The AptaReport™
Newsletter
Spring 2023

Development and Characterization of DNA aptamer against Retinoblastoma by Cell-SELEX

Retinoblastoma (RB) is a type of aggressive, malignant tumor inside the eye. It is the most prevalent type of ocular tumor affecting children, affecting about 1 in 16,000 children at birth. Medical advances in recent years have improved outlook for patients affected by RB. A recent paper from the Vision Research Institute in India seeks to add to these by using aptamers, which are short DNA or RNA molecules designed to bind to a selected molecule. They are useful in oncology for imaging, detection, and possibly even drug delivery. Researchers have developed a specific aptamer—in this case a DNA aptamer—that can target specific cells affected by RB, while avoiding healthy cells.

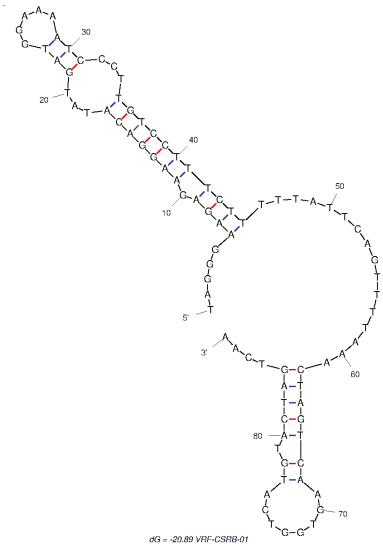

Figure 1: Aptamer VRF-CSRB-01 secondary structure predicted by mFold. Note: Structure may be inaccurate due to changes resulting from interactions with the target.

After testing for binding and sequencing the aptamers through Next Generation Sequencing (NGS)—a modern process in which one can find the order of the DNA building blocks in the larger molecule—the researchers set out to prove that their process worked and would be effective for use in medicine. They determined that the aptamer VRF-CSRB-01, could bind to upwards of 94% of cells affected by RB while avoiding healthy cells. To show this, they attached a light-emitting dye to the aptamer and incubated it with both healthy and cancerous cells. The green dye on the end of the aptamer shows clearly in the image of RB cells, while it is washed away from healthy cells because the aptamer did not bind to the tumor. The group hopes to do further research using this aptamer by attaching chemotherapeutic drugs to it for targeted drug delivery, which would send the drugs straight to affected cells.

*Reference: Apta-Index™ ID# 7299 -M.C.

Selection and identification of a novel ssDNA aptamer targeting human skeletal muscle

Skeletal muscles make up nearly 40% of our cells and allow us to move our bodies. They attach to bones by tendons and are voluntary, unlike other muscle cells in the body. Diseases that alter the function of skeletal muscles significantly decrease quality of life. Some examples include muscular dystrophy, cerebral palsy, and myasthenia gravis. The number of people with skeletal muscle degenerative diseases increases as our life expectancy increases. Standard treatments are often effective but untargeted, causing systemic side effects. Aptamer technology can be used to create a targeted therapeutic that can deliver drugs specifically to skeletal muscles.

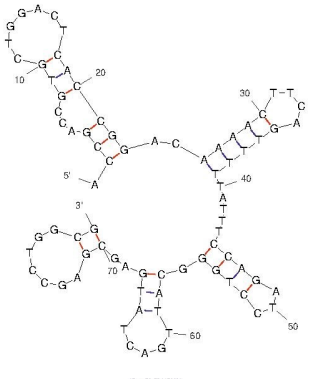

Figure 2: Aptamer HSM01 secondary structure prediction based on mFold, with respect to the ionic strength of the binding buffer. Note that secondary structure may be inaccurate due to other modifications, and changes resulting from interactions with the target.

