Computational Simulations of Bispecific Aptamer Targeting EpCAM and Transferrin Receptors for Treating Brain Cancer Metastases

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Secondary brain tumors, or brain metastases, occur when cancer cells spread from other parts of the body to the brain. These brain disorders are challenging to treat since the blood-brain barrier inhibits most small molecules from entering the brain. Aptamers are small segments of single-stranded nucleotides that specifically bind to a target biomolecule and are gaining popularity in diagnostics, therapeutics, and biosensing. Recently, bispecific aptamers have been designed to cross the BBB (by binding the transferrin receptor) and interact with the target cancer cell (via the EpCAM receptor). We hypothesize that the bifunctional nature of the aptamer could be due to the nucleotide conformations that bind to both receptors. In our investigation, we performed molecular docking simulations on both receptors. In total, nine aptamers were modeled using Vfold2D and Vfold3D software. The obtained 3D structures of aptamers were docked on the two receptors to understand the receptor-aptamer binding interaction. These structures were analyzed by computing the aptamer interactions and performing visual inspections, which showed that aptamer TENP binds strongly to the transferrin and EpCAM receptors. The current work can potentially improve drug delivery across the blood-brain barrier, paving the way for future advancements in cancer therapeutics and personalized medicine.

1. INTRODUCTION

One protein on cell membranes that helps with iron absorption into cells is called human transferrin receptor 1 (TfR1), or CD71 (1). Its main job is to make iron-bound transferrin easier to internalize, which controls the quantity of iron in cells (1). The blood protein transferrin binds to iron and transports it throughout the body (1). When transferrin combines with iron to form a complex, it can attach itself to TfR1 on the cell surface (1). Red blood cells, which require iron to produce hemoglobin, have the greatest amounts of TfR1 expression. TfR1 is

also found in many other cell types, such as hepatic and epithelial cells (2). The interaction between transferrin and TfR1 is tightly controlled for the body to distribute and absorb iron appropriately (2). Anomalies in TfR1 expression or function may lead to anemia brought on by either too much or too little iron (2). In addition to its role in iron uptake, TfR1 has been actively investigated because of its potential as a target for cancer therapy (2). TfR1 overexpression may result from cancer cells' higher iron needs, which they frequently display to obtain more iron (2). Scholars have explored the possibility of targeting TfR1 with certain ligands or antibodies to minimize damage to healthy cells while delivering deadly agents to malignant cells (2). Expression of the glycoprotein called epithelial cell adhesion molecule, or EpCAM, on epithelial cells (3). It plays a crucial role in cell adhesion and maintaining epithelial integrity (3). It is involved in various biological functions, including cell differentiation and proliferation (4). EpCAM is often overexpressed in malignant cells, making it a prominent marker in cancer research, particularly for epithelial cancers (3). Ongoing research is focused on its role in tumor development and its potential as a therapeutic target (3).

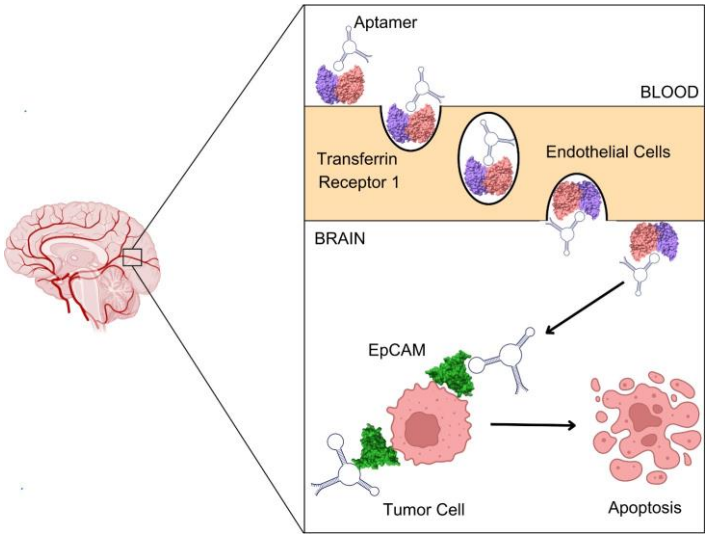

Fig. 1. Aptamer binding to transferrin (TR) and EpCAM receptor. The aptamer in this research initially binds to the TR, facilitating its transport across the BBB via receptor-mediated transcytosis. After crossing the BBB, it gets released to the brain and later binds to EpCAM, a cell surface protein overexpressed in various brain cancers. The figure was generated by BioRender.

