

# Partnering Aptamer and RNAi Technologies

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RNA interference (RNAi) is an endogenous mechanism for post-transcriptional gene regulation. There are two major arms of the RNAi pathway: regulation of translation by micro RNA (miRNA), and directed destruction of mRNA by small interfering RNA (siRNA) [1]. The process of RNAi involves enzymatic cleavage by the RNase III enzyme, Dicer, of precursor duplexes into siRNAs of between 21-23 bases in length with two-base 3' overhangs. These siRNAs are incorporated into an RNA-induced silencing complex (RISC) that selects the antisense strand that eventually serves as a guide for the identification of complementary target mRNAs. The net result of this process is targeted destruction of an mRNA complementary to the siRNA antisense component.

Most eukaryotic cells harbor the machinery for effecting RNAi, and it has thus become one of the more popular choices for sequence-specific inhibition of gene expression. RNAi has rapidly progressed from an interesting phenomenon to an essential epigenetic tool and promising therapeutic [2]. RNAi is now entering the clinic for the treatment of conditions ranging from adult macular degeneration to respiratory syncytial virus (RSV) infection.

The major impediment to the widespread use of RNAi as a therapy is delivery of the siRNAs to the appropriate tissues, since negatively charged polymers such as siRNAs do not readily traverse cellular membranes without some protective covering [3]. A number of delivery schemes for siRNAs have been proposed, and include cholesterol-conjugated siRNAs [4], bi-layer liposomes [5], transferrin-conjugated  $\beta$ -cyclodextrins [6], antibody fragments conjugated to basic protamine [7], and chitostatin nanoparticles [7]. With the exception of cholesterol, for each of these approaches, the siRNAs must be complexed with the carrier particles, requiring separate preparation of the carrier and the siRNAs. The cholesterol modification allows covalent attachment of the siRNA to a cholesterol moiety. The cholesterol in turn binds to proteins that traffic the complex primarily to the liver or jejunum [4].

Two recent publications provide exciting new data expanding the options for cell type-specific delivery of siRNAs by joining the siRNAs to RNA molecules called aptamers [8, 9]. Aptamers are nucleic acids derived with SELEX (systematic evolution of ligands by exponential enrichment), which generates high-affinity binding ligands for interactions with other macromolecules [10]. Depending upon the sequence complexity of the nucleic acid mixture used in the selection process, an astoundingly large variety of shapes are possible, which allows for selection of a specif-

ic shape or set of shapes that exhibit high-affinity binding to a selected ligand.

Each of the new studies made use of an aptamer selected for high-affinity binding to the prostate-specific membrane antigen (PSMA), a cell surface receptor that is overexpressed in prostate cancer cells and vascular endothelium. Both groups asked whether the PSMA-targeting aptamer could selectively deliver the siRNA to cells expressing this receptor. MacNamara *et al.* used the aptamer to deliver siRNAs targeting polo-like kinase 1 (*PLK1*) and *BCL2*, which are survival genes overexpressed in many human tumors [8]. Chu *et al.* chose to deliver siRNAs targeting the gene for lamin A/C or *GAPDH*, two genes routinely used to demonstrate proof-of-principle of new siRNA approaches [9]. In addition, the two sets of workers used different methods to join the PSMA aptamers to the siRNAs. MacNamara *et al.* joined the aptamers to the siRNAs covalently, whereas Chu *et al.* used streptavidin to join biotin end-labeled siRNAs to the aptamer. Both groups used two prostate cancer cell lines, LNCaP and PC3, to demonstrate selectivity in siRNA uptake as a consequence of aptamer binding to PSMA. LNCaP cells express PSMA whereas PC3 cells do not. Both reports demonstrated that the PSMA aptamers only delivered the siRNAs to the LNCaP cells, thereby verifying the selectivity.

Both groups of investigators took advantage of the observation that duplex RNAs long enough to be cleaved by Dicer can be effective triggers of RNAi in mammalian cells [11, 12]. Each group used these longer duplexes to tether the PSMA aptamer with the intent that the aptamer portion would be cleaved off of the siRNA once the complexes or chimeric molecules entered the cytosol where Dicer is present for cleavage. Chu *et al.* used 27-mer duplexes to attach the aptamer. Two different approaches were tested to join the siRNAs to the aptamer. The first used a 5' biotin on the sense strand, allowing direct interaction with streptavidin conjugated to the aptamer. The other approach used a di-thiol linker between the 5' base and the biotin such that the biotin moiety could be attached by reducing the di-thiols. MacNamara *et al.* carefully modeled the aptamer secondary structure so it could be covalently joined to the sense strand of a Dicer substrate siRNA.

There are pros and cons to each approach. The method of Chu *et al.* results in four siRNAs per streptavidin, since each molecule of streptavidin can bind four biotins. In theory, this would allow multiplexing of different siRNAs with the same streptavidin-aptamer carrier, but the requirement to join the

aptamer to the siRNA by non-covalent linkages could have some drawbacks for clinical applications, such as dissociation of the complex in serum. The covalent linkage of the aptamer with the siRNAs described by MacNamara *et al.* is a more straightforward method for preparing the chimeric molecules, but requires synthesizing fairly long RNAs containing the aptamer fused to the sense strand. Depending on the size of the aptamer, this could result in poor synthetic yields and high production costs. Nevertheless, it was refreshing to see very similar biological results obtained from the two groups using different aptamer-siRNA complexes.

In the study by Chu *et al.*, no backbone modifications for stabilization were utilized, yet the aptamer-siRNA complex was as effective as a cationic lipid encapsulated siRNA in cell culture. The effectiveness of the aptamer-siRNA complex in the LNCaP cells may result from the rapid uptake of these complexes by receptor-mediated endocytosis, which took place within thirty minutes of exposure to cells in culture. MacNamara *et al.* used 2' fluoro modification of the pyrimidines in the sense strand, which stabilizes the RNAs in serum. They showed that the backbone-modified aptamer-siRNA chimeras were effective in both cell culture and *in vivo* (see below). Furthermore, in neither case was there any indication that these complexes were activating type I interferon responses [13].

Both groups demonstrated the specificity of the targeting with the PSMA aptamer based on results in LNCaP versus PC3 cells, although the work by MacNamara *et al.* went further by demonstrating that downregulation of PSMA gene expression by 5- $\alpha$ -dihydrotestosterone, or by blocking the receptor with an antibody, both dramatically reduced the uptake and biological efficacy of the aptamer-siRNA chimeras. These investigators also conducted extensive FACS analyses of the treated LNCaP cells to verify that the siRNAs

targeting the anti-apoptotic genes *BCL2* and *PLK1* triggered apoptosis. Perhaps the most exciting aspect of the MacNamara *et al.* study is the demonstration that injection of the PSMA aptamer-Plk1-siRNA chimeras into LNCaP-derived tumors led to tumor regression in a mouse xenograft model.

Both of these studies represent important advances in siRNA delivery. The power of SELEX to develop aptamers capable of binding receptors should prompt the widespread use of aptamer delivery of siRNAs via other receptors. The carefully crafted experiments in these two publications provide a powerful proof-of-principle that selective cell- or tissue-specific targeting of siRNAs can be achieved by using the appropriate aptamer/siRNA combinations.

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