A miR-thless Perspective
How Asymmetric Dimethylarginine Impairs the Functions of Angiogenic Progenitor Cells

Ralf P. Brandes

Asymmetrical dimethylarginine (ADMA) is an endogenous derivative of the amino acid L-arginine that is formed by the proteolytic degradation of methylated protein residues. ADMA plasma levels are increased in patients with cardiovascular risk factors, although the mechanism underlying the increase may vary among individual risk factors: several enzymes involved in L-arginine methylation, demethylation, and ADMA transport are differentially regulated. It is generally believed that ADMA acts as a biologically significant endogenous inhibitor of the endothelial nitric oxide synthase (eNOS). The plasma concentrations of ADMA are, however, approximately 2 orders of magnitude lower than those of the eNOS substrate L-arginine. Thus, it is unlikely that a robust competitive antagonism to L-arginine at eNOS with a pronounced reduction in eNOS activity is the primary mode of action of ADMA.\(^1\) Rather, ADMA will induce a slight reduction of NO formation that appears to be sufficient to initiate processes that ultimately result in a massive breakdown of NO production. For example, ADMA promotes eNOS uncoupling, which, through a vicious cycle of reactive oxygen species (ROS) formation, tetrahydrobiopterin oxidation, and peroxynitrite formation, leads to overt endothelial dysfunction and an impaired vascular regenerative capacity.\(^2\) Moreover, it has been observed that ADMA has prooxidative and proinflammatory effects in cells lacking NO formation. This NO-independent component of the action of ADMA is mediated, at least in part, by the activation of the transcription factor nuclear factor κB.\(^3\)–\(^5\)

Bone marrow–derived progenitor cells, referred to as angiogenic progenitor cells (APCs), have gained considerable attention as a proangiogenic element. Despite ongoing discussions about the true nature of APCs, their precise mode of action, and the best nomenclature for these cells, their great importance for vascular regeneration has been convincingly demonstrated in a large number of studies. ADMA has a negative impact on APCs because it reduces their number in the circulation and their function ex vivo, but, to date, the mechanisms underlying this observation have been unclear.\(^6\)

In this issue of Circulation Research, Fleissner et al\(^7\) report how ADMA attenuates the function of APCs derived from monocytic blood cells. On the basis of a screen, the authors identified several differentially regulated microRNAs in APCs, and, among those, microRNA (miR)-21 was expressed under basal conditions and significantly induced by ADMA. Inhibition of miR-21 restored normal APC function, as determined by an assay for migratory capacity and formation of ROS. To identify the mechanism of action of miR-21, the authors performed a 2D differential in-gel electrophoresis (DIGE) approach and identified 51 targets of miR-21, among those were manganese superoxide dismutase (MnSOD) and the endogenous mitogen-activated protein kinase inhibitor sprouty-2. Subsequent siRNA experiments revealed that the ADMA-induced miR-21–mediated APC dysfunction was indeed a consequence of a downregulation of these 2 proteins. Importantly, a positive correlation between ADMA and miR-21 was observed in patients with coronary artery disease, and inhibition of miR-21 also increased MnSOD expression and enhanced the migratory capacity of these APCs.

