In Silico Design and Simulation of Aptamer-Mediated Enrichment Strategies for CD8αα+ CAR T-Cells

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Published Feb, 2025

CAR T-cell therapy treats malignant tumors by extracting T-cells from the patient's blood and modifying the receptors to kill cancer cells. Enrichment is the process in which CAR T-cells are separated from the unmodified T-cells. Aptamers are molecules of genetic information that connect the T-cell receptor to the B-cell receptor. Recently, Kacherovsky et al. have designed aptamers that specifically bind to the CD8αα+ CAR T-cells and can be used to enrich the receptor. We hypothesize that the aptamers can selectively bind to the CD8αα receptor on CAR T-cells and help their enrichment. The 3D structure and binding sites of CD8αα+ were identified by using the software AlphaFold3, P2Rank, and ChimeraX. To see which aptamer can bind best to CD8αα, aptamer structures were predicted using web servers VFold2d and VFold3D by using their respective DNA structures and the dot-and-bracket notations. Once all necessary structures were created, the docking process began to eliminate aptamers that don't bind to the binding site of CD8αα by using HDOCK and PLIP. Aptamers can further be decided for CAR T-cell therapy if they have a great number of interactions of hydrogen bonds and less binding energy. The A3 aptamer was applied to all three criteria and was concluded to be the most suitable aptamer for CAR T-cell therapy. By identifying the most effective aptamer, CAR T-cell therapy can be advanced to enrich normal T-cells to have the most suitable receptor and bind to the identified aptamer to show success in cancer removal efficiency.

1. INTRODUCTION

Chimeric Antigen Receptor T cells (CAR T cells) are a type of immunotherapy involving a patient's T-cells (1). These cells are genetically engineered to target specific proteins on malignant cells by changing them to express chimeric antigen receptors and being put back into the patient's cells. By detecting malignancy using the expression of receptors, CAR T cells can locate and kill cancer cells successfully (1). CAR T cell therapy is commonly used in hematologic cancers such as leukemia or lymphoma (2). Aptamers are short, single-stranded molecules made of artificial RNA or DNA that bind to targets to prevent specific actions, such as controlling the growth of cells in cancer (3). These are used to mimic a substrate in terms of structure to change the action of the substrate to identify and monitor differences in the environment and its effects on organisms (4). Aptamers are created by a process called SELEX, where random oligonucleotides are selected into sequences to have the correct binding properties as the substrate (5). Aptamers are similar to antibodies because of their functions in a cell.

Computationally, how these aptamers bind and interact with the CAR T cell can be explored by using molecular docking simulations (6). It is a method used to find the orientation of a molecule so that when bound to another molecule, it forms a stable complex (7-9). This process allows scientists to make models of the interaction between molecules (drugs) and target proteins at the microscopic level to create a protein corresponding to the interaction (6). The two steps of molecular docking are the search algorithm, which finds the potential binding locations of the ligand, and the scoring function, which identifies the strength and stability of the ligand-protein complex (6). With this docking process, potentially successful drugs can be created (6).

The CD8 protein is a glycoprotein on the surface of cytotoxic T-cells that reduces or potentially removes cancerous cells (10). This protein is a heterodimer and is composed of CD8α and CD8β chains (11). The extracellular portion of each chain has an immunoglobulin-like (Ig-like) domain. This section connects with major histocompatibility complex (MHC) class I molecules to identify the malignant cells (11). The domains of CD8α and CD8β interacting with MHC class I molecules is crucial as it will kill the target cells by the cytotoxic T-cells (11). Once the CD8 receptor binds to the cancer cell, the intracellular regions of CD8α and CD8β interact with other signaling molecules to

