The AptaReport™

Newsletter Summer 2022

Fighting the Huntington's Disease with a G-Quadruplex-Forming Aptamer Specifically Binding to Mutant Huntingtin Protein: Biophysical Characterization, In Vitro and In Vivo Studies

Huntington's disease is known for its neurodegenerative effects which result in a decrease of cognitive function and coordination along with behavioral changes. This disease comes from mutated Huntingtin, a protein characterized by a high amount of CAG repeats within the first exon of its gene. Numerous studies have been conducted for its treatment with very little promise. Researchers in Naples, Italy conducted experiments observing various properties and therapeutic effects of an aptamer called MS3. MS3 has a repeating four nucleotide sequence, GGGA, which allows for G quadruplexes to form that are believed to provide some resistance to degeneration via nucleases. This aptamer was able to enter neuronal cells without killing them and can exist within them for multiple hours. After in vitro studies, the researchers moved to see how the aptamer works in vivo using transgenic Drosophila melanogaster (small fruit flies) predisposed to Huntington's Disease as the model.

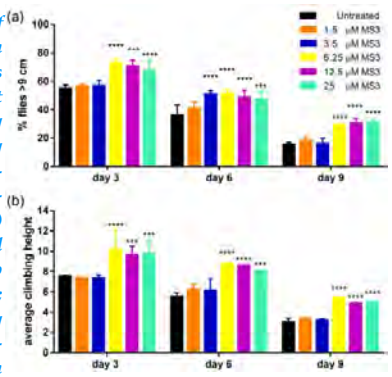


*Figure (adapted) Climbing ability of age-matched adult female flies grown on food supplemented with different doses of MS3, evaluated at four different points: presymptomatic (pre-HD; 1 day posteclosion, not shown), early symptomatic (early-HD; 3 days post-eclosion) and late symptomatic (late-HD; 6 and 9 days post-eclosion). (a) Percentage of treated flies that climbed over 9 cm was higher in comparison to sibling flies in a dose-dependent manner; (b) average climbing height reached by treated and untreated flies, both in pre- and late symptomatic periods. For each condition, the climbing ability of three groups of 20 flies was monitored (n = 60) for a total of 3 trials (n = 180). Analysis of data was conducted using ANOVA one way; data represents mean ± SEM (**** p < 0.0001; *** p < 0.001; compared with untreated as control).*

Flies were split into six groups which each had different concentrations of MS3 in their food and were tested on climbing height as a measure of motor ability.

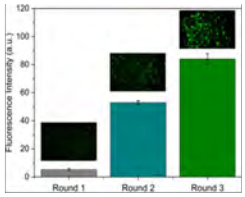
In vitro studies showed that the aptamer was not cytotoxic to cells at the observed concentrations. The researchers also observed a dose dependent increase in therapeutic effect from the in vivo study. Flies that had a higher concentration of aptamer treated food were able to fly higher on average than their lower concentration or untreated counterparts. Having observed promising data from both studies, MS3 may serve as a possible therapeutic for Huntington's patients.

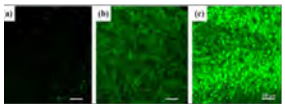
*Reference: Apta-Index™ ID# 7263

R.M.

Surface Acoustic Wave Assisted Microfluidic Isolation of Aptamers

Microfluidics have lately been used in SELEX operations to boost efficiency and reduce expenses. Surface acoustic waves (SAW) may be utilized to enhance affinity selection in microfluidic SELEX. The use of acoustic streaming improves the interactions between solution-based oligonucleotide molecules and microbead-immobilized target molecules, allowing for a more efficient search for high-affinity aptamer candidate. At the microscale level, SAW-aided mass transfer strengthens the interactions between oligonucleotide molecules and beads-bound target molecules.





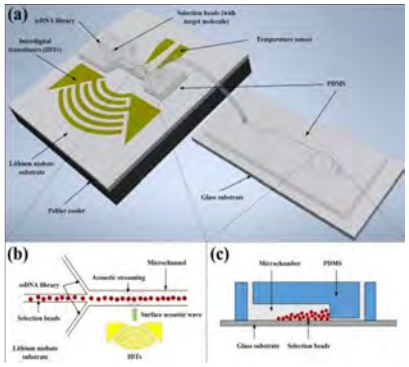


Figure (adapted): SAW-assisted microfluidic SELEX device. (a) Schematic of the device. (b) Acoustic-assisted incubation microchannel. (c) Separation microchamber. Figure (adapted): Fluorescence intensity of IgE-coated beads after incubation with the enriched ssDNA pool from each round. Figure (adapted) Fluorescence imaging of bead-immobilized IgE incubated on-chip with an ssDNA library doped with 1% D17.4 aptamer under different conditions: (a) without acoustic waves, (b) with 150 mVpp acoustic waves, and (c) with 250 mVpp acoustic waves.

