Published in final edited form as:

Angew Chem Int Ed Engl. 2010 August 9; 49(34): 5934–5937. doi:10.1002/anie.201001736.

Expanding the concept of chemically programmable antibodies to RNA-aptamers: a novel class of chemically programmed biotherapeutics

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Abstract

Chemically programmed antibodies represent a new class of biologic drugs that acquire their specificity through chemistry rather than through biology. To date, this approach has used small molecules and peptides to direct targeting and to extend the pharmacokinetics and otherwise enhance the biological function of the small molecule or peptide through Fc-based mechanisms of the antibody. However, other classes of therapeutically active molecules, such as aptamers, should benefit from antibody conjugation and the chemically programmed antibody approach. Aptamers are structured nucleic acid ligands often selected using the ‘selective evolution of ligands by exponential enrichment’ (SELEX) procedure. Although aptamers are a promising class of therapeutics because of their excellent binding and inhibitory properties, only a single VEGF-targeting aptamer is an approved drug. For *in vivo* applications, aptamers suffer from low chemical stability (these molecules are readily degraded by nucleases in serum) and poor pharmacokinetic properties (circulatory half lives are on the order of several minutes). Nuclease resistance can be enhanced significantly by incorporating 2' ribose modified nucleobases; 2'-O-methyl modified oligonucleotides have acceptable serum stabilities. Other oligonucleotide modifications are also being explored to solve this difficult problem.

Here, we demonstrate for the first time site-specific conjugation of an aptamer to the aldolase antibody 38C2 to produce aptamer programmed cpAbs. Conjugation of the VEGF-targeting aptamer ARC245 to the well-characterized chemically programmable antibody 38C2 resulted in a biologically active aptamer-antibody conjugate that had significantly increased functional affinity and circulatory half-life as compared to the free aptamer. The aptamer-cpAb strategy developed here should be general and readily transferable to other aptamers. Aptamer-based cpAbs of the type developed here represent a promising new class of aptamer immunotherapeutics that combine the favourable characteristics of aptamers with those of antibodies.

Keywords

angiogenesis; aldol reaction; aptamer; pharmacokinetics; enamine; bioconjugation; VEGF

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Supporting information for this article is available on the WWW under <http://www.angewandte.org> or from the author.

Key to understanding of the concept of chemically programmable antibodies is the catalytic antibody 38C2 that efficiently catalyzes aldol and related reactions through an enamine mechanism.[1] The reactivity of this and related antibody aldolases is provided by an exceptionally nucleophilic lysine residue located on the heavy chain variable domain. This lysine residue can be selectively and covalently labelled with 1,3 diketone- or β -lactam-equipped ligands such as small molecules or peptides to reprogram the binding specificity of the antibody (Fig. 1).[2] Thus, in contrast to classic monoclonal antibodies that acquire their specificity through biology (gene rearrangement and hypermutation), chemically programmed antibodies (cpAbs) acquire their specificity through chemistry. Conjugation to the antibody equips the small molecule or peptide ligand with the pharmacokinetic properties of the antibody and antibody effector functions mediated by the antibody Fc domain. Several studies have shown that this strategy increases the therapeutic efficacy of peptides and small molecules by several orders of magnitude in animal models.[2a,c,f,h] Currently four peptide-based cpAbs are in human clinical trials (www.clinicaltrials.gov).

To date, this approach has used small molecules and peptides to direct targeting. However, other classes of therapeutically active molecules, such as aptamers, should benefit from antibody conjugation. Aptamers are structured nucleic acid ligands often selected using the 'selective evolution of ligands by exponential enrichment' (SELEX) procedure.[3] Although aptamers are a promising class of therapeutics because of their excellent binding and inhibitory properties,[4] only a single VEGF-targeting aptamer is an approved drug.[5]

