The AptaReport™
Newsletter
Spring 2021

Six-Letter Self-Assembling Nantotrain Carries Deoxrubicin to Liver Cancer Cells

Liver Cancer has a five year survival percentage of 7% and can affect up to 800,000 people a year, in the United states alone. There are 33,000 people a year that get diagnosed and 27,000 a year die. Doxorubicin blocks topoi-somerase-2 helps prevent and block the division of cancer cells. However, it causes damage to non-cancerous cells too. The LZBH5 aptamer was initially selected and then truncated to LZH5B, which is shorter and has a binding affinity of 12 nM. The proposed aptamer is derived from a 6-letter (GACTZP) DNA. This provides for increased information density and prevents invasion from a standard four nucleotide-based DNA from a sample. The six-base DNA allows for orthogonal P:Z pairing, which is exploited to construct a nanotrain. When the LZH5B trigger is added to the five prime end, this allows for self assembly of the nanotrain. When the aptamer-nanotrain is loaded with doxorubicin, this allows for doxorubicin to be directly administered to liver cancer cells bound to the LZH5B aptamer.

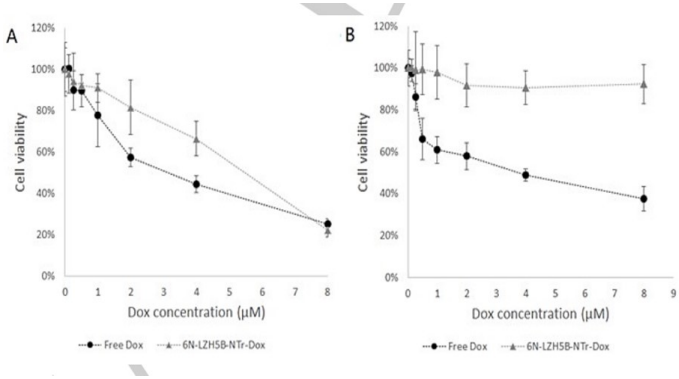

Figure 1 (adapted) from L. Zang 2020 When the Dox concentration is changing to 8 um, the free doxorubicin kills the cells, however, when turned up to 8 um with the aptamer the cells do not die.

As shown in the figure above, when doxorubicin is loaded on the aptamer, it has a similar potency as free doxorubicin. It is only exerted on the desired cells and no toxicity is observed on the non-cancerous cells.

*Reference:Apta-Index™ ID# 7080 -R.R

Cubane-Modified DNA Aptamer Selectively Binds to Malaria

Modification of aptamers beyond the four canonical bases can improve in vivo stability and residence time. Modifications can be done before or after the SELEX process. Post-SELEX modification often reduces the affinity and specificity of the aptamer. Conversely, pre-SELEX modification can confer the same benefits while simultaneously improving both affinity and specificity. Modified libraries can be used to find aptamers to solve problems unable to be solved by unmodified aptamers. A group of researchers recently evolved pre-SELEX modified aptamers to target the malaria biomarker protein Plasmodium vivax lactate dehydrogenase (PvLDH). This cubane-modified aptamer is capable of distinguishing between PvLDH and the very similar Plasmodium falciparum LDH, which no unmodified aptamer has previously been able to do. This pre-SELEX modification process uses the CuAAC reaction to functionalize deoxyuridine (dU) bases with cubane moieties.

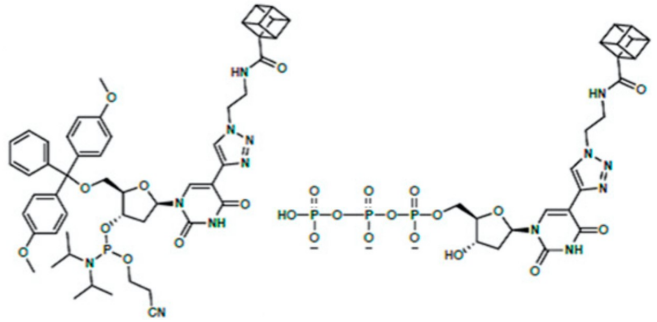

Figure 1 (adapted) from Yee-Wai Cheung 2020 Cubane-functionalized dU phosphoramdite (Left) and cubane-modified dU triphosphate (Right)

Cubane, an organic molecule named for its cube-like structure, has many advantages as an aptamer modification compared to aromatic groups commonly used to functionalize nucleobases. Unlike benzene, cubane is nontoxic and biostable. Additionally, cubane is more water-soluble than benzene because of its ability to form hydrogen bonds and disrupt π stacking.

