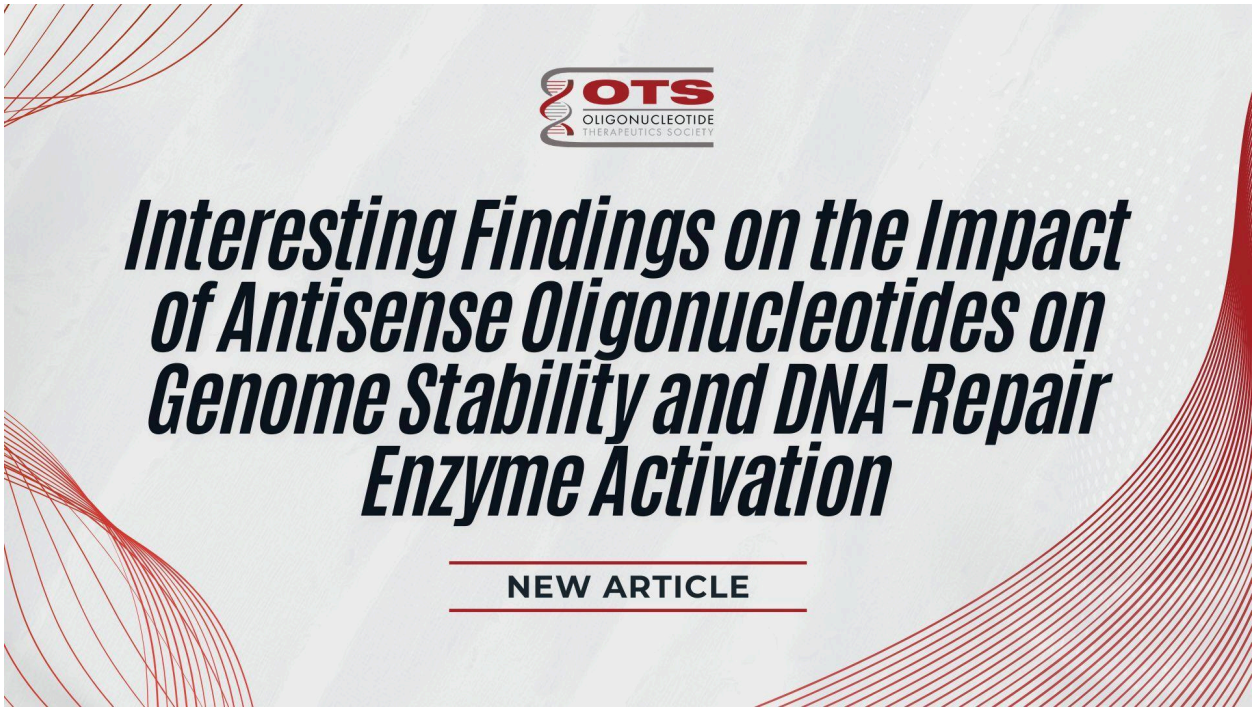
Interesting Findings on the Impact of Antisense Oligonucleotides on Genome Stability and DNA-Repair Enzyme Activation



Antisense oligonucleotides (ASOs) have emerged as promising drug candidates for the treatment of genetic diseases. Because of their ability to target virtually any disease-related gene product, several ASO drugs have been approved, and many more are in development (1). While they remain among the most precise therapies, investigations are ongoing to find improvements and increase safety. A recent study from Karolinska Institutet published in *Nature Communications* found that ASOs can activate a cell's DNA damage response even when no damage is present.

Background: chemistry and cellular behavior of ASOs

Most ASO therapeutics are chemically modified to include phosphorothioate (PS) backbone modifications, which improve their stability, cellular uptake, potency, and tissue delivery (2). Importantly, the PS modification also increases protein binding (2). Previous studies have shown that specific protein binding influences the localization of PS ASOs within the cell (3). These proteins can direct ASOs to specific cellular compartments, including the nucleus, where DNA repair proteins reside, as well as stress granules and paraspeckles, where they can accumulate and interfere with normal cellular processes (1, 3).

A 1998 study provided evidence that PS-modified ASOs can form abnormal nuclear structures by trapping proteins, regardless of their intended target (4). This helps explain that the PS bodies identified in the new study are a specific type that go a step further by mimicking DNA damage and activating repair pathways.

The discovery: ASOs and DNA repair disruption

Against this background, Marianne Farnebo and her team set out to better understand how ASOs might be influencing DNA repair. Farnebo, the research group leader at the Department of Oncology-Pathology at Karolinska Institutet and the senior author of the study, investigates the role of endogenous RNA in DNA repair. They previously found that RNA can bind to DNA repair enzymes and modulate their catalytic activity (5). Farnebo and her team often use ASOs to knock down RNAs of interest to study their functions. During these experiments, they noticed that their control ASOs unexpectedly affected DNA repair, making it difficult to distinguish specific effects from nonspecific ones. To continue using ASOs reliably, they needed to understand and resolve this issue.

Farnebo explained that synthetic ASOs, which closely resemble natural RNA as negatively charged, single-stranded nucleic acid polymers, can also bind to DNA repair enzymes and dysregulate their activity, suggesting that ASOs can mimic endogenous RNA and thereby modulate DNA repair pathways. The researchers found that when ASO molecules bind to DNA repair proteins, they form dense clusters called PS bodies inside the cell's nucleus. These clusters attract and trap DNA repair proteins, activating a false DNA damage response. This disrupts the natural repair process and, over time, causes real DNA damage.

