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Unraveling the epitranscriptome of small non-coding RNAs in vascular cells

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Small nucleolar RNAs (snoRNAs) are small non-coding RNAs that mediate RNA modifications at a post-transcriptional level. The nearly 400 snoRNAs encoded within mammalian genomes function in 2′-O-methylation, pseudouridylation, or processing of other cellular RNAs.[1] In this issue of *Molecular Therapy – Nucleic Acids*, van Ingen and colleagues have provided fundamental mechanistic knowledge on AF357425/SNORD113-6, showing for the first time that it can regulate tRNA expression in vascular fibroblasts by binding to their leucine anti-codon TAA (tRNALeu(TAA)), causing 2′O-ribose-methylation, and preventing the tRNA cleavage into tRNA fragment (tRF)$^{Leu47-64}$.[2] This process is essential because tRNA fragments have emerged as a new class of non-coding RNAs involved in regulating cell function, which is particularly relevant to cardiac pathophysiology.[3] Furthermore, these responses appear regulated by oxidative stress, which can be reconciled with previous studies reporting snoRNAs from the Rpl13a locus as unexpected regulators of reactive oxygen species (ROS) and oxidative stress.[4]

After the ascent of microRNAs (miRNAs) as key therapeutic targets and biomarker candidates in a variety of human diseases, other small non-coding RNAs (sncRNAs) are timidly gaining popularity across the biomedical community. Across several sncRNA species, small nucleolar RNAs (snoRNAs; which are 60–200 nt in length) are primarily invested in guiding nucleotide modifications of ribosomal RNA (rRNA), thus contributing to rRNA maturation. Moreover, snoRNAs target other non-coding RNAs and messenger RNAs (mRNAs) to affect distinct chemical modifications (2′-*O*-methylation or pseudouridylation), impacting RNA processing and function. snoRNAs are expressed in all somatic cells and are grouped into 2 classes: box C/D snoRNAs (SNORDs) and box H/ACA snoRNAs (SNORAs). snoRNAs have already been reported to be altered expressionally and to be functionally relevant in a handful of cardiovascular studies, and advancing their mechanistic understanding is essential to understand their translational potential.[5]

In humans, SNORD113-6 originates from the imprinted DLK1-DIO3 locus on chromosome 14 (14q32), which is particularly interesting for ncRNA biology given that it encodes a cluster of 41 CSNORDs, together with the long ncRNA (lncRNA) MEG3, one of the longest polycistronic miRNA clusters, and the endonuclease DICER, which processes silencing RNAs, miRNAs, snoRNAs, and tRNAs.[6] The Nossent group has already contributed significantly to understanding the regulation and function of the 14q32 miRNAs in cardiovascular disease.[7] They also reported a role for AF357425/SNORD113, focusing on its mRNA targeting capacity resulting in regulation of the integrin signaling pathway.[8] This new report expands the knowledge considerably, showing that AF357425/SNORD113-6 targets tRNAs, protecting the tRNA from cleavage into small fragments. Specifically, they demonstrated that AF357425/SNORD113-6 induces 2-O-methylation (2′Ome) of the mature tRNALeu(TAA), thereby protecting against site-specific tRNA fragmentation.

The authors have applied different techniques to confirm the expression of tRFLeu 47–64 and mature tRNALeu(TAA) and acknowledged the limitations of qRT-PCR to detect tRFs and tRNAs due to the presence of modifications on tRNAs. However, this approach is laborious and addresses only a few sites simultaneously. Therefore, to experimentally determine the global snoRNA target spectrum in vascular cells, integration of RiboMeth sequencing with crosslinking, ligation, and hybrids (CLASH)[7] could be employed in future studies.

The potential biological impact of tRNA fragmentation of tRF$^{Leu\ 47–64}$ on vascular homeostasis opens up the field of the biology of ncRNA and RNA fragmentation in cardiovascular pathophysiology with exciting perspectives. For example, an intriguing question would be to investigate whether these tRNA fragments are immunogenic and how primitive pattern recognition receptors sense them. Several groups reported on the recognition of tRNA by the endosomal TLR7, demonstrating that an immune activation via TLR7 can be triggered by any unmodified or modified tRNA.[9] Such a process would contribute to the general immunogenicity of sterile stress responses, including endoplasmic reticulum (ER) stress, which is critical to trigger an inflammatory response. Consistent with the possible immunogenicity of these cell-intrinsic responses, the accumulation of tRNA fragments could contribute to the chronic inflammatory state associated with the vascular remodeling in *in vivo* models of acute ischemic stroke and peripheral artery disease where the formation of tRNA fragments was enormously increased.[10]

Regarding the potential therapeutical aspects of this mechanism, the authors exploited an interesting approach based on third generation of antisense oligonucleotides (3GAs) to overexpress AF357425 (3GA-AF25). Mechanistically, the 3GAs directed against the 3′ ends of AF357425 (3GA-AF25) induced snoRNA overexpression, likely through protection from degradation by endonucleases. Whether tRF$^{Leu\ 47–64}$ fragmentation might

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be a potential future therapeutic target for the treatment and prevention of cardiovascular disease remains to be clarified; however, it is possible to speculate that 3GAs modulating SNORD113-6 could be exploited to control excessive inflammatory response due to tRNA fragmentation in response to ischemic stimuli.

Future studies should emphasize identifying molecular pathways, transcription programs, and genomic setups that lead to clarification of snoRNA function in cardiovascular diseases.

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