Molecular Therapy
Nucleic Acids
Commentary

CRISPR-Cas13d: RNA's own Jedi Master in the fight against viral darkness

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Type VI CRISPR-Cas systems feature programmable, RNA-guided ribonucleases that act as single-effector enzymes, enabling robust RNA knockdown and editing.[1] CRISPR-Cas13d has emerged as a powerful tool against viral pathogens.[2] This RNA-targeting system has garnered significant attention, especially in the context of challenging RNA viruses, like severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[3] Research has highlighted Cas13d′s unique potential to cut through complex, structured RNA targets, potentially transforming how we develop antiviral therapies.[4]

Hussein and colleagues[5] tackle a crucial challenge in leveraging this platform for RNA-targeting therapies: assessing the impact of RNA secondary structure on Cas13d-mediated RNA cleavage. To better understand this interaction, the researchers selected two potent anti-SARS-CoV-2CRISPR RNA (crRNAs) and modified the sequence surrounding the target site to create a perfectly base-paired hairpin structure. Subsequently, they introduced specific mutations to gradually destabilize the hairpin, allowing an insight into Cas13d′s performance across a spectrum of RNA structural stabilities.

By systematically varying the stability of hairpin structures containing SARS-CoV-2 sequences, the researchers uncovered Cas13d′s impressive capacity to effectively bind and cleave highly stable RNA structures. Notably, only RNA hairpins with perfectly matched base pairs in their stems, where the RNA strand folds back and pairs with itself, were able to avoid being targeted by Cas13d. Such perfectly matched hairpins are uncommon in nature, as hairpins are typically not perfectly paired. This potency to target highly stable RNA structures sets Cas13d apart from other RNA-targeting systems, particularly RNA interference (RNAi), which demonstrated greater sensitivity in direct comparisons.

To fully appreciate the significance of these findings, it is important to consider the challenges posed when targeting viral RNA genomes. Many clinically relevant viruses, including SARS-CoV-2 and hepatitis C, have highly structured RNA genomes. These complex structures have long posed difficulties for researchers developing RNA-targeting therapies, often interfering with the binding and activity of therapeutic molecules. Cas13d′s demonstrated ability to navigate and cleave these structured targets opens new avenues for antiviral strategies, potentially overcoming limitations faced by previous approaches.

The comparison with RNAi is particularly interesting. While both CRISPR-Cas13d and RNAi have shown promise as antiviral tools, their differing potency to target RNA structure could have significant implications for their broader application in therapy. The study revealed that RNAi was unable to perturb structures with a thermodynamic stability of −19.0 kcal/mol, whereas Cas13d remained active until confronted with structures of −31.7 kcal/mol or greater stability. This clear contrast, as simplified in Figure 1, underscores the potential advantages of Cas13d in targeting the structured RNA landscapes of viral genomes.

However, as with any emerging technology, challenges remain on the path to clinical application. Both CRISPR-Cas13d and RNAi face hurdles related to off-target effects, a critical consideration for any nucleic-acid-targeting therapy. While Cas13d has demonstrated remarkable specificity in some contexts, accurately predicting and mitigating unintended targeting remains a significant challenge. Moreover, the prokaryotic origin of the Cas13d system presents unique considerations for its use in human cells, including the need to deliver both the potentially immunogenic Cas13d protein and its guide RNA.

Despite these challenges, the findings of Hussein and colleagues[5] paint an optimistic picture for the future of Cas13d in antiviral therapies. Its ability to cleave structured RNA targets with high efficiency could prove invaluable in targeting a wide range of viral pathogens, potentially offering a more robust approach than existing RNA-targeting technologies. Furthermore, this structural resilience might find applications beyond virology, opening doors in fields such as RNA biology and gene therapy.

The potential applications of Cas13d extend well beyond antiviral therapies. In the field of RNA biology, Cas13d could be a powerful tool for studying the function of structured RNA elements, such as riboswitches or IRES (internal ribosome entry site) elements. Its ability to cleave through stable structures could provide new insights into RNA-mediated gene regulation and genetic compensation mechanisms.[6]

In gene therapy, Cas13d′s structural resilience could be particularly valuable. Many genetic disorders involve mutations in genes with complex RNA secondary structures. Cas13d′s ability to navigate these structures more effectively than other RNA-targeting