Single-stranded, tiny molecules of RNA or DNA called aptamers bind to their targets with extreme precision (5). They can fold into different three-dimensional shapes and were selected using the SELEX process (6). They can identify and stick to a variety of substrates, including proteins, small molecules, and cells (7). They are used in many different applications, such as pharmaceuticals, biosensors, and other diagnostic techniques (8). They differ from conventional antibodies in that they can be chemically modified to increase certain properties, have lower production costs, and are easier to synthesize (7,9).

A vital method in molecular biology and drug development, molecular docking may computationally predict the interactions between a small molecule

ligand and a target protein (10-13). By building and optimizing the ligand and protein structures, algorithms explore various ligand orientations to determine the optimal binding spot within the protein binding site (14). This approach provides valuable insights into the interactions between potential drugs and their biological targets, aiding drug design and optimization (11,12). Molecular docking sped up drug discovery, which rapidly and efficiently identifies potential therapeutic possibilities (12). It accomplishes this by screening massive chemical libraries quickly (12).

Despite various promising stories of aptamers, a significant limitation that aptamers face is transporting the blood-brain barrier. A bispecific aptamer crossing BBB and binding to the cancer cell has recently been developed by Macdonald et al (15). The transferrin receptor (TfR) and EpCAM, two proteins overexpressed in metastatic brain cancer cells, are the simultaneous targets of this aptamer (15). By increasing aptamer binding to the cancer cells and reducing off-target effects, the dual-targeting approach seeks to improve the treatment's specificity and effectiveness. The proposed binding mechanism of this aptamer is shown in Figure 1. The aptamer first binds to the TfR, enabling its transport across the blood-brain barrier (BBB) through receptor-mediated transcytosis. Once it crosses the BBB and enters the brain, the aptamer is released and subsequently binds to EpCAM, a cell surface protein overexpressed in several types of brain cancer. Thus, this aptamer can be linked to cancer drugs or fluorescent dyes for cancer treatment or detection. We hypothesize that the aptamer bispecific nature could be due to its shape, size, sequence, and location in which it binds to the receptor. Therefore, in the current work, we have used various in silico techniques to design the 3D structure of aptamers and later dock those aptamers to both transferrin receptor and EpCAM protein. We have found that the aptamer binds to the specific region of both TR and EpCAM. The current research will help understand the bispecific nature of aptamers binding and will aid in developing novel targeted treatments for brain disorders.

2. METHOD

Initially, the three-dimensional structures of the transferrin and EpCAM receptor were obtained from the Protein Data Bank (PDB). The primary nucleotide sequence of the aptamer served as the starting point for structural predictions. This sequence was first subjected to secondary structure prediction using Vfold2D (20), which provided the predicted secondary structure in dot-bracket notation. The tertiary structure was meticulously modeled using the advanced Vfold3D, and the resulting 3D structures were then visualized with the powerful Chimera (20,21). In cases where the aptamers formed multiple clusters, the first aptamer with the lowest energy state was selected for the subsequent molecular docking simulations. The HDock was then employed to dock the aptamers to the transferrin and EpCAM (16). The receptor and ligand structures were uploaded to the HDock server, and the docking process was initiated with default parameters to predict the binding modes of the aptamer to the EpCAM and transferrin receptor. The binding site on the receptor was identified using a graph neural network (GNN) called GrASP, designed explicitly for binding site prediction (22).

3. RESULTS

Delivering medications to the target cells is a significant difficulty in the treatment of brain-related disorders, mainly because of the blood-brain barrier's (BBB) restrictive nature. This obstacle severely restricts the delivery of drugs. To overcome this challenge, scientists have been consistently devising creative remedies. We have been studying aptamers that attach to proteins overexpressed in cancer cells and avoid the blood-brain barrier. These aptamers may be used in targeted cancer therapy in addition to being biomarkers for cancer detection.

