcinoma, squamous cell carcinoma, and large cell carcinoma (2). Unlike small cell lung cancer (SCLC), NSCLC typically grows and spreads more slowly (2). NSLC symptoms include persistent cough, chest pain, shortness of breath, and unexplained weight loss. Depending on the cancer's stage, several treatment options are available, such as immunotherapy, chemotherapy, radiation therapy, targeted therapy, and surgery. Early detection of cancer can significantly improve the prognosis (2).

Aptamers are short, single-stranded DNA or RNA molecules that bind to specific targets with high affinity and specificity, like antibodies. (3). Aptamers are selected using a process known as SELEX (Systematic Evolution of Ligands by Exponential Enrichment), which involves repeated rounds of binding, separation, and amplification to isolate the most efficient aptamers from a vast pool of random sequences (3). Aptamers have become valuable tools for diagnostics, therapeutics, and biosensing because of their distinctive properties, such as their small size, low im- immunogenicity, and ease of synthesis and modification (3). Their versatility and potential to target a wide range of molecules, including small molecules, proteins, and cells, make them a promising field of study in molecular biology and medicine (4).

A molecular docking computational method is used to predict the preferred orientation of one molecule when bound to a second to form a stable complex (5). Molecular docking

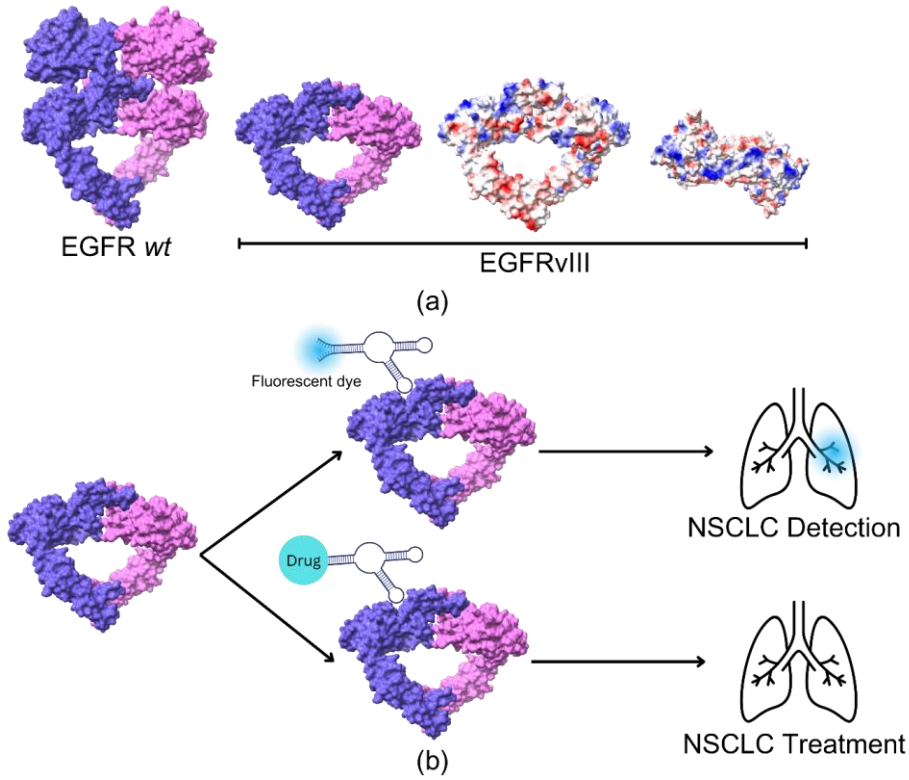

Fig. 1. Scheme of research. (a) The structure of EGFR and mutant EGFRvIII shows that the head domain of the mutant form gets truncated, and (b) the aptamer used in this study binds to the EGFRvIII receptor. It could help in NSCLC detection and treatment.

plays a crucial role in drug discovery due to its ability to model the interaction between small molecules (ligands) and their target proteins (5). By modeling the fit of a drug candidate into a protein binding site, molecular docking helps understand the binding affinity and specificity of the interaction (6). The process involves creating and scoring several potential binding orientations based on how well the logan fits into the protein's active site, considering factors including electrostatic interactions, shape complementarity, and hydrogen bonding(6). The goal is to expedite the drug development process by identifying the most promising compounds for additional experimental validation (6).