For *in vivo* applications, aptamers suffer from low chemical stability (these molecules are readily degraded by nucleases in serum [6]) and poor pharmacokinetic properties (circulatory half lives are on the order of several minutes [7]). Nuclease resistance can be enhanced significantly by incorporating 2' ribose modified nucleobases; 2'-O-methyl modified oligonucleotides have acceptable serum stabilities.[8] Other oligonucleotide modifications are also being explored to solve this difficult problem.[9]

To date, most strategies aimed at enhancing the pharmacokinetic properties of aptamers have focused on covalent attachment of ligands such as polyethylene glycol (PEG) to reduce renal clearance.[11] In one study, conjugation of a 40 kD PEG to an aptamer increased the circulatory half-life from several minutes to 23 h.[11d] Data from a phase I clinical trial with PEGylated aptamer ARC1779 indicate that the circulatory half life is 2 h in humans.[12] The extent and site of PEGylation must be evaluated for each aptamer since not all aptamers tolerate chemical conjugation to PEG molecules above a certain size.[13] Antibody programming could provide an attractive alternative to current strategies for extending aptamer half lives. By attaching an aptamer to the chemically programmable antibody the therapeutically valuable binding specificity of the aptamer should be combined with the bivalency, the long *in vivo* half-life and effector functions of the antibody. In order to explore the potential of aptamer-based programming of antibodies, we synthesized the β -lactam based heterobifunctional linker **3** (Fig. 2) with a reactive maleimide portion for attachment to a thiol modified aptamer.[14] The synthetic scheme for antibody aptamer conjugation is outlined in Figure 3. For our proof of concept experiments, we chose the thiol-modified anti-VEGF aptamer ARC245 since this aptamer is fully 2-O-methyl modified, highly nuclease resistant, and its binding and inhibitory properties are well characterized.[11d] Linker **3** was first reacted with the aptamer and after purification cp38C2 was specifically reacted with the lactam portion to yield an irreversible linkage. The antibody and aptamer-conjugated antibodies were analyzed by gel electrophoresis as shown in Figure 4.

The reactive lysine is located on the heavy chain of 38C2 and consistent with this and the crystal structure of the antibody complex with a β -diketone, we observed that only the heavy chain band was shifted to a higher molecular weight under reducing conditions indicative of site-specific labelling.[1c,o,r] Labelling was complete as determined by loss of aldolase activity (Supporting Information).

In order to evaluate the binding properties of our aptamer-cpAbs we performed a displacement ELISA in which either the unlabeled ARC245 or cp38C2-ARC245 competed for binding to surface-coated VEGF165 with biotinylated-ARC245.[14] Data from this assay is shown in Figure 5A. cp38C2-ARC245 showed a 60-fold lower EC_{50} value than the aptamer alone (0.69 nM vs. 41 nM). Based on these results, the affinities of aptamer and aptamer-cpAb were determined by the method of Oroz *et al.*[14] In this experiment, the affinity of ARC245 was determined to be 1.8 nM and the aptamer-cpAb had a 30-fold higher affinity of 66 pM (Fig. 5B). This result shows that by antibody conjugation, the functional affinity of an otherwise monovalent aptamer was increased significantly. Other reports indicate that bivalent aptamers have a higher functional affinity than their monovalent counterparts.[15]

The therapeutic target of ARC245 is VEGF, a proangiogenic factor excreted by many tumors to stimulate blood vessel growth into the tumor and to maintain a supply of oxygen and nutrients to rapidly dividing tumor cells.[16]

ARC245 is a potent anti-VEGF antagonist that prevents VEGF from binding to the VEGF receptor. To study the biological activity of cp38C2-ARC245, we performed cell migration assays using human umbilical cord endothelial cells (HUVEC) in a transwell assay format. Cells were seeded into the top chamber of 8- μ m transwell plates and allowed to migrate at 37 °C for 4 h towards the bottom chamber to which 10 ng/mL VEGF was added.