In this study, researchers identified an aptamer which binds specifically to skeletal muscle cells. The aptamer that was selected as the most efficient, HSM01, is a single stranded DNA molecule consisting of 80 nucleotides. The researchers elected to utilize cell SELEX in order to select for this aptamer, using human skeletal muscle cells as their positive target, and human umbilical vein endothelial cells as their counter target. This method amplifies DNA that binds to the muscle cells while those that bind to the endothelial cells are discarded in each round. After 13 rounds, a few candidates were identified, two of which continued to further in vitro and in vivo testing. Flow cytometry demonstrates an increased binding between both aptamers (HMS01 and HMS02) with human skeletal muscles when compared to the controls, while there is little to no binding to the endothelial cells. These results were confirmed by microscopy, which also demonstrated no aptamer binding to epithelial cells.

The researchers continued to perform in vivo tests in mice and tree shrews and determined that the HSM01 aptamer was most effective. Furthermore, the drug delivery device of liposomes (similar to isolated cell membranes) was tested and found to be an effective method, providing long-term circulation. These findings support the potential for this aptamer to be used for clinical applications for a variety of skeletal muscle pathologies.

*Reference: Apta-Index™ ID# 7300 -A.K.

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An antigen-targeting assay for Lyme disease: Combining aptamers and SERS to detect the OspA protein

Antibodies are our most reliable immune system response to detecting early stages of Lyme disease since they can be identified in clinical assays. However, current diagnostic tests, like the common clinical application of surface enhancing Raman scattering (SERS), only identify 40-60% of Borrelia infections from the Ixodid tick species. The red, erythema migrans (EM) rash commonly experienced is another response that has limitations for diagnoses. This has led to many Lyme disease cases being diagnosed after the optimal period for effective treatment and hence the quest for an improved diagnostic method has been underway.

A revised SERS method called orthogonal projections to latent structures discriminant analyses (OPLS-DA) allows spectra differentiation (the distinguishing of peaks on a spectrum) between Lyme samples and symptomatic control samples as well as the classification of unknown samples. This alone is not enough to gain significant results and led to the combination of this method with an aptamer, a molecule of high binding ability to a target. In this case, modified aptamer, Ion-OspA-59, was chosen since it achieved the highest affinity and specificity of six varieties towards the target, Outer Surface Protein A (OspA), which is active in Lyme disease infections. In the figure, modifications are shown at both ends of the aptamer: the DTPA labels the cytosine on the 5-prime end providing a site for the SERS substrate to bind and the 5OctdU, which is a shared component of multiple compounds, replaces the T on the 3-prime end to distinguish specific signals on a spectrum.

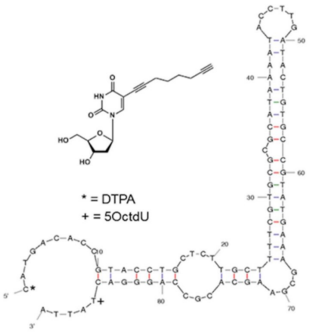

Figure 3: *Ion-OspA-59 DNA aptamer secondary structure prediction based on mFold, with respect to ionic strength of the binding buffer. Note that secondary structure may be inaccurate due to the presence of non-DNA bases, other modifications, and changes resulting from interactions with the target.*

With this set up, serum samples were collected from 23 Lyme disease patients and 24 symptomatic control (Lyme mimicking) patients to be run through the revised SERS plus aptamer method. Spectra data of the aptamer with different spectral features based on the two Lyme conditions were plotted and used to determine clinical sensitivity (% of correctly assigned OspA positive samples) and specificity (% of correctly assigned OspA negative samples). Twenty one of 23 of positive cases and 23 of 24 negative cases showed 91% sensitivity and 96% specificity of Ion-OspA-59 to the OspA even at the lowest detection concentration of 1 x 10 -4 ng/mL. These results suggest a new analytical method to diagnostically address Lyme and other diseases that are outside of our current methods.

*Reference: Apta-Index™ ID# 7304 - L.Z.

Potential Therapeutic use of Aptamers against HAT1 in Lung Cancer

Lung cancer is one of the leading causes of death worldwide and the most common of all cancer types. Histone acetyltransferase 1 (HAT1) is said to be a potential therapeutic target due to its involvement in multiple pathologies, including cancer. HAT1 overexpression can be due to viral infections, inflammatory diseases, and cancer, where it is associated with poor prognosis and low survival. Therefore, HAT1 is a potential therapeutic target.

