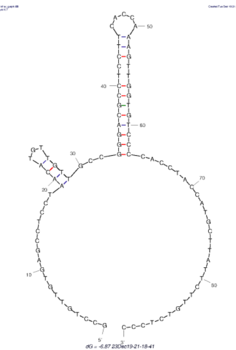
The AptaReport™ Newsletter

Winter 2024

Selection of aptamers against triple negative breast cancer cells using high throughput sequencing

Breast cancer presents an urgent challenge in diagnostics and treatment as it is among the most diagnosed cancers and a leading cause of death in women. Of the breast cancers categorized by receptors status, triple-negative breast cancer (TNBC) is the deadliest, most aggressive subtype. It lacks the three receptors used to classify breast cancer: those for estrogen, progesterone, and epidermal growth factor receptor 2. Therefore, TNBC does not respond to hormone therapy, limiting care to conventional treatment and select emerging therapies. More recently, aptamers are being explored for the specific targeting of tumors, with several potential advantages over other therapies. Here, researchers selected tumor-specific aptamers using cell-SELEX, an iterative process that evolves a random sequence library by repeatedly isolating and amplifying molecules that respond to the cultured cells of interest. To increase the chance of identifying a high-affinity aptamer and combat PCR bias, the researchers employed high-throughput sequencing (HTS) and phylogenetic analysis.



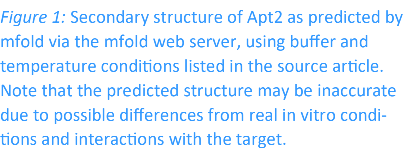


Figure 1: Secondary structure of Apt2 as predicted by mfold via the mfold web server, using buffer and temperature conditions listed in the source article. Note that the predicted structure may be inaccurate due to possible differences from real in vitro conditions and interactions with the target.

Cell-SELEX was performed using a library of ssDNA, with a human breast cancer cell line as target cells and human epithelial breast cells as a negative control and counter-target. Selection involved incubating the library with either target or negative control cells, centrifuging, and collecting the appropriate fraction. After the 8th SELEX round, the ssDNA was analyzed with HTS; only 9 sequences had over 50 reads. The two most abundant, Apt1 and Apt2, were selected for their frequency and because they shared conserved regions with many of the other top-25 sequences while representing two unique sequence families. A titration of fluorescently labelled Apt1 and Apt2 against cells revealed respective Kd values of 44.3 ± 13.3 nM and 17.7 ± 2.7 nM. Treating cells with either trypsin or proteinase K led to a loss of aptamer binding, indicating specificity towards cell-surface receptors; this was consistent with a co-localization experiment showing significant binding to the cell membrane. Apt1 and Apt2 also showed reduced binding to other TNBC cell lines and other breast cancer variants. Apt2 showed no toxicity, but was successful in staining breast cancer tissue sections, indicating potential for usage as a targeting moiety for anti-tumor drugs.

*Reference: Apta-Index™ 8538 -M.R.

A Non-G-Quadruplex DNA Aptamer Targeting NCL for Diagnosis and Therapy in Bladder Cancer

Bladder cancer (BC) is a highly aggressive malignant cancer characterized by its ability to spread, poor outcomes, and limited ability to successfully treat. Since nucleolin (NCL) is considered a reliable tissue-specific biomarker for BC, researchers have developed a DNA aptamer using tissue-based systematic evolution of ligands by exponential enrichment (SELEX) to diagnose and treat BC. The selected aptamer, TB-5, has high affinity and specificity for NCL. Previous NCL aptamers appeared to bind based on a G-quadruplex secondary structure that NCL recognizes nonspecifically. Because TB-5 binds NCL without assuming a G-quadruplex structure, its interaction with NCL is different, allowing it to reduce NCL interference with normal cellular maintenance processes.

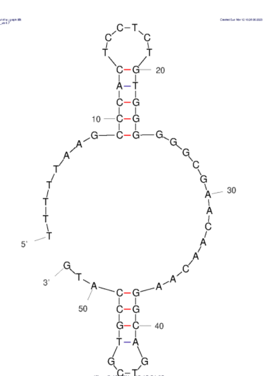


Figure 2: Secondary structure of Apt2 as predicted by mfold via the mfold web server, using buffer and temperature conditions listed in the source article. Note that the predicted structure may be inaccurate due to possible differences from real in vitro conditions and interactions with the target.

The selection utilized single-round SELEX strategy with "X-Aptamers," a bead-based process using a chemically-modified DNA library. TB-5 has demonstrated 165.9nM affinity to BC cells and good specificity towards a range of clinical BC tissues but not normal bladder tissues. TB-5 could also bind to other cancer tissues with high affinity including ovarian cancer, breast cancer, colon cancer, lung cancer and liver cancer. This shows the potential of TB-5 as a novel molecular probe for assessing various cancers. Based on molecular docking simulations, the researchers believed that TB-5 binds to NCL at the RNA-recognition motifs (RRMs) of NCL, specifically RRM2 and RRM3. TB-5 may act as a therapeutic by blocking the ability of NCL to recognize RNAs and therefore limit transcription of their associated proteins. By demonstrating specific cytotoxicity to NCL high-expressing cells, TB-5 has showed remarkable anti-tumor capacity by promoting autophagy and preventing cell migration and invasion of BC cells compared to AS1411.

