HIGHLIGHTED TOPIC | Physiology of the Aging Vasculature

The role of p66Shc deletion in age-associated arterial dysfunction and disease states

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Cardiovascular disease remains the most important cause of morbidity and mortality in the Western world. Although mortality declined steadily over the last 30 yr, it stabilized over the last decade and may even increase again due to the growing aging population (22). This is due to the fact that age per se represents a risk factor for the development of cardiovascular disease (Fig. 1) (16). The vast majority of cardiovascular disease is caused by dysfunction of the vasculature, in particular of the endothelium, in the coronary, cerebrovascular, renal, and peripheral circulation. Vascular dysfunction involves vascular smooth muscle cells, endothelial cells, and their interaction with circulating blood cells such as platelets, monocytes, and lymphocytes. In addition, the resulting vascular stiffening also includes adventitial cells as well as connective tissue.

The endothelium, the innermost part of the vessel, is in a strategic anatomic position to govern different cellular events, and it does so via the release of vasoactive substances such as nitric oxide (NO), reactive oxygen species (ROS), prostaglandins, and endothelin-1 (19–21) (Fig. 2). NO is produced by endothelial NO synthase (2) and is 1) a potent vasodilator, 2) an inhibitor of vascular smooth muscle proliferation and migration, and 3) an antiaggregant for platelets, and thus it represents an important protective factor in the vasculature. Its release and biological activity are profoundly impaired with age by the increasing levels of ROS (12). Indeed, NO interacts rapidly with superoxide (O2·−) to form peroxynitrite (ONOO−), a potent prooxidant (12, 38). Aging vessels are known to accumulate ROS and oxidative damage, and, as a result of this, they undergo functional impairment (13).

P66Shc: Role and Function

ROS are principally generated in mitochondria partly as a result of aerobic respiration, and in this context the mitochondrial adaptor protein p66Shc represents an interesting focus. In fact, genetic ablation of p66Shc in the mouse was shown to reduce production of intracellular oxidants and consequently prolong life span by 30% (26). The mammalian Shc locus encodes for three different adaptor proteins with respective molecular masses of 46, 52, and 66 kDa. The three isoforms share a Src-homology 2 domain, a collagen-homology region,
and a phosphotyrosine-binding domain. However, the splice variant p66Shc, which contains a unique NH2-terminal region, is the only isoform to play an important role as a redox enzyme implicated in mitochondrial ROS generation and translation of oxidative signals into apoptosis (5). p66Shc has been proposed to regulate ROS production by controlling the partition of ATP generation in the cell and by participating to the electron flow chain in the mitochondria (30). In fact, in the absence of p66Shc, mitochondrial oxidative phosphorylation is reduced in favor of glycolysis. Because mitochondrial electron flow, besides being the major source of ATP, also represents the major producer of cellular ROS, this may provide an explanation for the decreased production of ROS observed in p66Shc/−/− cells and organisms (30). In light of its pivotal role in ROS generation, p66Shc adaptor protein has been regarded as a key player involved in mediating age-dependent loss of endothelial integrity. In line with this hypothesis, p66Shc/−/− mice were shown to be protected from age-dependent endothelial dysfunction (13). Wild-type mice display age-dependent loss of acetylcholine-induced, NO-mediated vasorelaxation, whereas p66Shc/−/− mice do not (13). In line with this, aged p66Shc/−/− mice, unlike age-matched wild types, show increased endothelial bioavailability of NO, lower aortic O2 levels, and reduced aortic 3-nitrotyrosine content (13), thus suggesting a potential mechanism by which NO availability and vasorelaxant responses are preserved in aged p66Shc/−/− mice. Based on these findings, one could also speculate that this is one of the mechanisms involved in the extended life span observed in p66Shc/−/− mice.

Age-dependent endothelial dysfunction is not only explained by a steady loss of NO bioavailability caused by increased scavenging. Indeed, the vasculature is exposed to ROS damage, leading to endothelial cells death throughout its lifetime. In normal conditions, however, a balance between ROS damage and endothelial progenitor cells (EPC)-mediated repair exists (9) and guarantees endothelial integrity. This balance becomes disturbed with age due to an increased pro-

Fig. 1. In health, a balance between noxious insults and protective systems exists. With age, however, this balance is progressively lost in favor of the development of endothelial dysfunction and vascular disease. ROS, reactive oxygen species; NO, nitric oxide.

Fig. 2. Schematic representation of the endothelium and some of its key derived vasoactive substances. The p66Shc (p66) is principally located in the mitochondria, where it contributes to the production of superoxide anion (O2•−) radicals, which scavenge NO to form peroxynitrite (ONOO−). Different stress stimuli (e.g., glucose), increase in number and occurrence with age and enhance the production of O2•−, thus exacerbating endothelial dysfunction. oxLDL, oxidized low-density lipoprotein; ET1, endothelin 1; NOS, NO synthase, ONOO−, peroxynitrite; MCP-1, monocyte chemotactic protein-1; ICAM, intercellular adhesion molecule; VCAM, vascular cells adhesion molecule; NFkB, nuclear factor-kappa B; IkB, inhibitor to NFkB; NADPH, nicotinamide adenine dinucleotide phosphate-oxidase; PKCβ1, protein kinase C-β1; GPx, glutathione peroxidase.
duction of ROS, which in turn causes more damage and a decreased EPC’s function, eventually leading to organ dysfunction (1). In keeping with the theory of an enhanced age-dependent ROS production, aged mice display enhanced endothelial mitochondrial ROS compared with young mice. The increased ROS production observed in aged mice could be the explanation for 1) the decreased endothelial cells function and 2) the decreased EPC-mediated repair, ultimately leading to vascular dysfunction. This changes, however, are not observed in age-matched p66Shc−/− mice, which present lower levels of ROS and a preserved endothelial function (13).