Thomas et al. recently developed aptamers targeting the EGFR receptor in NSCLC.(7) These aptamers are specific to the wt EGFR receptor and can be used to treat NSCLC cells. We hypothesize that the aptamer sequence has a specific amino acid that binds to the EGFR, not the EGFRvIII receptor. In this investigation, we first predicted the 3D structure of aptamers. These aptamers were docked, and their interactions were analyzed by visual inspection, computing their interaction, and binding energy calculations. This result will help combat NSCLC and design novel therapeutics for the disease.

2. METHOD

The aptamer structures were predicted using the following steps: First, we converted the aptamer sequence from one-dimensional to two-dimensional structure using the UNAFold Web Server(8), which gave us a 2D image and a .ct file. Using the .ct file of our two-dimensional DNA sequence, we then converted the sequence into dot and bracket notation through the RNAStructure website (9). Next, the VFOLD software produced a three-dimensional DNA sequence by inputting the RNA sequence, dot and bracket notation, and several clusters (10). We applied this method to 10 aptamers and used the process of molecular docking on all 10 with the HDOCK software(11). This happened by inputting the input receptor molecule, EGFR, and the input ligand molecule, 3D DNA sequences we created from VFOLD (12). The molecular docking gave us the receptor-aptamer complexes, as shown in Figure 3. We used the ChimeraX 1.8 program to import our docking data and view our receptor-aptamer complex in three dimensions to create our structures(13). We then used the PLIP web server to view the receptor-aptamer interactions, such as hydrophobic interactions, hydrogen bonds, cation interactions, and salt bridges (14).

3. RESULTS

In this work, we identified unique aptamer interactions with the EGFR receptor while reducing binding to the EGFRvIII mutant receptor to create and computationally evaluate anti-EGFR aptamers as prospective targeted therapy for Non-Small Cell Lung Cancer (NSCLC). We predicted the druggable site using graph neural networks (GNN) through the Graph Attention Site Prediction web server. Traditional neural networks process structure input, such as images or sequences, but GNNs could capture the relationships and interactions between nodes in a graph. This approach viewed bonds as edges and atoms as nodes in graphs representing molecules. The most significant areas of the graph are emphasized due to the attention mechanism indicating potential sites for drug binding. Figures 2a and 2b show a predicted druggable area marked in green and yellow,

Table 1. Table 1: Aptamers used in this work. Sequences and dot-bracket notations of aptamers were used in this study.

Aptamer Name	Aptamer Sequences
A5 (EGFRvIII)	GGATAATACGACTCACTATAGGACGGATTTAATCGCGGACGGATTTAATCGCCGTA-GAAAAGCATGTCAAAGCCGGAACCGTCCGGACGGTTCCGGCTTTGACATG ....(((.((.((.....)).)).)))..........((((..........)))).....((((((((((((((((((....)))))))))))))))))))
A2 (EGFR)	GGATAATACGACTCACTATAGGCGTAGTGATTATGCTATAGGCGTAGTGATTAT-GAATCGTGTGCTAATACACGCCCGACGACGACGACGACGACGATCGTCGTCGTCGTCGTCGTCGG ....(.((((.((.....)).)))).)....((((.....))))..............(((((.....))))).((((((((((((((((((....)))))))))))))))))))
A4	GGATAATACGACTCACTATAGGGTTCTACGATAAAGGGTTCTACGATAAACGGTTAAT-GATCAGCTTATGGCTGGCAGTTCCCCGACGACGACGACGACGACGATCGTCGTCGTCGTCGTCGTCGGG .....((.....................))..............((.....))..........((((.....))))........((((((((((((((((((....)))))))))))))))))))
A5	GGACGGAUUUAAUCGCCGUAGAAAAGCAUGUCAAAGCCGGAACCGUCC ((((((.((.....(((.....((. .....)))))))))))
A6	GGCGUAGUGAUUAUGAAUCGUGUGCUAAUACACGCCCGACGACGACGACGACGA CGA ..((((.....))))...(((((.....)))))..((.((.((....)).)).)).
A8	GCCATTGCCATTGCCATTGCCATTGCCATTGCCATTGCCATTGCCATTGCCATTG .....((....))....((....))....((....))....((....)).....
A9	GGCUUUCGGGCUUUCGGCAACAUCAGCCCCUCAGCCCGACGACGACGACGACGACGA .....((((((...(((.....)))....))))))....((.((....)).)).
A10	GGGUUCUACGAUAAACGGUUAAUGAUCAGCUUAUGGCUGGCAGUUCCCCGACGACGACGACGACGACGA .....((.....))..........((((.....))))..........((.((.((....)).)).)).

