AI-Mediated Computational Analysis of RNA-Based Aptamers Targeting the CD133+ Glioblastoma Cells

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Glioblastoma multiforme (GBM) is a highly malignant brain tumor originating from glial cells, characterized by aggressive proliferation, infiltration, and resistance to conventional therapies. The blood-brain barrier (BBB), which protects the brain, makes targeting GBM cells even more challenging by blocking the passage of most drugs. CD133 is a transmembrane glycoprotein and a well-established marker for cancer stem cells, associated with tumor progression and therapy resistance. We hypothesize that if the aptamer binds to the CD133 receptor and inhibits its activity, it will block the glioblastoma's progres- sion. The CD133 receptor structure was predicted using AlphaFold 3 to generate a high-resolution model. Aptamers were designed with Vfold 2D for secondary structure prediction and refined with Vfold 3D for tertiary structure modelling. Docking simulations with HDOCK predicted interactions between the modelled aptamers and the CD133 receptor. PLIP analysis was then performed to identify and evaluate the molecular interactions between the aptamers and the receptor, including hydrogen bonds and salt bridges. The 3D-modeled aptamers were docked onto the TfR to understand the binding interactions using the HDOCK2.0 software, and this was further validated using the deep learning-based method ScanNet. The number of interactions (using PLIP) and binding affinity (utilizing PDA-Pred) were computed to select the aptamers. The results showed aptamer CD133a as a promising candidate, potentially enabling dual-targeting strategies to enhance drug delivery across the BBB. Virtual reality (VR) technology was also used to visualize the results. These results will pave the way for designing aptamers and further enhancing specificity and efficacy in glioblastoma therapy.

1. INTRODUCTION

Glioblastoma is an aggressive type of brain cancer that arises from glial cells, which support the brain's neurons [1]. It is the most common and deadly primary brain tumor in adults [1]. CD133 is a cell surface glycoprotein expressed on certain stem and progenitor cells. It maintains stem cell properties, like self-renewal and differentiation [2]. CD133 is often overexpressed on cancer stem cells within the tumor. These cells are believed to drive tumor growth, resistance to treatment, and recurrence, making CD133 a marker for identifying and targeting the aggressive stem-like cells in glioblastoma. Aptamers are short DNA or RNA molecules that bind to specific targets with high affinity, acting like antibodies but easier to produce and modify. In cancer treatment, aptamers target tumor cells for precise drug delivery, reducing harm to healthy tissue and improving treatment accuracy [2]. CD133, also known as Prominin-1, is a transmembrane glycoprotein primarily expressed on the surface of various stem and progenitor cells, including hematopoietic stem cells, neural stem cells, and cancer stem cells [2]. It plays a crucial role in maintaining stemness, cell differentiation, and self-renewal. CD133 is often used as a marker for identifying and isolating cancer stem cells, particularly in tumors such as glioblastomas, colorectal cancers, and liver cancers. Its presence is linked to tumorigenicity and treatment resistance, making it a key target for cancer research. Despite its importance, the exact biological function of CD133 remains somewhat elusive, and ongoing research seeks to clarify its role in normal tissue development and cancer progression, Figure 1.

Aptamers are short, single-stranded DNA or RNA molecules that can bind to specific targets, such as proteins, small molecules, or cells, with high affinity and specificity. [3]. They are used in various fields, including diagnostics, targeted drug delivery, and therapeutics, due to their ability to act like antibodies while being easier to produce and modify. [3, 4]. In cancer treatment, aptamers can target tumor cells, delivering drugs or imaging agents directly to the cancerous tissue and minimizing damage to healthy cells. Their versatility makes them valuable in precision medicine and molecular diagnostics.

