A Missing LNC in Vascular Diseases

John P. Cooke, Nicholas J. Leeper

Pericytes are vascular smooth muscle cells that stabilize the endothelial networks that comprise the microvasculature. There is increased interest in the role that these cells play in maintaining the architecture, permeability, and remodeling of the microvasculature. Furthermore, these cells may be a source of regenerative cells that participate in the response to injury and ischemia. Bischoff et al.1 have comprehensively characterized a novel determinant of pericyte function and suggest its possible role in heart failure.

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Pericytes and Vascular Homeostasis

Pericytes are important players on the vascular stage, finally getting the spotlight that they deserve in maintaining microvascular homeostasis. These cells comprise the mural layer of arterioles, venules, and lymphatics, and they bear great similarity to vascular smooth muscle cells of the conduit arteries, expressing contractile proteins and maintaining a close apposition to the endothelium. However, accumulating evidence indicates that these cells also play a critical role in the response to injury and ischemia. Accordingly, it is important to understand the molecular mechanisms that control the proliferation of pericytes and their interaction with the endothelial cells of the microvasculature. The Dimmeler group has contributed much to our understanding of vascular homeostasis and the mechanisms that go awry in the pathobiology of vascular disease. In the current investigation, they introduce an intriguing new determinant of pericyte behavior that becomes most apparent during hypoxia.

Dimmeler et al. began by characterizing their cells, to ensure that they were working with authentic human pericytes. Their human pericytes expressed proteins that have been used as markers for pericytes, including PDGF-Rβ (platelet-derived growth factor receptor β), desmin, α-SMA (α-smooth muscle actin), and NG2 (neural/glial antigen 2). Parenthetically, it may be argued that requiring all of these markers might define a subset of cells that is not reflective of all pericytes. Nevertheless, as expected of pericytes, in coculture with endothelial cells, these human pericytes became tightly associated with, and reduced the permeability of, the endothelial monolayer, a function which is important in maintaining microvascular homeostasis.

To obtain a global view of how pericytes respond to a specific form of tissue injury, they exposed the cells to normal or low oxygen (hypoxic) conditions and then isolated the total RNA from the cells for sequencing (RNA-seq). This is an excellent approach to obtaining a global view of the transcriptional activity of a cell. An analysis of RNA-seq data can reveal novel transcripts that are determinants of phenotype and function. However, the amount of data obtained from RNA-seq can be overwhelming. Accordingly, to focus their analysis, the Dimmeler group concentrated on the effect of hypoxia to upregulate long noncoding RNAs (lncRNAs).

What Is the Link?

IncRNAs are RNA transcripts >200 nucleotides that do not encode a protein (this somewhat arbitrary cutoff for IncRNA distinguishes it from smaller regulatory RNAs, such as microRNA). Although these IncRNAs do not generally encode proteins, they may affect cell phenotype and function by virtue of their epigenetic effects. For example, they may act as cofactors that permit the interaction of a transcription factor with a promoter region of a gene, thereby regulating transcription. Alternatively, they may interact with epigenetic modifiers that act on histone proteins to repress or activate gene expression.

IncRNAs are increasingly recognized as having a role in cardiovascular health and disease. Indeed, IncRNAs have been causally linked to vascular inflammation, lipid metabolism, atherogenesis, and other related processes. Well-described examples include MALAT1 (metastasis associated lung adenocarcinoma transcript 1; which regulates endothelial cell proliferation and angiogenesis), SENCR (smooth muscle and endothelial cell-enriched migration/differentiation-associated long noncoding RNA; which governs the differentiation status of vascular smooth muscle cell), and LeXis (liver-expressed LXR-induced sequence; which regulates cholesterol efflux and biosynthesis). The potential clinical significance of these factors is emphasized by the fact that the top overall genome-wide association study hotspot for cardiovascular disease is associated with variation in a specific IncRNA known as ANRIL. ANRIL resides at the chromosome 9p21 locus, and it suppresses the expression of the nearby cell cycle regulator, CDKN2B, possibly via the recruitment of polycomb complexes or post-transcriptional RNA processing. We and others have shown that carriers of the risk allele have altered expression of CDKN2B. The altered expression of CDKN2B can perturb the proliferation, viability, and fate of vascular cells. The 9p21 locus has been conclusively
linked to a wide variety of clinical phenotypes, including myocardial infarction, abdominal aortic aneurysm, and stroke. Accordingly, there is great interest in modulating lncRNA levels as a novel translational approach to cardiovascular disorders.

The RNA-seq studies performed by the Dimmeler group revealed the expected upregulation of genes known to be regulated by hypoxia and a novel lncRNA that they termed as hypoxia-induced endoplasmic reticulum stress regulating lncRNA (HypERlnc). To understand if this lncRNA was physiologically relevant, they did a classic loss-of-function study, knocking down its expression with an antisense locked nucleic acid GapmeR. These reagents are stable oligonucleotides that can base pair with their RNA target to induce its degradation by endogenous RNase H. Notably, knockdown of HypERlnc with the LNA GapmeR decreased pericyte surface markers and impaired their association with, and to reduce the permeability of, an endothelial monolayer. Furthermore, HypERlnc deficiency increased markers of endoplasmic reticulum stress and reduced the viability and proliferation of the pericytes.

**Pathobiological and Clinical Implications**

These in vitro studies indicated that the lncRNA discovered by Dimmeler’s group has a central role in pericyte function and in its adaptation to hypoxia and stress. These data suggested that in states of maladaptation to hypoxia or other stresses, HypERlnc may be deficient. This supposition was confirmed by a bioinformatics analysis of the transcriptional profile of the pericytes after knockdown of HypERlnc. The data suggested that a deficiency of HypERlnc activated a gene network involved in cardiovascular disease. Accordingly, the investigators surveyed tissues representative of cardiovascular disease. HypERlnc correlated with the expression of pericyte markers in healthy or diseased human lung. This finding was confirmatory of the correlation between HypERlnc and pericyte biology, but was not very illuminating with respect to its role in pulmonary vascular disease.

Of somewhat greater interest, the investigators found that HypERlnc was markedly reduced in the myocardium of patients with heart failure. This is an interesting finding that is difficult to interpret. If this finding is because of a reduction in the pericyte lncRNA, it could be consistent with an abnormal microvascular structure in heart failure. Because pericytes are critical for the stability of the microvasculature, this finding could explain the disseminated microfoci of fibrosis that are observed in heart failure. Specifically, a destabilization of the microvasculature could lead to endothelial-to-mesenchyme transition and fibrotic replacement of microvessels. Indeed, such a process participates significantly in the fibrosis that occurs in a preclinical model of the diabetic heart. In this regard, the most numerous cell in the heart is not the cardiomyocyte, but rather the endothelial cell. At least some forms of heart failure are primarily a dysfunction of the endothelium and vasculature, which secondarily impairs contractility and relaxation of the ventricle. Alternatively, the impairment in pericyte function and viability could directly impair cardiac myocyte regeneration. Beltrami and Madeddu have provided a compelling case that a subset of pericytes may comprise a reservoir of multipotent mesenchymal stem cells that could replace lost myocytes or other cardiovascular cells.

To conclude, this article reveals a novel determinant of the vascular response to hypoxia, the lncRNA, HypERlnc. The pathobiological relevance of HypERlnc to cardiovascular disease requires further study.

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**Disclosures**

None.

**References**


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