Imaging with aptamers

By Kai-Jye Lou, Staff Writer

Researchers at **Hunan University** have developed a method for synthesizing aptamer probes that fluoresce only when bound to their target.[1] The group thinks its approach could be used to generate aptamers that improve the speed and sensitivity of early cancer detection.

Conventional molecular probes typically require a waiting period of hours following injection to allow the probe to distribute to target tissues and for excess probe to exit the body before an imaging procedure. Despite that washout period, low levels of unbound probe remain in the body during the imaging procedure and decrease contrast and sensitivity.

To improve contrast, molecules such as antibodies and peptides have been engineered to activate via enzyme processing upon target binding to produce a signal. However, these molecules need to undergo a series of modifications to decrease immunogenicity; antibodies also are bulky molecules that take time to clear from the body.

Because of their small size, nucleic acid aptamers inherently offer rapid clearance and also have high specificity and affinity for target molecules. However, it has been challenging to engineer aptamers that only emit a signal upon binding a target, as existing methods that worked with larger compound classes were not compatible with aptamers.

The Hunan researchers have solved this problem. Their answer has its roots in studies from members of the current group and researchers at the **University of Florida** showing that aptamers can change conformation upon binding to their target.[2,3] The Hunan group sought to exploit this phenomenon as a molecular switch for turning on a fluorescent signal when an aptamer binds its target.

The team synthesized a series of aptamer probes consisting of five parts: a DNA sequence that binds to PTK7 protein tyrosine kinase 7 (PTK7; CCK4), a separate DNA sequence complementary to part of the first DNA sequence, a poly-thymine linker region, a fluorophore on one terminal of the probe and a molecule to mask fluorescence—called a quencher—on the opposite terminal.

In unbound aptamer, the targeting DNA sequence remains bound to the complementary DNA sequence and the fluorophore stays in close proximity to the quencher molecule. Upon binding to its target, the probe undergoes a conformational change that displaces the complementary strand and its attached fluorophore from the quencher molecule's proximity, thus activating the probe (*see* **Figure 1 "Conformational change upon binding to target"**).

PTK7 is an oncogenic extracellular protein upregulated in multiple cancers.[4]

In a PTK7-overexpressing human leukemia cell line, the lead aptamer probe produced stronger fluorescence intensity relative to background and had a signal-to-background ratio about 2.5 times higher than that of a conventional aptamer probe. In mice with PTK7-overexpressing tumors, i.v. injection of the lead probe produced the strongest fluorescence signal at the tumor site and had decreased background fluorescence compared with injection of the conventional aptamer probe.

Data were published in the *Proceedings of the National Academy of Sciences*.

"These aptamer probes might hold great potential as a versatile molecular probe for *in vivo* cancer imaging with their high sensitivity and specificity, which could facilitate the detection of cancer at an early stage," said Kemin Wang, corresponding author on the paper and a professor of the State Key Laboratory of Chemo/Biosensing and Chemometrics at Hunan University.

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> ***—Kemin Wang, Hunan University***

Wang added that the group's aptamer design methodology could be used to generate probes for monitoring tumor recurrence and metastasis and for assessing a tumor's response to treatment in clinical and preclinical studies.

"The introduction of this aptamer design into other clinical diagnosis imaging technologies, such as MRI and PET, will be essential to the clinical translation," he told *SciBX*.

"It is most interesting that this approach was successful in an *in vivo* cancer imaging model, which is quite complex and often unforgiving," said Shawn Lupold, director of the molecular biology core for small animal imaging resources program and an assistant professor at **The Johns Hopkins University School of Medicine**. "The manuscript convincingly demonstrates that this concept is possible," he told *SciBX*.

Lupold noted that the aptamer probes described in the paper only need to bind to their target for activation, whereas many other imaging probes require enzymatic processing to activate. "This property may therefore expand the application of imaging probes that become activated to diseases or tissues that don't have unique enzyme targets," he told *SciBX*.

Larry Gold, founder, chairman and CEO of aptamer diagnostics company **SomaLogic Inc.**, said that selecting the right targets for a probe remains of paramount importance. "For tumor imaging *in vivo*, one has to think very hard about what molecular targets ought to be imaged," he told *SciBX*.