*Reference: Apta-Index™ ID# 7081 -J.V.

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Identification of Aptamers for Plasmodium Falciparum Infected Erythrocytes

Plasmodium falciparum is the causative agent to the deadliest form of human malaria responsible for infecting nearly 228 million patients and causing 45 thousand deaths each year. Specifically, aptamers were selected against the surface of plasmodium falciparum infected erythrocytes with uninfected erythrocytes as the negative selection.

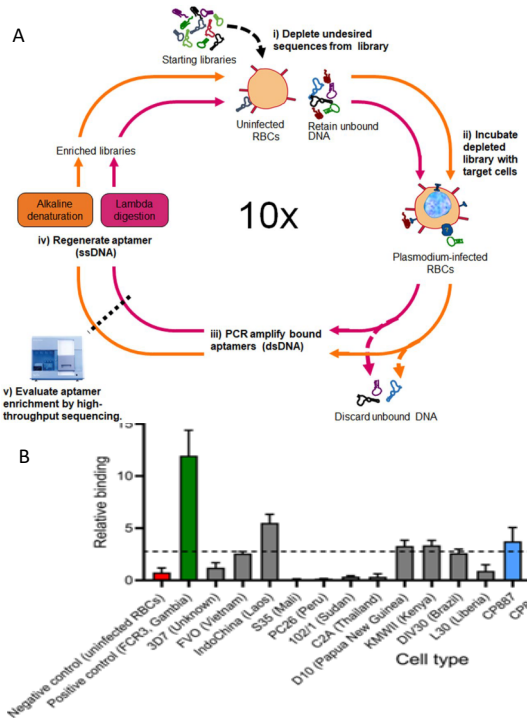


Figure (adapted) from Otengy 2020 A.) A parallel SELEX scheme was utilized to select aptamers to bind to P. falciparum – infected red blood cells (RBCs). B.) Shows the relative binding of Malaria.1 to various cell types. 5 million cells for each strain per well were incubated with

This comparative study by Oteng *et al*. runs two parallel selections in which the post amplification denaturation techniques, alkaline denaturation and lambda exonuclease digestion, are compared to decipher which technique is superior. Following 10 rounds of selection the sequenced aptamer pools shows that in the lambda exonuclease digestion assessment, the top three aptamer clusters account for 43.6, 1.8, and 0.9 percent of the total pool, whereas the top aptamer cluster from the alkaline denaturation only make up 0.004 percent of the total pool. Ultimately, Malaria.1 aptamer, which had the largest cluster in the exonuclease digestion and showed up 0 times in the alkaline denaturation, was determined to have a K_d of 60nM. The aptamer was screened against a panel of laboratory adapted Plasmodium falciparum, and it was found that the aptamer displayed somewhat broad binding, recognizing 9 out of the total 20 adaptations of the infected erythrocyte.

*Reference: Apta-Index™ ID #7110 -W.R.

In Vivo Bone Defect Repair Guided by Novel Aptamer Nanoparticles Exhibiting High Affinity and Specificity to Mesenchymal Stem Cells

Current bone fracture and defect repair methods are casting, open reduction with internal or external fixation. These methods require a long duration to heal during which non-union can form, especially when the bone breaks into many small fragments (comminuted fractures). Endogenous stem cell recruitment has been studied extensively as an alternative approach to repair bone fractures. A new method has been proposed by Wang *et al*. utilizing this theory in fabricating the first, feasible, economical, bio-compatible, and functional mesenchymal stem cell (MSCs) aptamer-directed nanoparticles that are called Nano-Aptamer Ball (NAB). These nanoparticles have been proven in vivo, when being immobilized on the defect sites, to bind and capture MSCs and promote osteogenesis generation.