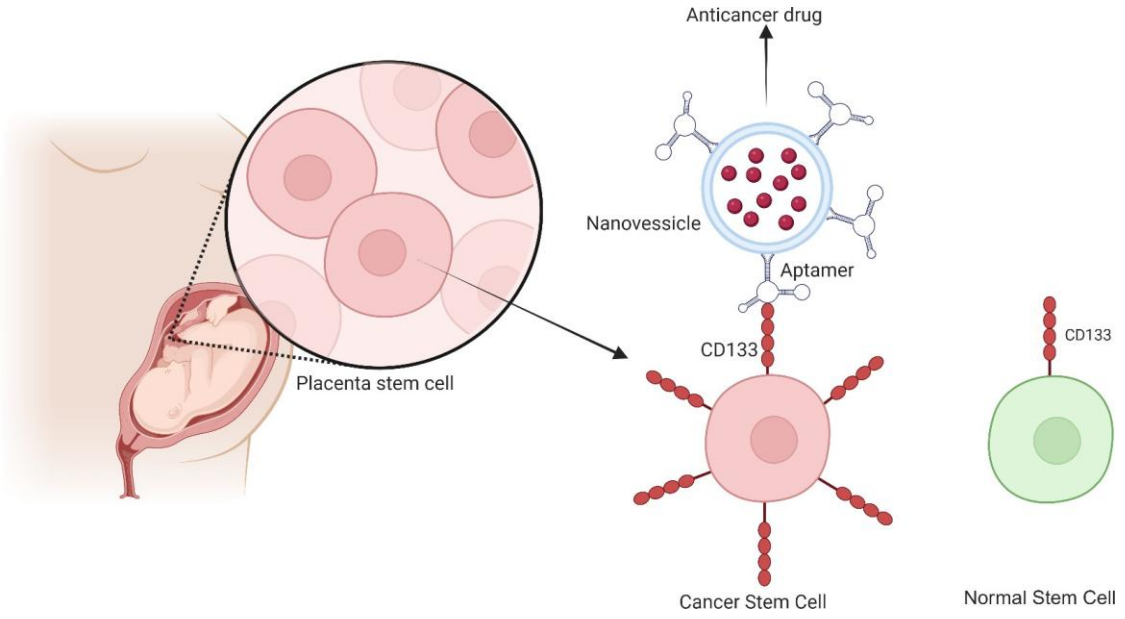

Fig. 1. The figure shows the schematic of aptamer-boundnano-vehicle-mediated CD133 cancer cell destruction. CD133 receptors are highly expressed on cancer stem cells, making them a valuable detection and therapeutic target for treating glioblastoma and medulloblastoma in children. While CD133 is minimally present on normal stem cells, shown in green, its overexpression on cancer stem cells creates a unique opportunity for aptamers to bind. These aptamers are displayed on the surface of a nanovehicle, which is loaded with anti-cancer drugs. The binding of the nanovehicle to CD133 facilitates targeted drug delivery to cancer stem cells, enhancing the precision and efficacy of the treatment.

Molecular docking is a computational technique to pre- dict the interaction between two molecules, typically a small molecule (like a drug) and a protein [5]. By simulating how these molecules fit together, docking helps to identify the pre- ferred orientation and binding affinity of the small molecule to the protein's active site [5]. This method is widely used in drug discovery to screen and optimize potential drug candidates before laboratory testing. Molecular docking is also employed in structural biology to understand the mechanisms of molec- ular interactions and in designing inhibitors for enzymes and other biological targets. Figure 2 illustrates the schematic representation of CD133-mediated cancer cell inhibition, comparing a normal cell and a glioblastoma cancer cell. While CD133 is expressed at low levels on normal cells, it is significantly overexpressed on glioblastoma cells, making it an effective marker for their detection. The schematic demonstrates two potential applications: drug-bound aptamers targeting the CD133 receptor for therapeutic purposes and fluorescent dye-bound aptamers for cancer cell detection. These approaches highlight the versatility of CD133 as a target for both cancer diagnosis and treatment.

Several techniques transport drugs to cancer cells, enhancing their effectiveness while minimizing side effects. Nanoparticles can be designed to deliver chemotherapy drugs directly to tumour cells, issuing surface ligands to target cancer-specific receptors. Liposomes are another method, encapsulating drugs to improve their delivery and reduce toxicity. Additionally, antibody-drug conjugates utilize monoclonal antibodies to specifically bind to cancer cells, releasing the drug upon attachment. These targeted approaches aim to improve precision in cancer treatment and reduce damage to healthy tissues. Previous studies have established the role of CD133+ cells in promoting glioblastoma progression and resistance to therapy. We hypothesize that if the aptamer binds to the CD133 receptor and inhibits its activity, it will block the progression of glioblastoma. We used computational modelling and docking to design aptamers targeting CD133 to test this. Results showed favorable binding interactions between the aptamer and CD133 receptor. This supports the potential of CD133-targeting aptamers as a therapeutic strategy for glioblastoma.