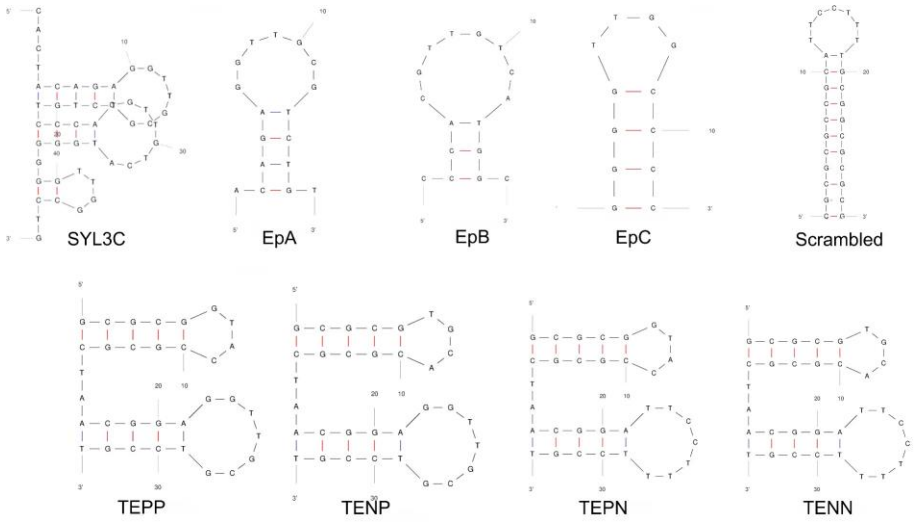

Fig. 2. Secondary structures of the aptamers. The image shows the base pair interactions (hydrogen bonds), crucial in deciding the aptamer 3D structure.

First, the aptamers' secondary structure and then tertiary structure were predicted. Molecular docking simulations using the HDOCK 2.0 software were then performed on both transferrin and EPCAM proteins (16). The transferrin receptor will help transport the aptamer across the BBB, and EPCAM is an increased protein on the surface of cancer cells. Therefore, these aptamers are bifunctional. Based on the secondary structure of the aptamer shown in Figure 2, EpA, EpB, EpC, and the scrambled aptamer have only one head and are linear, while the rest have two heads. The tertiary structure of the aptamers shows similar results, with EpA, EpB, and EpC exhibiting similar structures. In contrast, TEPP, TENP, TEPN, and TENN showed a different yet consistent structure. However, the scrambled aptamer and SYL3C displayed structures distinct from the rest of the aptamers. The 3D shape of the transferrin and aptamer reveals that all aptamers interact with the transferrin protein's binding region. Prior research has demonstrated an equivalent binding region. We hypothesize that when the aptamer binds to this region, it will trigger the transferrin receptor to transport the aptamer across the membrane. Consequently, in the aptamer transport process, only the aptamer is transported across the membrane, while transferrin is not.

Based on plip analysis, aptamer EPA formed six hydrogen bonds, a π-Cation interaction, and a salt bridge. EPB formed six hydrogen bonds and five salt bridges. EPC formed four hydrogen bonds and three salt bridges.

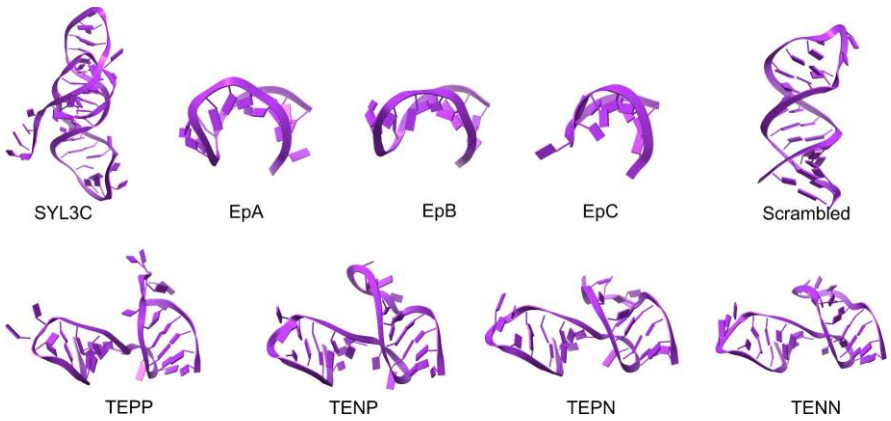

Fig. 3. Tertiary structures of the aptamers: The tertiary structures (3D) structures of aptamers were computed using Vinafold and RNA structure software.

SCRAMBLED formed five hydrogen bonds and two salt bridges. Aptamer SYL3C formed thirteen hydrogen bonds and six salt bridges. TENN formed nine hydrogen bonds and six salt bridges. TENP formed eight hydrogen bonds and six salt bridges. TEPN formed twelve hydrogen bonds and four salt bridges. TEPP formed ten hydrogen bonds and five salt bridges. EpCAM formed eleven hydrogen bonds. Since all these aptamers are derived from the truncation of SYL3C aptamers, they should likely bind to a similar region of the transferrin receptor, as shown in Figure 4. The TENN, TENP, TEPN, and TEPP showed a similar range of interactions; however, for further analysis, we have only used TEPN because, experimentally, it has demonstrated a successful transcytose of the BBB in vivo (15).

