H_2S to Mitigate Vascular Aging: A SIRT1 Connection

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H_2S is an endogenous gasotransmitter that plays an important role in physiological conditions. In this issue, Das et al. provide evidence that SIRT1-dependent angiogenesis is augmented by H_2S—findings reinforced by Longchamp et al., who demonstrate that H_2S-dependent angiogenesis is triggered by amino acid deprivation.

Endothelial cell (EC) dysfunction and impairment of angiogenesis are two important hallmarks of vascular aging. There is substantial evidence that vascular impairment plays a pivotal role in the development of age-related cardiovascular disease (CVD) and muscle mass loss (sarcopenia). While the mechanisms driving vascular aging and how this impairment leads to age-related pathogenesis remain poorly understood, several studies have shown that sirtuin deacetylase 1 (SIRT1) and hydrogen sulfide (H_2S) are critical modulators of vascular growth, remodeling, and homeostasis (Potente et al., 2007; Bai et al., 2016; Kanagy et al., 2017) (Figure 1).

In this issue of *Cell*, an elegant series of experiments by Das and colleagues demonstrate that stimulation of the NAD^+-H_2S/SIRT1 axis reverses age-associated EC dysfunction and promotes angiogenesis (Das et al., 2018). First, the authors showed that endothelial-specific *SIRT1* knockout mice (ESKO) presented some of the vascular defects associated with aging—namely a decline in endothelial migratory capacity and a reduced ability to form capillary-like structures. The latter was also linked to the low exercise capacity of ESKO mice, as these mice have an impaired capillary network. While it is recognized that exercise is a potent stimulator of vascular endothelial growth factor (VEGF) release and consequent angiogenesis (Morland et al., 2017), this study thus shows that SIRT1 is a critical factor for this exercise-induced muscle neovascularization. Furthermore, elegant experiments employing either SIRT1-deficient ECs or myocytes from muscle-specific PGC-1α (peroxisome proliferator-activated receptor gamma [PPARγ] coactivator 1-α, a master regulator of mitochondrial beiogenesis) overexpressing mice showed that SIRT1 in ECs was required for PGC-1α to enhance exercise tolerance, thus underscoring the critical role of vascular SIRT1 in endurance even in conditions where mitochondrial function was previously elevated.

Pro-angiogenic growth factors, such as VEGF and fibroblast growth factor (FGF), are known to enhance vascular integrity and promote angiogenesis. Das and colleagues also provide evidence that SIRT1 is required for pro-angiogenic growth factor signaling from myocytes to ECs. Small hairpin RNA (shRNA)-mediated targeting of *SIRT1* in human aortic ECs led to a reduced response to growth factors, and VEGF- or FGF-induced sprouting was reduced in aortic rings lacking SIRT1. In order to strengthen their findings, Das et al. employed an EC-specific SIRT1 overexpressing mouse strain (ESTO). These ESTO mice had higher VEGF serum protein levels and a 1.5- to 2-fold increase in capillary number and ran 1.8 times farther.

Nicotinamide adenine dinucleotide (NAD^+) levels are thought to decline as we age. SIRT1 requires NAD^+ for enzymatic activity, and NAD^+ precursors—such as nicotinamide mononucleotide (NMN)—are known to fuel SIRT1 activity (Nogueiras et al., 2012). Confirming this regulation, Das and colleagues also show that NMN significantly increased the number of outgrowths or sprouts from aortic rings taken from 18-month-old WT mice, but not in old SIRT1-iKOs (iKO, inducible knockout), in addition to showing that these NMN-induced effects were primarily mediated by SIRT1 and not by other Sirtuins. *In vivo* experiments showed that addition of NMN to their drinking water restored the number of capillaries and capillary density of old mice to those typically seen in young mice, and that SIRT1-iKO mice failed to respond to NMN-induced increase in capillary density.

In the final set of experiments, Das and colleagues tested the effect of exogenous H_2S on SIRT1 activity. While it was established that H_2S may promote SIRT1 activity and protect against pathological conditions (Suo et al., 2013), its effect on capillary density in aged animals was not known. Here, Das and colleagues establish that sodium hydrosulfide (NaHS) serving as an H_2S precursor increased intracellular NAD^+ levels, EC motility, and spheroid sprouting—effects that were SIRT1 dependent. These findings are consistent with another elegant article by Longchamp and colleagues in this issue, which shows that H_2S increases cell migration in a SIRT1-dependent but VEGF-independent manner (Longchamp et al., 2018).

It has been more than two decades since the observation that hypoxia induces VEGF-dependent angiogenesis (Shweiki et al., 1992). However, links between nutrient deprivation and angiogenesis have been less defined. Breakthrough findings made by Longchamp and colleagues in this issue elucidate mechanisms by which nutrient restriction acts

