Where Are All the Aptamers?

Geoffrey S. Baird, MD, PhD

DOI: 10.1309/AJCPFU4CG2WGJJKS

Oligonucleotide aptamers, first reported 20 years ago by Tuerk and Gold[1] and Ellington and Szostak,[2] are short, single strands of DNA or RNA that assume 3-dimensional configurations that facilitate specific binding interactions with other chemical species. In Tuerk and Gold's[1] seminal article, the nucleic acid target of an RNA-binding protein (specifically, the 8-base RNA sequence favored by T4 DNA polymerase) was selectively amplified from a pool containing all possible random 8-base RNA sequences. It is interesting that the technique found not only T4 DNA polymerase's native RNA target sequence in the pool but also another sequence that bound the protein with similar affinity, indicating that their technique was a generalizable method for finding nucleic acids that bound to nonnucleotide targets. This work and Ellington and Szostak's[2] demonstration that RNA ligands could be found for small molecule dyes were the first of countless studies showing that Systematic Evolution of Ligands by EXponential enrichment, or SELEX, can be applied to nearly any molecular target to generate high-affinity, specific oligonucleotide ligands.

In SELEX, one begins with a chemically synthesized pool of randomized oligonucleotides flanked on either end by defined primer-binding sequences, operating on the hypothesis that a high-affinity binding species is contained somewhere in that pool. This hypothesis may seem farfetched, especially when considering that there are only 4 natural nucleotide bases in DNA, in contrast with the 20 natural amino acids possible in an antibody's variable region. However, even small random DNA libraries can be incredibly diverse—a theoretical 25-mer random DNA oligonucleotide pool still contains 4^{25}, or more than a million billion, different species—implying that relatively short sequences of randomized DNA can be sufficiently diverse to contain a wide variety of potential binding ligands.

After preparing the SELEX pool, one proceeds to partition it into fractions that bind to or do not bind to a target, such as a protein immobilized onto beads. Fractions that bind (or that do not bind, if negative selection is desired) are then eluted from the target using harsh conditions such as high salt concentration or temperature, amplified, and repartitioned into binding and nonbinding fractions. The procedure is repeated iteratively several times (5-20 times in practice), using more and more stringent binding or washing steps until the pool converges to one or a few sequence families, at which point the library is cloned and the individual aptamers are sequenced. The original report of SELEX used a randomized RNA pool so that the amplification step required reverse transcription followed by polymerase chain reaction and in vitro transcription, but newer studies have demonstrated the feasibility of using single-stranded DNA pools to generate DNA aptamers to a wide variety of targets, thus removing the reverse transcription and transcription steps.[3] Although RNA aptamers are perhaps more biologically relevant, as they occur in nature (ie, the T4 DNA polymerase example used in the first report of aptamers), single-stranded DNA aptamers have proven just as robust as binding agents, with the added benefits of cheaper, easy production and increased stability.

Since the original report, SELEX has seen large methodological improvements. Currently, in a matter of days, one can create hundreds of aptamers in a highly parallel, automated process, evolving each aptamer to function in exactly the same biochemical conditions, with reagent sensitivities and specificities comparable to monoclonal antibodies.[4] (This work has been pioneered by SomaLogic, a biotechnology

company with which I collaborate and from which I receive research funding.) In contrast, antibodies require weeks or months and animals or cell lines for discovery and production. Because they do not occur frequently in nature, another advantage of aptamers is that it is unlikely that a patient would harbor an antireagent antibody, thus avoiding one of the significant drawbacks of antibodies in clinical immunoassays. Aptamers can also be produced on kilogram scales, reducing the downstream potential cost to fractions of a cent per assay; they can easily be derivatized regioselectively with detection reagents like dyes and purified with routine chromatographic methods; and they are stable essentially indefinitely when stored dry at room temperature. A clinical laboratory application based on aptamers might not even require a refrigerator, which could be a boon to assays intended for use in resource-poor environments. Perhaps the most important advantage of aptamers, however, is that they are produced in a controlled manner by combinatorial chemistry. While antibodies are in essence the hijacked by-product of an animal immune system used in applications for which they were never intended by nature, aptamers are designed from the beginning to act as diagnostic reagents.