As shown in Figure 6, addition of 50 nM 38C2-ARC245 conjugate potently inhibited VEGF-mediated cell migration, demonstrating that the biological activity of ARC245 was retained after antibody conjugation. It is well established that compounds that inhibit VEGF-mediated cell migration in a transwell assay also block proangiogenic signalling *in vivo* provided they demonstrate favourable pharmacokinetic properties.[17]

The pharmacokinetic properties of cp38C2-ARC245 were evaluated in athymic nude mice. cp38C2-ARC245 (100 μ g; 5 mg/kg) was injected intravenously and blood was collected at various time points up to 96 h post injection by tail bleeding. The concentration of aptamer ARC245 was analyzed by ELISA. In order to detect the intact antibody-aptamer conjugate an ELISA assay was developed in which h38C2 was first captured from serum. A biotinylated antisense oligonucleotide was then added that annealed to the aptamer and was detected with streptavidin HRPO. In all experiments the first time point (5 min) was defined as 100%. The hcp38C2-ARC245 conjugate showed a clearance half-life of approximately 21 h. In parallel, the concentration of the antibody 38C2 was determined from the same blood samples (Supporting Information). This half-life was approximately 68 h. The most likely explanation for the difference in half-lives of the antibody and the functional aptamer is either nuclease digestion of the aptamer portion or cleavage of the heterobifunctional linker. While we believe that the addition of a 5'-cap to the aptamer and modifications of the heterobifunctional linker might increase the overall stability of the conjugate, the increase in circulatory half-life of the antibody conjugate determined here (as compared to the free aptamer) is very significant. In accord with the results of Healy *et al.* on unconjugated aptamers, the circulatory half-life of the fully 2'-O-Me modified ARC245 was determined here to be less than 30 min following intravenous dosing.[13a] Thus the cpAb approach

provides aptamers with a very substantial increase in circulatory half-life that should translate into less frequent dosing regimens for aptamer drugs in a therapeutic setting.

In summary, we demonstrate for the first time site-specific conjugation of an aptamer to the aldolase antibody 38C2 to produce aptamer programmed cpAbs. Conjugation of the VEGF-targeting aptamer ARC245 to the well-characterized chemically programmable antibody 38C2 resulted in a biologically active aptamer-antibody conjugate that had significantly increased functional affinity and circulatory half-life as compared to the free aptamer. The aptamer-cpAb strategy developed here should be general and readily transferable to other aptamers. Aptamer-based cpAbs of the type developed here represent a promising new class of aptamer immunotherapeutics that combine the favourable characteristics of aptamers with those of antibodies. This approach might also be applicable to chemically programmed vaccines.[18]

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the NIH R01 CA104045 and The Skaggs Institute for Chemical Biology for funding.

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Figure 1.
Chemical reactivity of catalytic antibody 38C2 and active site structure showing enamine formation with Lys H93 [1r]

Figure 2.
Synthesis of heterobifunctional linker 3.

Figure 3.
Irreversible programming of aldolase antibody 38C2 with aptamer ARC245.

Figure 4.

Representative SDS acrylamide gel after conjugation of antibody and aptamer. Lane 1, unmodified 38C2; lane 2, human 38C2 (hu38C2) after conjugation to aptamer; lane 3, mouse 38C2 (38C2) after conjugation to ARC245; lanes 1', 2', and 3', samples as in first three lanes under reducing conditions.

Figure 5.

A) Competitive ELISA with increasing amounts of either unmodified ARC245 or hcp38C2-ARC245 incubated with 20 nM biotinylated ARC245. Biotinylated ARC245 was detected with streptavidin HRPO and percent displacement was plotted. **B)** Resulting linearization scheme according to Oroz *et al.*[14] From the slopes, the K_D s for ARC245 and hcp38C2-ARC245 were determined to be 1.81 nM and 66 pM.

Figure 6.

Transwell migration assay of HUVEC cells migrating toward 10 ng/mL recombinant human VEGF165. cp38C2-ARC245 was applied at a concentration of 50 nM. Cells were allowed to migrate for 4 h; $n=3$, $p=0.014$.