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Invited commentary

Vitamin D puts the brakes on angiotensin II-induced oxidative stress and vascular smooth muscle cell senescence

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ABSTRACT

Signaling via both vitamin D (VitD) and the renin-angiotensin system (RAS) plays important roles in physiological processes. Evidence has mounted linking cardiovascular disease to both increased activity of the RAS and VitD deficiency. Although several studies have established functional relationships between the RAS and VitD, many aspects of their complex interaction remain unknown. In this issue of Atherosclerosis, Valcheva and colleagues show that defective VitD signaling can promote vascular damage by inducing premature senescence of smooth muscle cells due to elevated local production of angiotensin II and reactive oxygen species, and upregulation of the tumor suppressor $p57^{Kip2}$.

The main function of the renin-angiotensin system (RAS) of mammals is to regulate blood pressure and electrolyte balance, and dysregulated activation of the RAS has been linked to the pathogenesis of different cardiovascular alterations, including hypertension, atherosclerosis, restenosis post-angioplasty, aortic aneurysm, heart attack, and hypertrophy of the left ventricle and vascular smooth muscle cells (VSMCs) [1,2]. Key components of the RAS are renin and the renin-like enzyme cathepsin D (CatD), which cleave angiotensinogen to produce angiotensin I (AngI); angiotensin converting enzyme (ACE), the carboxydipetidase that converts AngI into AngII, primarily in the lung; and AngII receptors [1,2]. Adverse cardiovascular events have also been linked to deficiency of vitamin D (VitD) leading to reduced signaling through its receptor VDR, a member of the nuclear receptor superfamily that mediates the action of the active form of $VitD_3$ (1,25-dihydroxyvitamin D_3, also called calcitriol) [3]. In addition to their implications in cardiovascular disease, both vitamin D/VDR and the RAS play important roles in physiological processes [2,4].

The tissue distributions of VitD receptors and the RAS overlap almost exactly, and previous studies established functional relationships between the RAS and VitD/VDR systems [5]. For example, the plasma VitD level correlates inversely with plasma renin activity and blood pressure [6–8], and VitD supplementation can reduce blood pressure in hypertensive patients [9,10]. The inverse relation between vitamin D and activity of the RAS could be at least partly explained by VitD/VDR–dependent suppression of renin transcription [11,12]. In agreement with these findings, renin expression and plasma AngII production are elevated in VDR-null mice, correlating with the development of hypertension and cardiac hypertrophy and above-normal water intake independently of effects on the levels of blood calcium or parathyroid hormone [12]. Recent studies have improved our understanding of the crosstalk between VitD and the RAS, but many aspects of their complex interaction remain unknown [5].

In a previous issue of *Atherosclerosis*, Valcheva et al. [13] add new pieces to the puzzle of the VitD-RAS relationship (Fig. 1). By analyzing primary cultures of wild-type and VDR-null VSMCs, they demonstrate that VDR deficiency enhances production of AngII and reactive oxygen species (ROS), leading to premature cell senescence; these are characteristic features of the pathological vascular remodeling that occurs during atherosclerosis, hypertension and aging [14–16]. The authors first examined primary VSMCs for the expression of several factors involved in AngII-mediated signaling. They could not detect renin expression in wild-type and VDR-null VSMCs; however mutant VSMCs expressed high mRNA and protein levels of both the renin-like acid protease CatD and AngII type 1 receptor (AT1), and this correlated with a higher AngII level in the culture medium. Fukuda et al. were similarly unable to detect renin

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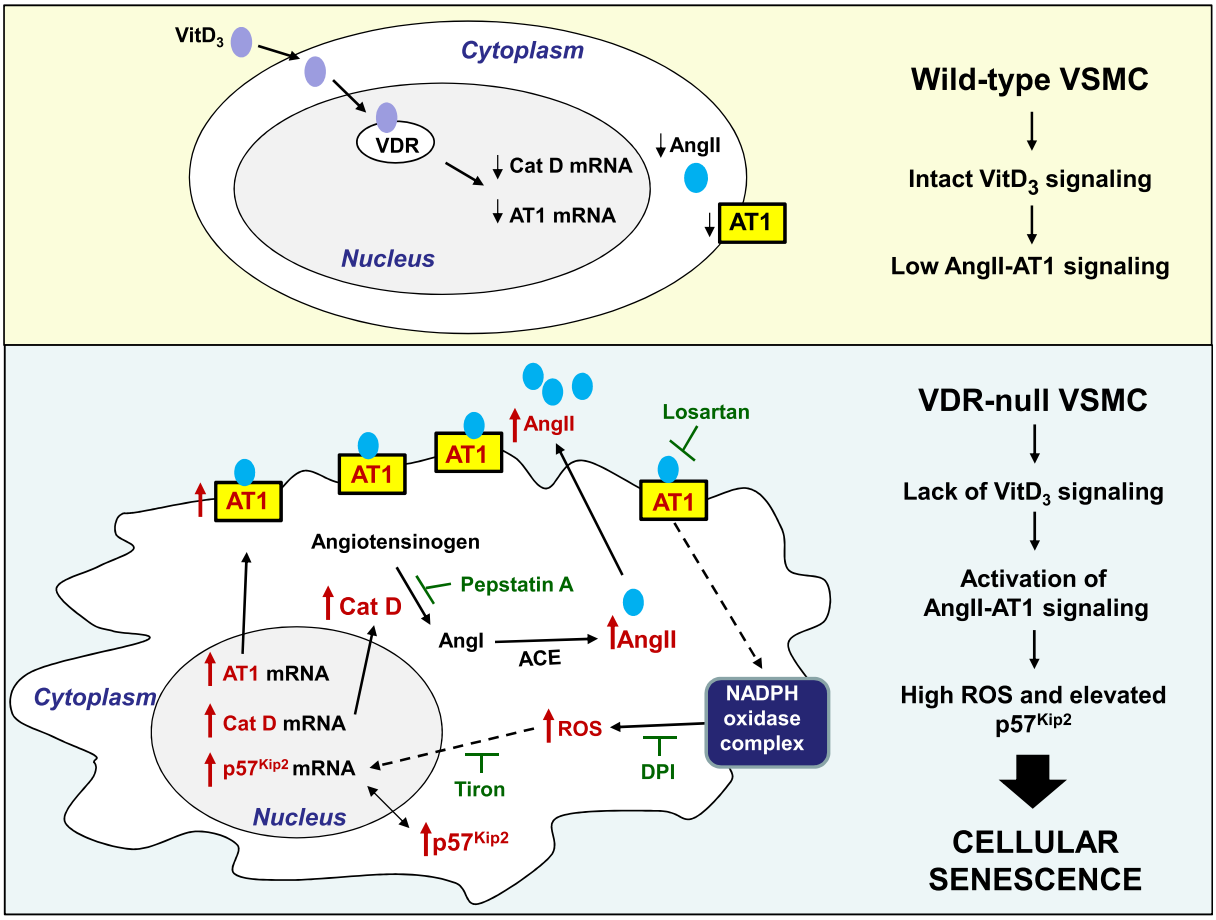