demonstrating the aptamer binding site. The druggable site successfully displayed the protein's capacity to bind and target certain drugs. Druggable sites must be discovered to design treatments that can attach to these biomolecules.

Next, we performed molecular docking simulations to find the interactions between the EGFR and aptamers. Figure 3 displays the 3D structures of EGFR-aptamers. Based on these complexes, most of the aptamers bind to the EGF binding site of the EGFR receptors. Additionally, we docked these aptamers on the mutant form of EGFR (EGFRvIII) to compare the specificity of these aptamers to the EGFR receptors. Only E5 could attach to the predicted binding side of the protein. This demonstrates that the aptamers are more specific to the EGFR receptor, binding exclusively to EGFR NSCLC cells, not EGFRvIII NSCLC cells. Based on the docked structure shown in Figure 3, only seven aptamers (A2, A4, A5, A6, A7, A9, A10) bind to the EGF binding site, while aptamers A8 and A11 were removed. Based on the interaction graph depicted in Figure 4, we expect that each aptamer created an equivalent quantity of hydrogen bonds and bridges. As a result, we did not choose the aptamers based on the number of interactions. The aptamers' binding energies were also calculated, and the results indicate that aptamers established the most substantial interactions.

Aptamer selection was based on the following criteria: visual inspection, number of salt bridges, and binding energy. To begin with, we used the ChimeraX software to view where the aptamers would bind on the NSCLC cell. If the aptamer binds to the EGFR site, it would be ruled as an effective aptamer. As shown in Figure 2, A2, A4, A5, A8, and A9 all binded to the EGFR site, while A6, A7, and A10 did not. After, we viewed the number of salt bridges between the aptamer and protein. In proteins, salt bridges are formed between positively and negatively charged amino acids. They are powerful bonds, like ionic bonds. A4 and A7 showed the greatest salt bridges (Figure 4). Lastly, to decide which aptamer was most effective, we looked at binding energy, which is how strong the aptamer binds to the EGFR binding site on the NSCLC cell. A4 had a binding energy of -16.61, while A7 had a binding energy of -15.98. Thus, we picked A4 as our aptamer.

EGFR-aptamer interactions and binding energy. The table shows the hydrogen bonds formed between the protein and the aptamer.

4. DISCUSSION

The structural difference between the EGFR and EGFRvIII receptors is mainly due to deletion mutation with the extracellular domain of EGFRvIII. The mutation removes the intact extracellular mind-binding domain. The deletion disrupts part of the ligand-binding domain, resulting in a truncated extracellular region. In the current research, we have worked on a third type of the EGFR mutant in which the mutation is present at the L858R position of the EGFR receptor. This mutation is prevalent in NSCLC cancer cells, and the EGFR L858R receptors are overexpressed on the cancer cell surface. Therefore, this property can be exploited to design inhibitors specifically binding to this receptor.

Given our research, we can apply our findings on aptamers to the medical field in multiple ways. In the larger category of lung cancer, which includes both small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC), our aptamers target the EGFR receptor in NSLC. These aptamers help diagnose EGFR-positive tumors. For example, we can target NSCLC cells with the aptamers by attaching a drug to the aptamer, and when the aptamer binds to the NSCLC cell's binding site, it will kill the cancer cell. Additionally, doctors can improve cancer detection by attaching fluorescent dyes to the aptamers. Aptamers bind to the EGFR receptor, as most connect to the receptor's active site, allowing for more accurate targeting and potential treatment options for NSCLC.