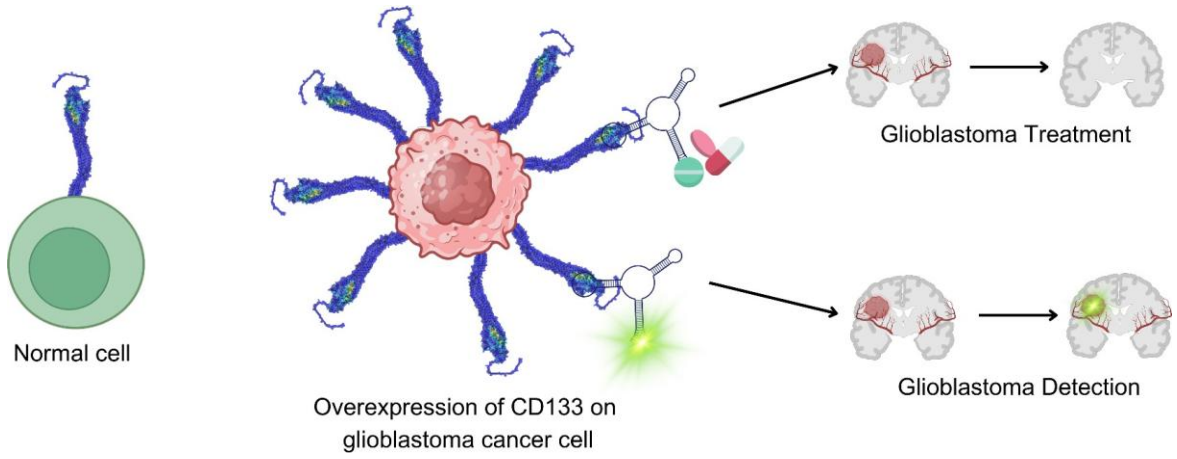

Fig. 2. The figure depicts a normal cell with low CD133 receptor expression and a glioblastoma cancer cell with significant CD133 overexpression. This overexpression can be leveraged using the aptamer developed in this research. The aptamer can either be conjugated with an anti-cancer drug to bind to the cancer cell and induce apoptosis or fluorescently labeled to facilitate selective detection of glioblastoma cancer cells. These strategies highlight the potential of CD133-targeted aptamers for both therapeutic intervention and diagnostic applications in glioblastoma.

2. RESULTS

Molecular docking simulations were conducted to analyze the interactions between the aptamers and the CD133 receptor, Figure **3.** All aptamers successfully bound to the predicted binding site of the receptor, indicating their strong affinity for the target. The aptamer-CD133 complex was analyzed further using protein-ligand interaction profiler (PLIP) software, which revealed that the CD133A aptamer formed the most stable interaction due to its higher number of hydrogen bonds and salt bridges. These interactions made the CD133A aptamer the most suitable candidate for further study.

Next, the aptamers' secondary and tertiary structures were predicted to assess their potential for binding to CD133, Figure 4 and 5. The primary base sequence of the aptamer was uploaded to the UNA web server, which generated the 2D secondary structure. This was subsequently used to predict the 3D tertiary structure using UNAFold. The resulting structure was then utilized for molecular docking simulations, where the aptamer was docked onto the CD133 receptor for further interaction analysis.

Molecular Docking Simulations: Molecular docking simulations were conducted to analyse the interactions between the aptamers and the CD133 receptor. The results revealed that all aptamers successfully bind to the predicted binding site of the receptor. The aptamer-CD133 complex is depicted in Figure 6. A protein-ligand interaction profiler (PLIP) analysis was performed using PLIP software, which detailed the interactions between the CD133 receptor and the aptamers to identify the most suitable aptamer (Figure 7). Based on this analysis, the CD133A aptamer exhibited the highest number of hydrogen bonds and salt bridges, making it the most appropriate candidate for further study.

Crossing the blood-brain barrier (BBB) presents a significant challenge in drug delivery, particularly for targeting glioblastoma. Nanoparticles offer a promising solution for transporting aptamer-conjugated drugs across this barrier. These tiny particles can encapsulate or conjugate therapeutic agents, enhancing their stability and bioavailability. In our research, we utilized CHARMM-GUI, a molecular modelling tool, to design and simulate the nanoparticle structure, ensuring its effectiveness in delivering the drug.

