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Bivalent Aptamers Deliver the Punch

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Aptamers, sometimes termed "chemical antibodies," have been engineered into multimerized versions for therapeutic application. The groups of Gilboa and Sullenger now report the development of a bivalent aptamer-molecular device as a receptor agonist that has the same functional properties, but stronger avidity than a corresponding antibody.

Aptamers are in vitro selected nucleic acids that assume specific and stable three-dimensional shapes, thereby providing highly specific, tight binding to targeted ligands. Many aptamer properties are comparable to those of protein monoclonal antibodies, but the nucleic acid nature of aptamers offers more exciting advantages (Nimjee et al., 2005), including the potential for chemical synthesis, convenient modification, chemical versatility, stability, and lack of immunogenicity. Therefore, aptamers can be utilized for a variety of applications ranging from diagnostics to therapeutics (Pestourie et al., 2005; Famulok et al., 2007). Recently, bivalent or multivalent aptamer-based molecules have been engineered via different methods to serve as diagnostic probes (Fredriksson et al., 2002), delivery vectors (Chu et al., 2006), antiviral agents (Darfeuille et al., 2001), and receptor agonists (McNamara et al., 2008).

Aptamers targeting cell surface receptors have been demonstrated to modulate immune responses in vivo, hence attracting renewed attention in the field of aptamer development. In particular, multimerized versions of aptamers have been successfully engineered and these have enhanced efficacy over monovalent aptamers. This has been attributed to local cooperative interactions of multimeric aptamers and their cognate receptors. One such approach for aptamer multimerization has been described by Santulli-Marotto et al. (2003). These investigators used four cytotoxic T cell antigen (CTLA) aptamers annealed to a complementary DNA scaffold which resulted in enhanced binding affinity without changing the functionality of the individual aptamer units. Similarly, McNamara et al. (2008) described that multivalent configurations of the 4-1BB aptamers costimulated T cell activation in vitro and promoted tumor rejection in vivo (Figure 1A), thus exploiting the biological role of 4-1BB as a T cell costimulatory receptor that prolongs cell survival. In this study aptamer dimers were generated by adding short complementary sequences to the 3′-ends of the aptamers and allowing them to anneal in pair-wise fashion. However, the 21 nucleotide linker in this molecular device limited the molecular distance and structural flexibility as well as in vitro transcribed RNA yields.

In this issue, Sullenger and colleagues (Dollins et al., 2008) describe multivalent configurations of the OX40 aptamer (Figure 1B) which costimulated T cell activation in vitro and promoted tumor rejection in vivo. In this study, 2′-fluoro pyrimidine RNA aptamers with nanomolar binding constants for the OX40 receptor were isolated using a standard bead-based SELEX method. Initially, although capable of binding OX40, the selected monomeric aptamers were unable to stimulate OX40 function. This was not surprising since the crystal structure of the OX40-OX40 ligand complex revealed multiple binding sites for its ligand, whereas an average of one aptamer was bound to a single receptor.

Considering the features of multiple ligand binding sites on OX40, these researchers developed a malleable, DNA oligonucleotide-based molecular scaffold which was able to bind two copies of the aptamer. A polyethylene spacer (18 carbons in length) was inserted between the aptamer annealing sites on the scaffold to provide flexibility (Figure 1B). With this scaffold, two OX40 aptamers were arranged in a flexible conformation, spaced

