p66\text{Shc} as the Engine of Vascular Aging

Francesco Paneni\textsuperscript{1} and Francesco Cosentino\textsuperscript{2,*}

\textsuperscript{1}IRCCS Neuromed, Pozzilli; and \textsuperscript{2}Cardiology, Department of Clinical & Molecular Medicine, University "Sapienza", Rome, Italy

Abstract: The present work is addressing the latest advances made in understanding the molecular mechanisms of vascular aging. Increased production of reactive oxygen species (ROS) is the common denominator of vascular aging, endothelial dysfunction and atherosclerosis. ROS are generated by different intracellular molecular pathways. In view of its role in determining the redox state of the cells and their responses to free radicals, mitochondrial p66\text{Shc} protein has been regarded as part of a putative transduction pathway relevant to endothelial integrity. Future efforts should translate our knowledge of the mechanisms of aging and its interaction with risk factors into the development of new therapeutic strategies to prevent age-associated cardiovascular disease.

Keywords: p66\text{Shc}, aging, endothelial dysfunction, oxidative stress.

INTRODUCTION

Demographic data demonstrate that the proportion of elderly people is growing [1]. Aging is an independent risk factor for the development of cardiovascular (CV) diseases [2]. It will impose a major burden on health care resources, since it is estimated that by the year 2035, nearly 1 in 4 individuals will be 65 years of age or older. CV disease death rate over 65 shows a rapid, almost exponential rise becoming the most common cause of death among the elderly [3]. Furthermore, most people with diabetes mellitus (DM) in the developed world are ≥65 years of age, whereas most of those in developing countries are only 45 to 64 years old [4]. Many individuals with DM in developing nations will suffer this condition during their most productive years, and those who will age are at risk of acquiring the serious chronic complications that accompany this disease [4].

Based on current trends, ischemic heart disease will continue to be worldwide the leading cause of disease burden, including mortality and disability. Aging together with diabetes are considered the utmost contributors among traditional risk factors to the epidemic of atherosclerotic CV disease. The triad of CV disease, diabetes and aging will dominate the scenario of future global mortality and burden of disease in developing and western countries [5]. All forms of CV disease show higher frequency with age, even in the absence of other CV risk factors [6, 7, 9]. These observations prompted research efforts dealing with the vascular biology of aging to determine mechanisms underlying the increased risk conferred by aging per se. Mounting data suggest that CV structure and function change with time as result of the "aging process" characterized by endothelial dysfunction, wall thickening and stiffness, increased left ventricular mass due to enlargement of myocyte size and focal proliferation of the matrix, occurring also in adventitial cells as well as connective tissue. The age-associated changes in cardiac and vasculature structure intertwine and modulate superimposed traditional cardiovascular risk factors to determine the occurrence of CV diseases in the elderly [6-8].

BIOLOGY OF VASCULAR AGING

Endothelial dysfunction is one of the most important age-associated CV changes [6]. Vascular aging is characterized by the transition of the endothelium from an antiatherosclerotic to a proatherosclerotic state [10]. Indeed, nitric oxide (NO) bioavailability, which is crucial for endothelial integrity and function, decreases with age [11]. Several animal models have demonstrated that aging vessels exhibit an increased production of reactive oxygen species (ROS) and in turn undergo functional impairment as a result of loss of NO bioavailability [12-16]. Indeed, while under physiological conditions the production of NO is not substantially affected by superoxide anion (O\textsubscript{2}\textsuperscript{-}), an excessive generation of O\textsubscript{2}\textsuperscript{-} rapidly inactivates NO leading to the formation of peroxynitrite (ONOO\textsuperscript{-}), a powerful oxidant [17, 18]. ONOO\textsuperscript{-} is able to penetrate across cellular membranes and inactivate by substrate nitration a number of regulatory receptors and enzymes, including free radical scavengers [19, 20]. The identification of molecular pathways modulating the endothelial cell redox state is therefore relevant to our understanding of mechanisms linking vascular aging to endothelial dysfunction and atherosclerosis. In view of its originally reported role in determining the redox state of the cells and their responses to free radicals [21], mitochondrial p66\text{Shc} adaptor protein has been regarded as part of a putative transduction pathway relevant to endothelial integrity. This hypothesis was further strengthened by the observation that p66\textsuperscript{Shc/-} mice have an approximately 30% increase in life span compared to wild type littermates [21]. Accordingly, p66\textsuperscript{Shc/-} mice are protected against age-dependent endothelial dysfunction [22]. Indeed, wild type mice display age-associated blunting of endothelium-dependent relaxation to acetylcholine, whereas p66\textsuperscript{Shc/-} mice did not [22]. Compared with age-
matched wild type mice, old \( p66^{Shc-/} \) mice show increased endothelial bioavailability of NO, lower aortic \( O_2^- \) levels and reduced aortic 3-nitrotyrosine content in the absence of any difference in the expression of Mn SOD and Cu/Zn SOD [22]. Of interest, the expression of the inducible form of NO synthase (iNOS) increased significantly in the old WT mice, whereas no age-dependent changes were found in the \( p66^{Shc-/} \) mice [22]. This suggests a potential mechanism by which NO availability and vasorelaxant responses are preserved in aged \( p66^{Shc-/} \) mice. Indeed, age-dependent upregulation of iNOS is involved in ONOO⁻ formation and, hence, may lead to increased oxidative vascular damage [13, 23]. Based on these findings, it is tempting to conclude that prevention of endothelial dysfunction and hence protection against aging-associated vascular diseases might contribute to the extended life-span of \( p66^{Shc-/} \) mice [24].

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 [25, 26]. Hyperglycemic states in this setting play a central role in ROS generation, leading to arterial endothelial dysfunction and later to atherosclerosis [27, 28]. 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 [29-31]. In conditions of raised glucose plasma levels, \( p66^{Shc} \) is known to oxidize cytochrome \( c \) and in turn to generate proapoptotic ROS through a PKC-dependent pathway [32, 33]. In line with this concept, peripheral blood monocytes from patients with diabetes mellitus were shown to have increased \( p66^{Shc} \) mRNA expression compared with healthy subjects [34]. The putative role of \( p66^{Shc} \) in hyperglycemia-induced, ROS-mediated vascular dysfunction was investigated further in a mouse model of type 1 diabetes [32]. In this study, we demonstrated that \( p66^{Shc-/} \) diabetic mice, unlike WT, are protected from endothelial dysfunction by means of an unaltered ROS production, which resulted in a preserved NO bioavailability. Interestingly, \( p66^{Shc} \) protein expression was increased in aortas from wild-type hyperglycemic mice compared with controls, thus underlining a causal relationship between high glucose, ROS, \( p66^{Shc} \) and vascular dysfunction.

**CONCLUSIONS**

Endothelium-derived NO has received a great deal of attention over the past years becoming a major factor in cardiovascular homeostasis. Vascular biology successfully brought together basic and clinical sciences unmasking the key unifying role of oxidative stress in endothelial dysfunction and atherosclerosis. We believe that future efforts should translate our current knowledge of the molecular mechanisms of aging and its interaction with risk factors into the development of new therapeutic strategies to prevent age-associated CV disease.

**CONFLICT OF INTEREST**

The authors confirm that this article content has no conflicts of interest.

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Fondazione, Roma, Italy.

**REFERENCES**