The possible role of p66Shc in the aging process of humans was recently investigated (32). Both p66Shc protein and messenger RNA were assessed in young people, elderly, and centenarians. Paradoxically, in this study, the expression of p66Shc was shown to increase with age (32), thus creating an apparent contradiction in terms deserves more scientific efforts to be fully comprehended. For instance, it would be interesting to investigate whether human p66Shc protein remains functional in aging or perhaps undergoes an adaptive response to age-dependent cellular damage in vascular tissue. In any case, this observation does not preclude that p66Shc indeed mediates aging also in humans.

The initial theory that p66Shc may be at the crossroad between ROS production and arterial dysfunction drove one of the earliest investigations meant for elucidating its role in arterial atherogenesis (29). Wild-type and p66Shc−/− mice were fed a normcholesterolemic diet or a high-fat diet and then systemic vs. vascular levels of oxidative stress, as well as the extent of arteriosclerosis, were investigated. In this experimental setting, the authors found that wild-type mice fed a high fat diet showed much enhanced early aortic lesion formation compared with p66Shc−/−. This finding was recorded despite the fact that the lipid profile was unchanged in the two strains. In addition, p66Shc−/− mice exhibited a decrease in intimal foam cells formation, arterial oxidized LDLs as well as systemic plasma isoprostanes (29).

A later report once again proposed a correlation between plasma levels of LDL cholesterol, early markers of arterial dysfunction and p66Shc in pacemaker-implanted patients (6) thus confirming that p66Shc plays a role in translating the effects of different age-related risk factors into arterial dysfunction and early atherosclerotic lesions.

Abnormal glucose metabolism predominantly affects older individuals; indeed, 35% of the aged population presents, to some degree, abnormal glucose tolerance and shows signs of insulin resistance (17, 18). Hyperglycemic states frequently encountered in such conditions are thought to play a central role in generation of ROS, leading to arterial endothelial dysfunction and later to atherosclerosis (34, 39). Indeed, high levels of glucose induce a cascade of cellular events that increase the production of free radicals, thus decreasing NO bioavailability and eventually leading to vascular dysfunction (10, 15). In conditions of raised glucose plasma levels, p66Shc is known to oxidize cytochrome c and in turn to generate proapoptotic ROS through a PKC-β-dependent pathway (7, 30). In line with this concept, peripheral blood monocytes from patients with diabetes mellitus were shown to have increased p66Shc mRNA expression compared with healthy subjects (31). The putative role of p66Shc in hyperglycemia-induced, ROS-mediated vascular dysfunction was investigated further by separate studies. An interesting study by Menini et al. (25) demonstrated that p66Shc−/− mice are protected against diabetic glomerulopathy, a leading cause of chronic renal failure (25). Indeed, p66Shc−/− mice did not show high glucose-induced, ROS-dependent increase in glomerular cell apoptosis and extracellular matrix deposition, thus underlining once more the pivotal role of p66Shc in translating ROS-related insults into apoptosis.

The role of p66Shc in mediating hyperglycemia-induced, ROS-dependent endothelial dysfunction was recently addressed in a mouse model of Type 1 diabetes (7). In this study, p66Shc−/− hyperglycemic mice, unlike wild types, were shown to be protected from endothelial dysfunction by means of an unaltered ROS production, which resulted in a preserved NO bioavailability (7). Interestingly, p66Shc protein expression was increased in aortas from wild-type hyperglycemic mice compared with normoglycemic controls, thus underlining a causal relationship between high glucose, ROS, p66Shc- and vascular dysfunction (7).

**FUTURE RESEARCH DIRECTIONS**

Epidemiological studies demonstrated that even in the absence of other risk factors such as diabetes or high cholesterol, aging per se increases cardiovascular morbidity and mortality. A better understanding of the molecular mechanisms of aging and its interaction with risk factors could, in the future, lead to the development of therapeutic interventions aimed at decreasing the functional decline of the cardiovascular system.

From an evolutionary perspective, ROS pathways have developed for enhancing energy metabolism and host defense. However, in the modern world (i.e., fewer infections, high caloric intake), the accumulation of ROS observed in aging appears to be heavily implicated in age-associated cardiovascular diseases. Thus redox-sensitive molecular pathways such as p66Shc are under intensive investigation as the common denominators of the pathophysiology of several cardiovascular risk factors. In this view, the concept that p66Shc regulates ROS production, thereby determining cellular and organ decline raises the question whether pharmacological modulation of its expression and/or activity may be effective in delaying the onset of age-dependent vascular disease. Thus research efforts should persist in the current direction to fully elucidate the exact relationship between all the factors (namely ROS, aging, risk factors and redox pathways), that cause the unrelenting decline in efficiency of the cardiovascular system.

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**REFERENCES**

