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Review

Double-Face of Vasohibin-1 for the Maintenance of Vascular Homeostasis and Healthy Longevity

Yasufumi Sato

Department of Vascular Biology, Institute of Development, Aging and Cancer, Tohoku University, Sendai, Japan

The structural and functional integrity of endothelium is essential for the maintenance of vascular health. Vasohibin-1 (VASH1), originally isolated as an endothelium-derived angiogenesis inhibitor, has another function to promote stress tolerance of endothelial cells (ECs), and these functions are critical for the maintenance of vascular homeostasis preventing both pathological angiogenesis and stress-induced vascular diseases. The expression of VASH1 is downregulated during replicative senescence of ECs by the alteration of microRNA expression, and this age-associated downregulation of VASH1 might be a risk of deterioration of vascular homeostasis and age-related vascular diseases. Contrary to this expectation, the lack of *Vash1* gene in mice exhibited healthy longevity. Thus, VASH1 has double-face for the maintenance of vascular homeostasis and healthy longevity. This feature of VASH1 and its mechanism will be described in this mini review.

Key words: Vasohibin-1, Angiogenesis inhibition, Vascular stress tolerance, Healthy longevity

Introduction

Cardiovascular diseases (CVDs) are the major cause of death in industrialized countries. Aging is the critical risk factor of CVDs, as the prevalence of CVDs is significantly increased in the older population[1]. Aging is associated with the progressive deterioration of the structure and function of all cell types, including vascular endothelial cells (ECs). Aging is influenced both by replicative senescence and by environmental factors. When various cellular stresses cause DNA damage, cells arrest their cell cycle for the repair of DNA damage, which leads to premature cell senescence. Replicative senescence is the result of telomere shortening, whereas premature cell senescence does not require such an event. Nonetheless, replicative senescent cells are more prone to DNA damage and the resultant premature cell senescence[2].

ECs on the vascular wall receive cumulative damages during aging, and this causes the age-dependent impairment of the vascular endothelium[3]. In order to maintain the integrity of vascular structure and function, the vascular endothelium possess a self-defense system. Vasohibin-1 (VASH1) is initially isolated as an endothelium-derived angiogenesis inhibitor, and is further revealed to promote stress tolerance of ECs for the maintenance of vascular homeostasis[4, 5].

Address for correspondence: Yasufumi Sato, Department of Vascular Biology, Institute of Development, Aging and Cancer, Tohoku University, 4-1, Seiryo-machi, Aoba-ku, Sendai 980-8575, Japan
E-mail: yasufumi.sato.b3@tohoku.ac.jp
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Isolation of Vasohibins

To search for angiogenesis regulators expressed in ECs, we stimulated ECs in culture with VEGF and then performed DNA microarray analysis to characterize VEGF-inducible genes in these cells. We focused our attention on genes whose functions were undefined at that time. Recombinant proteins of such genes were made and applied to functional bioassays of angiogenesis. This attempt led to the isolation of a gene having anti-angiogenic activity, which we designated as vasohibin (VASH). By examining in a database, we found a gene homologous to VASH, and designated the homolog as vasohibin-2 (VASH2) and we rename VASH as VASH1. The overall homology between human VASH1 and human VASH2 is 52.5% at the level of the amino-acid sequence. Moreover, it is now revealed that lower organisms possess one ancestral vasohibin gene, whereas vertebrates have VASH1 and

VASH2. The common ancestral vasohibin gene is divided into VASH1 gene and VASH2 gene during the evolution. The conserved amino-acid sequence across species indicates the importance of the biological role of the vasohibin family[6].

VASH1 for Angiogenesis Inhibition and Stress Tolerance of ECs

VASH1 is preferentially expressed in ECs of newly formed blood vessels behind the sprouting front where angiogenesis terminates[7]. When angiogenesis was induced in *Vash1* KO mice, angiogenesis was maintained in the area where angiogenesis should be terminated. Moreover, when the adenoviral vector encoding human VASH1 (AdhVASH1) was injected from tail vein of *Vash1* KO mice, this sustained angiogenesis was abrogated[7].