A crucial component of this system is the protamine-aptamer complex, which plays a key role in protecting the aptamer during delivery. By forming this complex, the aptamer remains stable and can efficiently reach the negatively charged tumor microenvironment in glioblastoma. This targeted approach enhances drug efficacy while minimizing degradation and off-target effects, making it a potential strategy for overcoming the limitations of traditional treatments.

3. DISCUSSION

The CD133 receptor is overexpressed in childhood cancers such as glioblastoma and medulloblastoma, making it a valuable target for cancer detection and treatment. Its presence on cancer stem cells allows for early identification, crucial for improving treatment outcomes. This study developed aptamers that specif-

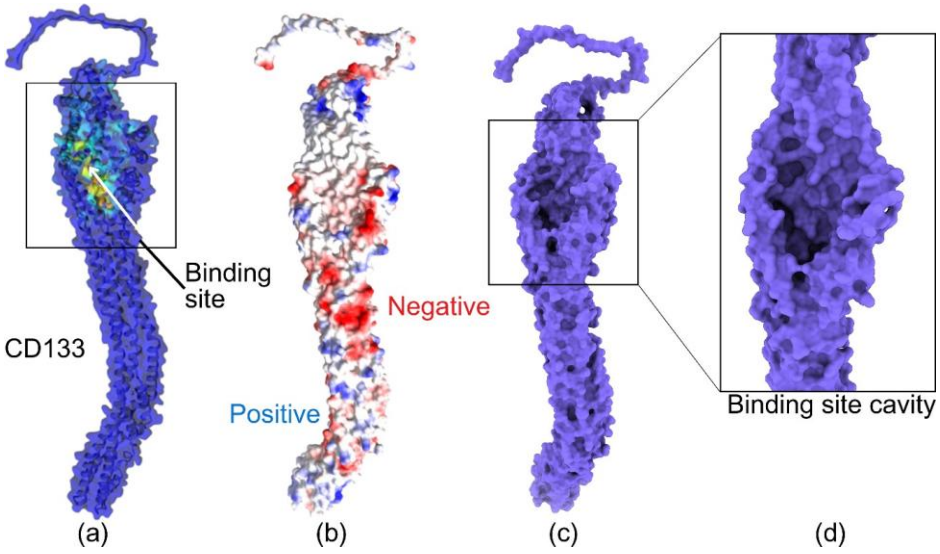

Fig. 3. Surface properties of CD133: (a) binding site prediction on the surface of CD133 protein; (b) Electrostatic Surface Potential (ESP) tells us the charge of the CD133; and (c) shows the two monomers of CD133.

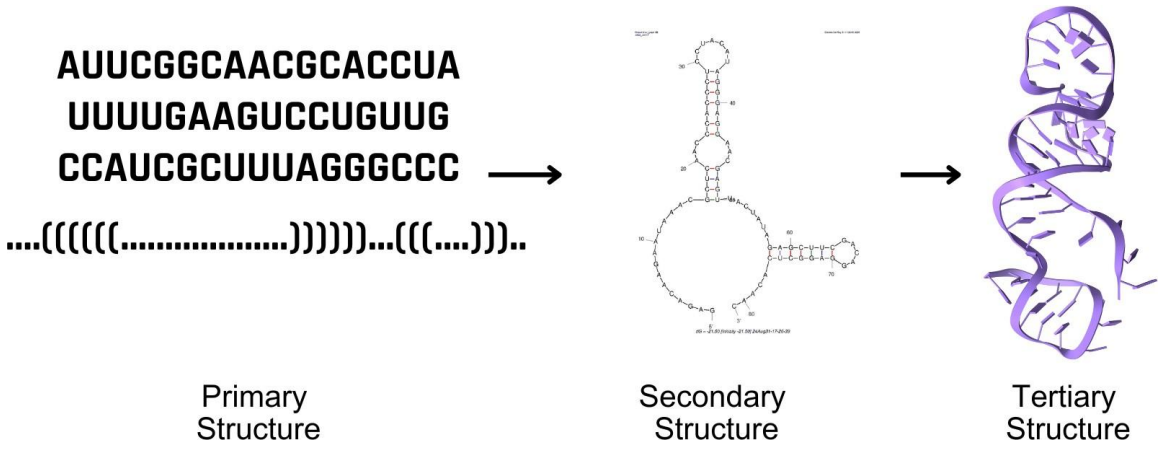

Fig. 4. Aptamer Structure Prediction: Begin with the nucleotide sequence of the aptamer. Dot-Bracket Notation: Predict secondary structure using dot-bracket notation to represent base pairs and loops. Tertiary Structure: Build the 3D structure by modelling the secondary structure into its spatial conformation.