"Our results show that ASOs can trigger a repair response that should not normally be activated, and this affects the cell's normal handling of DNA damage," said Farnebo in the news release.

Mechanism: phase separation and protein activation

To understand how these clusters form and why they activate repair enzymes, the researchers examined their physical properties. They found that these structures behave like liquid droplets, in a process known as phase separation where molecules can condense into dense, dynamic compartments. This environment can concentrate proteins and alter their activity, effectively switching on DNA repair enzymes (such as DNA-PKcs, ATM, and PARP1) simply by bringing them together, even in the absence of damaged DNA (1).

Previous studies have also demonstrated that ASO-protein aggregates can undergo liquid-to-solid phase transitions. In one such study, researchers found that while some aggregates form with both nontoxic and toxic ASOs, toxic ASOs can also form unique nucleolar aggregates associated with dysfunction and cell death (6).

The role of the PS backbone

Farnebo and her team's findings point to the importance of the chemical properties of ASOs themselves. The study found that both DNA- and RNA-based ASOs with a phosphorothioate (PS) backbone can induce PS body formation in cells, suggesting that this chemical feature alone is sufficient to drive clustering. This raises the possibility that similar interactions, if present naturally, could aid in the formation of droplet-like clusters of DNA repair proteins (1).

Nuclear organization and protective mechanisms

Interestingly, however, ASOs do not behave uniformly across all regions of the nucleus. “We find that ASOs are not enriched at DNA break sites, but instead excluded, even though the concentration of repair factors is high at these sites,” the authors stated (1). This exclusion may serve as a protective mechanism to prevent disruption of critical repair processes (1).

Together, these findings suggest that ASOs can influence nuclear organization and cellular function in multiple ways. Similar effects have been reported in other studies, where fully PS-modified oligonucleotides were shown to induce structured nuclear inclusions, redistribute nuclear proteins, and alter gene expression patterns (7). The work by Farnebo and colleagues suggests that when such reorganization involves DNA repair proteins, it may lead to inappropriate activation of DNA damage signaling pathways.

Clinical context and emerging risks

These findings raise important questions about how ASOs behave under clinically relevant conditions. Farnebo noted that it is important to distinguish between the ASO treatment used in the study and clinically used methods, in which significantly lower concentrations of ASOs reach the cell nucleus. Importantly, she said their in vivo data indicate that ASOs can activate DNA damage signaling even at lower, clinically relevant concentrations (1).

Doctoral student and first author Linn Hjelmgren explained, “Our results show that the impact on DNA repair can occur in several distinct ways, not just through the clusters formed in the cell nucleus.” The authors pointed to several possible pathways, including direct interactions with repair enzymes and broader transcriptional changes induced by ASO treatment (1).

“With ongoing clinical use and the rapid development of ASO-based drugs - especially those designed to enhance cellular uptake and bypass endosomal trapping - the potential risk of interfering with DNA repair is likely to grow,” she explained. “This underscores the need to communicate these findings broadly within both the RNA therapeutics field and the pharmaceutical industry.”

Broader implications for biology and therapeutics

According to Farnebo, the realization that synthetic oligonucleotides can mimic endogenous RNA and affect RNA-dependent processes has broad implications for both basic research and therapeutic applications.

“Our demonstration that ASOs can strongly activate DNA damage signaling and suppress DNA repair reveals a previously unrecognized mechanism of ASO-induced toxicity,” she explained.

“These findings advance the fundamental understanding of RNA-mediated DNA repair and are highly relevant for the safety assessment and future development of RNA-based therapeutics,” said Farnebo.

Future directions

Farnebo and her team hope the field will further investigate how synthetic oligonucleotides interact with DNA repair pathways, and that future design and safety testing of next-generation RNA therapeutics take this into consideration.

“A key open question is how ASOs influence DNA repair in vivo, particularly across different tissues and delivery methods,” said Farnebo. “This is essential for a thorough safety evaluation.”

The researchers are continuing their work by examining ASO-induced dysregulation of DNA repair in vivo using mouse models, including multi-organ analysis following treatment with fluorescent ASOs. Their future work will focus on identifying strategies to prevent or mitigate ASO-driven effects on DNA repair.

Farnebo said collaborations enabled the team to evaluate ASO effects in an ASO-treated mouse model, in which they observed activation of the DNA damage response in cells with high ASO uptake. Although she said these findings should be validated in larger cohorts, they provide in vivo proof-of-principle that ASOs can influence DNA repair under clinically relevant delivery conditions. “These findings suggest that ASOs can mimic natural RNA and alter RNA-dependent DNA repair functions, potentially contributing to unintended therapeutic side effects.”

Despite these findings, ASOs remain a powerful and clinically validated therapeutic platform. Their ability to precisely target disease-causing genes has transformed treatment options for several rare conditions. However, as newer ASO therapies are designed to improve cellular uptake and increase nuclear delivery, the potential for interference with DNA repair may also rise. Given the growing clinical use of ASOs, these findings highlight the importance of carefully evaluating their effects on genome stability (1).

References:

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