The AptaReport™
Newsletter
FALL 2021

An Aptamer That Binds To Vitamin C Could Help Alleviate Certain Forms Of Dementia

Vascular dementia is a cognitive impairment caused by insufficient blood supply to the brain. It damages brain cells via oxidization and inflammation, resulting in gradual memory loss and other neurological issues. Vitamin C is an essential nutrient that reduces and prevents oxidative tissue damage, like the damage vascular dementia causes. However, its benefits are short-lived. Vitamin C itself oxidizes very quickly and often depletes before it can do any real help. NXP031 is a DNA-based aptamer that has a strong affinity for for vitamin C. When NXP031 binds with vitamin C, it prevents the vitamin's oxidization and allows it to last longer in the body.

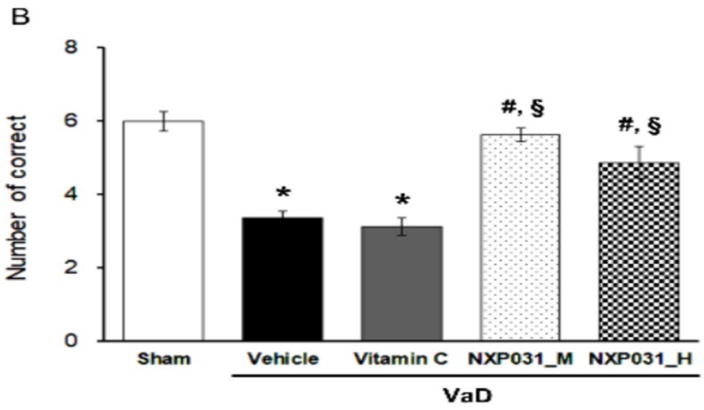

Figure 1 (adapted). Lee, J. M., Lee, J. H., Song, M. K., & Kim, Y. J. (2021). NXP031 Improves Cognitive Impairment in a Chronic Cerebral Hypoperfusion-Induced Vascular Dementia Rat Model through Nrf2 Signaling. International Journal of Molecular Sciences, 22(12), 6285.

Researchers at Kyung Hee University in Korea tested this newly discovered aptamer on rats with vascular dementia. They injected the rats with two separate forms of NXP031 and tested their working memory in a maze. The results were compared to those of healthy rats, rats injected with a placebo, and rats injected with vitamin C alone. Aptamer-treated rats showed memory comparable to healthy rats, and significantly better than placebo and vitamin-treated rats. Tissue necropsies also showed that NXP031 decreases damage to blood vessels in the hippocampus. By increasing the lifespan of vitamin C, NXP031 makes the nutrient much more effective at the treatment of tissue damage within the brain.

*Reference: Apta-Index™ ID #7199 -H.R.

Design of Aptamers with Improved Target-Affinity to Pb^{2+} Ions

The SELEX technique generates aptamers for aptasensor design, but it's a time-consuming, costly and specialist-dependent method. This study introduces a screening method to complement the SELEX process using molecular dynamics (MD) simulation to obtain the aptamers with improved specificity to the intended target. The proposed screening method has been applied to introduce mutants with higher specificity to Pb^{2+}. The T30695 aptamer was chosen via SELEX as the most specific aptamer to the ion among the previously produced aptamers. Using the T30695 aptamer as a starting point, mutations were introduced throughout its sequence to explore all possible variants. The screening by the MD simulation proves that the M4, M16, and M4-16 mutants possess improved specificity to Pb^{2+} compared to the T30695 aptamer. For experimental confirmation, the T30695, M4, and M4-16 aptamers have been applied to detect Pb^{2+} by a simple colorimetric assay.