While our method has its benefits, it also comes with some limitations. We cannot confirm our finding with only computational results; we need experimental lab results. Computational results help predict how the aptamers interact with receptors; however, they are only sometimes accurate in real-world results. Additionally, molecular docking is an extensive process that

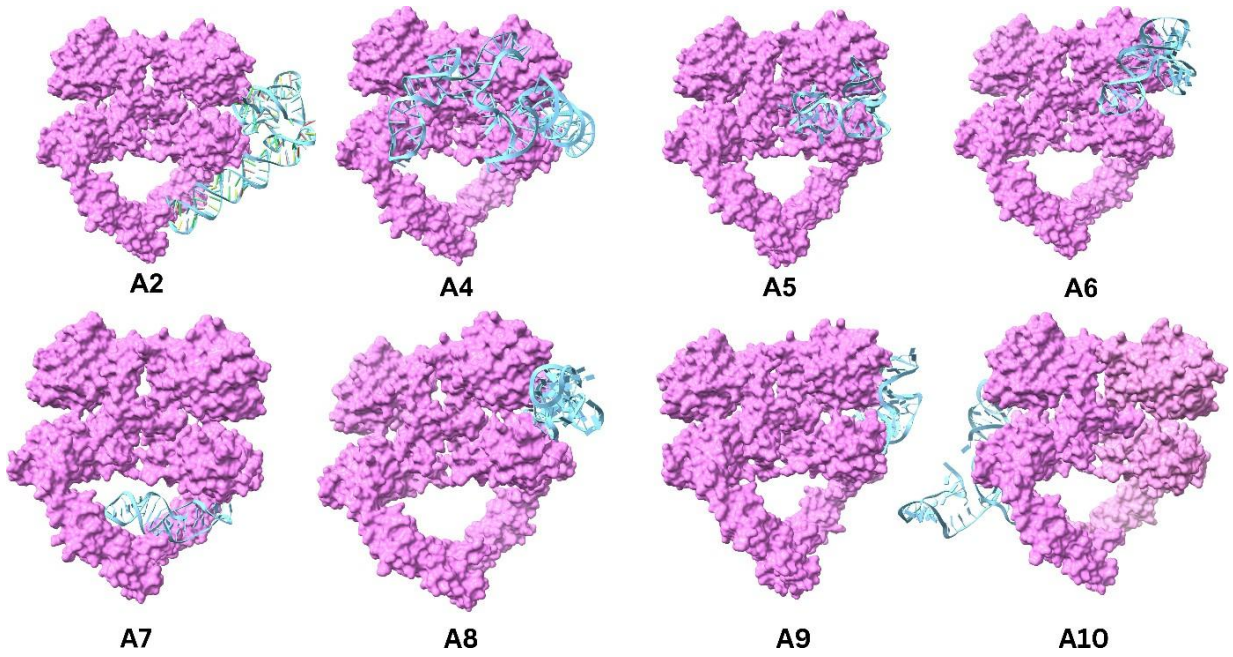

Fig. 2. Structure of EGFR-aptamers docked structures.

