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Commentary

SPRINGing forward: Advancing RNA editing efficiency and precision with engineered ADAR2

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INTRODUCTION

The field of RNA editing has gained significant attention in recent years due to its potential as a therapeutic approach for correcting genetic mutations. Unlike CRISPR-Cas9 and other DNA-targeting genome editing technologies that introduce permanent modifications, RNA editing offers a reversible and tunable alternative, minimizing risks associated with genomic alterations, off-target effects, and immune responses.

One of the most studied RNA editing mechanisms involves adenosine deaminases acting on RNA (ADAR), which catalyze the conversion of adenosine (A) to inosine (I), functionally interpreted as guanosine (G) during translation.[1]

A major challenge, however, has been the relatively low enzymatic activity and specificity of RNA editing platforms, which limit their clinical applicability.

A recent study by Ai et al., published in *Molecular Therapy Nucleic Acids*,[2] presents an innovative approach to improving RNA editing efficiency and specificity using engineered ADAR systems. Their work introduces the Strand Displacement Responsive ADAR System for RNA Editing (SPRING), which employs a "blocking sequence" to form a hairpin guide RNA, enhancing both the efficiency and specificity of site-directed RNA editing. This study represents a significant advance in RNA-based therapeutics, addressing key limitations of existing ADAR-based platforms.

Ai et al. demonstrate that incorporating a blocking sequence into guide RNAs enhances RNA editing efficiency by preventing the formation of non-functional RNA-protein complexes. Their SPRING system improves editing efficiency by over 2.2-fold compared with conventional MS2-MCP-ADAR systems, achieving up to 67% efficiency at specific target sites. Notably, transcriptome-wide analysis reveals an ∼60% reduction in off-target effects compared with BoxB-λN-ADAR systems. In addition, the SPRING system is adaptable for C-to-U editing through integration with the ADAR-RESCUE system, broadening its therapeutic potential for transition mutations.[2]

BROADER IMPLICATIONS FOR THE FIELD

RNA editing is emerging as a powerful tool for correcting disease-causing mutations, modulating protein function, and offering an alternative to gene therapy.[3] Hence, this study has significant implications for basic research, as well as molecular and cellular therapies, particularly in precision medicine. The SPRING system's integration of hairpin guide RNAs represents a novel approach to enhancing ADAR-based platforms. Ai et al.'s findings have the potential to drive safer and more effective RNA-based therapies, with promising implications for treating neurodegenerative diseases, metabolic disorders, and certain cancers.

One promising application of SPRING is genetic compensation, particularly transcriptional adaptation, i.e., the regulation of compensatory gene activation in response to mutations by mRNA. Without altering the DNA sequence, the SPRING system could serve as a powerful research tool to investigate the mechanisms of transcriptional adaptation, providing insights into how cells respond to genetic perturbations and maintain functional stability.[4,5] Given its ability to fine-tune RNA without altering the genome, the SPRING system could be a valuable tool for studying adaptive mechanisms in response to genetic disorders.

In addition, RNA editing offers a promising strategy for immune modulation. ADAR-mediated RNA editing plays a crucial role in innate and adaptive immune regulation, and reduced editing of dsRNA has been linked to heightened interferon responses and chronic immune-inflammation, which are key drivers of autoimmune diseases such as multiple sclerosis and neuromyelitis optica.[6,7]

By selectively modifying mRNA transcripts involved in cytokine signaling, immune checkpoints, and regulatory pathways, SPRING could help restore immune balance and mitigate autoimmune pathology with greater precision than conventional therapies.

In addition, this approach could extend to dermatological applications, such as modulating inflammatory responses in skin disorders or enhancing wound healing by fine-tuning key regenerative pathways. For instance, RNA editing could be used to regulate cytokine expression, promoting a

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balanced inflammatory response, or to boost the activation of genes involved in collagen production and tissue repair, thereby accelerating wound healing and improving skin regeneration. Moreover, RNA editing could also be leveraged in anti-UV creams to enhance the skin's natural defense mechanisms, such as DNA repair and antioxidant responses, providing an additional layer of protection against UV-induced damage.[8]

The SPRING system also has potential applications in regenerative medicine, oncology, infectious disease research, and synthetic biology.

In stem cell therapy, transient RNA editing could modulate immune-related genes (e.g., HLA molecules) to improve graft compatibility, following thorough quality control of iPSCs,[9] to avoid introducing permanent genetic changes.[10] In cancer therapy, it could fine-tune tumor suppressor or oncogene expression for targeted treatments. In addition, SPRING could introduce protective modifications in host transcripts or disrupt viral RNA replication in infectious diseases. Its tunable capabilities in synthetic biology could enable dynamic gene regulation in engineered cells, expanding possibilities for both therapeutic and biotechnological innovations. While this study primarily enhances RNA editing efficiency, future research should explore these broader applications to maximize its clinical and industrial impact.

LIMITATIONS OF THE STUDY

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Despite the promising findings, some limitations remain. First, while the SPRING system enhances specificity, off-target effects are not entirely eliminated. Additional improvements, such as incorporating split ADAR deaminases or refining nuclear localization strategies, could further improve specificity. Second, the study primarily focuses on *in vitro* experiments using HEK293T cells. While these findings are encouraging, validation in primary cells and *in vivo* models will be essential to determine the system's effectiveness in a therapeutic setting.

Third, scalability and delivery remain critical challenges for RNA editing platforms. The development of efficient *in vivo* delivery methods for SPRING-based RNA editors, particularly for targeting tissues with low endogenous ADAR expression, is therefore an area that warrants further investigation.[3]

CONCLUSION

Ai et al. provide an elegant solution to a long-standing challenge in RNA editing by leveraging strand displacement-responsive guide RNAs to enhance both efficiency and specificity. The SPRING system offers a promising RNA-editing platform with potential applications in gene therapy, biotechnology, and basic research. While challenges remain, this study marks a significant step forward in the development of precise and efficient RNA therapeutics, potentially transforming the treatment landscape for genetic disorders.

Future work should focus on refining delivery systems, validating findings in disease-relevant models, and expanding applications to a broader range of RNA modifications. The ability to adjust RNA function without making permanent changes to the genome highlights the game-changing potential of this approach, making RNA editing a key player in the future of molecular medicine.

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AUTHOR CONTRIBUTIONS

A.R. drafted and wrote the commentary with input from all authors.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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