Engineered RNA Nanorings for Efficient Delivery of siRNA

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Statement of Purpose: Self-assembling nanomaterials constructed from biological macromolecules are increasingly being harnessed as carriers for drugs and small molecule therapeutics for the treatment of various diseases. Particularly, short synthetic RNA duplexes called small-interfering RNAs (siRNAs) have shown considerable promise for gene modulation and silencing specific biological pathways in cancer[1]. Currently, more than 20 different therapeutic siRNAs are in clinical trials[2]. Besides specific siRNAs (or micro-RNAs), several other promising therapeutically potent RNA classes such as antisenses, aptamers, and ribozymes are worthy of consideration. However, non-modified siRNAs cannot be used practically to silence genes since they are unstable in the blood stream, thus having a short half-life, and encounter difficulties in crossing membranes due to their negative charges. Here we propose the use of computationally designed RNA nanorings as vehicles for efficient delivery of siRNA's for potential use in cancer therapy. The use of RNA nanorings functionalized with siRNAs provides a precise control over the formulation and higher local concentration of siRNAs, which in turn may improve the loading of RNA induced silencing complex (RISC), presented only in specific cytoplasmic locations. Such designed RNA scaffolds have multiple advantages such as – 1) tight control of structural homogeneity and targeting; 2) programmability; 3) precise control over folding and self-assembly; 4) simple conjugation with different natural nucleic-based functionalities (e.g. RNA aptamers, siRNAs, and proteins; 5) thermal and chemical stability; 6) biocompatibility and biodegradability; 7) relatively low immunogenicity; 8) relatively low cost of production; and 9) improved stability of complexes with cationic carriers.

Methods: The design strategies of RNA nanoscale scaffolds employ assembly principles borrowed from natural RNA structures. We functionalized RNA nanoscaffolds with six therapeutic siRNAs, visualized the structures with electron cryo microscopy, and tested these therapeutic constructs in several cell lines and mice. The detailed methods for the computational design, synthesis, characterization, functionalization with siRNA, and the therapeutic efficacy of siRNA delivery in several cell lines and mice have been described in Ref. 1. To study the potential use of nanorings as scaffolds for simultaneous delivery of siRNAs and assess the release of siRNAs from the functionalized nanorings upon dicing inside the cells, experiments with human breast cancer cells stably expressing enhanced green fluorescent protein (GFP) were carried out as described in Ref. 1.

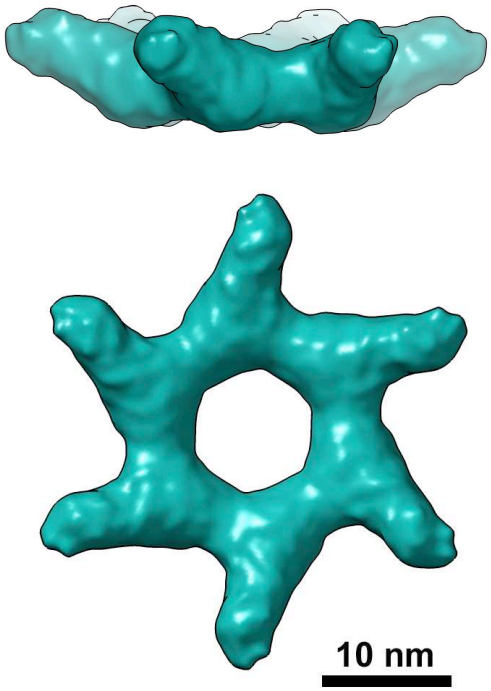

Figure 1. Functional RNA nanoparticle. RNA nanoring was functionalized with siRNA arms. Functionalized siRNA arms in the nanoring point upward creating a crown shape (top) and arms in the siRNA nanoring are positioned in a pinwheel fashion (bottom).

Results: Characterization of RNA containing nanorings using single-particle cryo-electron microscopy (cryo-EM) revealed that siRNA arms do not pointing straight out as expected from the model. After reconstruction, it was found that siRNA arms in the nanoring point upward creating a crown shape (Fig. 1 top). Also, looking from the top, the siRNA arms were positioned in a pinwheel fashion around the ring (Fig 1 bottom). Cells transfected with small amounts of siRNA functionalized nanorings (1 nM final) or DS RNA duplexes (6 nM) showed significant levels of silencing of GFP. The effect of gene silencing persisted over a nine-day period.

Conclusions: In conclusion, the data suggest that RNA nanorings are attractive scaffolds for the delivery of siRNA and may have potential applications as nanocarriers for other drugs and small molecule therapeutics for a number of applications in biotechnology and nanotechnology.

References:

1. Afonin KA et al. *Nano Lett,* 2014 14: 5662-5671.
2. Zhou et al. *Pharmaceuticals*, 2013 6:85-107