Cytoglobin at the Crossroads of Vascular Remodeling

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Since its discovery 15 years ago, cytoglobin has been studied extensively. Because it is found outside the red cell, cytoglobin is categorized as a nonerythroid globin, along with (in humans) proteins, such as myoglobin, neuroglobin, androglobin, and hemoglobin α. The putative functions of these nonerythroid globins are linked to tissue protection from conditions such as hypoxia, ischemia, and oxidative stress. Like other heme globins, cytoglobin not only fulfills these functions but also has been related to other roles, including tumor suppression and the regulation of fibrosis in cell and animal models. Like other heme globins, cytoglobin can reversibly bind oxygen and other small molecules. The ability of cytoglobin to store and sense oxygen, as well as its involvement in nitrite and nitric oxide (NO) metabolism, being able to both scavenge NO and produce NO from nitrite, is probably key to its function(s). However, in spite of significant progress in understanding the structure, localization, and functional characteristics of cytoglobin, the central physiological roles of this protein have yet to be fully elucidated.

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In this issue of Arteriosclerosis, Thrombosis, and Vascular Biology, Jourd’heul et al examine the role of cytoglobin in controlling apoptosis and vascular remodeling after injury. Cytoglobin seems to be the predominant globin in vessel walls of humans, rats, and mice with expression levels substantially higher than those of myoglobin. The protein is found in medial smooth muscle cells (SMCs), and dedifferentiation of SMCs by culture or vascular injury leads to a loss of cytoglobin expression, although this loss is only temporary after injury, with cytoglobin expression recovering after several days.

The authors use 2 different injury models: a rat model of unilateral carotid artery balloon angioplasty and a mouse model of unilateral carotid artery ligation. After either vascular injury, animals that do not express cytoglobin show substantially impaired remodeling, specifically decreased neointima formation (Figure). Analyses of apoptotic and proliferative markers suggest higher levels of apoptosis and cell death with cytoglobin loss but unchanged levels of proliferation, indicating increased apoptosis as the primary cause of disrupted neointima formation.

In experiments with rat aortic SMCs, the authors observe increased cytoglobin expression under hypoxic conditions or after treatment with inflammatory cytokines. Although hypoxia is known to increase cytoglobin expression, the induction of cytoglobin by cytokines has not been documented before and suggests cytoglobin may modulate inflammatory responses to nonischemic tissue damage. Cytoglobin silencing significantly increases rates of cell death, indicating a cytoprotective role.14 1400W—a selective NOS2 inhibitor—largely reversed this increase in cell death, suggesting NO-dependent cytotoxicity that can be prevented by cytoglobin expression. Worth mentioning, cytoglobin-knockout mice show exacerbated expression of NOS2 and inflammation markers, suggesting a link between cytoglobin function and immune response. The increase in cell death was also reversed with the use of the reducing agent N-acetyl cysteine or a pan-caspase inhibitor, implicating oxidative stress in promoting apoptosis. Specifically, cytoglobin loss seems to activate caspase-3—a finding that had previously been observed in animal models of brain ischemia-reperfusion injuries but is novel in the context of vascular injury.

The implication that cytoglobin protects cells from NO-dependent toxicity is particularly compelling because numerous researchers have explored cytoglobin’s NO dioxygenase activity, which was first proposed by Halligan et al. NO dioxygenation occurs when oxygen-bound cytoglobin reacts with NO, resulting in the production of nitrate and the oxidation of the heme iron from the ferrous to the ferric state. This reaction is extremely rapid for globins (nearly diffusion limited). NO dioxygenation is considered to significantly contribute to NO metabolism in vivo, with physiological effects, including cytoprotection and regulation of vascular tone.

Catalytic NO dioxygenase activity is limited by the reduction of the heme iron. A reducing system has been characterized for hemoglobin α; inhibition of CYB5R3 (cytochrome b5 reductase type 3)—a reducing enzyme present in endothelium—slows NO consumption by hemoglobin α. Recent data from our group and others show a highly efficient reduction of cytoglobin by the NADH/CYB5/CYB5R3 reducing system; in fact, the reduction of cytoglobin is at least an order of magnitude faster than that of other heme globins. Taken together, these results suggest the existence of a cytoglobin/CYB5/CYB5R3 metabolon in vascular SMCs, enabling rapid consumption of NO and thus modulating NO bioactivity and signaling. Interestingly, myoglobin in vascular smooth muscle has previously been shown to contribute to hypoxic...
vasodilation via nitrite reduction to NO. The responses of myoglobin and cytoglobin to oxygen and the relative efficiency of their reducing systems could underlie different roles for both proteins on vascular-wall NO signaling.

This work by Jourd’heuil et al\textsuperscript{13} showcases a new role for cytoglobin as an important regulator of apoptosis and vascular remodeling in SMCs after injury, acting independent of other globins. This role is supported by the novel observation that inflammatory cytokines trigger re-expression of cytoglobin in dedifferentiated SMCs in vitro, preventing NO2-dependent cytotoxicity and promoting proper remodeling of vascular tissue. Rapid NO consumption by cytoglobin in vascular SMCs, which has previously only been shown to influence vascular tone and blood pressure, may mediate this effect. This work provides compelling evidence that cytoglobin may be a key regulator of vascular function under both normal and pathological conditions, indicating that cytoglobin and other non-erythroid globins may have value as therapeutic targets or agents for myriad disease states. The particular properties of the non-erythroid globins keep revealing new possibilities, from the potential of modulating NO signaling to their use as potential carbon monoxide scavengers or oxygen carriers.\textsuperscript{22,28}

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None.

**References**


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