Besides its emergence there on the vascular wall for the termination of angiogenesis, the VASH1 protein is immunohistochemically detectable in certain ECs unrelated to angiogenesis[8]. The additional role of VASH1 was subsequently revealed by the knockdown of VASH1 in cultured HUVECs, which treatment significantly increased the number of premature senescent cells. Alternatively, when VASH1 was overexpressed in HUVECs, such cells became resistant to stress-induced premature cell senescence[5]. This protective function of VASH1 was confirmed under *in vivo* conditions by the treatment of mice with paraquat, which generates superoxide and causes acute organ damage including lungs. When compared with wild-type mice, *Vash1* KO mice died in greater numbers due to acute lung injury caused by paraquat. Alternatively, when VASH1 was overexpressed in the lungs of *Vash1* KO mice by the intra-tracheal administration of AdhVASH1, the paraquat-induced acute lung injury was almost completely prevented[5]. The protective role of VASH1 from vascular diseases was further clarified by comparing the development of STZ-induced diabetic nephropathy, or the development of intimal thickening of femoral artery in cuff-injury model in *Vash1* KO mice with that in their wild-type mice. The results indicated that diabetic nephropathy or intimal thickening of femoral artery was significantly exacerbated in *Vash1* KO mice[9, 10]. The protective activity of VASH1 was further documented in murine diabetes models, as human VASH1 protein delivered from the liver infected with AdhVASH1 prevented diabetic nephropathy[11, 12] or intimal thickening of femoral artery[13].

Thus, these loss-of-function and gain-of-function studies have clearly demonstrated the role of VASH1 is to maintain vascular homeostasis by both angiogenesis inhibition and stress tolerance of ECs.

Dual Regulation of VASH1 Expression for the Maintenance of Vascular Homeostasis

Gene expression begins with the transcription of DNA for the generation of mRNA and ends with its translation to protein. When ECs are exposed to pro-angiogenic stimuli such as VEGF, ECs increase their expression of VASH1 mRNA and VASH1 protein synthesis over a 24-hour period[4]. This VEGF-mediated increase of VASH1 mRNA is not influenced by the actinomycin D treatment[14], indicating that this increase of VASH1 mRNA is mediated by the transcriptional activation.

Post-transcriptional regulation controls the fate of mRNAs at the steps of splicing, export, stabilization, and translation; and these processes are regulated by the interaction of *cis*-regulatory elements on mRNA and *trans*-acting factors such as RNA binding proteins (RBPs) and microRNAs that bind to these elements[15]. When HUVECs are exposed to cellular stresses such as H_2O_2 treatment or serum starvation, HUVECs promptly increase the VASH1 protein level, and this is not accompanied by the increase of VASH1 mRNA. This prompt increase in VASH1 protein synthesis without transcription is mediated by an RBP, namely, HuR[5]. HuR belongs to the embryonic lethal abnormal vision (ELAV) family of RBPs that bind to U-rich and/or AU-rich elements in the 3′UTRs of their target mRNAs, and it prevents their degradation and enhances translation[15]. There are four members of the ELAV protein family, i.e., HuB, HuC, HuD, and HuR. HuB, HuC, and HuD are selectively expressed in the nervous system and play roles in neuronal differentiation and plasticity, whereas HuR is ubiquitously expressed and involved in numerous cellular responses[15]. HuR is present predominantly in the nucleus under normal conditions. When cells are exposed to cellular stresses, HuR is translocated to the cytoplasm, where it interacts with mRNAs and elicits cellular stress responses[16].

Thus, VASH1 protein synthesis is induced transcriptionally for angiogenesis inhibition or post-transcriptionally for stress tolerance of ECs, and these functions are critical for the maintenance of vascular homeostasis (**Fig. 1**).

Age-Associated Downregulation of VASH1

It is well documented that a variety of functional classes of genes are downregulated with age, often in a tissue specific manner[17]. We found that the expression of VASH1 was decreased with replicative senescence of ECs[10]. To explore the mechanism of this downregulation of VASH1, the expressions of micro-

