Small Nuclear RNAs (snRNAs) Enhance RNA Base Editing

Gene editing technologies are transforming molecular medicine, offering unprecedented potential to treat — and even cure — a range of genetic disorders. However, as tools like CRISPR enter clinical settings, concerns about its safety, specificity, and long-term side effects, such as off-target edits or increased cancer risk, have prompted scientists to improve the technology or find alternatives. With these drawbacks in mind, a team of scientists led by Aaron Smargon, Ph.D., explored a gene editing method that may provide improved safety.

While scientists are pursuing workarounds to make CRISPR safer, Smargon's work in Dr. Gene Yeo's lab at UC San Diego sidesteps these concerns altogether by repurposing RNA-editing proteins in cells using engineered small nuclear RNAs (snRNAs) from human noncoding RNAs. These naturally occurring snRNAs, which do not produce proteins and are found inside the nuclei, primarily function to edit and process other RNA molecules. With the study results published recently in *Nature Chemical Biology*, Smargon and his team aimed to determine whether snRNAs could improve base editing efficiency in mammalian cells.

To start, the team focused on two uridine-rich snRNAs to guide editing to specific positions in target RNAs: U1 and U7smOPT snRNAs, which have shown promise in preclinical studies. With backbone sizes of 153 and 45 nucleotides, both snRNAs are comparatively small and can easily be encoded in different genetic delivery vehicles like lipid nanoparticle or an adeno-associated virus (1).

The researchers compared the snRNA system to other "state-of-the-art" methods, like circular ADAR-recruiting RNAs (cadRNAs), hypothesizing that because of their shared nuclear location with ADAR, U snRNAs may be more efficient adenosine-to-inosine (A to I) editors than cadRNAs

(1). After comparing the snRNA technique to the best current editing tools, the team found that their method showed clear advantages.

snRNA provides improved editing on complex genes

After testing the two U snRNAs as well as cadRNA at several different spots in human genes to determine their adenosine-to-inosine (A to I) editing capacity, they found that U1 snRNA performed more poorly than U7smOPT snRNA. The researchers believe this is due to its greater molecular complexity and tendency to recruit splicing machinery. They also found that U7smOPT snRNA outperformed cadRNA in four gene locations, particularly those with more exons. To further test this, they compared U7smOPT snRNA and cadRNA performance on eight new gene locations with progressively higher exon counts. On all target genes—except one—U7smOPT snRNA outperformed cadRNA, showing that the snRNA worked better on complex RNAs. Based on these findings, the team decided to focus on U7smOPT snRNA for the remainder of the study (1).

“Given that genes with high exon count tend to be larger and more prone to accumulating disease-relevant mutations, ... U7smOPT snRNAs present an attractive new modality for treating PTC diseases,” the authors state (1).

snRNA reduces off-target effects and edits noncoding and pre-mRNA

The researchers then examined how U7smOPT snRNAs compared to cadRNAs regarding off-target genetic perturbations. They found that A to I snRNAs caused four to eight-fold fewer unwanted changes or side effects in other genes than cadRNAs, with fewer splicing variations (~1.5-2 fold less) and no evidence of innate immune activation or recombination artifacts seen in cadRNAs (1).

“We attribute this unexpected finding not to directly guided splicing perturbations but rather to pleiotropic effects stemming from cadRNA-mediated downregulation of splicing factors,” the authors state (1).

However, when examining the number of off-target A to I editing events, the researchers found that U7smOPT snRNA consistently produced more off-target edits than cadRNA. While this might seem concerning, these extra edits did not appear to contribute to transcriptome-wide genetic perturbations.

The team proposed that the seemingly paradoxical results — higher cadRNA-mediated gene perturbations but more off-target edits from U7smOPT snRNA — could be explained by where each editing system resides inside the cell. U7smOPT snRNAs remain largely within the nucleus, where ADAR enzymes are most active, allowing them to efficiently edit RNA without causing widespread disruptions. In contrast, cadRNAs persist longer and are exported to the cytoplasm, where they can inadvertently bind and reduce other RNAs, leading to broader gene activity changes (1). This idea, called the localization hypothesis, also explains why U7smOPT snRNA

outperforms cadRNA in editing genes with many exons, which spend more time in the nucleus before export. Supporting this, the researchers found that A to I snRNAs localize about 70% to the nucleus, compared to 25% for cadRNAs, and are on average ~2 µm closer to nuclear ADAR enzymes, enabling more efficient and precise editing (1).