After reading the previous paragraph, astute pathologists should be asking themselves the following questions: If aptamers are so great, and it's been 20 years since they were invented, then *where are they*? Why are they not being used in the clinical laboratory? I am a practicing pathologist who also runs a research laboratory devoted to developing aptamer-based diagnostic reagents, and I ask myself these questions often. To my knowledge, there are no Food and Drug Administration–approved clinical diagnostic tests using aptamers available in the US market, and neither are there any aptamers in clinical use in laboratory-developed tests in the United States. Does the dearth of aptamers in the clinical laboratory mean that they simply are not ready for prime time, or are practicing pathologists simply ignoring a technology that could revolutionize their practice?

There is no single good answer to why aptamers have not yet penetrated into the clinical laboratory, but several factors are clearly at play. First, aptamers are a change from antibodies, and change for its own sake is not always welcome in the clinical laboratory. Our hesitancy to switch away from tried, although not necessarily true, antibody-based assays is undoubtedly due to some aversion to risk inherent in laboratory directors. More fundamentally, though, neither anatomic nor clinical pathology training curricula include any dedicated effort to teach residents about aptamers, so only pathologists who have made a concerted individual effort to learn about aptamers would be expected to know they even exist. My personal impression, based on giving talks on aptamers around the country, is that only a minority of pathologists have ever heard of aptamers, and an even smaller minority could satisfactorily explain what one is, how it could be made, or how it could be used.

Another reason why aptamers are not yet common in the clinical laboratory is one more germane to this editorial, which can be summed up as the "thrombin problem." The thrombin problem refers to the well-known thrombin aptamer[5] and the fact that hundreds of investigators have focused their attention on studying this aptamer or designing a clever detection strategy for this target,[6] rather than developing assays for more clinically relevant targets. In this way, aptamers have become, in some sense, the victims of their own success. Because it is relatively easy to manipulate DNA and to develop creative detection methods, and because it is so easy to obtain an aptamer—a reasonable quantity of the thrombin aptamer or a variant can be purchased from an online oligonucleotide vendor for a few dollars and delivered the next day by express mail—the barrier to getting a successful report published is much lower if one chooses to modify the existing thrombin aptamer rather than use SELEX to create and validate a new aptamer. To be clear, many aptamers to interesting and clinically relevant proteins have been reported in the literature (Lee et al[7] describe a database of these aptamers, maintained by the Ellington laboratory), but only few have taken that reagent all the way through to a clinical application. It is for this reason that the article in this issue of the *Journal* by Zhang et al[8] is such a promising development.

In this article, Zhang et al[8] report the application of an RNA aptamer to CD4 to flow cytometric phenotyping. By using an RNA aptamer to CD4 first described many years ago, they show comparable performance between CD4 aptamers and antibodies in detecting surface CD4 expression. If there were a flaw with the study, it would be that they had to focus on characterizing reagent stability, a significant concern with RNA-based reagents that could have been largely ameliorated if they had chosen to create and use a DNA aptamer. Nevertheless, they demonstrate similar flow cytometric scatter grams resulting from CD4 aptamer– or CD4 antibody–based profiling of clinical samples, and they show that the aptamer can be used simultaneously with other reagents for multiplex detection. Although their effort stops short of the full validation one would require before unleashing a method in the clinical laboratory, their data clearly support the contention that an aptamer can replace an antibody in at least one targeted application. Whether aptamers can replace antibodies in other flow cytometric applications remains to be seen, but there are no obvious reasons why it would not be feasible. Wholesale replacement of all antibodies in flow cytometry with aptamers may be too large a leap to contemplate from this single study and may not be necessary or helpful, but at least we now know it may be possible.

What might we gain if studies of this sort continue and more aptamer reagents were validated for other pathology

DOI: 10.1309/AJCPFU4CG2WGJJKS

applications? Aside from very low reagent cost, one significant advantage could be the ease with which one can attach aptamers to detection moieties. Small-molecule dyes are usually attached to antibodies through amine-reactive intermediates, leading to antibodies that harbor dyes at one or more locations in their primary sequence. Thus, antibody-dye conjugates can vary in their properties quite significantly lot to lot, and some antibody-dye conjugates do not function well at all and require secondary antibodies. Aptamers, on the other hand, can be unambiguously derivatized at one or many prespecified locations along their sequence, and the resulting conjugate can be purified to homogeneity and characterized with mass spectrometry, facilitating reagent quality control. Aptamers can also be easier than antibodies to conjugate to fluorophores that are not small molecules, such as quantum dots.[6] Quantum dots are semiconductor particles that emit fluorescent light in very narrow bandwidths, such that many different signals can be assessed simultaneously without spectral overlap, a problem that limits the number of dyes that can be detected on a single laser channel in a flow cytometer.

