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Abstract:

Overcoming aptamer-resistance in HIV-1 inhibition with broad-spectrum RNA aptamer

RNA aptamers that bind HIV-1 Reverse Transcriptase (RT) inhibit HIV-1 replication in human cells. Because RT must interact with diverse nucleic acids during HIV-1 replication, the genetic threshold for eliciting resistance to some aptamers may be high. To evaluate the impact of RT-aptamer binding specificity on virus replication, we engineered proviral plasmids to encode phylogenetically diverse RT within a constant HIV-1NL4-3 (Subtype B) background. Viruses that were inhibited by pseudoknot aptamers from the F1Pk family were rendered resistant by the previously recognized R277K point mutation, providing the first demonstration of aptamer-specific resistance. Naturally F1Pk-resistant viruses were rendered sensitive by the inverse K277R mutation, thereby establishing RT as the genetic locus responsible for aptamer-mediated HIV-1 inhibition. In contrast, aptamers with 6/5 asymmetric loop ("6/5AL") or "UCAA" structural motifs exhibited broad-spectrum inhibition of replication across the entire recombinant panel. Inhibition was only observed when virus was produced in aptamer-expressing cells, but not in target cells, indicating that prior encapsidation is required. The magnitude of HIV-1 suppression correlated with the number of encapsidated aptamer transcripts per virion, especially at low expression levels, with saturation occurring around 1:1 stoichiometry with RT. Encapsulation specificity for inhibitory RNAs suggests that it likely arises from cytosolic encounter with dimerized GagPol during viral assembly. High expression levels promoted non-specific encapsidation of control RNAs without inducing inhibition. Overall, this study provides important new insights into HIV-1’s capacity to resist aptamer-mediated inhibition, the utility of broad-spectrum aptamers to overcome that resistance and molecular interactions that occur during viral assembly.
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Bio-summary:

Dr. Burke obtained his B.A. degree in Chemistry/Biology from the University of Kansas in 1986. His formal training included Ph.D. studies in biophysical chemistry and microbial genetics at the University of California, Berkeley (with John Hearst) 1986-1992 and postdoctoral training in RNA biochemistry and aptamer selection at the University of Colorado, Boulder (with Larry Gold) 1992-1998.

From 1998 until 2005 Dr. Burke was an Assistant Professor of Chemistry & Biology at Indiana University in Bloomington, IN. He currently serves as Professor, in the Department of Molecular Microbiology & Immunology, University of Missouri School of Medicine and holds joint appointments in the Departments of Biochemistry and Biological Engineering. He has been a Present Bond Life Sciences Center Investigator at University of Missouri since 2005 and is Associate Chair of Department of Molecular Microbiology & Immunology at the University of Missouri School of Medicine.

Dr. Burke has a broad background in the biology and biochemistry of RNA, with emphasis on 1) understanding the limits of RNA function and 2) developing novel RNA molecules for biomedical and synthetic biology applications. A long-term objective of his lab's research is to develop RNA aptamers for control and study of viral pathogens, cancer, and host immunological responses to infection. As PI of several projects funded by NIH, NASA, NSF and private foundations, Dr. Burke’s group has developed extensive expertise and infrastructure for carrying out work in this area. One current project (NIH) evaluates antiviral aptamers for potential gene therapy treatment of HIV. Another current project (NASA) is developing new ribozymes that directly interface with the small molecule metabolome. The current project is part of a major new initiative to develop new aptamers that bind surface markers on viruses and on defined subsets of cells to aid efforts to study, detect and treat human and animal diseases.