Using microbeads, an acoustic incubation channel, and a separation microchamber, the SELEX microfluidic system can select for high affinity aptamers. The separation microchamber successfully selected weakly bound ssDNA molecules using IgE protein as a model target. Fluorescence microscopy was used to monitor the enrichment of the target-binding ssDNA pool directly in the microchamber. Three rounds of SELEX yielded an IgE aptamer with high affinity (Kd = 22.6 nM) and specificity in 5 hours from an initial ssDNA library. When compared to non-SAW microfluidic approaches, there was a considerable improvement in efficiency and time, highlighting the promise of SAW-assisted continuous flow microfluidic SELEX for accurate and faster synthesis of aptamers against biological targets.

*Reference: Apta-Index™ ID# 7265

V.R.

The AptaReport™

Newsletter

The Identification of Single Strand DNA Aptamers Which Specifically Bind to Platelets Using Cell-SELEX Technique

Platelets, also known as thrombocytes, are the second most abundant cell type in blood. Though small, they are vital to important processes like blood clotting and wound healing, and are known to play a role in inflammation, tumor growth, and metastasis. Platelets are also important in atherosclerotic disease, where it is important to prevent clots from occurring. Current anti-platelet medications are limited in scope and have no antidotes, increasing potential risk of severe bleeding. However, aptamer technology offers an opportunity to create more specialized therapeutics.

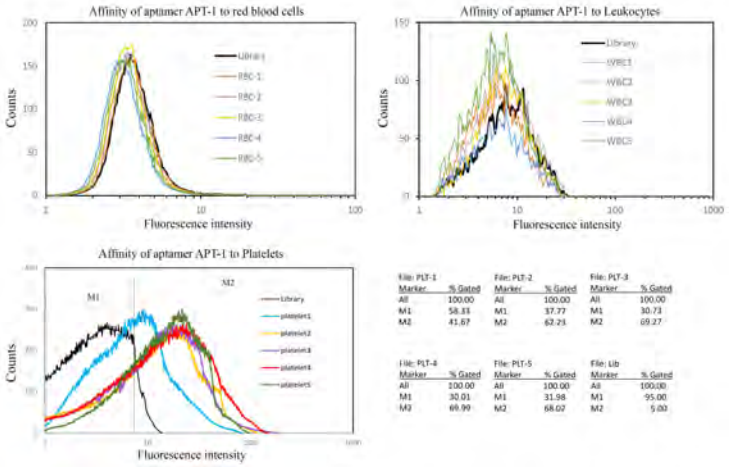


Figure (adapted): These flow cytometry charts show the affinity of APT-1 to platelet samples (bottom left), compared to samples of red blood cells (RBC; top left) and leukocytes (WBC; top right). The RBC and WBC fluorescence intensities match the control library (black), indicating no affinity to these cells. In contrast, platelet intensities were noticeably higher, demonstrating APT-1 preferentially binds to platelets.

In this study, researchers identified the first known aptamer designed to bind specifically to platelet membranes. The aptamer, APT-1, is a single-stranded DNA oligo 79 nucleotides in length. The SELEX process, which involved whole cells, included red blood cells and leukocytes as counter-targets. This allowed for potential aptamers that bound to blood components other than platelets to be removed. Thus, APT-1 has greater affinity for platelets than other blood cells (Figure 7). They also determined it does not have a significant impact on platelet function, but its binding does protect platelets from protease digestion. The researchers intend to continue their work by identifying which cell surface proteins APT-1 binds to. For now, the introduction of a platelet aptamer provides a potential tool for forensic identification of human platelets and for development of future therapeutics.

*Reference:Apta-Index™ ID# 7264 -S.S.

An Antibody-Aptamer-Hybrid Lateral Flow Assay for Detection of CXCL9 in Antibody-Mediated Rejection after Kidney Transplantation

Kidney transplantation is the most common transplant procedure performed globally. Despite the prevalence of the operation, the long-term success of a renal allograph is generally restricted by the effects of chronic antibody-mediated rejection (AMR). To limit the effect of rejection on kidney function, transplant recipients are monitored regularly for indicators of rejection such as the presence of donor-specific antibodies (DSA), and serum creatinine elevation. However, as these indicators are not entirely specific to transplant rejection, the presence of DSA or elevated creatinine requires a more invasive procedure such as a biopsy for a certain diagnosis. This study describes a new lateral flow assay used to detect a biomarker CXC-motif chemokine ligand (CXCL9) which was found to indicate AMR when present in urine. The Aptamer used in this study is G123, an 84 nucleotide aptamer that was discovered using SELEX. It was found to have a binding affinity to CXCL9 with a KD of 92 ± 14 nM. To facilitate use in the test strip the aptamer was modified with a 5′ disulfide linker, extended through a hexaethylene glycol spacer, and conjugated with gold nano-particles.

