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Commentary

Bridging siRNA strands for better function

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It has been 25 years since Fire and Mello first reported that double-stranded RNAs trigger a gene silencing event via Watson-Crick base pairing with a complementary sequence in mRNAs.[1] Shortly thereafter, Elbashir et al. demonstrated that the triggering agents are 21 base pair duplexes, termed small interfering RNAs (siRNAs), in which one strand is complementary to the target mRNA while the other strand has the sequence as the target.[2] The mechanism of RNA interference is primarily a process by which endogenous RNA hairpin structures are processed into duplexes termed microRNAs.[3]

Before endonuclease processing, microRNAs are configured with a covalent loop joining the guide and passenger strands. In a recent issue of *Molecular Therapy – Nucleic Acids*, Shibata et al. report that chemical bridging of the two strands of siRNAs results in higher potency and reduces an undesired complication of siRNAs termed "off-target" effects.[4] Off-target effects occur when the passenger strand guides untargeted cleavage of partially complementary sequences. This novel chemical bridging of the 5′ end of the passenger strand with the 3′ end of the guide strand via 2′ backbone chemistries effectively eliminates off-target effects.[4]

The net effects of this somewhat unusual configuration is an RNAi trigger with enhanced on-target efficacy and reduced off-target passenger strand activity. On-target and off-target effects were monitored by dual luciferase assays in which the RNAi activity of each strand is determined. Importantly, from a therapeutic perspective, this bridging enabled the investigators to inhibit a fusion oncogene message that has been difficult to target with conventional siRNAs. In contemplating why this strand crosslinking enhances RNAi function, one can best surmise that it engages Argonaute 2 more efficiently perhaps by forming a better fit into the Argonaute active site. However, this is only speculation that needs to be analyzed experimentally, perhaps using X-ray crystallographic analyses. It is important to independently confirm these results before we accept this approach as a new standard for siRNA design. Aside from the enhanced utility of the bridged siRNA strands, understanding why they function more optimally as siRNAs will enhance our understanding of the molecular events in RNA interference.

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