The AptaReport™
Newsletter
Summer 2021

Aptamer RA16 Inhibits Growth of Non-Small Cell Lung Cancer

Lung cancer is the leading cause of cancer-related mortality in men and the second leading cause in women worldwide. Thus far, the two main types of lung cancer are non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). NSCLC accounts for 85–90% of all lung cancer cases. Chemotherapy is the primary treatment of choice for NSCLC; however, there are side effects such as gastrointestinal distress, organ damage, and even death.

Aptamers are a class of single-stranded oligonucleotides (RNAs or ssDNAs) that can serve as ligands that recognize and bind to their targets with specificity and high affinity. A previous study has demonstrated the potential of a NSCLC-specific RNA aptamer selected via in vivo SELEX. The aptamer, named RA16, was capable of binding to and inhibiting NSCLC human large cell lung cancer cell line NCI-H460 cells in vitro and in vivo. This may be applied to tumor imaging technique and targeted therapies. A major advantage of RNA aptamers is that they can be chemically synthesized for use in diagnosis, treatment, and biomarker discovery. Therefore, the binding and inhibitory activity of the synthesized RA16 (syn-RA16), as well as the potential mechanisms should be further investigated. Furthermore, a smaller aptamer size could facilitate large-scale chemical aptamer, it has a similar potency as free doxorubicin but it is only exerted on the desired cells and no toxicity is observed on the non-cancerous cells.

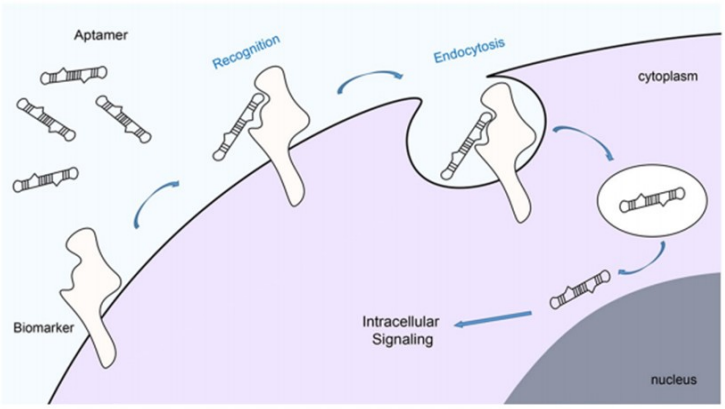
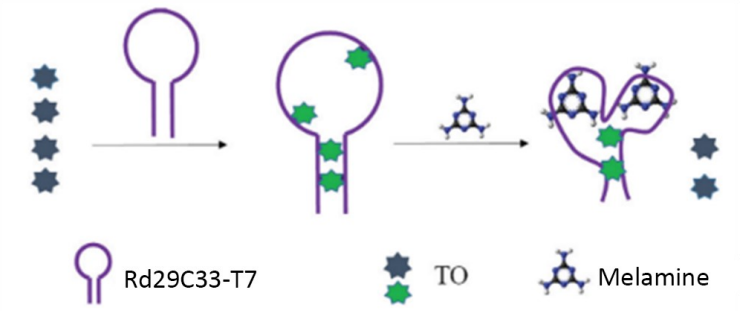


Figure: Aptamer RA16 binds to the biomarker, then penetrated into the cytoplasm and resulted in intracellular signaling pathway.

*Reference: Apta-Index™ ID #7113 - T.H.

Detecting Melamine with Modified Aptamer

Melamine is an industrial thermosetting plastic with flame retardant properties. It is mainly used in white boards, pesticides, heat resistant cooking utensils, and fertilizer. However, when ingested it causes kidney failure and kidney stones in humans and animals when it reacts inside the body with cyanuric acid (1:1 mixture), an event that happened in 2008 with China's milk scandal. Melamine was sold to baby formula companies and was advertised to boost the overall protein content in formula. Melamine was found to be about a thousand-fold more than the FDA approved amount in the most popular brands. A solution had been proposed by Gu *et al.* utilizing aptamer Rd29C33 with fluorescence nucleic acid dye to detect the amount of melamine in milk products for the concerned consumers.