Fig. 1. Model of AngII-dependent induction of premature VSMC senescence upon disruption of VDR. The model is based on the results reported in this issue of *Atherosclerosis* by Valcheva et al. [13]. Primary cultures of wild-type VSMCs *(top)*, with intact VitD/VDR signaling, exhibit repressed transcription of AngII type 1 receptor (AT1) and cathepsin D (CatD, which converts angiotensinogen into AngI), and low production of AngII. In contrast, primary VDR-null VSMCs *(bottom)* have high expression of CatD and AT1 mRNA and protein and elevated AngII production. This is accompanied by activation of NADPH oxidase and high ROS production leading to elevated $p57^{Kip2}$ mRNA and protein levels and the premature onset of VSMC senescence. Red text indicates factors that are upregulated in VDR-null VSMCs and green text drugs used by Valcheva and colleagues to block different steps in the pathways that link VDR deficiency to AngII-AT1-induced ROS production and VSMC senescence. Higher expression levels of the AngII signaling components CatD and AT1 and the growth suppressor $p57^{Kip2}$ were also observed in the aorta of VDR-null mice.

in rat VSMCs and found that higher production of AngII in VSMCs from spontaneously hypertensive rats was due to increased CatD expression [17]. Collectively, the observations by Valcheva et al. suggest that VDR deficiency in VSMCs causes local RAS activation due at least partly to induction of CatD and AT1. VitD suppresses renin gene transcription by blocking the activity of the cyclic-AMP-response element (CRE) directly in the renin gene promoter [11]. Since the CatD promoter contains a putative CRE [18], the authors suggest that CatD may be subject to the same inhibitory regulation by VitD as renin. Testing this and other possible pathways is necessary in order to understand how VitD regulates CatD and AT1 expression, which may reveal new therapeutic targets.

Cell senescence is activated upon tissue damage in diverse pathological contexts in response to different stimuli, such as DNA damage and telomere loss, derepression of *CDKN2a*, oncogenic signaling, inactivation of tumor suppressors, and accumulation of ROS [19]. Bearing in mind that systemic RAS activation augments ROS generation and VSMC senescence [15,16], Valcheva and colleagues next examined the intracellular production of superoxide anions and the activity of senescence-associated-β-galactosidase (SA-β-GAL), the most widely used assay for cell senescence. Compared with wild-type cells, VDR-null VSMCs had higher levels of intracellular superoxide anion, and this was accompanied by a significant accumulation of cells with SA-β-GAL activity. This excess of ROS could be decreased by treatment with either pepstatin A (an inhibitor of aspartyl proteases, including CatD), losartan (an AT1 antagonist), or diphenyleneiodonium (an NADPH oxidase inhibitor), suggesting that the increment in VSMC free radical content induced by the lack of VitD is caused by elevated AngII/AT1-dependent activation of NADH oxidase. Importantly, treatment of VDR-null VSMCs with pepstatin A and the AT1 antagonist losartan over a period of 14 days in culture also prevented the accumulation of SA-β-GAL. Of note in this regard, increased VSMC senescence in low-density lipoprotein receptor-null mice lacking VDR is associated with augmented formation of atherosclerotic plaques, and inhibition of renin by aliskiren reduces atherosclerosis burden in these double-mutant mice, consistent with the idea that the RAS aggravates atherosclerosis in the absence of VDR at least partly by promoting cellular senescence [20].

During active tissue repair, senescent cells arrest their own proliferation [19]. Using growth curve studies and cell-cycle analysis, Valcheva and colleagues found below normal proliferation