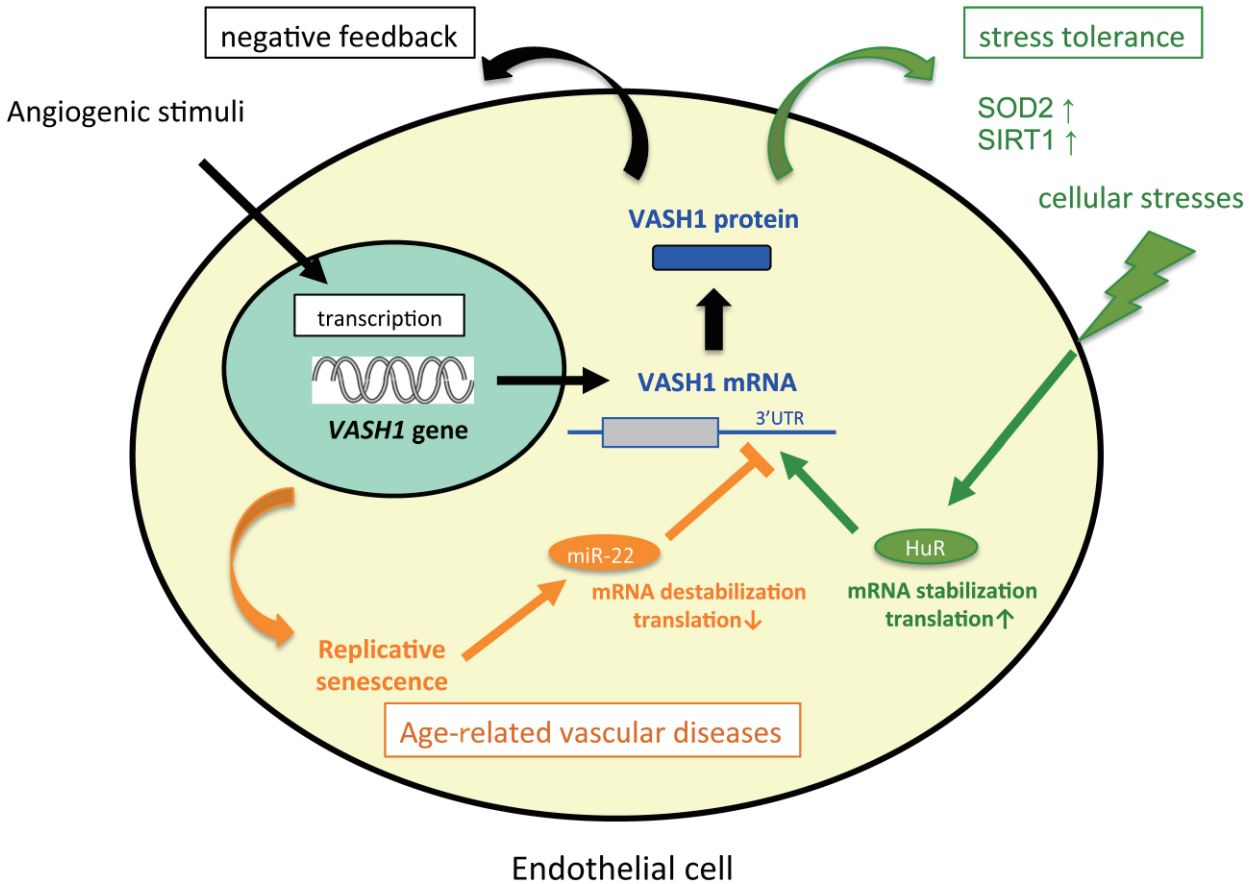

Fig. 1. Regulation of VASH1 expression in EC

VASH1 is induced transcriptionally by angiogenic stimuli for angiogenesis inhibition or post-transcriptionally by RNA binding protein HuR for stress tolerance of ECs, and these functions are critical for the maintenance of vascular homeostasis. VASH1 is downregulated with replicative senescence of ECs by the increase of miR-22, and this age-associated downregulation of VASH1 might be a risk of deterioration of vascular homeostasis and age-related vascular diseases.

RNAs in old HUVECs (49 population doublings) and young HUVECs (5 population doublings) were compared by performing microarray analysis[10].

MicroRNAs are small non-coding RNAs, which regulate gene expression at the post-transcriptional level via target mRNA degradation and/or translational suppression via binding to the 3′-untranslated region (3′-UTR)[18]. It is estimated that approximately one-third of all animal genes are controlled by microRNAs, and thus microRNAs exhibit a wide range of biological processes including aging[19]. Indeed, increasing evidences suggest that changes in the expression profile of microRNAs contribute to cellular senescence, aging and aging-related diseases, and several studies were directed to examine the relationship between certain microRNAs and cardiovascular aging[20, 21]. Our analysis revealed that among the top 20 microRNAs that were expressed at a higher level in old HUVECs, the third highest microRNA, namely, miR-22-3p, had its binding site on the 3′-UTR of VASH1 mRNA. Experiments with microRNA mimic and anti-miR revealed that miR-22-3p was involved in the downregulation of VASH1 in ECs during replicative senescence[10]. MiR-22 is previously reported to promote senescence of endothelial progenitor cells by targeting AKT3[22]. We added VASH1 to the list of miR-22 targets that were downregulated with replicative senescence of ECs, and that might be responsive to aging-associated vascular diseases (**Fig. 1**).