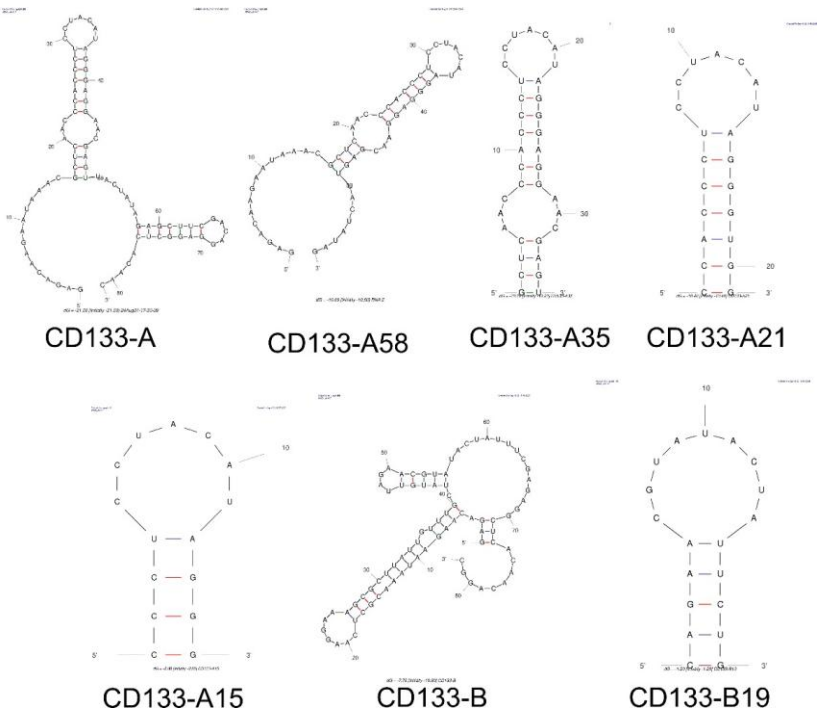

Fig. 5. The image illustrates the aptamers' two-dimensional (2D) or secondary structure, highlighting the base pairs forming hydrogen bond interactions. These 2D structures are critical for determining the three-dimensional (3D) structure, as they define the hydrogen bond interactions between the base pairs. The structure includes key features such as bulbs, stems, and roots, essential for the aptamer's overall folding and functionality.

