Molecular Therapy
Commentary

Isolating Functional (Iso)miRNA Targets During Ischemia

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Long regarded as junk, non-coding RNAs—and more specifically microRNAs (miRNAs)—are now recognized to have important regulatory functions in numerous biological processes and disorders. Various miRNAs have been shown to be differentially regulated in cardiovascular diseases and during adaptation to, e.g., tissue ischemia/hypoxia. In this issue of *Molecular Therapy*, van der Kwast et al.[1] further unravel the role of the 14q32 miRNA cluster during vascular growth by measuring the expression of miR-411 (WT-miR-411) and its isomiR (ISO-miR-411) in tissue samples from patients with peripheral artery disease (PAD). The authors also provide *in vitro* and *in vivo* evidence for functional regulation of ISO-miR-411 and its selective production during chronic ischemia.

miRNAs usually comprise ±22 nt and can target many mRNAs simultaneously.[2] At the same time, a given mRNA can be targeted by many miRNAs. Hence, the discovery of the regulatory functions of miRNAs has added significantly to our understanding of biological networks and pathways. Although miRNAs are usually annotated as a single sequence, deep sequencing experiments have actually shown a wide range of length and/or sequence variations for individual miRNAs.[3] Initially, these variants were often dismissed as technical sequencing artifacts. Today, isomiRs are recognized as true miRNA variants. Yet, the resulting functional implications so far remain largely unexplored.

isomiRs come in different forms. Polymorphic isomiRs diverge from the canonical or wild-type (WT) miRNA in their nucleotide sequence, but have the same length and are supposedly rather rare. Most known isomiRs differ in length by post-transcriptional processing through shortening via exoribonucleases or lengthening due to imprecise cleavage or post-transcriptional nucleotide addition, either at the 5′ (5′ isomiR) or the 3′ (3′ isomiR) end.[4] Depending on whether these changes result in sequence alterations compared to their respective pre-miRNA, they are further classified as templated (i.e., matching nucleotide sequence) or non-templated (i.e., non-matching nucleotide sequence). 5′ isomiRs are thought to be less prevalent than 3′ isomiRs and evidence for context-specific differential protein expression through selective isomiRs has been limited so far. However, the lack or addition of nucleotides on the 5′ end can result in a shift of the seed sequence and can thus change the identity of the targeted mRNAs (targetome).

Adaptive neovascularization (i.e., angiogenesis, arteriogenesis, and vasculogenesis) has gained much attention as a would-be alternative to current medical and interventional therapies in cardiovascular disease patients. However, current approaches aiming to promote vessel growth still suffer from potentially devastating side-effects, in particular by increasing atherosclerosis and destabilizing existing atherosclerotic plaques.[5] The discovery of miRNAs and the development of miRNA antagonists (e.g., antagomirs) has resulted in new targets of possible clinical utility. Several miRNAs have since been tested, including miR-155 and miR-143-3p.[6,7] The latter is upregulated in young healthy volunteers by high intensity exercise training during artificial blood flow restriction. Furthermore, miR-143-3p positively correlates with lactate concentrations leading the authors to pinpoint lactate as the driving force behind miR-143-3p expression. This is in line with miR-143 role in arteriogenesis in a rat ischemic hind limb model (K. Troidl et al., 2013, Eur. Surg. Res., abstract) as high intensity exercise training and the resulting increased blood flow is a known stimulus for collateral artery growth (arteriogenesis) in healthy and diseased subjects.[7] In the quest for stimulation of beneficial vascular growth without worsening of the underlying atherosclerotic disease, miRNAs with selective pro-arteriogenic effects may prove to be an escape out of the current dilemma.

The 14q32 miRNA cluster is a promising miRNA target for adaptive neovascularization. In humans, it comprises 54 miRNA genes and inspired the work of van der Kwast et al.[1] in the current study.[8] This group has previously shown that inhibition of 4 of the cluster's miRNAs (i.e., miR-329, miR-487b, miR-494, and miR-495) increases blood flow recovery after ischemia.[9] At the same time, silencing of miR-495 using oligonucleotides (GSOs) reduces atherogenesis and improves plaque stability.[10] While it may seem paradoxical not to move further along this road, the current work reminds us of our—still—limited understanding of miRNA biology as it adds another layer of complexity by showing a functional role for isomiRs in the intricate regulation of protein expression. isomiR expression can be tissue- and/or cell-specific. Next to their possible functional impact, the mere fact that certain isomiRs are expressed, irrespective of their expression levels, can already convey valuable medical information. Using machine learning, an algorithm based on binary isomiR expression profiles (i.e., "on/present" versus "off/absent") has recently been published and is

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able to discriminate 32 TCGA (The Cancer Genome Atlas) cancer types.[11]

In their current study, van der Kwast et al.[1] measured ISO-miR-411, a 5′ isomiR, expression under acute and chronic ischemic conditions in different tissues. While acute ischemia resulted in a lower isomiR expression compared to the canonical miRNA, both *in vivo* and *in vitro*, chronic ischemia led to a higher ISO-miR-411/WT-miR-411 ratio in human blood vessels. This context-specific isomiR expression, differentially regulated at the level of post-transcriptional pri- and pre-miRNA processing, may not only serve as a marker of chronic ischemia/hypoxia but also has significant functional consequences. The resulting shift in the seed sequence (nt 2–8) caused by the additional adenosine on the 5′ end in this particular isomiR significantly affects its targetome. ISO-miR-411 targets approximately twice as many genes as the canonical WT-miR-411 with the majority being targeted either by the isomiR or the WT-miR and with only ∼48% being targeted by both forms. Among the non-overlapping target genes, the authors selected genes known to be involved in adaptive responses to hypoxia/ischemia: TGFB2 (transforming growth factor-beta 2), predicted to be targeted by WT-miR-411, and ANGPT1 (angiopoietin-1), predicted to be targeted by ISO-miR-411. Overexpression experiments confirmed differential protein expression regulation and thus the unique targetome of ISO-miR-411.

The current findings may shed new light on previous work on miRNA expression profiles, predicted targets and miRNA silencing effects due to—unknowing—disregard of isomiR presence and/or expression. Hence, although the article by van der Kwast et al.[1] provides novel insights into the role of WT-miR-411 and ISO-miR-411 and its targetome in adaptive neovascularization, its main and most important merit lies in showing another important factor in protein expression regulation. What earlier was considered to be a straightforward process from gene to mRNA to protein, recognized to be more complex due to our knowledge on the influence of miRNA, has gained yet another level of complexity by the functional role of isomiRs and their regulation by post-transcriptional processing.

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