The AptaReport™

Newsletter

Fall 2023

Aptamer Based Diagnosis of Crimean-Congo Hemorrhagic Fever from Clinical Specimens

Crimean-Congo Hemorrhagic Fever (CCHF) is an acute viral zoonotic disease with a widespread geographic distribution. Due to an increase in its incidence from new regions and its potential to cause public health emergencies, there is a critical need for rapid and accurate diagnostic methods, especially in rural and remote areas where most cases occur. Aptamers, which are specific and sensitive molecular tools, have been identified as promising candidates for rapid diagnostic methods. In this report, we summarize a research paper titled "Aptamer-based Diagnosis of Crimean-Congo Hemorrhagic Fever from Clinical Specimens," which outlines the development of an aptamer-based diagnostic test for CCHF using the Nucleoprotein (NP) of the CCHF virus (CCHFV) as the target. The primary objective of the research was to develop a simple, specific, and sensitive diagnostic assay for the rapid detection of CCHFV infection. To achieve this, the researchers aimed to isolate aptamers targeting the NP of the CCHFV and then utilize the most effective aptamer to design an aptamer-antibody ELASA (Enzyme-Linked Aptamer Sorbent Assay) test for the detection of CCHF NP in clinical specimens.

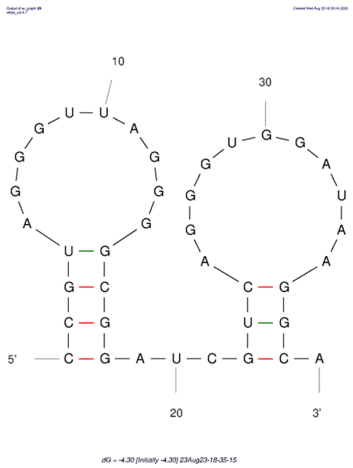

***Figure 1**: Aptamer 33 secondary structure predicted by mfold. Note that secondary structure may be inaccurate due to other modifications, and changes resulting from interactions with the target.*

The researchers employed Systematic Evolution of Ligands by Exponential Enrichment (SELEX) to isolate aptamers targeting the NP of the CCHF virus. They used a single-stranded DNA (ssDNA) aptamer library containing a random 40-nucelotide region between primer binding sites. This library under-went nine rounds of SELEX to enrich for aptamers with high affinity for the NP. The binding affinity of the obtained aptamers was calculated using Surface Plasmon Resonance technique. Among the isolated aptamers, Apt33, exhibiting the highest affinity to NP, was selected for further development.

Apt33 was utilized to design the aptamer-antibody ELASA test. This test successfully detected CCHF NP at a concentration of 90 ng/ml in human serum. The diagnostic accuracy of the test was then evaluated using clinical samples. The aptamer-antibody ELASA test demonstrated 100% specificity and sensitivity, indicating its potential as a reliable diagnostic tool for CCHF virus infection. The research results suggested that Apt33 can be incorporated into various rapid diagnostic tests for the early and accurate diagnosis of CCHF. The high sensitivity and specificity of the test make it a valuable tool for early detection and containment of outbreaks.

*Reference: Apta-Index™ ID# 7310 -S.J.

Selecting Small Molecule DNA Aptamers with Significant Conformational Changes for Constructing Transcriptional Switches and Biosensors.

The use of aptamers with substantial conformational changes within biosensors as well as transcriptional switches of target genes has grown over time. Researchers wanted to come up with a new SELEX method of creating high affinity aptamers while also maintaining large conformational changes. Some of the SELEX processes create aptamers that don't have significant conformational changes as the target molecules mostly bind to the stem-loop sections of the aptamers. The researchers focused on creating an aptamer that would target uridine-5'-diphosphate (UDP) which is a molecule that is the result of glycosylation of UDP-glucose, an important molecule in medicinal plants such as *Anoectochilus roxburghii*. After a few rounds of CIVT -SELEX the researchers found 6 aptamers that had relatively high affinity. Out of those 6 aptamers chosen for further testing Aptamer 3-83 was found to have the highest conformational change when in the presence of UDP.