The hairpin structure Figure 1 (adapted) is the modified aptamer that will fluoresce from binding of Thiazole Orange inside. Melamine molecules are then added to the solution, taking the place of the two other fluorescence molecules, and changing the structure of the aptamer. The aptamer changes shape to a G-quadruplex structure which still allows for the binding of two Thiazole Orange molecules.

Thiazole Orange was chosen for detecting melamine and the process required the aptamer to be truncated for use as a biosensor. The researchers found truncated aptamer Rd29C33-T7 to exhibit a higher affinity and better selectivity for melamine over Rd29C33. The addition of Rd29C33-T7 and Thiazole Orange enhanced the fluorescence intensity of the nucleic acid dye. When melamine was added last, as demonstrated in the above figure, it displaces the fluorescent dye from its position. This changes the configuration of the aptamer to a more stable form, resulting in a decrease in fluorescence intensity. This aptamer could potentially be used for applications of melamine detection at home or for suppliers to test their products before selling them.

*Reference: Apta-Index™ ID #7127 - F.K.

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Optimization of Apta-based Biomarker Discovery

Aptamers, synthetic antibodies, are easy to synthesize and have high flexibility for modification. Making aptamers highly promising to be used in the detection of biomarkers, informative molecules that differ in the expression level between diseased cells and healthy cells. However, because of the unstable bonds that form between aptamers and targets, it is still a big challenge to accurately identify these biomarkers. Bi et al. proposed to site-specifically incorporate a photoreactive group in the aptamer structure to help stabilize these bonds. They added a ssDNA sequence to the aptamer to form what they called a "binding probe BP". They also modified another ssDNA that is complementary to the ssDNA of the binding probe by adding a photoreactive group to one end and either a fluorescent label or biotin at the other end. They called this modified ssDNA "capture probe (CP)".

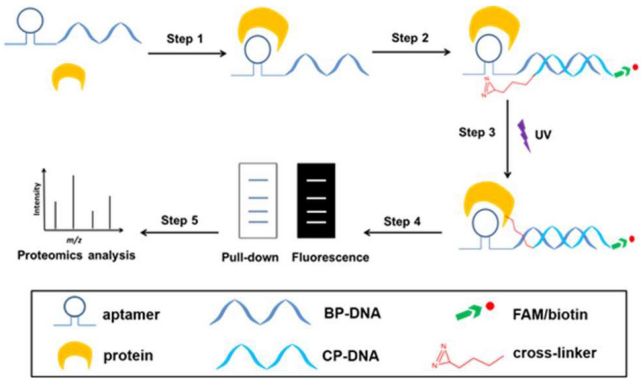

Figure 1. Schematic representation of the design strategy to increase the stability of the aptamer – target binding in order to optimize the biomarker discovery.

As shown in Figure 1, their experimental design consists of five steps. First, they incubated the target biomarker with the BP overnight to let them bind. Second, they added the CP the mixture and incubated to let the diazirine group on the CP to get closer to the target, and the ssDNA of the CP bind to its complementary strand on the BP. Third, they used UV light on the mixture to induce strong binding between the diazirine group and the target. Fourth, they measured the fluorescence intensity to quantify target/BP/CP complex for the fluorescently labeled CP. For the CP labeled with biotin, they used a pull-down technique to separate the target from the target/BP/CP complex and use mass spectrometry to identify the target. They were able to accurately detect lysozyme while implementing this experimental design.

*Reference: Apta-Index™ ID #456 -L.B.

ssDNA Aptamers to Diagnose and Treat Soft-Shell Turtle Iridovirus in Chinese Soft-Shelled Turtles

Chinese soft-shelled turtles (*Trionyx sinensis*) are valued for both economic reasons, as they are a food source in Asia, as well as for reasons related to biological research. These turtles frequently experience outbreaks of soft-shelled turtle iridovirus (STIV), causing economic problems. There is a need for both diagnostics and therapeutics which can combat STIV for *T. sinensis*.