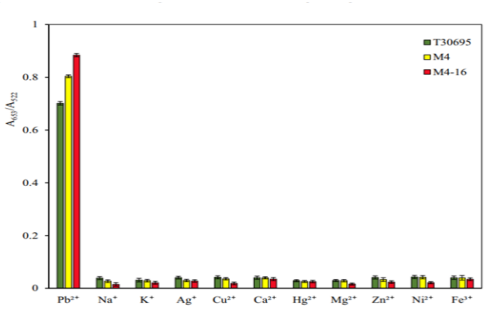

Figure 1: Selectivity of the aptamers in the presence of different metal ions (10nM) under the optimal conditions. The error bars represent the average standard errors for three measure-

This paper proposes a screening technique for designing novel aptamers with better target selectivity: first, use MD simulation to find the aptamer most specific to the target among the SELEX-introduced aptamers. Second, design mutants by changing the nucleotides of the selected aptamer. Third, choose mutants with enhanced specificity for the target during the screening process. Finally, using an experimental technique, determine the specificity of the chosen mutants toward the target. As a case study, the method was employed to introduce several mutants toward Pb^{2+}. Among the aptamers previously developed using SELEX, the T30695 aptamer was chosen as the most specific to the ion. The MD results show that among the developed mutants, M4, M16, and M4-16 have high specificity for Pb^{2+}. A simple colorimetric test based on gold nanoparticles was used to assess the mutants' selectivity. The detection limits for the T30695, M4, and M4-16 aptamers were 192, 177, and 65 pM, respectively, according to the experimental data. The colorimetric measurements further demonstrate the mutants' preference for Pb^{2+} over other metal ions.

*Reference: Apta-Index™ ID #7221 -T.S.

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Mirror Image Aptamer Shows Promise to Treat Anemia in Phase 2 Clinical Trials

Anemia is the most prevalent hematological disorder in humans, the most common cause of which is known as anemia of chronic inflammation (ACI). ACI affects about 77% of elderly anemic patients in which there is no clear cause of disease, suggesting that the pathogenesis of the disease is multifactorial (Madu, A. and Ughasoro, M. 2016). Hepcidin is a 2.8-kDa peptide that is responsible for maintaining iron homeostasis. ACI can occur when hepcidin is overproduced during inflammatory conditions and is often the case in anemic patients with chronic kidney disease, cancer, and Castleman disease. Due to the etiological role that hepcidin plays, scientists have determined that a structured mirror-image L-RNA aptamer, often referred to as a Spiegelmer, that could potentially target hepcidin as an inhibitory agent, thereby ameliorating the disease.

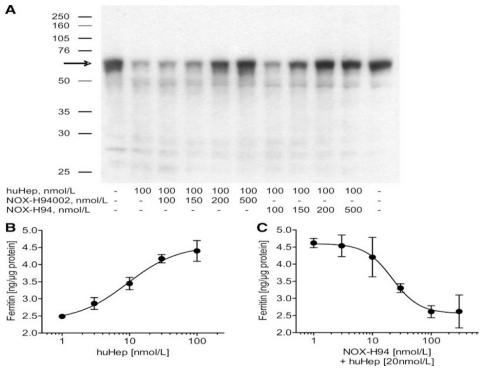

Figure (adapted) from Schwoebel et. al. showing (A) Ferroportin western blot analysis from mouse J774A.1 macrophages after stimulation with huHep alone or in combination with different concentrations of the l-oligoribonucleotide NOX-H94002 or its PEGylated variant NOX-H94. (B) Ferritin content of lysates obtained from macrophages after stimulation with different concentrations of huHep. (C) Ferritin content of lysates obtained from macrophages after stimulation with 20 nM huHep and different concentrations of NOX-H94.

NOX-H94 is the L-oligoribonucleotide that has been found to bind to human hepcidin with high affinity. This binding blocks the function of hepcidin, thereby stopping the degradation of ferroportin as well as reducing the concentration of ferritin in vitro. Both ferroportin and ferritin play an integral role in the development of ACI. In one study involving an acute cynomolgus monkey model of interleukin 6 (IL-6)-induced hypoferremia, NOX-H94 inhibited serum iron reduction completely. Additionally, a sub-chronic model of IL-6 -induced anemia with a NOX-H94 treatment showed that the Spiegelmer inhibited the decrease in hemoglobin concentration (Schwoebel, F. *et al.* 2013). NOX-H94 has shown to be safe and effective in phase 1 and phase 2 studies (Ni, S. *et al.* 2020).

Reference: Apta-Index™ ID #7220 -T.F.