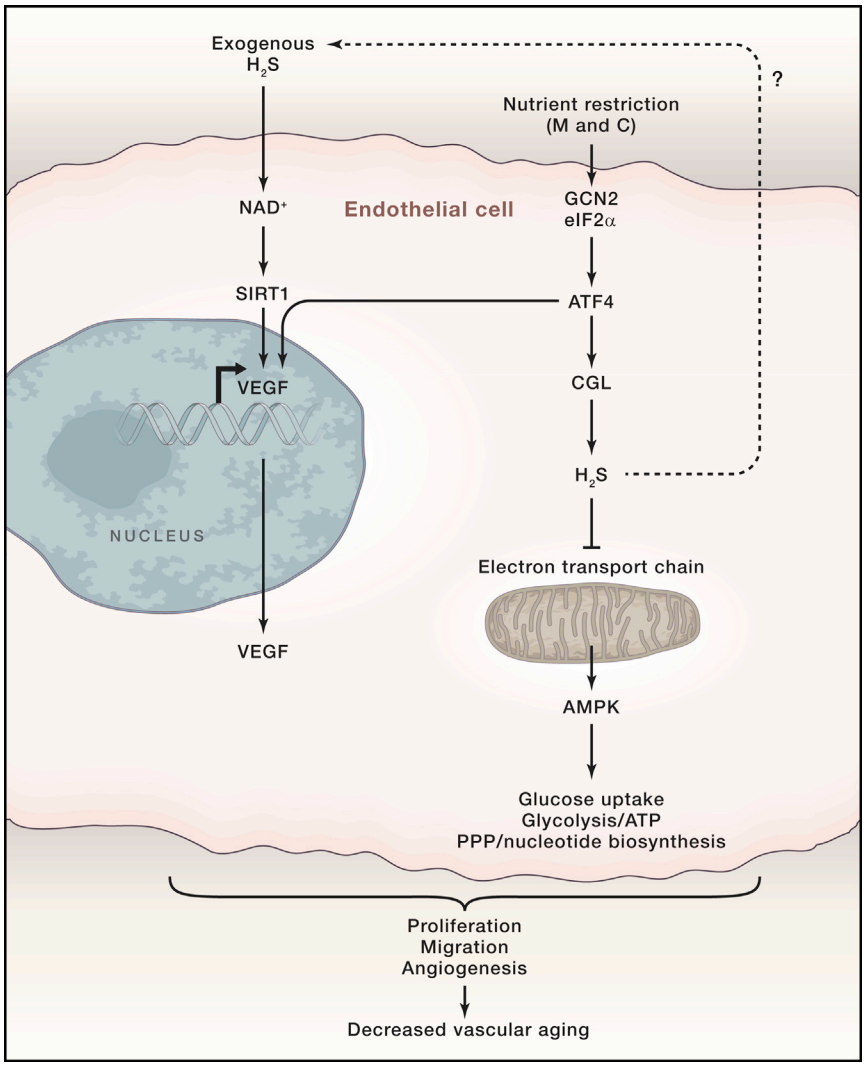

Figure 1. Mechanisms by which Exogenous H_2S and Nutrient Restriction Reverse Vascular Aging

Nutrient deprivation (M&C) increases VEGF via the amino-acid-sensing eIF2α kinase GCN2 and the transcription factor ATF4. GCN2/ATF4 activation increases CGL expression and H_2S production. H_2S boosts glucose uptake and glycolysis. Exogenous H_2S promotes NAD^+/SIRT1-dependent VEGF production. Collectively, this leads to EC proliferation, migration, and angiogenesis, as well as reversal of vascular aging. Of note, the link between intracellular H_2S production and its action in other cells in an exogenous manner is unknown at the moment.

as a pro-angiogenic trigger independent of hypoxia or HIF-1α. Culture media lacking methionine (M) and cysteine (C) led to increased VEGF and enhanced pro-angiogenic potential. Inhibition of SIRT1 significantly reduced angiogenic potential upon M and C deprivation, establishing that pro-angiogenic nutrient deprivation of ECs was dependent on both VEGF and SIRT1 activity. A sequence of *in vivo* experiments further established the fact that dietary sulfur amino acid restriction indeed increased VEGF-dependent vascular density. Nutrient-deprived animals also responded well to ischemic injury with improved revascularization.

As VEGF expression was unaffected by HIF-1α deletion or PGC-1α overexpression following M and C deprivation, Longchamp et al. explored other mechanisms, finding that eIF2α and ATF4 control VEGF expression during nutrient deprivation (Longchamp et al., 2018). Deletion of general control nonderepressible 2 (GCN2) kinase, upstream of eIF2α and ATF4, abolished M and C deprivation-induced VEGF expression and angiogenesis.

An earlier landmark study by the same team previously established that sulfur amino acid levels controlled transsulfuration enzyme cystathionine-gamma-lyase (CGL) expression and H_2S production (Hine et al., 2015). Here, these findings are extended, with CGL shown to be required for angiogenesis induced by M and C deprivation *in vitro* and CGL-mediated H_2S production essential for VEGF-mediated angiogenesis *in vivo*. Furthermore, H_2S and M and C deprivation increased glycolysis and pentose phosphate pathway (PPP) intermediates, which were shown to play a role in EC proliferation. Finally, the authors discovered the mechanism(s) by which H_2S promotes glucose uptake and utilization in ECs, establishing that H_2S transiently inhibits mitochondrial respiration by targeting the electron transport chain, resulting in increased glycolysis.

Both of these studies are significant in terms of our understanding the role of dietary restriction (DR) and the SIRT1/H_2S axis in aging and age-related vascular diseases. As vascular diseases, such as CVD or vascular dementia, contribute to significant health issues in the aging population, the question that arises is whether modulating H_2S benefits age-related vascular diseases and improves healthy lifespan in humans. Moreover, both studies make important contributions to our understanding of aging, as they stitch together pathways previously linked to aging, such as a decline in NAD^+ levels, to aging-associated pathologies. Determining how aging pathways interact is an important goal as the field moves toward an integrated model of human aging.

DECLARATION OF INTERESTS

B.K.K. is Scientific Director of Affirmativ Health, Board Chairman of Mt. Tam Biotechnologies, and on the board of directors at L-Nutra and Ponce de Leon Pharmaceuticals.

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