Impaired coronary collateral growth: miR-shaken neutrophils caught in the act

Zsolt Bagi

Vascular Biology Center, Medical College of Georgia, Georgia Regents University, Augusta, Georgia

CHRONIC MYOCARDIAL ISCHEMIA and heart failure remain the most common cause of morbidity and mortality in patients with metabolic syndrome (MetS). MetS is associated with a clustering of cardiovascular risk factors that greatly increase the risk of developing occlusive coronary artery disease. Despite the major advances in rapid restoration of myocardial perfusion, achieved mainly through acute coronary interventions and thrombolysis, development of effective therapies for myocardial ischemia-associated heart failure remains a major challenge.

Considerable experimental and clinical evidence indicates that the healthy coronary circulation efficiently adapts to increased hemodynamic and metabolic challenges (2, 8). In MetS, such adaptive coronary arterial mechanisms become compromised, which ultimately leads to a mismatch between myocardial blood supply and metabolic demand (5). Collateral vessel growth is believed to be one of the important aspects of coronary arterial adaptation to chronically developing myocardial ischemia (13). This special type of arterial-arterial connection helps to maintain myocardial perfusion when critical narrowing of proximal coronary arteries gradually occurs over time. The problem is that development of coronary collateral vessels is seriously compromised in the diseased heart (1). The exact mechanisms that underlie the impaired coronary collateral growth remain largely unknown in MetS.

Existing coronary collaterals can enlarge their lumen in response to various stimuli that trigger the adaptive vascular remodeling in an outward direction. Increased luminal wall shear stress is one of the key driving forces for collateral vessel expansion, which via endothelial mechanotransduction induces release of vasodilator mediators, such as nitric oxide (NO). NO acutely dilates coronary arteries (7) and facilitates coronary collateral growth (11). Solid line of experimental evidence indicates that MetS impairs shear stress-dependent release of NO in coronary arteries, which is mainly due to increased production of reactive oxygen species (ROS) (3, 4, 7). Oxidative stress has also shown to be detrimental to coronary collateral development in MetS (14), but the underlying mechanisms remain poorly understood.

In MetS, activated leukocytes, including neutrophils, are the major sources of ROS in the circulation. Several fundamental questions, however, remain, such as whether or not neutrophils directly (transiently or permanently) interact with the luminal aspect of coronary arterial endothelium and to what extent this interaction contributes to the development of ROS-mediated endothelial dysfunction. The current study by Hutcheson et al. (10) in this issue of the American Journal of Physiology–Heart and Circulatory Physiology provides a new aspect of impaired collateral growth in MetS. Key results from this study show that because of the inadequate apoptosis and consequent accumulation of neutrophils within the arterial lumen, the outward luminal expansion of coronary collateral vessels is greatly diminished in the J. C. Russel (JCR) rat, a rodent model of MetS. It has been long recognized that monocytes and neutrophils comprise the main inflammatory cell population, which transiently increases during the collateral vessel growth (12). The study by Hutcheson et al. demonstrates that in the JCR rats the infiltrating inflammatory cells are predominantly neutrophils with only very little involvement of monocytes. More importantly, neutrophils in the JCR rats tend to accumulate and persist in the lumen of small collateral vessels. This happens, the authors argue, because neutrophils normally undergo apoptosis during the late phase of collateral expansion, but neutrophil apoptosis (as indicated by terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling and Gr-1, neutrophil marker positive cells) is apparently absent in the JCR rats. Apoptosis, as a possible component of physiological collateral vessel development, is generally presumed to be antithetical to growth processes. However, the authors found maximal cytochrome-c release and caspase-3/9 activation (indicative of apoptosis) in neutrophils of normal Sprague-Dawley rats 6 days after performing the repetitive ischemia protocol, exactly at the time, which represents the transition from inward to outward remodeling. Based on these observations, the authors argue that apoptosis of neutrophils is a fundamental component of the lumen expansion phase in ischemia-induced collateral development. On the other hand, a seriously diminished neutrophil apoptosis results in their intravascular accumulation, a pathology, which via increased ROS production leads to impaired collateral vessel expansion in MetS.