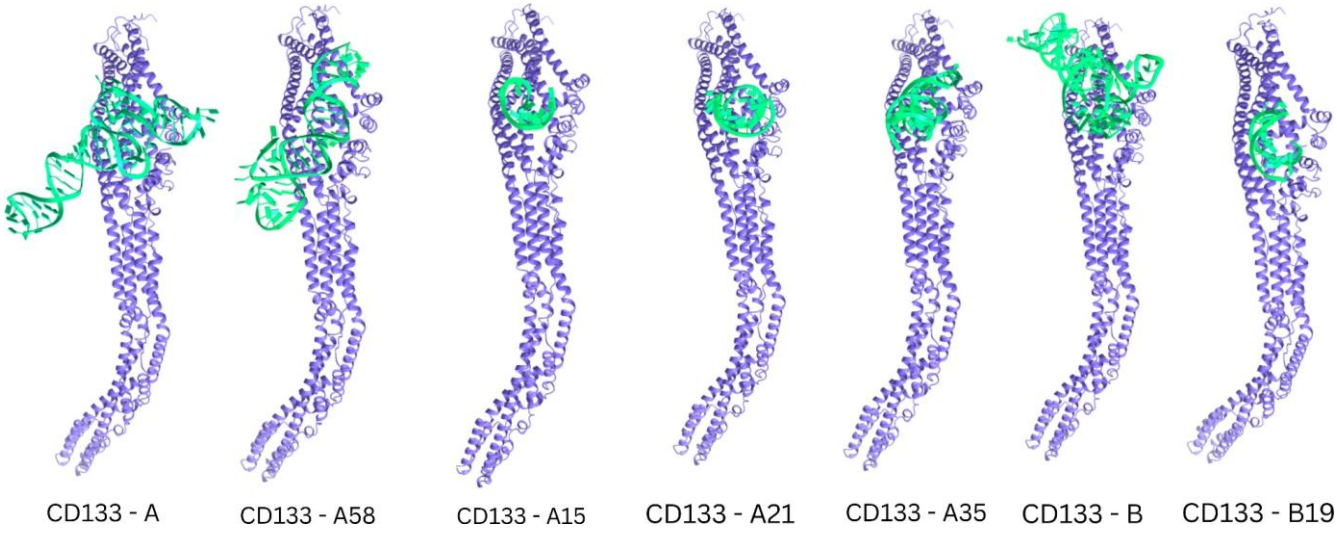

Fig. 6. Various predictions of how the aptamer would bind to CD133 using the HDOCK2.0 software. The CD133 protein is in purple and the aptamers are shown in green.

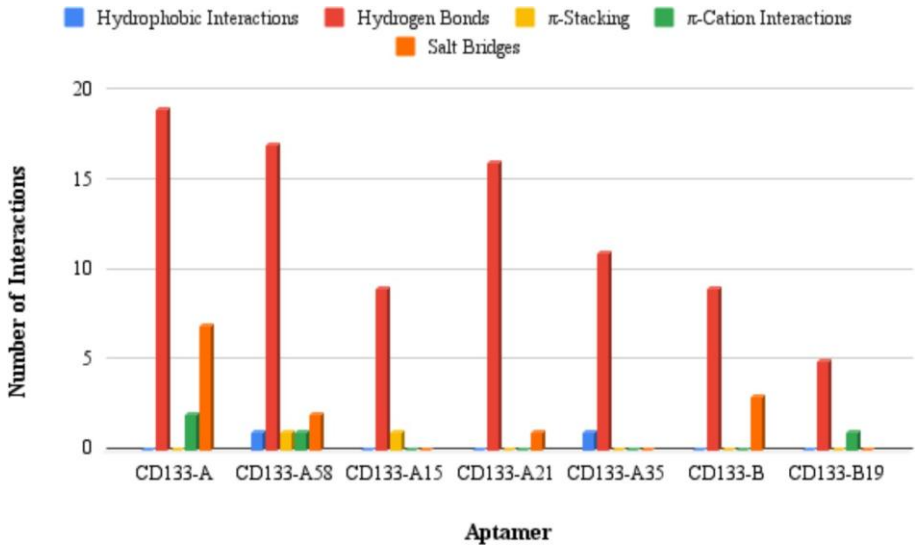

Fig. 7. Number of interactions formed between mature CD133 and various aptamers. The graph illustrates that hydrogen bonds and salt bridges were the predominant interactions, with a higher frequency than van der Waals and other non-covalent interactions.

ically bind to CD133, with the potential for incorporation onto nanovesicles encapsulating therapeutic agents. This targeted drug delivery method offers a promising strategy for early treatment, particularly in neonatal cancers. Additionally, CD133 is present in placental stem cells, further supporting its diagnostic utility in neonatal patients. Despite the promising applications, this study's computational nature is a key limitation. While molecular docking simulations provide insights into aptamer binding, experimental validation is required to confirm these interactions. Techniques such as surface plasmon resonance (SPR) or isothermal titration calorimetry (ITC) are necessary to verify aptamer-receptor binding. [6] Further validation through in vitro and in vivo studies is essential to assess the efficacy of these aptamers in glioblastoma treatment. Another constraint is the limited number of aptamers tested due to resource constraints. Future research should expand the aptamer library by introducing chemical modifications to the base pairs and conducting additional molecular docking simulations, increasing the likelihood of identifying the most effective aptamers for therapeutic and diagnostic applications.