4. DISCUSSION

In this research work we have worked on the designing of aptamers that could potentially cross the blood brain batter and bind to the cancer cells. To achieve this, we have targeted transferrin receptors present on the surface of the epithelial cells of BBB and EpCAM receptor present on the surface of cancer cells. Solid tumors are challenging to treat because many therapeutic medications have trouble getting beyond the blood-brain barrier (BBB) and into the central nervous system, mainly when the cancer is in the brain (17). Researchers are examining bispecific aptamers—tiny nucleic acid molecules that can bind to two targets—as a potential cure (18). Because these aptamers can specifically target cancer cells and cross the blood-brain barrier, they present a viable therapeutic approach for the treatment of brain tumors and other cancers of the central nervous system (18). Bispecific aptamers can bind simultaneously to a tumor-associated antigen and a target on the blood-brain barrier (18). Giving them the ability to precisely deliver cytotoxic medications or redirect immune cells to the tumor enhances the efficacy of treatment (19). The current research focus is optimizing target combinations, improving aptamer designs, and expanding

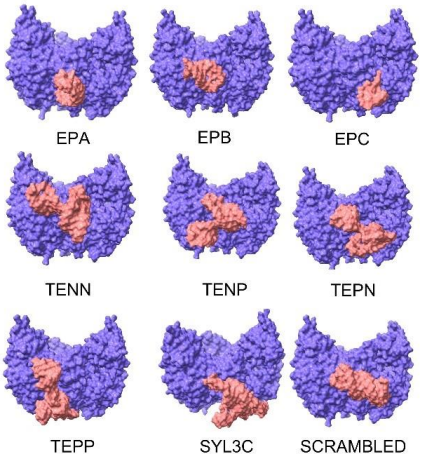

Fig. 4. Docked structure of receptor-aptamer complexes. All these structures show that the aptamer binds to the specific region of the receptor. This region is a transferrin (protein that binds to transferrin receptor and induces Fe transport across the BBB) binding region. We assume that the aptamer mimics the transferrin protein to cause its transport across the BBB.

these novel agents' therapeutic potential.

However, a significant challenge in this research is ensuring the stability and efficacy of aptamers in the complex biological milieu of the human body, especially in the microenvironment of the brain. Though designed to target cancer cells and cross the blood-brain barrier, their poor binding affinity, fast degradation, and off-target effects may limit their utility. Furthermore, additional in vitro and in vivo studies are required to corroborate the biological relationships and therapeutic efficacy. The work is based on in silico methods such as molecular docking simulations. These challenges emphasize the need for additional research to validate the therapeutic utility and enhance aptamer designs.

Furthermore, the study demonstrated bifunctional aptamers that bind to the epithelial cell adhesion molecule (EpCAM) and link to the transferrin receptor (TfR) to penetrate the blood-brain barrier (BBB) to target cancer cells. Molecular docking simulations demonstrate that these aptamers successfully connect to both proteins, indicating that they could be employed as specific and targeted therapeutic agents for brain cancer. The findings suggest that these aptamers may be used as biomarkers and therapeutic agents, providing a fresh approach to blocking BBB trafficking and a focused way to treat brain tumors.

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Table 1. Base pair-amino acid-binding. This lists the amino acids involved in aptamer interaction.

EPA		
	Amino acids	**Distance (Å)**
Hydrogen Bonds	**Arg510**	**2.87**
	Tyr524	**3.13**
	Arg532	**2.24**
	Ser535	**2.80**
	Arg536	**3.12**
π-Cation Interactions	**Arg536**	**2.72**
	Arg536	**4.44**
Salt Bridges	**Arg527**	**4.92**
EPB		
Hydrogen Bonds	**Gln66**	**3.23**
	Asn1146	**2.54**
	Ser1158	**2.29**
	Gln116	**2.17**
	Ser1164	**2.95**
Salt Bridges	**Arg1149**	**4.92**
	Arg1149	**5.22**
	Arg1166	**5.37**
	Arg1166	**4.98**
	Arg1171	**4.34**
	EPC	
Hydrogen Bonds	**Thr385**	**2.74**
	Asn496	**2.30**
	Ser497	**3.13**
	Thr534	**2.19**
Salt Bridges	**Glu493**	**4.53**
	Arg494	**4.65**
	Arg494	**4.07**
	Scrambled	
Hydrogen Bonds	**Arg510**	**3.95**
	Gln521	**3.28**
	Ser525	**3.46**
	Asn639	**3.37**
	Gln705	**3.08**
Salt Bridges	**Arg504**	**5.14**
	Arg532	**4.92**
SYL3C		
Hydrogen Bonds	**Gln1031**	**2.88**
	Ser1046	**2.83**
	Arg1133	**2.48**
	Arg1133	**2.78**
	Asn1135	**3.13**
	Arg1149	**2.95**
	Arg1149	**2.32**
	Lys1153	**3.01**
	Ser1158	**2.52**
	Leu1159	**3.25**
	Gln1160	**2.27**
	Arg1166	**3.30**
	Thr1173	**3.39**
	Glu1027	**3.72**

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