Collectively, there appears to be a series of events mediated by neutrophils, including those described by Hutcheson et al. (10), that have to be taken into account when discussing their pivotal role in collateral vessel development. However, data from the present study strongly suggest some level of interaction between neutrophils and coronary arterial endothelium, dynamics and molecular mechanisms of endothelium-leukocyte interactions, especially those that determine normal or pathological collateral development remain unclear. The authors in the current study exclusively employed immunohistochemistry in unperfused and perfused heart samples of normal Sprague-Dawley and JCR rats so as to identify adherent inflammatory cells within the lumen of small collateral arteries. Whether cell-to-cell interactions were firm adhesions or were merely transient events have yet to be determined. Also, quantification of endothelium-leukocyte interaction, identifying the underlying adhesion molecules for this interaction as well as functional consequence (e.g., increased ROS and diminished NO production) are difficult, if possible at all, to assess in postmortem histology sections. To overcome some of the aforementioned limitations, the authors employed combination of antibodies against CD11b/CD18/CD44 adhesion receptors in vivo and found a remarkable improvement in collateral growth in the JCR rats. Although results and conclu-
sions from these experiments carry limitations, they clearly provide rationale and feasibility for future studies, in which neutrophils will eventually be “caught in the act” during the impaired coronary collateral expansion.

To provide the mechanistic insight for their observations, the authors propose that the increased neutrophil survival in MetS is mainly mediated by a specific microRNA (miRNA), miR-21. miRNAs are ~20-22 nucleotides, single-strand RNAs, which have been recently recognized mediators of gene regulation and have become a major interest in biology and medicine. miRNAs are abundant in the vascular system, where they play key roles in vessel development and are considered to be important contributors of various vascular pathologies (6). miR-21 is among the major proproliferative and antiapoptotic miRNAs, which promotes cell proliferation and cell survival, primarily via downregulating phosphatase and tensin homolog and upregulating phosphatidylinositol 3-kinase and Akt signaling (15). A previous study by Hutcheson et al. (9) has found miR-21 to be upregulated in the ischemic heart of JCR rats. Increased miR-21 levels were associated with the accumulation of synthetic type of vascular smooth muscle cells in small collateral arteries (9). Intracardial administration of anti-miR-21 blocked smooth muscle cell proliferation and significantly improved coronary collateral growth in the JCR rats (9). New data from the current study by Hutcheson et al. (10) take this observation further while bringing even more complexity into the sequence of events of coronary collateral development. Results from this study show that intracardial injection with anti-miR-21 also promotes neutrophil apoptosis, which in turn leads to augmented lumen expansion of coronary collaterals in the ischemic heart of JCR rats. Interestingly, treatment with anti-miR-21 significantly reduced ROS production in the ischemic myocardium to the level similar to that of achieved by neutrophil adhesion-blocking anti-CD11b/CD18 antibodies.

Taken together, it seems miR-21 plays a unique pathological role during ischemia-induced collateral vessel development likely through affecting several cell types in the heart, including but apparently not limited to its role in recruiting neutrophils. While results from therapeutic delivery of anti-miR21 is remarkable, little is yet known about what determines the cell specificity of intracardial-delivered anti-miR constructs and, more importantly, how it evokes the observed, well-timed cellular effects. One possible explanation is that cells take up anti-miRs depending on their proliferation status and proliferation rate, and hence anti-miRs could regulate overall function in a cell-specific and also a sequential manner. Indeed, collateral vessel growth is characterized by at least two phases of main cellular events in which specific cell types, e.g., endothelium, vascular smooth muscle, and recruited inflammatory cells, are undergoing proliferation and apoptosis with varying dominance and rates (12). Results from this study provide a nice example how an anti-miR therapy may be harnessed to interrogate complex series of pathological events, such as manifested during the early and late phases of coronary collateral growth. In general, this unique feature of anti-miR therapy may also be considered when applying to other physiological processes and pathologies that are facing the ultimate challenge of selectively targeting cells with varying proliferation and apoptosis rates.

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