The Decrease of *Vash1* Gene for Healthy Longevity

Since VASH1 has a characteristic of promoting stress tolerance in ECs, we speculated that the lack of the *Vash1* gene should result in a short lifespan. However, to our surprise, *Vash1* KO mice lived significantly longer with a milder senescence phenotype than wild-

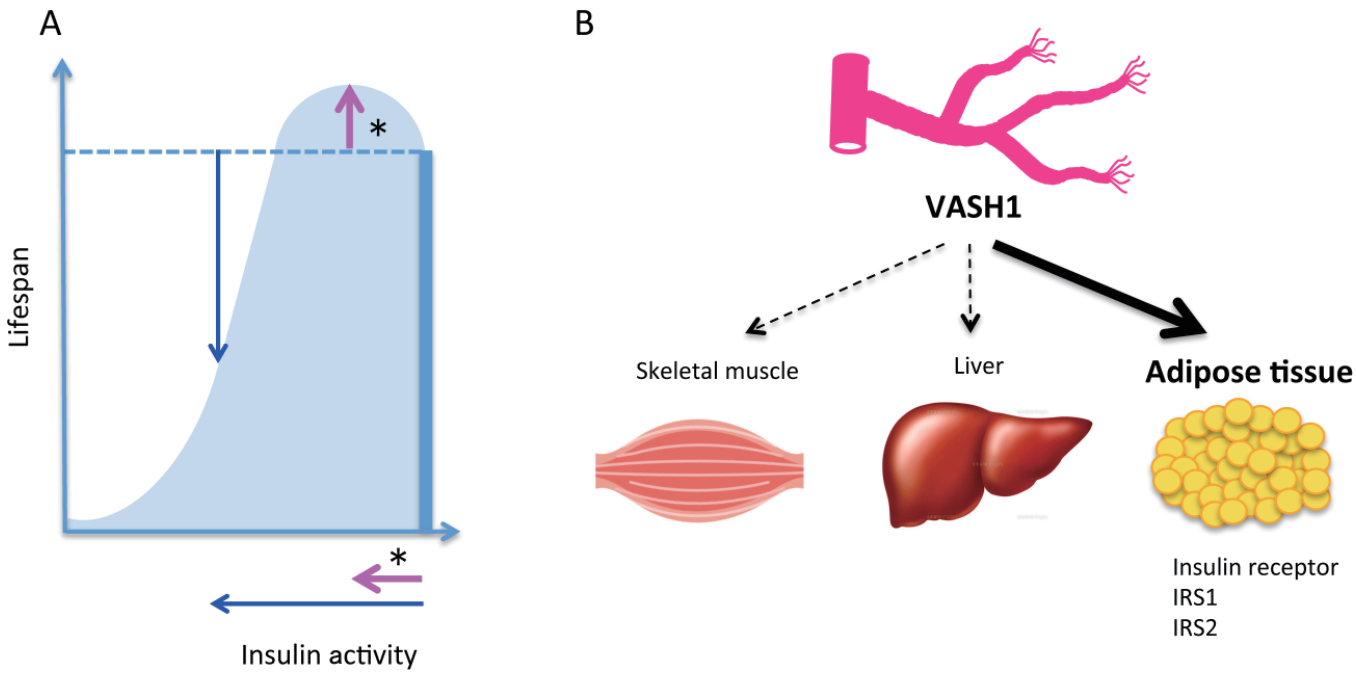

Fig. 2. The decrease of *Vash1* gene for healthy longevity

A: Reduced insulin signaling can increase longevity, but its profound reduction can result in diabetes and short life. The lack of *Vash1* gene caused mild insulin resistance without the outbreak of overt diabetes and that might contribute to healthy longevity (asterisks). B: Skeletal muscle, liver, and adipose tissue are three major target organs of insulin for glucose homeostasis. The expression of Vash1 dominated in the vasculature distributed to WAT among three major target organs of insulin, and the expression of Vash1 is involved in the regulation of the expression of Insr, IRS1, and IRS2.

type mice[23].

Numerous studies have being focusing on the mechanism of aging, and so far they have revealed certain genes and signaling pathways that contribute to the regulation of the lifespan[24]. Among them, insulin signaling plays a crucial and evolutionally conserved role in longevity[25]. Decreased insulin signaling for longevity was first demonstrated in *Caenorhabditis elegans*, next *Drosophila*, and further confirmed in mammals[26-28]. However, as insulin signaling is essential for glucose homeostasis, this decreased insulin signaling should cause diabetes mellitus. So-called insulin paradox has raised a challenging question as to how the preserved insulin signaling for glucose homeostasis and the reduced insulin signaling for longevity can be balanced[29].