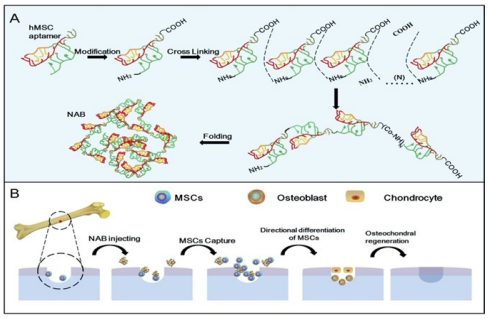
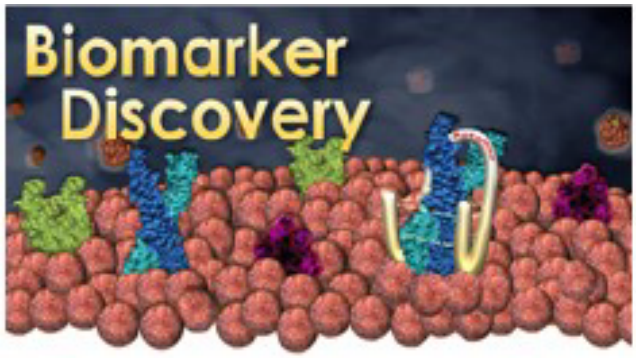
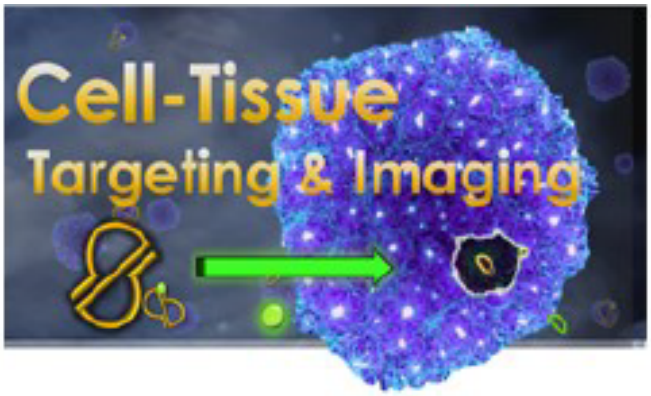


Figure (adapted) from Wang 2019. (A) Fabrication of NAB using aptamer HM69 where HM69 was modified with -NH2 and -COOH and individual aptamers were linked together under NHS/EDC system and generate -NH-CO- chemical bond. In an aqueous solution, poly-HM69 tended to fold into a nanoparticle. (B) Schematic diagram of bone defect. Upon injection, NAB can recognize the MSCs in bone defects and be anchored. NAB could then capture and recruit MSCs to the defect site where they may differenti-ate into chondrocyte and osteoblast.

The aptamers specific to MSCs were selected via whole-cell SELEX. Human ESC cells were used as the target cell line and 293FT as the control cell line. The aptamer termed as HM69 demonstrated high specific binding with MSCs at high purity while having minimal cross-reactivity to other cells. NABs were constructed by NHS/EDC reaction. Non-specific cytotoxicty and cell-proliferation induction was evaluated using CCK8. In vitro, NAB could bind and capture MSCs effectively and did not cause obvious cytotoxicity. In vivo, NAB introduction shows signs of repair and osteogenesis generation in rat bones. Defect healing where NAB group is present was significantly better than control groups. The aptamer HM69 and NAB structure shown here demonstrates the possibility of utilizing aptamer-functionalized bio-nanoparticles for the restoration of bone defects via recruitment of MSCs.

*Reference: Apta-Index™ ID #7079 -T.L.


Biomarker
Discovery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