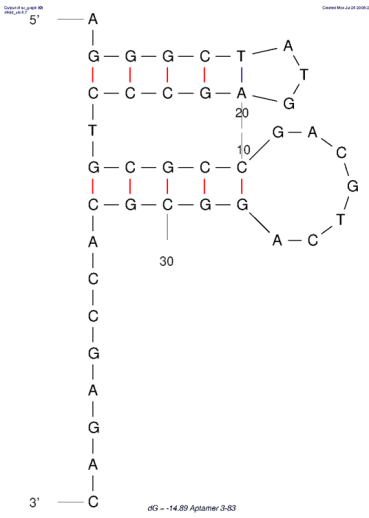

***Figure 2:** Prediction of Aptamer 3 -83 secondary structure created by mfold (Secondary Structure Program) utilizing papers buffer and temperature guidelines.*

The use of this aptamer between a T7 promoter and the target gene allowed the researchers to test whether the aptamer was able to downregulate or upregulate the transcription of the target gene with or without UDP present. They determined that in the presence of UDP Aptamer 3-83 was changing its conformation enough to allow the T7 polymerase to begin transcription more often than if no UDP was present. The ability to be a biosensor for the presence of UDP and the screening of glycotransferases' was also discovered by the researchers as this would alleviate the workload of Liquid Chromatography – Mass Spectrometry to determine the accuracy of the glycotransferases. They added a fluorescent probe SYBR Green I (SGI) that they believe attaches into the stem loop of the aptamer structure and when in the presence of UDP the structure of the aptamer was loosened. The inclusion of exonucleases decreased fluorescence because SGI was unable to bind to the loosened step-loop structure. Overall Aptamer 3-83 is a great representation of the possibilities of future biosensors and transcriptional switches using aptamers.

*Reference: Apta-Index™ ID# 7311 -J.K.

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A Novel Aptamer LL4A Specifically Targets Vemurafenib-Resistant Melanoma through Binding to the CD63 Protein

Melanoma is considered the most dangerous type of skin cancer and it occurs when the cells that produce melanin, melanocytes, become malignant. Various factors increase the risk of melanoma, including older age, genetic predisposition, and prolonged exposure to ultraviolet light from sources like the Sun. About half of patients that suffer with melanoma experience mutations in the BRAF gene. This gene is responsible for the continuous activation of the BRAF protein that leads to uncontrolled cell growth, contributing to the progression and spread of melanoma in individuals. The drug vemurafenib (PLX4032) was developed as a treatment for advanced melanoma patients as a BRAF inhibitor but its effectiveness has reduced over time due to adaptive resistance developed by melanoma cells, causing resistance towards the drug. Due to their high specificity, low immunogenicity, and ease of modification, aptamers are a great therapeutic solution for drug resistance in cancer.

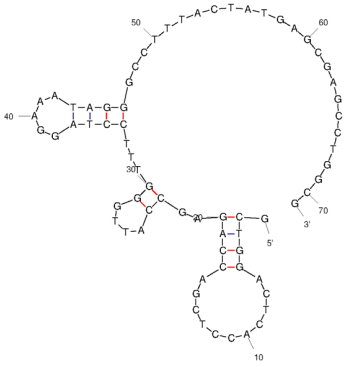


***Figure 3**: Aptamer LLKA secondary structure prediction based on mFold, with respect to the ionic strength of the binding buffer. Note that secondary structure may be inaccurate due to other modifications, and changes resulting from interactions with the target.*