*Reference: Apta-Index™ 8587 -M.R.

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Investigation of the Relationship between Aptamers' Targeting Functions and Human Plasma Proteins

Aptamers are single-stranded DNA or RNA molecules capable of recognizing targets via specific three-dimensional structures. Taking advantage of this unique targeting function, aptamers have been extensively applied to bioanalysis and disease theranostic. However, the targeting functionality of aptamers in the physiological milieu is impeded compared with their in vitro applications. To investigate the physiological factors that adversely affect the *in vivo* targeting ability of aptamers, the researchers systematically studied the interactions between human plasma proteins and aptamers and the specific effects of plasma proteins on aptamer targeting. Microscale thermophoresis and flow cytometry analysis were some of the methods employed to highlight the plasma interacting with aptamers, restricting their affinity toward targeted tumor cells.

To identify plasma proteins of interest, a magnetic-separation-based pull-down technique was developed. Aptamers with biotin labeling were preincubated with plasma for different time periods. Next, streptavidin-coated magnetic beads (SMBs) were utilized to capture aptamers with high affinity During this process, the bound proteins were stripped and isolated from plasma by magnetic separation. The direct interplay between plasma and SMBs without aptamers was considered the control group. Preliminary SDS-PAGE results revealed that proteins with around 260 kDa MW showed higher abundance on AS1411 aptamer, while proteins with 16–30 kDa MW exhibited higher abundance on AS1411, MJ5C, and SL1 aptamers, suggesting that the adsorption performed by plasma proteins was highly dependent on the individual properties of aptamers. The structures of AS1411 and its optimized sequences were modeled and visualized in RNA composer. The 3D structures of aptamers were then obtained by substituting bases T for U and removing the hydroxyl group on the five-carbon sugar. Although aptamer-based therapeutics have achieved a series of successes, clinical translation of aptamers is slow. It is for this reason that the aptamer of interest was further modified to investigate the competitive binding between FN1 and AS1411 aptamer's targeting ability in vivo.

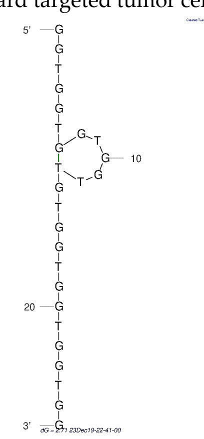


Figure 2: Secondary structure of Apt2 as predicted by mfold via the mfold web server, using buffer and temperature conditions listed in the source article. Note that the predicted structure may be inaccurate due to possible differences from real in vitro conditions and interactions with the target.

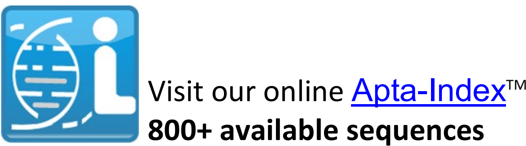
*Reference: Apta-Index™ 8486 -K.S.

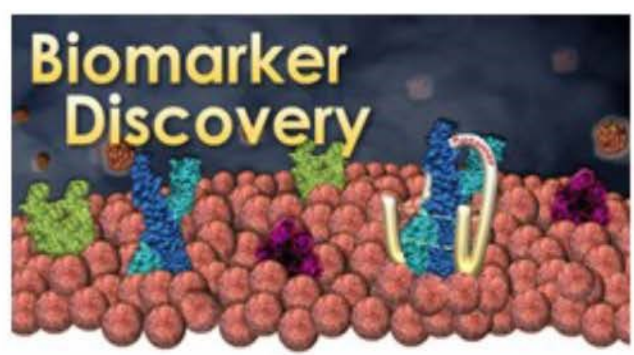
Modified Aptamers Enable Quantitative Sub-10-nm Cellular DNA-PAINT Imaging

Advances in super-resolution microscopy have been limited by the large size of fluorescently-labeled affinity reagents. The microscopes themselves are capable of magnifying down to only a few nanometers in diameter, but the fluorescent labels used to differentiate and identify distinct targets are much larger than this. This has resulted in a peculiar situation in which magnification power is sufficient enough to generate highly detailed images of nanoscale molecules, but not actually achieved due to the size limitations of fluorescent labels. Because aptamers are much smaller than conventional fluorescent labels, Jungmann and colleagues hypothesized that aptamers could be used for this purpose to generate highly detailed images at the nanoscale. In his recent study, Jungmann demonstrated the use of modified aptamers as fluorescent labels in microscopy. Compared to conventional fluorescent labels, these chemically-modified DNA aptamers enable enhanced resolution in super-resolution fluorescence microscopy by allowing for the closer and more accurate tagging of proteins, given their smaller size and specific binding capabilities.

16 SOMAmers against various protein targets were used to develop this technology. Sequences for these molecules were not disclosed, although modifications to the core binding sequence were listed.

-A.Y.

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