One of the significant challenges in glioblastoma treatment is crossing the blood-brain barrier (BBB), which restricts the delivery of therapeutic agents to the brain. Nanoparticle-based drug delivery provides a potential solution, as aptamer-functionalized nanoparticles could enhance transport across the BBB. Future studies should investigate the stability and efficiency of aptamer-nanoparticle complexes in facilitating drug delivery to glioblastoma cells. Computational models such as CHARMM-GUI may aid in optimizing nanoparticle formulations to ensure effective targeting and drug release. Additionally, protamine-aptamer complexes could be explored to improve aptamer stability and enhance therapeutic efficacy. Addressing these challenges will translate computational findings into viable clinical applications. Aptamers also present a promising alternative to conventional therapeutic strategies such as mRNA therapy, monoclonal antibodies (mAbs), and small-molecule drugs due to their high specificity, stability, and ability to penetrate biological barriers like the BBB. mRNA therapy, which delivers genetic instructions for protein synthesis, has shown success in vaccines and personalized medicine. However, its application in brain cancers is limited by its instability, susceptibility to enzymatic degradation, and difficulty crossing the BBB (Karimi-Sani et al., 2024). Particularly, monoclonal antibodies (mAbs) have large molecular sizes that prevent efficient BBB penetration, making drug delivery to glioblastoma cells particularly challenging. Additionally, mAbs require costly production in mammalian cell systems and can elicit immune responses, reducing their therapeutic viability (Rodríguez-Nava et al., 2023). Small-molecule drugs, though capable of crossing the BBB due to their size, often suffer from poor specificity, leading to off-target effects and systemic toxicity. In contrast, aptamers are small, synthetic, single-stranded nucleic acids that fold into three-dimensional structures, allowing them to bind with high affinity to specific targets. Their small size and chemical flexibility make them ideal for crossing the BBB, enabling targeted drug delivery to glioblastoma cells while minimizing off-target effects. Furthermore, aptamers can be synthesized at a lower cost compared to mAbs and exhibit greater stability than mRNA therapies, making them a viable approach for glioblastoma treatment and imaging.

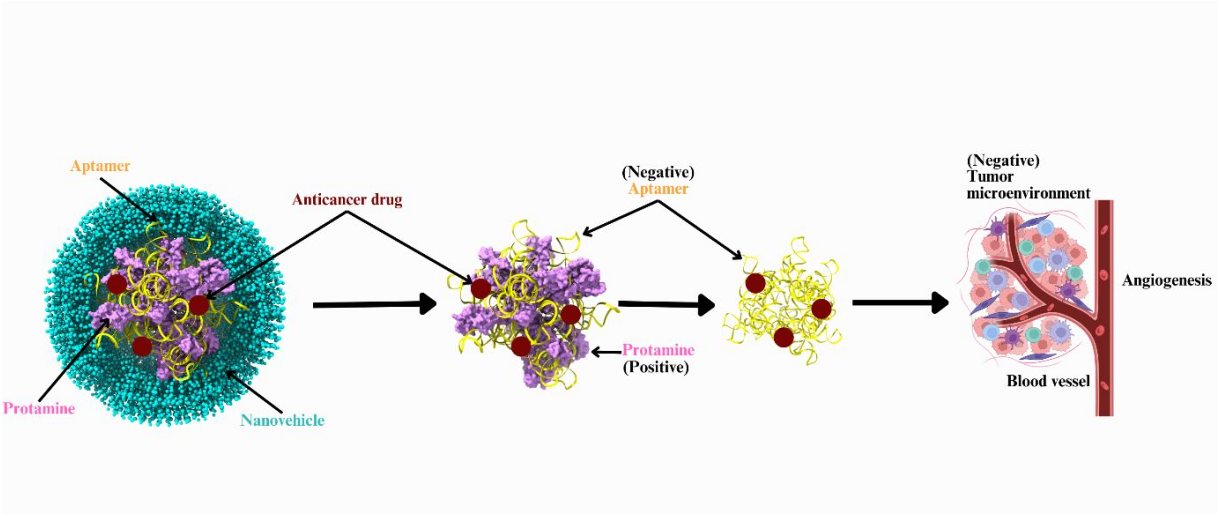

Fig. 8. Schematic representation of a nanovehicle-mediated drug delivery system targeting the tumor microenvironment. The nanovehicle, composed of protamine (pink) and encapsulated with aptamers (orange), carries anticancer drugs (red) for targeted therapy. Upon release, negatively charged aptamers interact with positively charged protamine, forming a complex that targets the negatively charged tumor microenvironment. This approach inhibits angiogenesis and effectively delivers anticancer drugs to tumor sites.