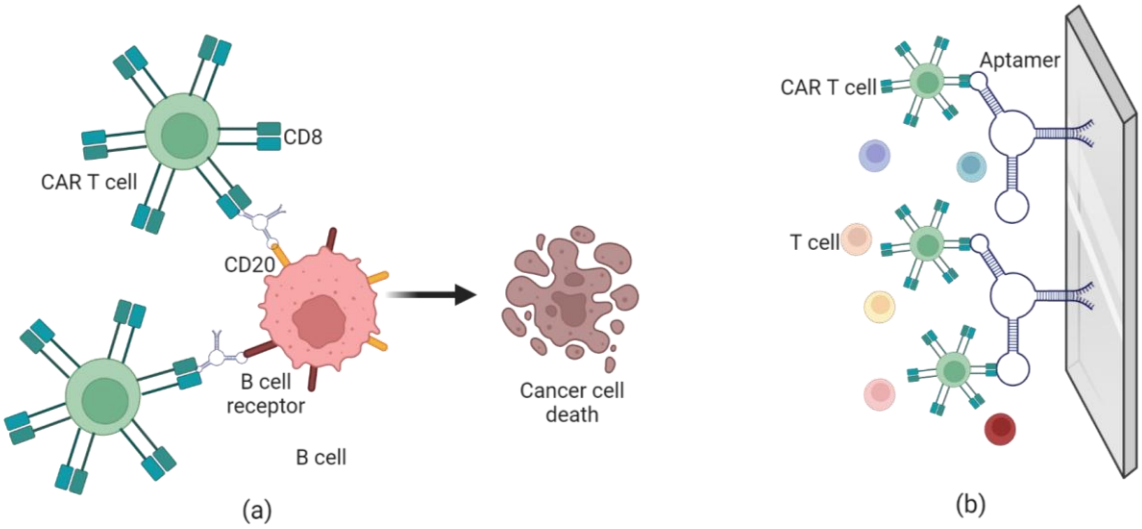

Fig. 1. CAR T-cell in cancer therapy.(a) Aptamers used in this research can specifically bind both CAR T-cell (via CD8 receptor) and malignant B cell (via CD20 and B cell receptors), leading to the induction of apoptosis in the target cells; (b) the aptamers utilized in this study will also facilitate the selective enrichment of CAR T-cells from a heterogeneous population of normal T-cells.

activate the T-cells (11). These receptors plays a crucial role in allowing and maintaining proper immune surveillance and response (11).

To perform this type of therapy, T cells are extracted from blood cells using leukapheresis (12). One CAR T-cell therapy method uses magnetic beads to enhance the purity of the T cells (12). Another technique, Streptamer-based technology, uses reversible binding, which is still expensive but reduces bead retention, slightly reducing the cost (13). Since both methods require high fees, Kacherovsky et al. have developed a reversible aptamer-selection method for selecting and isolating CAR T-cells containing CD8 receptors (14). These DNA aptamers can specifically bind to the CD8 receptors and could be used to isolate selective cells from the system. We hypothesize that the aptamers will specifically bind to the α and β domains of the CD8 receptor. Therefore, we have used computational simulations to understand the binding mechanism of aptamers to CD8 receptors. First, the aptamers' tertiary structures were elucidated and used to perform molecular docking simulations. The results show that aptamer A3 binds strongly to the CD8 receptors and agrees with previous experimental research. The current study will help design aptamer-based CAR T cell enrichment methods.

2. RESULTS

The surface structure of CD8$\alpha\alpha$ receptor: AlphaFold 3 is a software used to obtain 3D molecules of the receptors we are testing by entering codes of the receptor into the system. The models of CD8$\alpha\alpha$ were formed and then used to identify efficient binding sites of the receptor using the web server P2Rank with the created AlphaFold structure. P2Rank generated the location of the binding site and stated its charge as positive. The structure was then put into ChimeraX to get the electrostatic surface potential, which is finding the charges of each part of the receptor, indicated by colors: blue was positive, red was negative, and white was neutral. The binding site was marked with blue, which meant it was positive (see Figure 2). Since the aptamer is highly negative in charge, it can be concluded that it can bind to the positive binding site.

Criteria to select the best aptamer: To identify the best aptamer that combines the cancer cell and the CAR T cell, we used ChimeraX software to determine the binding site on the CD8$\alpha\alpha$ receptor, which P2Rank predicted, Figure 2. These binding sites are the desired places for binding with the aptamer, and to identify which aptamer will bind in the located binding site, ChimeraX was also used to find the binding area, Figure 3. From this, A1, A3, and A7 are all bound in the selected region for bind-