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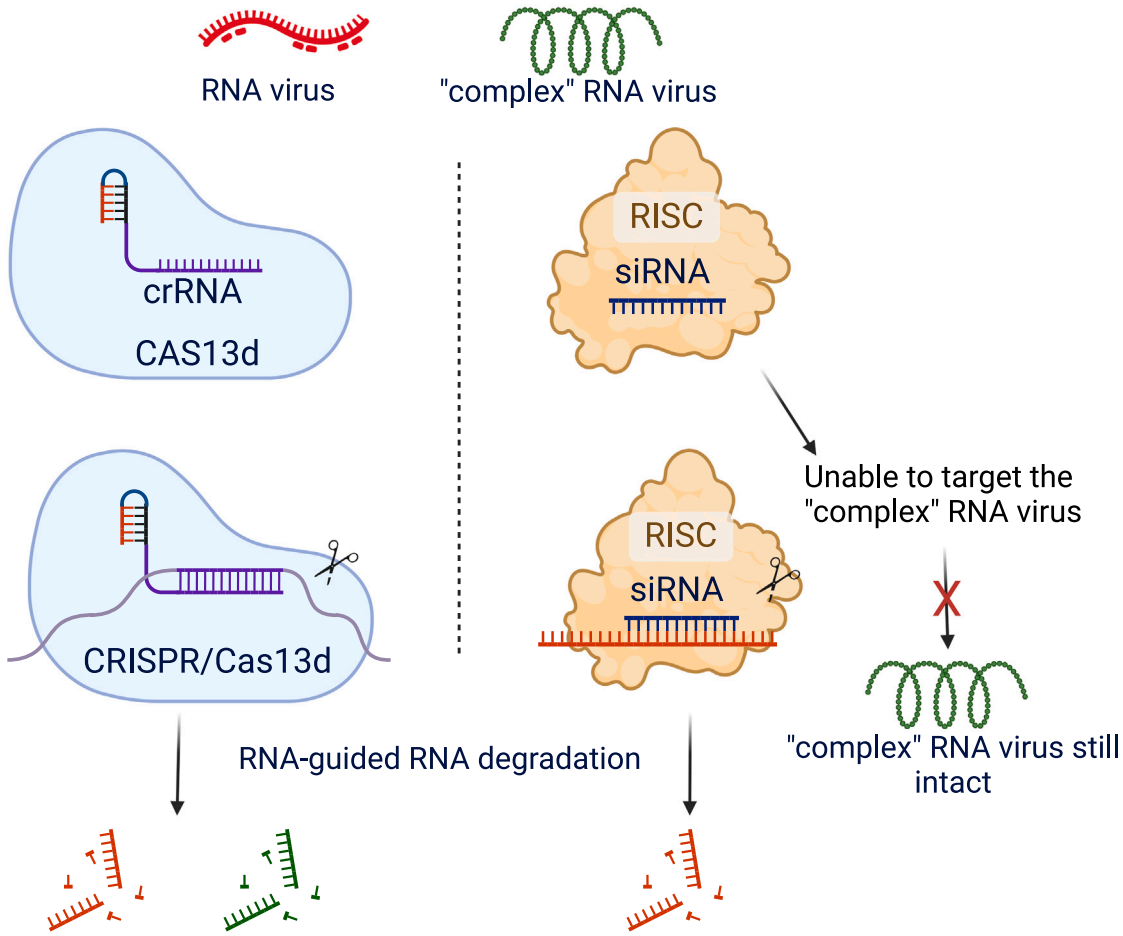

Figure 1. CRISPR-Cas13d: A promising strategy for overcoming siRNA limitations against complex RNA viruses

CRISPR-Cas13d's ability to target and degrade both simple and complex RNA structures could translate in action against RNA viruses with highly structured genomes (left). In contrast, siRNA-RNA-induced silencing complex (RISC) complexes are limited to degrading simpler RNA structures and thus might fail to degrade viruses with complex RNA structures due to their inability to effectively bind these targets (right).

technologies could lead to more efficient therapeutic strategies for conditions like myotonic dystrophy or fragile X syndrome, where targeting structured RNA is crucial.

Looking ahead, several key questions emerge from this work. First, further investigation into the specific mechanism behind Cas13d′s low sensitivity to stable RNA structures could yield insights for optimizing the system or developing new RNA-targeting technologies. Second, strategies for mitigating potential off-target effects and optimizing delivery methods will be crucial for translating these promising results into clinical applications.

In conclusion, the demonstrated ability of CRISPR-Cas13d to effectively cleave highly structured RNA targets represents a significant advance in the field of antiviral therapeutics. By overcoming the hurdle of target RNA structure more effectively than RNAi, Cas13d positions itself as a powerful tool in the fight against viruses with complex RNA genomes.

As research advances, we could be on the brink of a new era in antiviral therapy, equipped with tools to tackle even the most complex viral RNA structures. The journey from laboratory to clinic is often challenging, but this study provides a clear path forward in our fight against viral pathogens, bringing balance to the Force in our battle against these microscopic foes.

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AUTHOR CONTRIBUTIONS

Z.K., O.R., and A.R. drafted and wrote the commentary.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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