4. CONCLUSION

This study employed computational simulations to identify aptamers that bind to the CD133 receptor on glioblastoma cells. These aptamers have two primary applications: serving as targeted drug delivery agents and acting as biomarkers for cancer detection. Aptamers can facilitate drug delivery by selectively binding to CD133-expressing cells, thereby enabling the direct transport of therapeutic agents to tumor sites while minimizing off-target effects. As biomarkers, aptamers offer high specificity in detecting cancerous cells, aiding in early diagnosis and intraoperative imaging. One critical challenge in glioblastoma treatment is distinguishing cancerous cells from healthy tissue during surgery. By conjugating fluorescent dyes to these aptamers, they can be utilized for blue light fluorescence imag- ing, allowing for more precise tumor resection. Additionally, residual cancer cells infiltrating surrounding tissues could be degraded using aptamer-based therapeutics. Although promising, further experimental validation is required to confirm the binding affinity of these aptamers. Surface plasmon resonance and isothermal titration calorimetry should be performed to verify their interaction with the CD133 receptor. Moreover, investigating their ability to penetrate the blood-brain barrier and effectively deliver therapeutic agents is necessary for clinical applications. Despite these challenges, this research lays the foundation for novel therapeutic strategies against glioblastoma, potentially improving early detection and treatment approaches.

5. METHOD

CD133 is a glycoprotein and a marker primarily used to identify cancer stem cells. It is commonly found on the surface of stem cells in tissues like the brain, liver, pancreas, and blood. AlphaFold 3 is an AI model by Google DeepMind that predicts the 3D structures and interactions of biological molecules, aiding drug discovery and biological research [7]. The process involves several steps to generate a 3D model of the RNA aptamer from its primary sequence. First, the RNA sequence is converted from 1D to a connectivity table (CT) file. This begins by visiting the RNA folding server at mfold, uploading the RNA sequence, and saving the PNG image of the folded structure using Canva [8]. The CT file is downloaded for further processing. Next, to convert the CT file to a 2D structure, the file is uploaded to the RNAstucture CT2Dot server [9]. The dot and bracket notations are generated and can be copied into the research paper to represent the 2D structure. For 3D modeling, the RNA sequence and its dot-bracket notations are entered into the Vfold3D server [10]. The number of clusters is set to one, an email ID is provided for notifications, and the job is submitted to generate the 3D structure.

REFERENCES

1. C. Alifieris and D. T. Trafalis, “Glioblastoma multiforme: Pathogenesis and treatment,” Pharmacology & Therapeutics **152**, 63–82 (2015).
2. P. Vora, C. Venugopal, S. K. Salim, N. Tatari, D. Bakhshinyan, M. Singh, . . Singh, and S, “The Rational Development of CD133-Targeting Immunotherapies for Glioblastoma,” Cell Stem Cell **26**, 832–844 (2020).
3. M. R. Dunn, R. M. Jimenez, and J. C. Chaput, “Analysis of aptamer discovery and technology,” Nature Reviews Chemistry **1**, 76–76 (2017).
4. L. Yang, X. Zhang, M. Ye, J. Jiang, R. Yang, T. Fu, . . Tan, and W, “Aptamer-conjugated nanomaterials and their applications,” Adv Drug Deliv Rev **63**, 1361–1370 (2011).
5. J. Fan, A. Fu, and L. Zhang, “Progress in molecular docking,” Quantitative Biology **7**, 83–89 (2019).
6. G. K. Krishnamoorthy, P. Alluvada, S. H. M. Sherieff, T. Kwa, and J. Krishnamoorthy, “Isothermal titration calorimetry and surface plasmon resonance analysis using the dynamic approach,” Biochemistry and Biophysics Reports **21**, 100712–100712 (2020).
7. J. Abramson, J. Adler, J. Dunger, R. Evans, T. Green, A. Pritzel, . . Jumper, and J. M, “Accurate structure prediction of biomolecular interactions with AlphaFold 3,” Nature **630**, 493–500 (2024).
8. N. R. Markham and M. Zuker, “UNAFold,” in “Bioinformatics: Structure, Function and Applications,” J. M. Keith, ed. (Humana Press, 2008), pp. 3–31.
9. J. S. Reuter and D. H. Mathews, “RNAstructure: software for RNA secondary structure prediction and analysis,” BMC Bioinformatics **11**, 129–129 (2010).
10. J. Li, S. Zhang, D. Zhang, and S. J. Chen, “Vfold-Pipeline: a web server for RNA 3D structure prediction from sequences,” Bioinformatics **38**, 4042–4043 (2022).