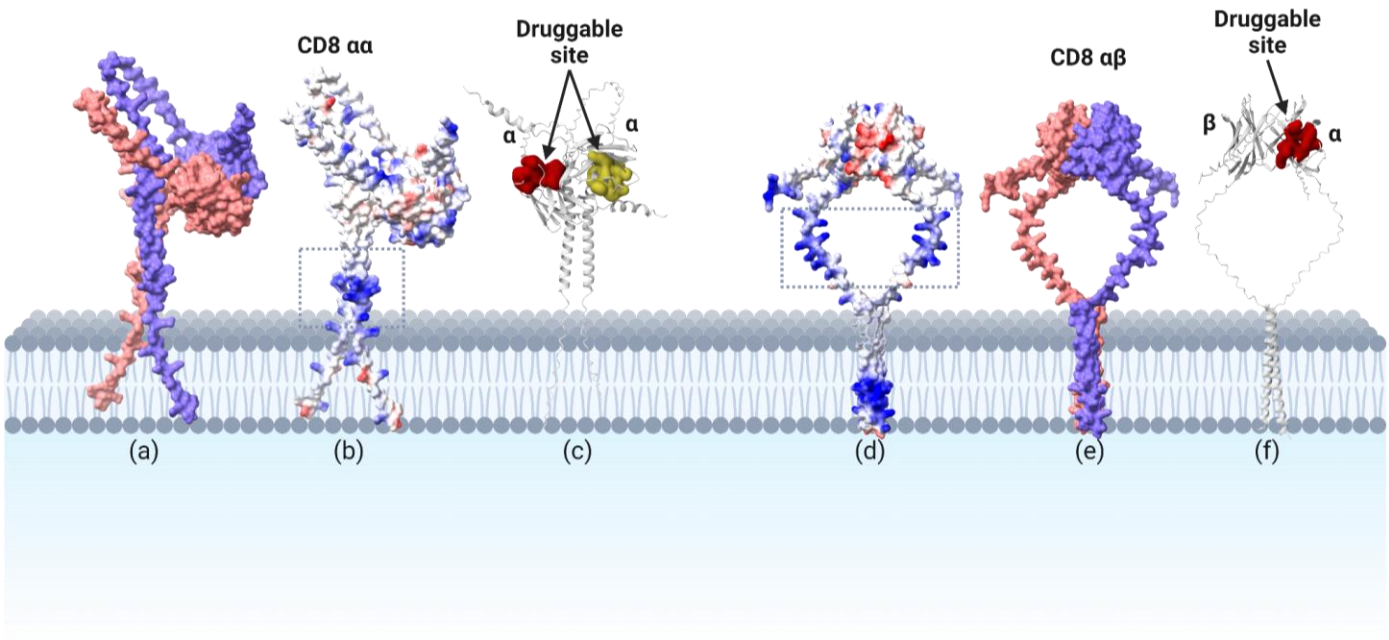

Fig. 2. 3D structure of CD8 receptors derived from AlphaFold 3.(a) and (e) show the CD8*αα* and CD8CD*αβ* protein in pink and purple, respectively; (b) and (d) electrostatic surface potential (ESP) of both receptors shows that protein is primarily neutral (white) in nature while the stem of the protein is positive (blue); and (c) and (f) shows the druggable site of the protein which shows that in CD8*αα* both head has druggable site but in CD8*αβ* only alpha subunit has a druggable site.

ing on the CD8*αα* protein. To further recognize the aptamer that will perform its function the best, hydrogen bonding analysis was used. PLIP was used to get the number of interactions of hydrogen bonds between CD8*αα* and the aptamers, as shown in Figure 4. A3 had the most significant interactions of hydrogen bonds out of the aptamers bound to the CD8*αα* binding site, so A3 would be the most coherent aptamer to use when the goal is to bring the CAR T and B cells together. A3 was applied to both scenarios of visual inspection and hydrogen binding strength, and to indicate that A3 is the aptamer that can be used in CAR T cell therapy, binding energies were analyzed using PDA-PRED, and the greatest absolute binding energy out of the aptamers that bound to the CD8*αα* binding site was A3, as well, Table 1. All three criteria applied to A3, making it the preeminent aptamer for CAR T cell therapy.

3. DISCUSSION

One of the significant challenges with CAR T cell production is the time and cost associated with it. Various studies have reported that aptamers can be used to select and elute the CAR T-cells. Aptamers provide multiple advantages over other elution methods. They have high affinity and specificity towards the receptors and can choose precisely CAR-T cells. In addition, aptamers provide label-free detection and decrease the possible interference from labels or tags. A significant advantage of using aptamers is they provide reversible binding; i.e., aptamers can bind and release CAR-T cells under certain conditions. Moreover, aptamers allow traceless enrichment of the cells. All these properties make aptamers a powerful tool in developing CAR-T cell therapies. Despite these characteristics, aptamer faces various challenges. For instance, the binding affinity of aptamers highly depends on the pH, temperature, and buffer composition. Furthermore, nuclease in biological fluids can degrade aptamers, and protective modifications in the structures are crucial to protect aptamers. They were, finally, attaching aptamers to the solid support while maintaining their formal conformations.