SomaLogic develops Slow Off-rate Modified Aptamers (SOMAmers) for biomarker discovery and diagnostics to analyze clinical samples. The company expects to launch a lung cancer diagnostic in 3Q11 and a pancreatic cancer diagnostic in 4Q11.

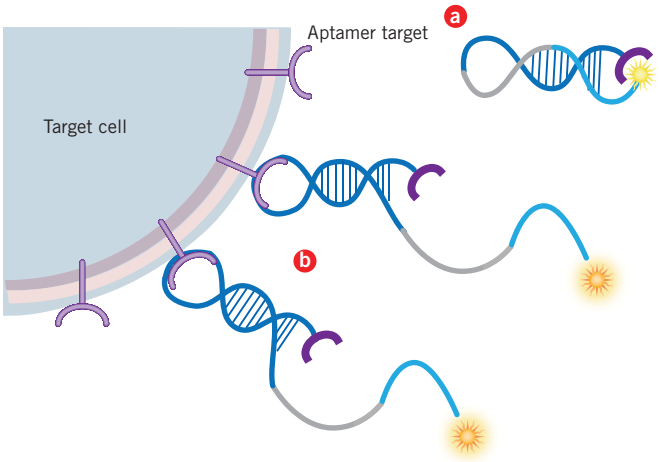

Figure 1. Conformational change upon binding to target. Nucleic acid aptamers are small, single-stranded oligonucleotides that can bind to various targets. **Hunan University** researchers reported in the *Proceedings of the National Academy of Sciences* a method to synthesize aptamer probes that activate after binding to their target.

These target-activated aptamer probes consist of three oligonucleotide parts: a DNA sequence that binds to its target (dark blue strand), a poly-thymine linker region (gray strand) and a separate DNA sequence that is complementary to part of the first DNA sequence (light blue strand). A fluorophore (yellow star) and quencher molecule (dark purple crescent) are attached to the opposite terminals of the aptamer.

When the aptamer probe is not bound to its target, the quencher molecule stays in close proximity to the fluorophore and masks the fluorescent signal [**a**]. However, upon binding to its target, the probe undergoes a conformational change that displaces the fluorophore from the quencher, which results in a detectable fluorescent signal from the probe [**b**].

Stable relationships

Wang said his group is now evaluating the serum stability of the aptamer probes. The team also is designing additional aptamers specific for extracellular targets on other tumor types.

Hisataka Kobayashi, chief scientist of the molecular imaging program at **NIH**'s **National Cancer Institute**, said it may be desirable to increase the time that the aptamer probe remains bound to its target, which would extend the window for imaging studies. He said this could be done by synthesizing a multivalent aptamer that has multiple binding sites for its target.

"From a chemistry standpoint, this activated aptamer probe has a beautiful design, and the researchers show that the molecule works well *in vitro*," said Kobayashi. "But before we could consider using these probes in the *in vivo* and clinical settings, it will be necessary to evaluate the stability of both the aptamer and its quenching state and to determine how efficiently the probe is activated at its target."

Kobayashi also wanted to know whether "other processes in the body, such as liver catabolism, could activate the probe and how this probe is cleared from the body."

Wang acknowledged that probe stability needs to be tested but said there are multiple strategies to slow *in vivo* degradation and increase probe half-lives.

He noted that aptamer degradation by nucleases could be slowed with a supplementary injection of unlabeled random oligonucleotides, which was the strategy his group used in the mouse imaging study. He added that probe half-lives could be further increased with various synthetic chemistry strategies like the use of locked nucleic acids (LNAs) and 3′ capping.

Wang said the university has filed patents covering the work described in the paper. The IP is available for licensing.

Lou, K.-J. *SciBX* 4(11); doi:10.1038/scibx.2011.299
Published online March 17, 2011

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   e-mail: kmwang@hnu.cn
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COMPANIES AND INSTITUTIONS MENTIONED

Hunan University, Changsha, China
The Johns Hopkins University School of Medicine, Baltimore, Md.
National Cancer Institute, Bethesda, Md.
National Institutes of Health, Bethesda, Md.
SomaLogic Inc., Boulder, Colo.
University of Florida, Gainesville, Fla.