Given this discovery, the team wondered if A to I snRNAs could be used to edit long noncoding RNAs (IncRNAs) and pre-mRNAs. The results showed that A to I snRNAs could indeed achieve higher editing on IncRNAs, achieving 50% editing versus 20% for cadRNA. Additionally, the A to I snRNA also edited pre-mRNA sites more efficiently, with editing rates ranging from 10 to 30%, and translating to improved exon skipping over both cadRNA and antisense snRNA (1).

snRNA shows improved U to Ψ editing without overexpression

After the team's success in localizing A to I snRNAs to the nucleus for enhanced A to I editing, they applied a similar approach to see if snRNAs could improve uridine (U) to pseudouridine (Ψ) RNA base editing. The researchers hypothesized that attaching programmable H/ACA box small nucleolar RNAs (snoRNAs) —which catalyze uridine (U) to pseudouridine (Ψ) editing of rRNAs and snRNAs — to a U7smOPT snRNA backbone would shift their location from the nucleolus to the nucleoplasm. This relocation, they hypothesized, would allow the snoRNAs to reach and modify protein-coding RNAs more effectively, improving U-to-Ψ editing efficiency. The results showed that U to Ψ snRNAs increased pseudouridylation by up to 20% to 40% on three endogenous loci in HEK293T cells. Additionally, this boost was achieved without DKC1 overexpression (1).

Additionally, the authors suggest that U to Ψ snRNAs could be administered to patients via nonviral delivery methods, such as lipid nanoparticles. Furthermore, the authors state that the snRNA backbone may facilitate nuclear delivery, as it spends part of its life cycle at the cytoplasmic SMN complex before returning to the nucleus (1).

snRNA shows encouraging results in a cystic fibrosis model

Finally, they tested the U to Ψ snRNA in a cystic fibrosis model, where the stop signal in the cells results in little to no CFTR protein. The researchers tested the snRNA tool on human bronchial epithelial cells carrying a common cystic fibrosis mutation. The results demonstrated improved efficacy, specificity, and nuclear localization. Specifically, they found that the cells treated with U into Ψ snRNA showed about a two-fold increase in CFTR RNA expression (1).

"Encouragingly, this enhanced activity of U to Ψ snRNAs will enable lower dosage and, thus, safer therapeutics for the same PTC suppression efficacy," the authors state (1).

Collaboration, key takeaways, and unanswered questions

Aaron Smargon, the lead in this study, has been working in the gene editing field since his Ph.D. research, during which he led the discovery and characterization of CRISPR-Cas13b in Dr. Feng

Zhang's lab at the Broad Institute. Smargon explains, "At that time, there was enormous enthusiasm for the translation of CRISPR systems to patients. Since then, we have seen that CRISPR has drawbacks in terms of immunogenicity and genotoxicity. Scientists are pursuing workarounds to make CRISPR safer, and I hope that they are successful."

As the first author, he led the team, performed or contributed intellectually to all experiments and analyses, and wrote the manuscript with guidance from Yeo. "The Yeo lab is an incredibly supportive and collaborative environment with researchers of many different types of expertise," he says. "It was natural to find collaborators within the lab and figure out how they could contribute to the paper, while also ensuring that their contributions furthered their own personal research goals."

Additionally, Dr. Yeo helped facilitate a collaboration with Dr. Wendy Gilbert's lab at Yale University to address important reviewer comments about pseudouridylation quantification.

According to Smargon, the most important takeaway from the research is that snRNA can be combined with other RNA scaffolds to improve the efficacy and safety of targeted RNA base editing by endogenous enzymes.

"We showed this both for adenosine-to-inosine and uridine-to-pseudouridine editing, demonstrating the versatility of engineered small nuclear RNAs beyond splicing perturbation."

However, the question of how snRNAs improve the efficiency of targeted pseudouridylation by snRNAs remains unanswered, Smargon says. For some targets, it can be explained by higher construct stability, but for others, he says the mechanism is less clear.

"There may be altered localization within the nucleoplasm," he says. "Figuring out the reason could help us boost the efficiency even further, which would be helpful in developing therapeutics to treat premature termination codon diseases."

The future of snRNA as a gene editing tool

The development of small nuclear RNA (snRNA)-guided base editing marks a significant advance in the quest for safer, more precise genetic therapies. By leveraging endogenous RNA molecules, the approach effectively addresses some of the key limitations of previous gene-editing technologies, including off-target effects and potential long-term safety concerns. The snRNA system excels at editing complex genes, noncoding RNAs, and pre-mRNA splice sites while reducing unintended edits—attributes that potentially position it as a versatile tool in molecular medicine and for base editing therapies for diseases like Duchenne muscular dystrophy and Hurler syndrome.

Smargon and his team are optimistic that the demonstrated cellular safety profile and utility of engineered snRNAs will increase their future adoption in RNA research and development. "My hope is that other researchers in both academia and industry will continue to work to improve

the efficiency of engineered snRNAs, find new applications for their use, and ultimately turn this knowledge into therapeutics that save lives."

References:

1. Smargon AA, Pant D, Gomberg TA, Fagre C, Glynne S, Nguyen J, Naritomi JT, Gilbert WV, Yeo GW. Enhancing RNA base editing on mammalian transcripts with small nuclear RNAs. Nat Chem Biol. 2025 Sep 18. doi: 10.1038/s41589-025-02026-8. Epub ahead of print. PMID: 40968292.