A new class of antisense oligonucleotides targeting regions downstream of genes directs mRNA for degradation and decreases gene expression

Pawel Grzechnik1 , Kinga Winczura1 , Lars Joenson2 , Erich Koller3 , Łukasz Kiełpiński2
1University of Manchester, School of Biological Sciences, Manchester, United Kingdom, 2Roche Innovation Center Basel, Pharma Research and Early Development, Therapeutic Modalities, Basel, Switzerland, 3Roche Innovation Center Basel, Pharma Research and Early Development, Pharmaceutical Sciences, Basel, Switzerland

Background: Antisense oligonucleotides (ASOs) are short, chemically modified nucleic acid molecules designed to hybridize to target RNAs and thus modulate gene expression. Widely used ASO gapmers contain a central DNA region, which forms a heteroduplex with the target RNA, triggering RNase H1-dependent RNA degradation. Method: Using human cells, transcriptomics and molecular biology approaches, we investigated whether gapmer ASOs can act on nascent RNA synthesised in transcription termination windows located downstream of annotated genes. Results: We describe a new class of gapmer ASOs that expand the range of target sequences and reveal a critical blind spot in current ASO off-target assessment. We identify non-coding RNAs transcribed from transcription termination windows as previously unrecognized ASO targets. We show that downstream-of-gene ASOs (DG-ASOs) induce RNase H1-dependent cleavage, which impairs mRNA 3' end processing and ultimately directs the unprocessed mRNAs for degradation, leading to a pronounced decrease in expression of the corresponding upstream gene. Conclusion: This novel mode of antisense regulation uncovers downstream-of-gene termination windows as genuine ASO targets that can be exploited to suppress gene expression. Importantly, it also reveals an underappreciated source of off-target effects that may arise from ASOs binding in these regions. Our findings indicate the need to expand ASO design and offtarget assessment guidelines to include termination-window sequences to improve therapeutic efficacy and safety.