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Resizing the Genomic Regulation of Restenosis

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With the introduction of drug eluting stents (DES) for percutaneous coronary interventions, restenosis appeared to be a problem of the past. However, the biology of arterial injury has returned to the limelight of clinical cardiology because of increased late-stent thrombosis associated with DES.[1] As DES use initially increased, concerns were raised about altering the normal responses to arterial injury with antiproliferative drugs based on animal and human data.[2] Since 2006, this concern has translated to dramatic decreases in DES deployment in catherization laboratories across the U.S. These events serve to underscore the importance of continuing to incorporate new clinical and basic experimental data for improving patient management.

In a different realm, developmental biologists working on the primitive earthworm *Caenorhabditis elegans* discovered a novel regulatory mechanism involving short pieces of RNA (microRNA or miRNA) for which they were awarded the 2006 Nobel Prize in Physiology/Medicine. Over 500 different miRNAs have now been identified in the mouse and human genomes (miRBase: http://microrna.sanger.ac.uk/); a schematic overview of miRNA biogenesis is provided in the Figure (A). miRNAs generally act on their target messenger RNAs (mRNA) by promoting RNA degradation or inhibiting protein translation. They are expressed in a tissue- and condition-specific manner indicating their potential role in normal development and disease pathogenesis.[3] They appear to regulate diverse processes such as cell proliferation, cell death, metabolism, hematopoiesis, angiogenesis, and tumorigenesis.[3–6] Emerging genetic evidence also suggests an essential role of miRNAs in normal cardiogenesis and abnormal stress responses such as hypertrophy and arrhythmogenesis.[7–9] There are also reports of miRNA regulation of skeletal muscle, but there are no studies on miRNA expression in proliferating vascular smooth muscle cells thought to be responsible for arterial restenosis.[10] In this issue of *Circulation Research*, Ji et al now report a novel association between miRNAs and neointimal proliferation in the well-studied carotid artery balloon catheter-induced injury model.[11]

Ji and colleagues describe a dynamic profile of miRNA changes several days after injury of the rat internal carotid artery.[11] They report 113 differentially expressed miRNAs of the 140 detectable in arterial tissue. Indicative of a chronic response, 102 miRNA species continue to be expressed at significantly different levels 28 days after injury. After confirming the differential expression of the most significantly altered miRNAs by real-time PCR and northern blotting, they focus on miR-21 as the most robustly induced miRNA to determine its biological significance. The selection of miR-21 is also notable because it has recently been found to promote tumor growth implicating its role in cell proliferation, a hallmark of restenotic neointimal lesions.[12]

Returning to the original carotid injury model, the authors tested the biological effect of knocking down miR-21 using modified antisense oligonucleotides locally delivered in a special pluronic gel. They confirm localization of the oligonucleotide in the vessel wall and knockdown of miR-21 within 3 to 7 days. Though the knockdown of miR-21 was modest, there was a dramatic 50% reduction in neointimal thickness. In addition, miR-21 inhibition resulted in decreased proliferation and increased death of vascular smooth muscle cells. They next replicate their in vivo observation in cultured rat vascular smooth muscle cells where in vitro dedifferentiation and proliferation of freshly isolated smooth muscle cells upregulates miR-21 expression. Conversely, the genetic depletion of miR-21 increases cell death and decreases proliferation.

To determine the molecular targets of miR-21, the authors use bioinformatics to identify 2 putative miR-21 targets, the known tumor suppressor PTEN and the well-studied oncogene Bcl-2. It should be noted that it is difficult to unambiguously identify miRNA targets because of their short length and potential for hybridizing with imperfect complementary target sequences. Nonetheless, the authors investigated these candidate targets in their in vitro vascular smooth muscle cultures. Their results support the notion that miR-21 negatively regulates PTEN and subsequently increases the phosphorylation of the PTEN downstream target, the Akt protein to p-Akt (Figure, B). These findings are consistent with the known mechanism of miRNA action and PTEN signaling. In contrast, the positive regulation of Bcl-2 protein by miR-21 is consistent with the observed in vivo data but cannot be explained by the direct targeting of Bcl2 mRNA by miR-21 as the authors had initially proposed. Identifying other targets of miR-21 in vascular smooth muscle by proteomic approaches, for example, may be useful for further elucidating the function of the miRNA.