The study by Fleissner et al\(^7\) is important for several reasons. It is the first report of on the impact of ADMA on microRNA expression, as well as one of the very few studies performing a microRNA screening on APCs. The study is also an exemplary demonstration of how rigorous utilization of unbiased screening technology can be used for mechanistic research. Indeed, with our present understanding of signal transduction, none of the links uncovered by Fleissner et al would have been postulated. For example, it is unclear how and by what mechanism ADMA affects miR-21 expression. The transcription of this RNA is positively influenced by several transcription factors, including activator protein 1 (AP-1),\(^8\) nuclear factor κB,\(^9\) and Stat-3,\(^10\) whereas Foxo3a inhibits miR-21 expression.\(^11\) Therefore, this microRNA is induced by cytokines\(^12\) and cellular stress in general. A link to NO formation, however, has not been previously observed. Instead, a proinflammatory effect of ADMA could be postulated. This should coincide with an increase in the antioxidative defense and an upregulation of the stress-responsive MnSOD. It was therefore exceedingly surprising that the second unbiased approach used by Fleissner et al (the 2D DIGE method) identified MnSOD as a protein strongly decreased by miR-21. As MnSOD is of central importance for the cellular antioxidative defense such a positive-feedback loop should, however, lead to a pronounced exaggeration of stress situations. Realizing this problem, Fleissner et al excluded MnSOD as a direct target of miR-21. Thus, the dangerous cycle uncovered by the authors has to be of an indirect nature. The mechanism underlying miR-21–induced
downregulation, however, remained unaddressed by Fleissner et al. Experiments performed in cancer cells have linked miR-21 to several aspects of carcinogenesis like increased cellular proliferation, migration, and invasion.\textsuperscript{13} miR-21 downregulates the cell cycle inhibitor Btg2, as well as the tumor suppressor phosphatase PTEN. This aspect is quite remarkable because PTEN limits AKT signaling. In endothelial cells, vasoprotective continuous laminar shear stress not only increased NO formation but also enhanced AKT phosphorylation via an induction of miR-21 and subsequent downregulation of PTEN.\textsuperscript{14} Also in endothelial cells, AKT predominately increases eNOS activity, although PIP<sub>3</sub>, which is upstream of AKT, can also stimulate the NADPH oxidase to increase the cellular ROS formation. Although APCs contain some eNOS, they express also NADPH oxidases,\textsuperscript{15} (in particular the leukocyte homologue), which produces high levels of ROS, contributes to the signal transduction of APCs,\textsuperscript{16} and even mediates APC dysfunction in response to angiotensin II.\textsuperscript{17}

This complex scenario illustrates an important problem of mechanistic research of epigenetic phenomena like microRNAs: The reaction patterns observed are often exceedingly complex, sometimes indirect in nature, and thus unexpected and cannot be derived from a hypothesis-driven assumption. Indeed, a large body of literature reporting individual effects of microRNAs has been assembled within just a few years. On the basis of specific inhibitors like antagonists, many of these studies are mechanistic. Nevertheless, the data collection as a whole is still largely incomprehensible. What is missing are those caved paths of cell signal transduction that are most appealing to the researcher’s way of thinking.

In keeping with these considerations, the discovery of a specific role of MnSOD for the effects of ADMA is not only a surprise but also counterintuitive. MnSOD is only one of several systems involved in the degradation of ROS, and these usually act in concert. Nevertheless, MnSOD appears to be of particular importance in APCs. It was previously noted that APCs express unusually high amounts of MnSOD.\textsuperscript{18,19} Although the induction of MnSOD in response to cytokines was more pronounced in APCs than in endothelial cells,\textsuperscript{18} for example, the mechanism underlying the high basal expression of MnSOD in APCs is unclear. Nevertheless, MnSOD is essential for normal APC biology. Downregulation of the protein largely reduced the oxidative stress tolerance\textsuperscript{19} and attenuated migratory and proangiogenic capacity of APCs.\textsuperscript{18,19} Interestingly, induction of MnSOD was previously observed to underlie the beneficial effect of nifedipine on APC migration.\textsuperscript{20}

Currently, we can only speculate why MnSOD is so important for APCs. The enzyme is exclusively located inside of mitochondria and specifically suited to degrade superoxide anions generated by the respiratory chain. Although APCs have relatively few mitochondria, these organelles are nevertheless important to their function. For example, downregulation of the mitochondrial antioxidant protein p66ShcA reduced the survival of APCs and increased the apoptosis sensitivity in diabetes.\textsuperscript{21}

In conclusion, by 2 consecutive screening approaches of APCs and specific inhibitor strategies, Fleissner et al\textsuperscript{7} could uncover how ADMA negatively affects the function of APCs. The highly indirect nature of the action of ADMA illustrates that screening approaches can be superior to the traditional pathway-based analysis when it comes to signaling via epigenetics.

**Sources of Funding**

Supported by grants from the Deutsche Forschungsgemeinschaft (SFB815/TP1 and SFB834/TPA2) and the excellence cluster cardio-pulmonary system (ECCPS).

**Disclosures**

None.

**References**


A miR-thless Perspective: How Asymmetric Dimethylarginine Impairs the Functions of Angiogenic Progenitor Cells
Ralf P. Brandes

Circ Res. 2010;107:12-14
doi: 10.1161/CIRCRESAHA.110.224022

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/107/1/12