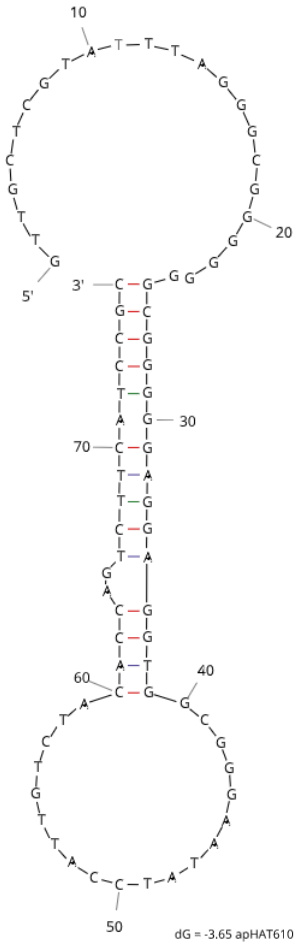

Figure 4: *apHAT1 aptamer prediction of secondary structure and G-quadraplexes based on mFold and QGRS Mapper programs. Red and blue boxes indicate the G-quadraplex groups, a kind of nucleic acid secondary structure comprising two or more G-quartet blocks bounded by Hoogsteen hydrogen bonding in G-rich oligonucleotide sequences. Note: The secondary structure may be inaccurate due to the presence of non-DNA bases, other modifications, and changes resulting from interactions with the target.*

In this work, aptamers against HAT1 are identified by performing SELEX. After six rounds of screening, two specific aptamers are obtained and subsequently characterized and optimized. Both aptamers and one derivative based on modified sequences recognized HAT1 with high affinity and specificity and can inhibit the acetyltransferase activity of HAT1 in vitro. In addition, the application of the apHAT610 aptamer produces reduced cell viability, induced apoptosis and cell cycle arrest, and inhibited colony formation in the lung cancer cell lines. What is more, the apHAT610 aptamer inhibited HAT1 activity in these three cell lines, in a way that produces a decrease in histone H4 acetylation and HAT1 protein levels. According to the results published in this paper, the apHAT610 aptamer could be considered a potential drug for the treatment of lung cancer.

*Reference: Apta-Index™ ID #7303 - P.B.

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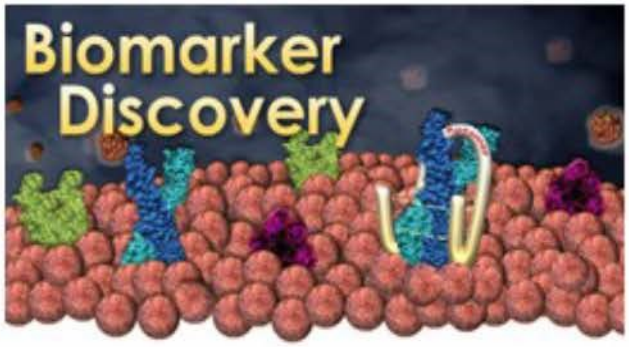

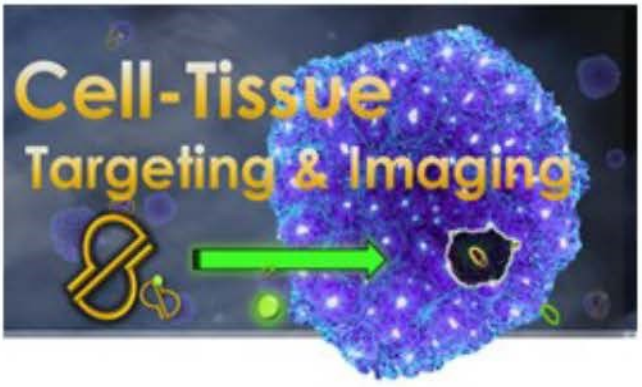

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