Using Radiolabeled DNA as an Imaging Agent to Recognize Protein Targets

In the article by Hicke et al. (*1*) in this issue of *The Journal of Nuclear Medicine,* a method to target tumors by a novel class of imaging probes (aptamers) is developed and validated in a small-animal model.

Aptamers are synthetic, single-stranded DNA or RNA molecules that have a unique 3-dimensional geometry that enables them to bind with other target molecules, including proteins (*2,3*). Since their discovery approximately 15 years ago, aptamers have been used to target many different molecules, including small molecules, amino acids, peptides, and proteins. A key feature of the cell machinery is that proteins do specifically interact with DNA and RNA in addition to interacting with each other. For example, many proteins are involved in regulating the transcription of a specific gene through regulatory regions of genes. At the heart of this regulation is the interaction of specific RNA or DNA sequences with specific amino acids within a protein. Therefore, it is possible to take advantage of this recognition characteristic in order to develop imaging agents in which a small oligonucleotide (DNA or RNA) is used to target a protein on the cell surface or in the extracellular matrix. Instead of using a protein (e.g., antibody) to recognize a protein target, one uses an aptamer to recognize the same protein target. The pharmaceutical industry has been studying aptamers for many years as potential drugs; thus, as is often the case, one can learn from these attempts in order to develop novel aptamer imaging agents.

See page 668

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Aptamers exhibit relatively high affinities for their target, with dissociation constants ranging from low nanomoles to picomoles. Aptamers can even discriminate between closely related isoforms of the same target molecule. They have also been used to discriminate between human and murine versions of the same protein. They are relatively robust to modification and can retain binding affinities even with different functional groups attached. DNA or RNA aptamers can routinely be isolated from synthetic combinatorial nucleic acid libraries by in vitro selection. This technique is known as systematic evolution of ligands by exponential enrichment (SELEX). A large library of up to 10^{15} different DNA molecules is initially generated by chemical synthesis and polymerase chain reaction amplification. For RNA libraries, in vitro transcription is used. The library can be modified to enhance nuclease resistance, as is usually needed for serum stability. The library can then be used with an appropriate selection strategy that allows separation of target-bound aptamers from everything else. This small portion of the original library is then amplified by reverse-transcriptase polymerase chain reaction, and the whole process is repeated iteratively. SELEX has now become developed enough so that, by using automation, aptamers for multiple targets can be selected and characterized in parallel within only a few weeks.

The first aptamer drug to be approved by the Food and Drug Administration was pegaptanib sodium (Macugen; Pfizer and Eyetech). It is used for the treatment of macular degeneration. Two further aptamers have been clinically used: an aptamer for inhibiting HIV replication and an antithrombin aptamer for use in coronary artery bypass surgery. Aptamers are poor antigens, likely because of their small size and similarity to endogenous molecules. Clinical studies have demonstrated that aptamers are of low or no immunogenicity and are nontoxic molecules. There will likely continue to be more drugs that are aptamers reaching clinical trials in the near future.

The first report in the literature of using an aptamer specifically for imaging was by Charlton et al. (*4*). In that study, the target molecule was human neutrophil elastase. An aptamer labeled with ^{99m}Tc was shown to image neutrophils in a rat inflammation model with a peak target-to-background ratio of approximately 4 at 2 h after injection. Comparisons were made to IgG, which, in the same model, had a slower clearance but a 2- to 3-fold greater absolute uptake than did the aptamer tested. Absolute values for percentage injected dose (%ID) per gram of tumor were not provided in the paper by Charlton et al., but the %ID in the tumor was approximately 0.5% at about 4 h. The aptamer tested was not modified for protection against serum nucleases, and the amount of target molecules was relatively low. In addition, significant nonspecific binding was observed in this model and thought to be related to neutrophil binding of DNA. Nevertheless, this work was the first demonstration that aptamers had some potential as imaging probes.

In the study by Hicke et al. (*1*), an extracellular matrix protein, tenascin-C, present in relatively high copy number

in tumors, was targeted with TTA1, an aptamer. Both fluorescently labeled TTA1 and ^{99m}Tc-labeled TTA1 (RNA) were studied in mouse xenograft models. TTA1 was previously identified using SELEX procedures against tumor cells and purified TN-C. It binds with a dissociation constant of 5 nmol/L to the fibrinogenlike domain of TN-C. As a target protein, TN-C has both high abundance (1–10 μmol/L) and accessibility to circulating ligands such as TTA1. TN-C is overexpressed in many tumor types and is therefore an ideal target. TTA1 has chemical modifications (including 2′-OMe purine substitutions) to minimize nuclease degradation. Significant serum degradation was still noted, but apparently, the aptamer in the tumor tissue remained intact and did not degrade. The absolute %ID/g achieved in the tumor was approximately 3% at 60 min after injection but decreased and stabilized to approximately 1% over longer periods. The rapid pharmacokinetics of TTA1 support labeling with ^{18}F and PET. Importantly, modifications of the MAG_2 radiometal chelator markedly altered the biodistribution and pharmacokinetics of the aptamer.

As important as the study by Hicke et al. (*1*) is, many issues still remain to be investigated. Studies now need to be pursued with other aptamers to determine how well this approach performs with targets that are lower in abundance. Not only did the study use a very high abundance target, but there also were up to 6 binding sites per target TN-C for the TTA1. The location of the target may also prove to be somewhat of a challenge. The current target is extracellular; if one targets a cell-surface protein, one may encounter additional limitations. The potential for appropriately modified aptamers to cross the cell membrane and potentially target an intracellular protein may open other important imaging applications but will require significant research investments. A key experiment that is needed is a head-to-head comparison of aptamers and engineered antibodies to determine the true advantages of the one over the other. Both can potentially have high affinity and rapid blood clearance. Both are amenable to general labeling strategies as well, but aptamers may provide somewhat better flexibility. The %ID/g achieved for engineered antibodies (e.g., minibodies) is markedly higher (>10-fold) with various cell-surface targets (e.g., carcinoembryonic antigen) than what is shown with aptamers in the current work by Hicke et al. However, before any conclusions can be drawn, comparisons are needed in which the same model is used for testing each type of imaging probe. The effects of aptamer dose on imaging assays will also have to be explored in more detail and in different models. Overall, much more work remains to be done, but aptamers definitely warrant further research as novel imaging probes with many potential advantages.

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REFERENCES

1. Hicke BJ, Stephens AW, Gould T, et al. Tumor targeting by an aptamer. *J Nucl Med.* 2006;47:668–678.
2. Crawford M, Woodman R, Ko Ferrigno P. Peptide aptamers: tools for biology and drug discovery. *Brief Funct Genomic Proteomic.* 2003;2:72–79.
3. Nimjee SM, Rusconi CP, Sullenger BA. Aptamers: an emerging class of therapeutics. *Annu Rev Med.* 2005;56:555–583.
4. Charlton J, Sennello J, Smith D. In vivo imaging of inflammation using an aptamer inhibitor of human neutrophil elastase. *Chem Biol.* 1997;4:809–816.