Skeletal muscle, liver, and adipose tissue are three major target organs of insulin for glucose homeostasis. We sought the cause of healthy longevity of *Vash1* KO mice and found that *Vash1* KO mice exhibited mild insulin resistance along with reduced expression of the insulin receptor (Insr), insulin receptor substrate 1 (Irs1), and insulin receptor substrate 2 (Irs2) in their white adipose tissue (WAT) but not in their liver or skeletal muscle. The expression of Vash1 dominated in the WAT among those three organs of WT mice, and its expression was selective in ECs. Importantly, *Vash1* KO mice never developed diabetes when fed a high-fat diet[23]. This is the first demonstration that endothelial cell-expression of Vash1 in adipose tissue is required for the normal expression of Insr, Irs1, and Irs2, and insulin sensitivity of the WAT (**Fig. 2**). Although the decrease of Vash1 may be responsive to aging-associated vascular diseases, which also causes mild insulin resistance without the outbreak of overt diabetes and that may contribute to healthy longevity.

Concluding Remarks

This mini review summarized a molecular basis for vascular homeostasis and healthy longevity regulated by VASH1. VASH1 is a negative-feedback regulator of angiogenesis synthesized by ECs, which also increases stress tolerance of ECs. Besides its transcriptional induction by angiogenic stimuli, the synthesis of VASH1 receives post-transcriptional regulation. Cellular stresses increase the synthesis of VASH1 via HuR, whereas replicative senescence of ECs decreases the synthesis of VASH1 via an increase of senescence-associated microRNA, namely miR-22. As a decrease

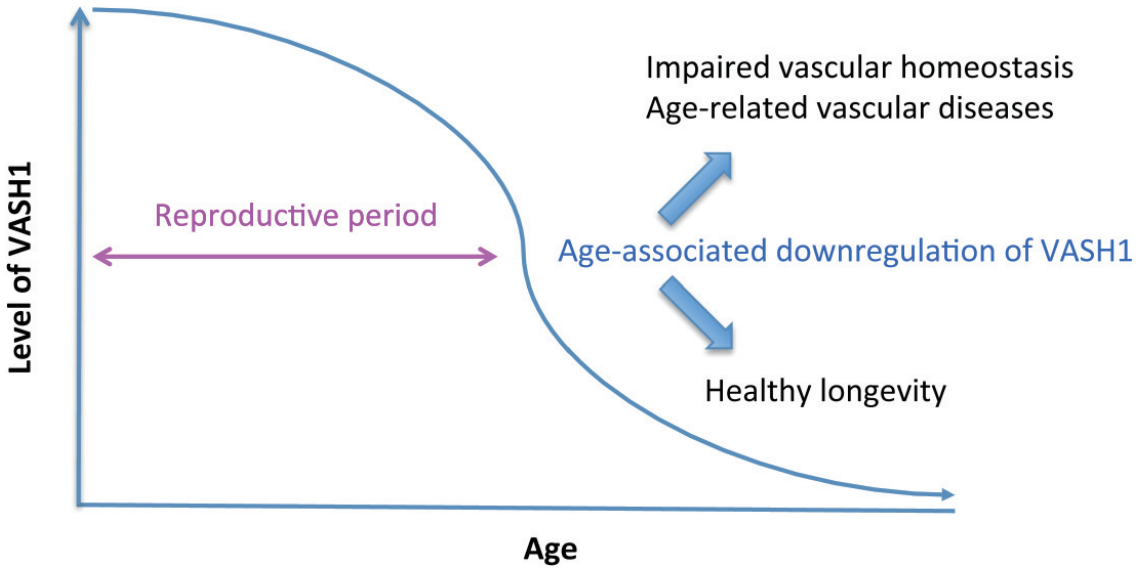

Fig. 3. Double-face of VASH1 for the maintenance of vascular homeostasis and healthy longevity

The expression of VASH1 is maintained in reproductive period but downregulated during aging, and this age-associated downregulation of VASH1 might be a risk of deterioration of vascular homeostasis and age-related vascular diseases. VASH1 has an additional role that influences the insulin signaling, and another face of age-associated downregulation of VASH1 might be for healthy longevity.

in VASH1 expression makes the vasculature vulnerable to cellular stresses, we were puzzled and wondered why nature would allow a decrease in the expression of such a valuable protein with aging. We then found that the lack of *Vash1* gene resulted in health longevity in mice. In accordance with this observation, we now propose the novel role of VASH1 that influences the insulin signaling, and that one purpose of age-associated downregulation of VASH1 might be to afford healthy longevity (**Fig. 3**).

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Nothing to declare.

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