Eight aptamers (ssDNA) were initially developed which target STIV. These aptamers were chosen via the SELEX process and all had high binding specificity. The individual binding specificities were validated using both gel shift assays and fluorescent localization. These eight segments of ssDNA ranged from 26% frequency to 2% frequency in the 8th selected pool during SELEX. Four aptamers, QA-9, QA-12, QA -36, and QA-92 made up 88% of the aptamer pool; the remaining four made up the other 12%. Therefore, QA-9, QA-12, QA-36, and QA-92 were chosen for a more detailed study.

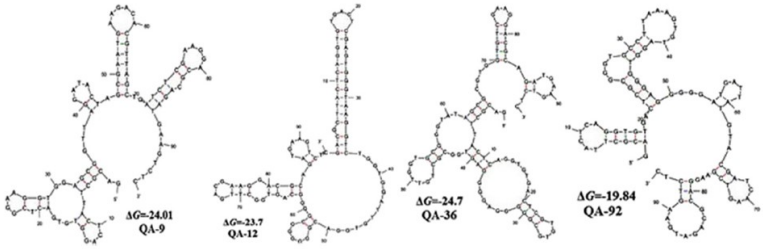

Figure 1 (Adapted). The four most-frequent ssDNA aptamers from the SELEX pool at round 8, chosen for more in-depth study. QA-36, with the lowest ΔG value (-24.7), has the most stable secondary structure.

The dissociation constants (K_d) were calculated for each aptamer, and of the four, QA-12 had the largest (80.7 nM) and QA-36 had the smallest (53.8 nM). This indicates that QA-36 had the highest binding affinity. The QA-36 aptamer also had the greatest inhibitory effect against STIV. The study also found that aptamer QA-12 was able to identify and bind to cells which were infected with STIV, providing promising results for aptamers to be used in diagnostics.

It was shown that the aptamers had no toxic effects on the treated turtles, and there was no change in liver or spleen tissue either. The lack of toxicity makes Aptamers a good option for diagnostics and treatment.

*Reference: Apta-Index™ ID #7146 -L.K.

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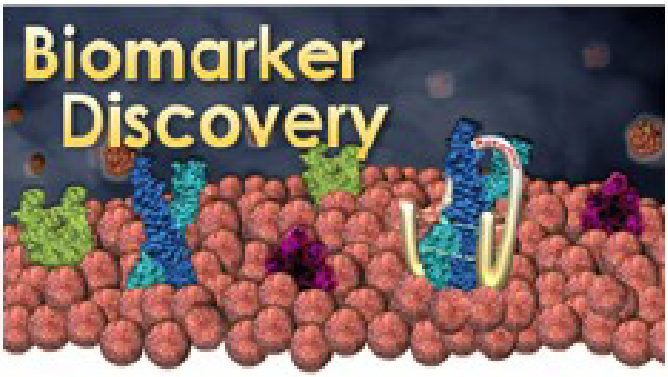

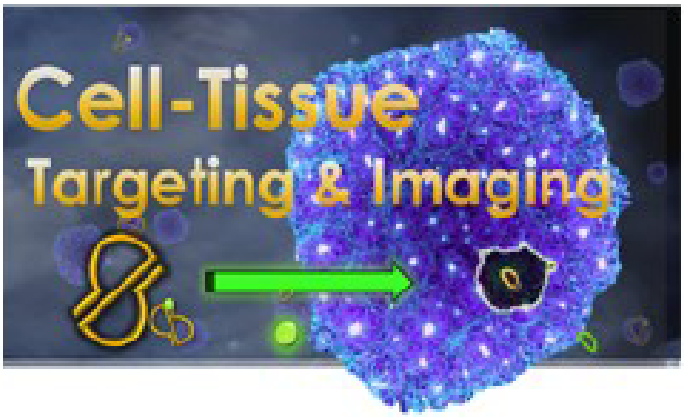

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