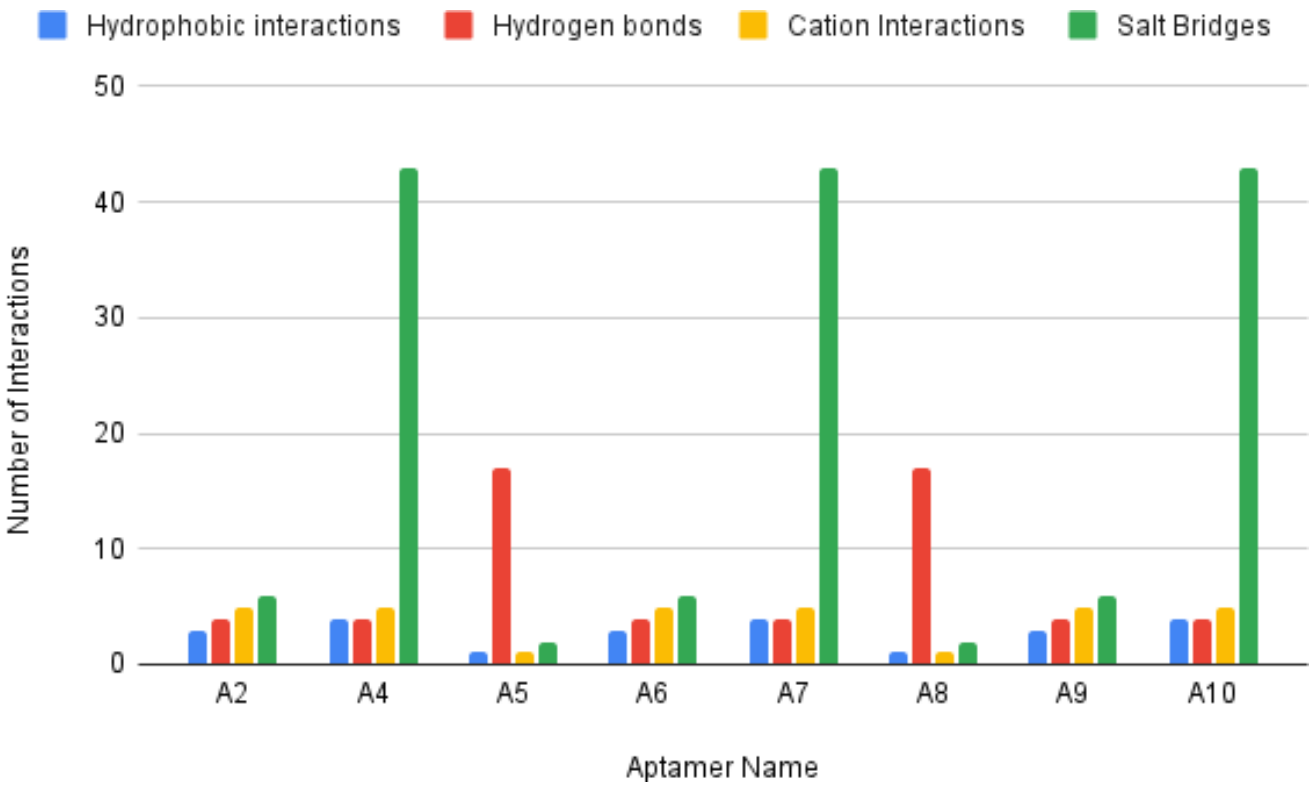

Fig. 3. Number of interactions formed between EGFR and aptamers. This shows that the interactions.

Table 2. Table 2: EGFR-aptamer interactions and binding energy. The table shows the hydrogen bonds formed between the protein and the aptamer.

	Hydrophobic interactions	Hydrogen bonds	Cation Interactions	Salt Bridge	Binging Energy (kcal/mol)
A5 (EGFRvIII)	1	17	1	2	-18.61
A2 (EGFR)	0	25	0	4	-17.35
A4	0	12	0	6	-16.61
A5	1	19	1	0	-21.24
A6	1	17	1	7	-16.15
A7	0	17	0	2	-15.98
A8	1	25	1	5	-14.87
A9	0	14	0	10	-17.62
A10	0	18	0	11	-16.88

takes a minimum of a day to achieve results due to the software's complex calculations for modeling the aptamer and protein interaction. To address this, incorporating machine learning would decrease the time spent docking. Protein-aptamer binding can be rapidly predicted using machine learning algorithms, reducing the need for time-consuming docking simulations.

The study shows how computational simulations can effectively design aptamers that target the EGFR receptor in NSCLC. We have found that these aptamers specifically bind to the EGF binding site of the EGFR and interact minimally with the mutant EGFRvIII form. We have seen the interactions of these aptamers to the EGFR receptor using molecular docking simulations. In addition, we have also computed the binding energy to select the best three aptamers that can be used for further research. These results opened the door for new therapeutic approaches towards treating NSCLC and will also accelerate the drug development process against the disease.

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