Novel Selex Method Leads to the Discovery of an RNA Aptamer that Specifically Targets Breast Cancer Exosomes

Despite the emergence and advancement of various treatment options over the course of decades, breast cancer is still one of the leading causes of cancer-based mortality in women. However, new research has identified exosomes (secreted vesicles that can affect cell function once absorbed) as potential diagnostic tools to identify cancerous cells. Furthermore, exosomes have also been shown to play a critical role in the communication between cancer cells and tumor tissues. This study by Esposito *et al.* identified a new aptamer, ex-50.T, that can exploit these unique attributes of cancerous exosomes for therapeutic purposes. Additionally, this aptamer was discovered using a novel method of SELEX, dubbed by the research team as "Exo-SELEX", which makes use of exosomes as selection tools.

Through this robust process, the research team was able to develop an aptamer can bind to breast cancer exosomes with high affinity (approximately 0.8 nM) but showed no binding to other types of exosomes. This aptamer was also shown to prevent cellular uptake of these cancerous exosomes, and significantly blocks the potential cell migration caused by these exosomes. Furthermore, the aptamer performed these tasks just as well in human serum, where the conditions are harsher, and the exosomes present are much less homogenous. All of these attributes make the ex-50.T aptamer a great potential candidate for the detection, targeting, and treatment of breast cancer.

Reference: Apta-Index™ ID #7200 -D.W.

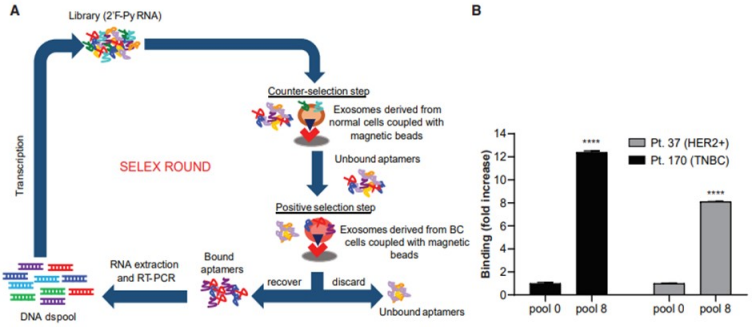

*Figure (adapted). (A) Scheme of the exo-SELEX strategy. (B) Binding by quantitative real-time PCR of the starting (pool 0) or the final (pool 8) pools from Exo-SELEX were analyzed on exosomes derived from primary breast cancer (BC) cells (Pt.37, HER+ ; Pt.170, TNBC) at 200 nM concentration. Results are expressed as folding increase over pool 0. Error bars depict means ± standard deviations (SDs). Significance of pool 8 versus pool 0 was measured by t test: ***p < 0.0001.*

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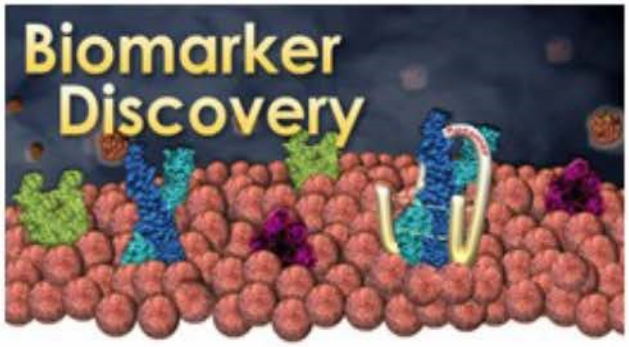

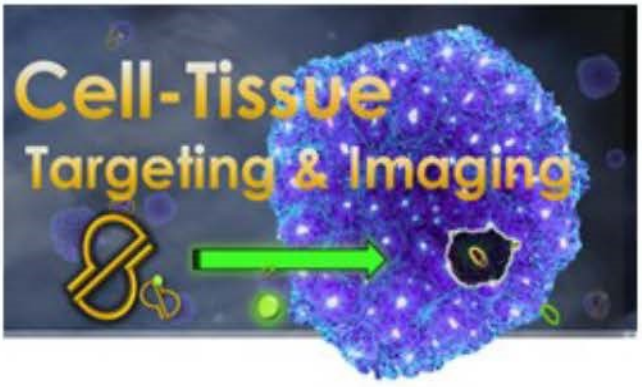

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