Flow cytometric applications aside, it is clear that aptamers hold much promise as reagents for clinical laboratory assays. For example, work in my own laboratory (in collaboration with SomaLogic) has demonstrated the usefulness utility of modified DNA aptamers for fast histochemical localization of HER2 and EGFR proteins, and the same group that reported this CD4 study[8] recently reported another study outlining the use of another RNA aptamer for histochemical localization of CD30.[9] Thus, aptamers can probably be used as replacements for antibodies in many diagnostic assays.

A PubMed search for "aptamer assay" on August 2, 2010, yielded 939 hits, clearly indicating that the knowledge base we need to help us continue to bring aptamers into our clinical laboratories is out there waiting to be tapped. The original patents on aptamer technology are expiring soon as well, which should encourage additional efforts on the part of academic and industrial pathologists who want to bring online laboratory-developed aptamer assays without fear of infringing on patents. Bringing aptamers into the clinical laboratory will be a long-term goal, however, and involve more than just those who do the innovative development work. As a community, it will be the responsibility of pathologists everywhere, first and foremost, to keep abreast of these new methodological developments and then to support the implementation of useful technologies without regard to whether they fall within a currently accepted paradigm.

In a climate of increasing health care costs, however, science itself might not be the only thing to consider when assessing the viability of aptamers in the clinical laboratory. I am not sure, for example, whether anyone would want to switch from antibodies to aptamers for clinical flow cytometric CD4 assays based solely on the results of the study by Zhang et al.[8] However, if I told you that you could purchase a lifetime supply of a DNA aptamer reagent for a similar assay for a few hundred dollars and store it at room temperature forever, would you be interested in hearing more about it? I know I would.

From the Department of Laboratory Medicine, University of Washington, Seattle.

References

1. Tuerk C, Gold L. Systematic evolution of ligands by exponential enrichment: RNA ligands to bacteriophage T4 DNA polymerase. *Science*. 1990;249:505-510.
2. Ellington A, Szostak J. In vitro selection of RNA molecules that bind specific ligands. *Nature*. 1990;346:818-822.
3. Ostroff RM, Bigbee WL, Franklin W, et al. Unlocking biomarker discovery: large scale application of aptamer proteomic technology for early detection of lung cancer. *NaturePrecedings*. 2010. http://dx.doi.org/10101/npre.2010.4537.1. Accessed August 24, 2010.
4. Gold L, Ayers D, Bertine J, et al. Aptamer-based multiplexed proteomic technology for biomarker discovery. *NaturePrecedings*. 2010. http://dx.doi.org/10101/npre.2010.4538.1. Accessed August 24, 2010.
5. Griffin L, Tidmarsh G, Bock L, et al. In vivo anticoagulant properties of a novel nucleotide-based thrombin inhibitor and demonstration of regional anticoagulation in extracorporeal circuits. *Blood*. 1993;81:3271-3276.
6. Cho E, Lee J, Ellington A. Applications of aptamers as sensors. *Annu Rev Anal Chem (Palo Alto Calif)*. 2009;2:241-264.
7. Lee J, Hesselberth J, Meyers L, et al. Aptamer database. *Nucleic Acids Res*. 2004;32(database issue):D95-D100.
8. Zhang P, Zhao N, Zeng Z, et al. Combination of an aptamer probe to CD4 and antibodies for multicolored cell phenotyping. *Am J Clin Pathol*. 2010;134:586-593.
9. Zeng Z, Zhang P, Zhao N, et al. Using oligonucleotide aptamer probes for immunostaining of formalin-fixed and paraffin-embedded tissues [published online ahead of print August 6, 2010]. *Mod Pathol*. In press.

DOI: 10.1309/AJCPFU4CG2WGJJKS