Computational Design and Delivery of Functional Ribonucleic Acids

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Abstract
Genetic therapy is hampered by limitations with regard to the design of active and specific nucleic acids as well as by delivery issues. We developed computational tools which enable identification, characterization, and design of functional RNA secondary structures. We found that unstructured complementary RNA is most efficiently binding to its respective target sequence. Applying this general principle, we demonstrated that unstructured antisense RNAs and guide siRNAs outreach the knockdown potential of structured RNAs by several orders of magnitude. Recently, we applied this approach towards the design of target binding domains of trans-splicing RNAs in a HSVtk/GCV suicide gene therapy approach targeting virus-transduced or cancer cells. Suicide RNA featured with unstructured target binding domains exhibited up to 30-100-fold improved on-target activity/specificity, and selectively triggered death of HPV-16-transduced cells, HIV-1-infected cells, and hepatoblastoma-derived human tissue culture cells without evidence for off-target cell killing. For cellular delivery, we explore non-viral, non-integrating, dumbbell-shaped DNA minimal vectors. We developed novel methods for cheap and efficient dumbbell-vector production and demonstrated that dumbbells exhibit superior cellular and nuclear delivery properties, high intracellular stability, and are not silenced in human primary cells. Thus, these vectors are most suitable for therapeutic applications that require profound transient expression in primary cells ex vivo or in vivo but for which integrating vectors are neither required nor desirable. Our largest CRISPR/Cas-expressing dumbbells are >6 kbp in length and our small RNA expressing dumbbells are with 130 bp the smallest expression vectors ever reported. As a second delivery vector system we developed permeability-tunable, capsule-like, polymeric, micron-sized, core-shell particles. These particles effectively release rod-shaped small DNA but selectively retain the RNA-encoding DNA template to avoid any contact between recombinant and genomic DNA. Capsule-mediated siRNA delivery triggered superior target gene knockdown in human tissue culture cells as compared with liposome-based delivery. Among primary human PBMCs, up to 50% of the monocytes internalized capsules ex vivo, indicating these capsules can be explored for genetic vaccination. Among all cellular membranes, the mitochondrial membranes represent the most insurmountable barriers for recombinant nucleic acids. We developed an efficient, modular and scalable mitochondrial delivery vector system which is based on structural subdomains of the long non-coding HCMV β2.7 RNA. Targeted mitochondrial delivery of antisense RNA triggered efficient knockdown of mitochondrial genes mtATP6 or mtATP8 leading to a reduction of mitochondrial ATP levels and cell viability. Nuclear transcription of β2.7-mRNA chimera gave rise to intra-mitochondrial EGFP expression. This new enabling technology may facilitate mitochondrial gene therapy. In summary, we explore novel vectors for the delivery of computationally designed RNA towards genetic therapy of human diseases.

Selected Relevant Publications for Reference