The study concluded that aptamers could bind and work with CD8 receptors on CAR T cells to provide functional therapy. Using molecular docking established the binding between the aptamer and the CD8 receptors. Findings from molecular docking are similar to those of prior experiments, which concluded that aptamers can locate CAR T cells from normal T cells through selective enrichment. The advantages of using aptamers in CAR T cell therapy are the cytotoxic effect on cancer cells, limited traces, and high specificity. Even though the downsides are pH-

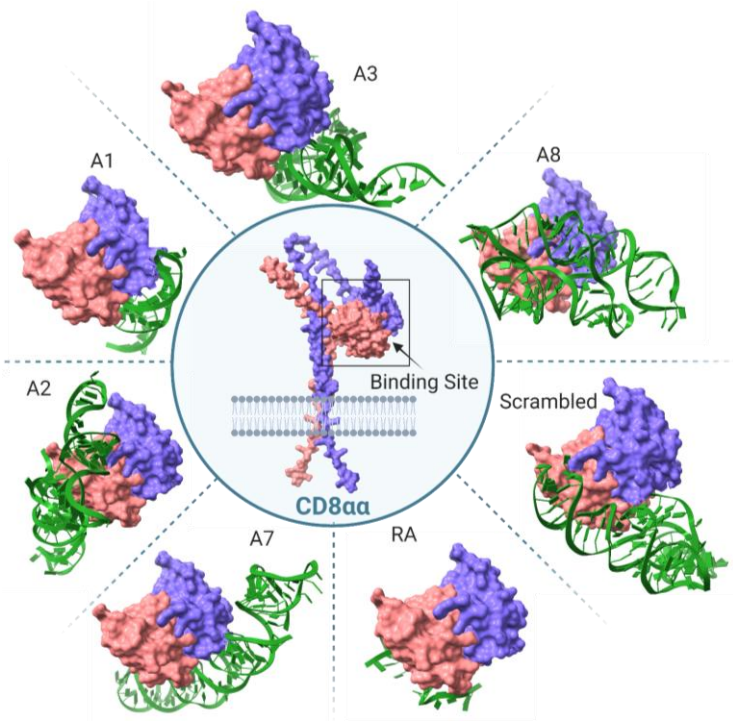

Fig. 3. Shows the different potential binding sites on the CD8*αα* protein, which the aptamer can bind to.Models of the CD8*αα* receptor were created using ChimeraX, and the part analyzed of the receptor was the head, as the bottom and center of the head are the desired binding sites. The rest of the CD8*αα* structure is considered to be in the cell. P2rank was the software used to find the locations of binding sites. Each aptamer has a unique location for binding to the CD8*αα*, and by using this software, the proper binding site was determined by the location of the DNA structure (green) representing each aptamer. From this, A1, A3, and A7 were selected as the most applicable aptamers to bind to CD8, as the DNA structure was bound primarily to the bottom of the head and between the pink and purple sections. Since only three were selected, the rest of the aptamers can be ruled out from being potential aptamers to use for CAR T-cell therapy.