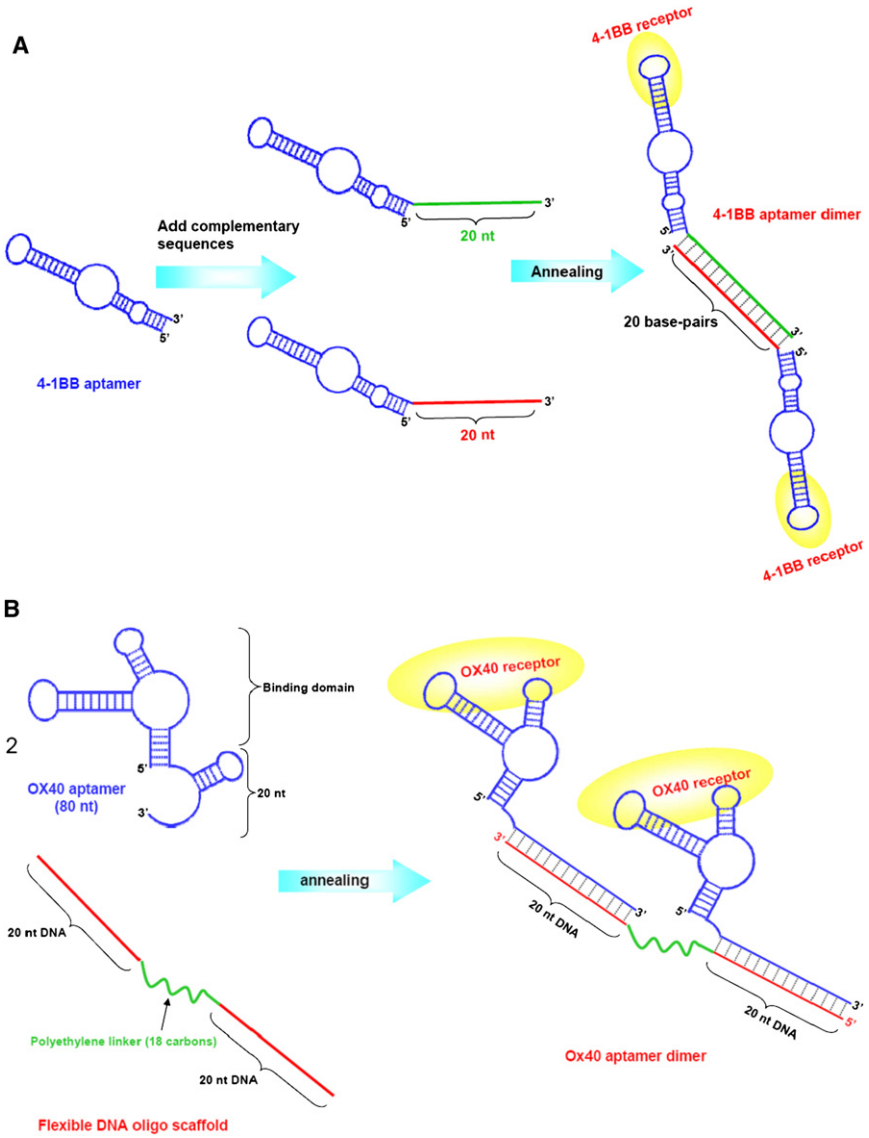

Figure 1. Engineering Aptamer-Based Molecular Devices

(A) The formation of 4-1BB aptamer dimer. The 3′-terminus of the 4-1BB aptamers was attached to additional complementary sequences (20 nt in length; red and green lines shown), which were subsequently annealed together.

(B) The formation of OX40 aptamer dimer. The 3′-end sequences (20 nt) of OX40 aptamers were annealed to a flexible DNA scaffold. A tandem repeat of 20 nt DNA oligos (red lines) was connected by a flexible polyethylene spacer (green).

appropriately apart to bind to the OX40 receptor and trigger multimerization, thereby activating receptor function. This attractive scaffold-based approach can also be employed to accommodate various intermolecular distances and conformations. In this strategy, the 20 nt DNA scaffold is annealed with the 3′-end of the aptamer. As part of the aptamer, this sequence of 20 nt when annealed with the scaffold should not affect the active structure and hence binding affinity of the aptamer. Generally, if the selected aptamers cannot be truncated without affecting the original binding affinity, an additional tail can be attached to the aptamer for annealing with a molecular scaffold.

Fortunately, after the selected OX40 aptamers were annealed with the scaffold, they were still capable of binding the OX40 receptor, which activated the OX40 receptor on primed T cells in culture. By contrast, a monomer aptamer or mutant aptamer had no stimulatory effects. Furthermore, the dimerized aptamer binding to OX40 significantly enhanced the antitumor potency of dendritic cells in vivo.

Although the work described by Dollins et al. (2008) focuses on development of OX40 agonists, structurally flexible, size-tailored aptamer-molecular devices could provide a useful means of regulating many biological processes where dimerization or multimerization is required. As simple and active building blocks, the various aptamers can also be assembled with other therapeutic nucleic acids (like antisense oligonucleotides, ribozymes and siRNAs) into versatile molecular devices with multiple functions. With the publication of this creative aptamer dimerization method, expect to see many more applications of aptamers as agonists for receptor activation or antagonists for blocking receptor function.

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