Though the authors have nicely characterized their initial observation, other significant issues remain to be clarified. Foremost is conclusively establishing whether changes in miR-21 expression are the cause or result of arterial injury. This may require tissue-specific and temporal regulation of miR-21 in transgenic animal models. Though challenging, comprehensively examining the functional significance of the

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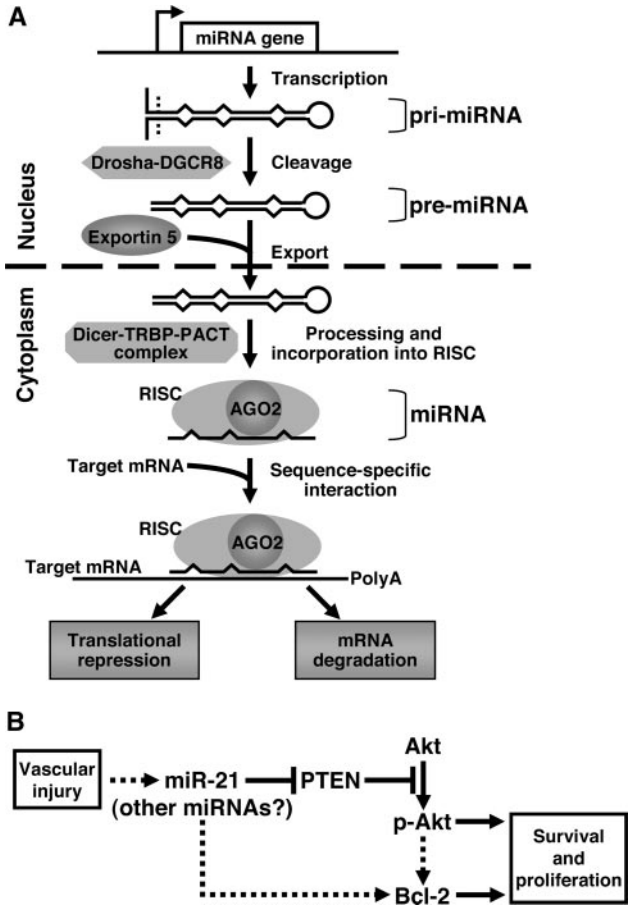

Biogenesis of miRNA and model of miR-21 activity in vascular smooth muscle. A, Primary miRNA (pri-miRNA) transcripts are processed into 60 to 70 nucleotide-long stem loop intermediates called precursor miRNAs (pre-miRNAs) by a complex of the nuclear RNase III (Drosha) and the DiGeorge Syndrome Critical Region Gene 8 (DGCR8).[3,21] The nuclear membrane transporter Exportin 5 facilitates the translocation of pre-miRNAs into the cytoplasmic space where they are further matured into miRNAs by another processing center comprised of RNA helicase Dicer, TAR RNA Binding Protein (TRBP), and protein activator of protein kinase PKR (PACT). This results in a ribonucleoprotein (RNA-Induced Silencing Complex [RISC]) containing the core catalytic protein Argonaute 2 (AGO2) and mature miRNA capable of recognizing target mRNA sequences and destabilizing it or inhibiting protein translational. B, Vascular injury results in the differential expression of various miRNAs including miR-21 which is proposed to target both tumor suppressor (antiproliferative) gene PTEN and oncogene (promoting survival/proliferation) Bcl2. The negative regulation of PTEN and the increased phosphorylation of Akt (p-Akt) protein are consistent with the known mechanism of miRNA action and PTEN signaling. The mechanism by which Bcl2 is increased is less clear (dashed lines). The net effect of increased miR-21 is to promote neointimal smooth muscle cell survival and proliferation after vascular injury.

remaining 112 differentially expressed miRNA through an unbiased screen may be more revealing than the current approach of selecting one highly expressed species that may or may not be the key biological mediator of vascular injury. Finally, measuring miR-21 expression in human restenotic coronary or peripheral atherectomy specimens would be a first step toward generalizing this preliminary observation to human studies.

There are some potential translational applications based on this interesting report. From a diagnostic perspective, a range of different cancers have been shown to express unique sets of miRNA that may serve as diagnostic or prognostic markers.[13] Similarly, given the dynamic profile of miRNAs after arterial injury and the importance of immune cells in this process, the measurement of specific miRNAs in blood may help risk stratify those at increased risk of restenosis or other proliferative vascular diseases such as cardiac allograft vasculopathy.[14,15] Patients with high risk miRNA profile may benefit by closer follow-up and more intensive medical management. A number of limited-scale studies examining genetic markers of cardiovascular diseases in blood have been reported, but it appears more comprehensive expression and single nucleotide polymorphism (SNP) genome-wide association studies are needed.[16–18] Given the findings of the current and other emerging reports, there is now additional impetus to include miRNAs in future genomic studies of cardiovascular diseases.

Although treatment strategies to knockdown miR-21 using exogenously delivered antisense miR-21 or "antagomirs" may be feasible in model systems, there is much work ahead to address the various issues of in vivo safety, delivery, and targeting before this therapeutic strategy can be considered.[19] As recently reviewed, there are some promising inhibitory RNA-based therapies using viral vehicles or nonviral carriers, such as liposomes or nanoparticles, but first selecting the correct therapeutic target based on the strongest level of experimental evidence is essential.[20] As the recent experience with drug-eluting stents have taught us, the long term consequences of altering the biological response to vascular injury need to be carefully considered to ensure that the surrogate clinical or basic experimental end points actually translate to long term improvement in patient outcome. The current observation suggesting miRNA involvement in restenosis has important clinical implications and affords a new opportunity to better understand the complex biology underlying vascular injury responses.

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Disclosures

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