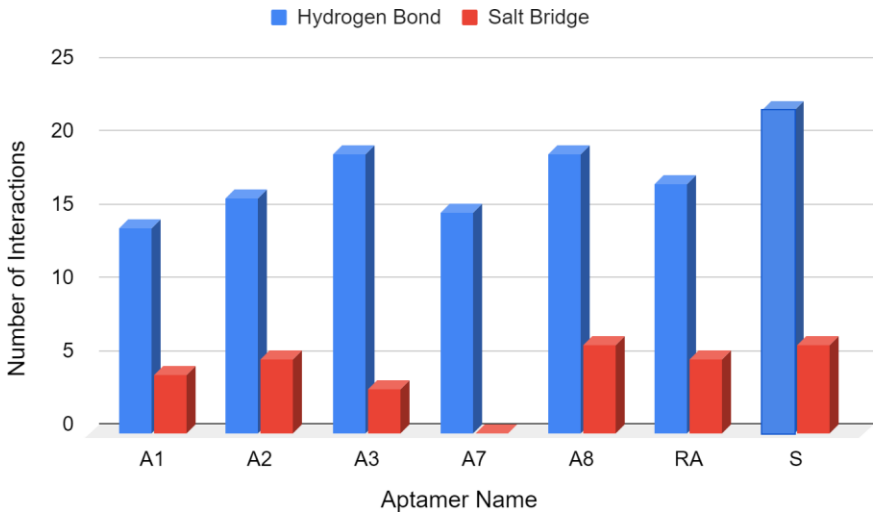

Fig. 4. This graph compares the number of interactions between the hydrogen bond and salt bridge of each tested aptamer. A3, A8, and Scrambled are the aptamers with more excellent interactions for hydrogen bonds; therefore, the aptamers bind more vital to the receptor. A8 has the most significant interactions for salt bridges, making it the most qualified aptamer to bind the receptors together. However, A8 and Scrambled aptamers were eliminated from the list of aptamers bound to the CD8*αα* binding site. Out of the aptamers that bound correctly on the receptor, A3 had the most significant number of hydrogen bonds, which would increase the strength of the bonds and further bring together the CAR T cell and the B cell to perform the function of getting rid of the B cell.

dependency, temperature, potential nuclease degradation, and challenges with buffer composition, using aptamers for CAR T cell therapy promises cell enrichment methods to reduce the time and cost of CAR T cell production.

4. METHOD

The AlphaFold 3 is the source of CD8's three-dimensional structures (15). The graph-neutral network GrASP models druggable site prediction to determine the best site (16). The RNA aptamer sequences are used in VFold2D to transform the structure from one-dimensional to two-dimensional, as represented in the Kekule structure. After converting the sequences into two-dimensional, pictures represent the aptamer's 2D structure. Along with the pictures, the software creates a unique dot-and-bracket sequence. To make the three-dimensional structure of the aptamer, the same RNA sequence and the dot-and-bracket sequence are put into VFold3D, with the number of clusters being one. Once the three-dimensional structures are made, the process of docking begins. Using the HDock software, pictures of molecules are created and have different possible locations of aptamer attachment, showing which points are successful in aptamer binding. Molecular docking simulations were performed using the CD8$\alpha\alpha$ subunit with all the aptamers to study their binding interactions so aptamers can be used to enrich the aptamer. To study the interaction of these aptamers for bi-specific interaction to both CAR T cell and Cancer cell.

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Name	Aptamers	Binding Energy (kcal/mol)
A1	ATCCAGAGTGACGCAGCACGCAGCACCCGTGGTAGTGTATCAGGGA GACACTACGTGATGCAGCTTGAAATGGACACGGTGGCTTAGT **.((((.((((((.((((((((((((.(((.((((((((((...)))))))))))))))))))))..))))))))..))))).....**	-14.93
A2	ATCCAGAGTGACGCAGCACGCAGCAAGGTGGCTGTGGGCGGATGGT GGGCTCGCGTGGGCGGCCACCTGATGGACACGGTGGCTTAGT **((((((((((((((((.((((....))))))))))))((...))).)))))...)))))(((((((((...))).)))))).... **	-16.87
A3	ATCCAGAGTGACGCAGCAACAGAGGTGTAGAAGTACACGTGAACAA GCTTGAAATTGTCTCTGACAGAGGTGGACACGGTGGCTTAGT **....((((((((((((.((..((((((....))))))))....)))))....(((((((((....))).)))))))))))))).. **	-15.08
A7	ATCCAGAGTGACGCAGCATTAGGAGGTGGGCTCGCGTGCACCAATC CATGGTCGGCGGGAATTTTAAGGGTGGACACGGTGGCTTAGT **(((((.(((((....)))))))))((((((.((((((.(((.(((((((..((((....)))))))))))))))))).)))))))). **	-13.17

A8	ATCCAGAGTGACGCAGCAGCTCGATCGTATAGCCGTGACGCAGCTT GAAATGGGATCGCGTCCACAGTTTTGGACACGGTGGCTTAGT **....((((((.((((((((((.....))))))).))))(((....))))((((((((((....)))))))))))).. **	-21.18
Scrambled	CCAGAGTGACGCAGCAACAGAGGTGTAGAAGTACACGTGAACAAGC TTGAAATTGTCTCTGACAGAGGTGGACACGGTGGCTTTTAGT **.((((((((((((((.((..((((((....))))))))....)))))....(((((((((....))).))))))))))...**	-16.58
RA (reversal agent)	ACTAAAAGCCACCGTGTCCACCTCTGTCAGAGACAA **.............(((((..((((...)))))))))**	-18.37

Table 1: Aptamer sequence used in this study. The dot-bracket notation is used for 2D structures of the aptamers.