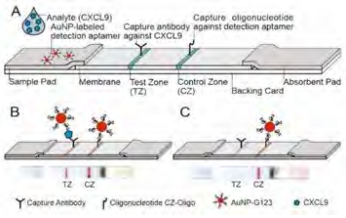
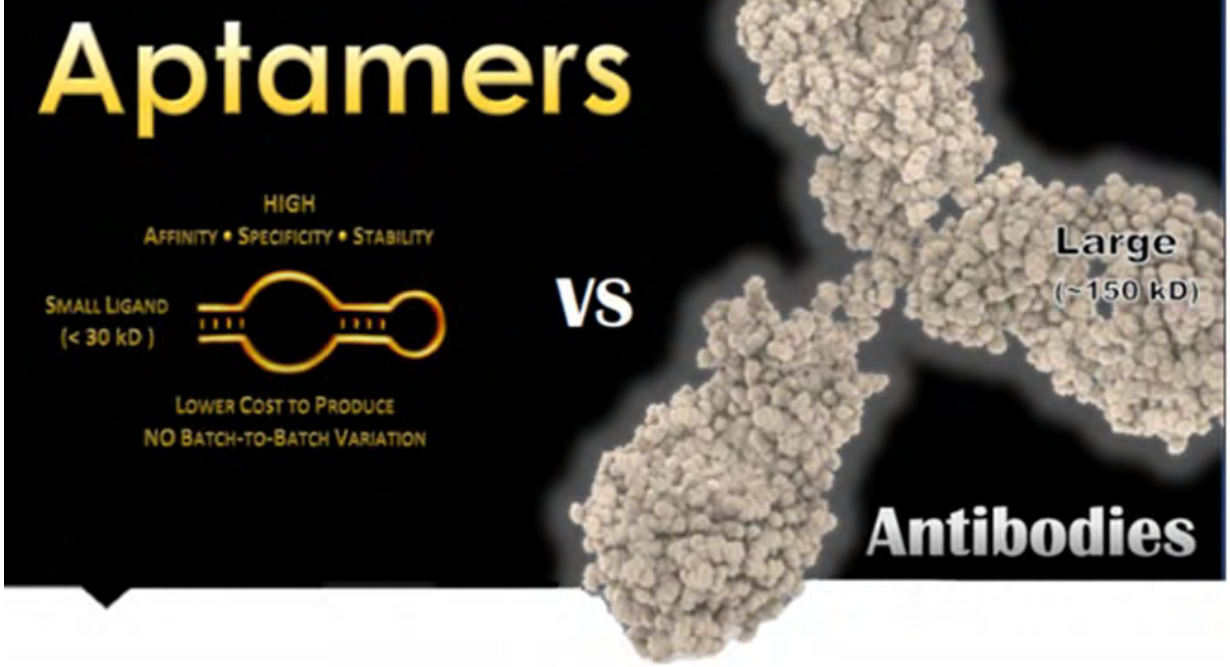


Figure (adapted): The proposed aptamer-antibody hybrid lateral flow assessment. Analyte is bound to the detection aptamer, which is passed across a specific capture antibody targeting the CXCL9 analyte. If a CXCL9-aptamer complex is present, the labeled aptamer presents in the test area. To prevent false negatives, the control zone contains an oligonucleotide specific to the labeled aptamer. Aptamer presence in each zone is indicated with a visual red line.

To analyze the results of the test strip developed in this study, test results for the aptamer-antibody sandwich assay were compared to results from serum creatinine and an alternative test strip developed using only antibodies. All of these results were subsequently confirmed via patient biopsy. Compared to the antibody only test strip, the aptamer assay showed a similar specificity, but a higher sensitivity (71% vs 53%). When compared to the results indicated by serum creatinine under ROC analysis, the hybrid-LFA test strip exhibits an AUC of 0.799, while patient eGFR (calculated from serum creatinine) showed an AUC of only 0.428. While the results of this study show great promise for a preliminary screening method for transplant rejection utilizing the G123 aptamer, more work can be done to further increase the test strip's specificity or alternatively learn more about rejection indicating biomarkers.

*Reference: Apta-Index™ ID #7266 -A.H.



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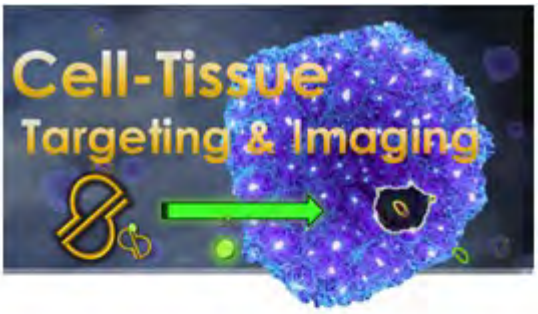

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