In this study, researchers were able to obtain the DNA aptamer LL4 with high affinity and specificity towards vemurafenib-resistant melanoma cells both *in vivo* and *in vitro*. Cell-based SELEX was used to generate DNA aptamers that can bind specifically to PLX4032-resistant melanoma cells, using Mel28-PLX cells for positive selection and parental Mel28 cells for counterselection. After 15 rounds of selection, six candidates were identified, and LL4 was identified as the aptamer that could specifically bind the target with the highest affinity. Through optimization, the researchers were able to develop LL4A, a shorter form of the LL4 aptamer. Flow cytometry assays demonstrated the binding capacity and stability of the LL4A aptamer. *In vivo* studies in mice models with tumors in which fluorescent-labeled LL4A aptamer was injected through the tail vein revealed that the aptamer was able to recognize and target PLX4032-resistant melanoma cells via higher levels of fluorescent signals in Mel28-PLX tumors than in Mel28 tumors. This remarkable molecule targets a transmembrane protein called CD63 that is involved in the signaling pathways responsible for disrupting cell growth and overcoming drug resistance in melanoma cells. It was determined that CD63 is the binding target for LL4A by combining the liquid chromatography-tandem mass spectrometry method with a pull-down assay to examine the direct interactions between the CD53 protein and LL4A aptamer. These findings were able to establish LL4A as an aptamer with potential therapeutic uses against PLX4032-resistant melanoma while effectively targeting CD63 molecules.

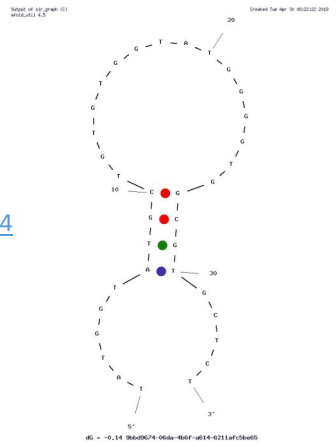
*Reference: Apta-Index™ ID# 7309 -I.M.

Reproductive Disorder Diagnostics Using Aptamer-Based Sensing

Abnormal levels of luteinising hormone (LH) secretion has been linked to multiple reproductive disorders. Monitoring these levels would be highly beneficial in the diagnosis and treatment of these disorders but current methods are expensive and time consuming. Liang et al. have developed a Robotic APTamer-enabled Electrochemical Reader (RAPTER) to measure the LH levels in blood samples.

This new method incorporates electrochemical aptamer-based (E-AB) sensing to test patient blood samples without needing to be washed or changed between readings. The E-AB is also cheaper than current tests making it an attractive alternative to use. The authors also hypothesize that the E-AB system could be adapted to other targets in the blood and it might be possible to incorporate the sensor in vivo to get real-time data from patients.

***Figure 4:** The secondary structure has been predcited to form a G-quadruplex. Note that secondary structure may be inaccurate due to other modifications, and changes resulting from interactions with the target.*



*Reference: Apta-Index™ ID# 644

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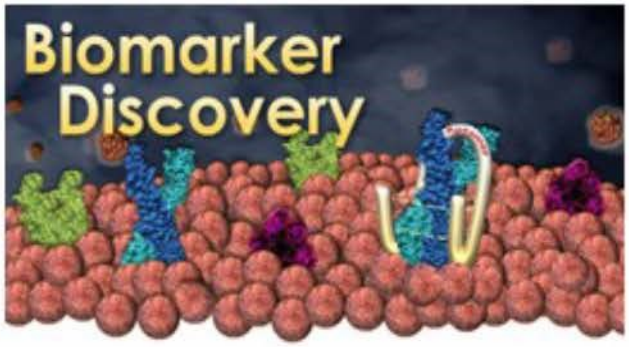

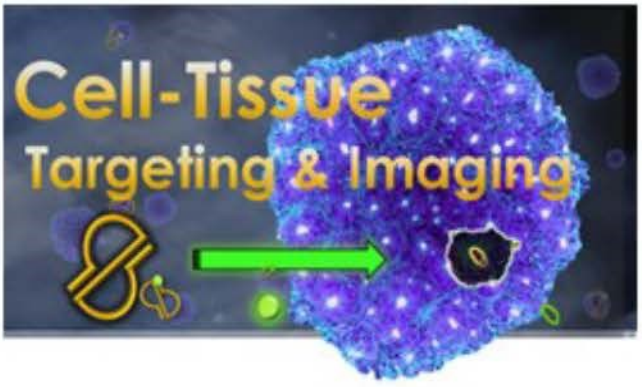

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