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 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 this issue of *Atherosclerosis*, Valcheva et al. [13] add new pieces to the puzzle of the VitD-RAS relationship. 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 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 rates in VDR-null VSMCs, while the proportion of dead cells was the same as in wild-type controls. Consistent with the observation that AngII enhances VSMC growth through AT1 receptor signaling [1], treatment with losartan significantly inhibited the proliferation of wild-type VSMCs. In contrast, in VDR-null VSMC cultures losartan inhibited SA-β-GAL activity and increased proliferation, in

agreement with the notion that VitD deficiency promotes senescence in VSMCs via increased AngII/AT1-dependent signaling. A key process in the regeneration of damaged tissues is the clearance of senescent cells through the recruitment of phagocytic immune cells [19]. Whether senescent VSMCs lacking VDR have an impaired capacity to induce their own elimination as a result of elevated AngII production and reduced recruitment of immune cells is an intriguing possibility that deserves further investigation.

Valcheva and colleagues went on to elucidate the mechanisms underlying cell-cycle arrest and senescence in VDR-null VSMCs, and found significantly elevated expression of $p57^{Kip2}$, a cyclin-dependent kinase inhibitor capable of inhibiting the growth of healthy cells and cancer cells [21]. The protection against senescence in VDR-null VSMCs achieved by pharmacological inhibition of CatD and AT1 was accompanied by reduced levels of $p57^{Kip2}$ mRNA. These results suggest that higher expression of the cell-cycle inhibitor $p57^{Kip2}$ is implicated in the induction of growth arrest and senescence in VSMCs with defective VitD signaling. Additional studies are warranted to examine the mechanisms regulating $p57^{Kip2}$ in this context, and to assess whether VitD signaling affects the expression or activity of other tumor suppressors known to promote cellular senescence, such as $p16^{Ink4a}$, ARF, p53, $p21^{Cip1}$, $p15^{Ink4a}$, $p27^{Kip1}$ and hypophosphorylated retinoblastoma protein [19]. Particularly interesting candidates are $p21^{Cip1}$, which is required for AngII-induced premature senescence of VSMCs and atherosclerosis development [15], and $p27^{Kip1}$, an important regulator of VSMC phenotype and vascular occlusive lesion development [22] that is modulated by both AngII [23, 24] and VitD [25, 26]. Two other notable observations are that AngII accelerates telomere attrition and induces DNA damage in human VSMCs via AT1 receptor-mediated induction of ROS [27] and that, conversely, treatment with vitamin D reduces oxidative stress damage and chromosomal aberrations and prevents telomere shortening in animal models and a variety of cell types [28, 29]. In light of these observations, future studies are warranted to examine whether the crosstalk between VitD/VDR and the RAS involves the regulation of key components of the DNA damage response and telomere function.

Valcheva and colleagues confirmed some of their in vitro findings by demonstrating higher CatD, AT1 and $p57^{Kip2}$ protein levels in aortic tissue of 6-month-old VDR-null mice, as assessed by western blot and immunohistochemical analysis. Although the results of these mouse studies are encouraging, additional work is needed to ascertain whether defective VitD signaling leads to increased local production of AngII and ROS and accumulation of senescent VSMCs in the artery wall. Since the aged vasculature is characterized by increased AngII and ROS production and the accumulation of senescent cells [16], studies are warranted in older wild-type and

VDR-null mice (18-24 months old), which may reveal an age-associated exacerbation of pathological responses in the absence of VitD signaling.

Valcheva and colleagues' study shows for the first time that defective VitD signaling can promote vascular damage by inducing premature VSMC senescence as a result of elevated local production of AngII and ROS and upregulation of the tumor suppressor $p57^{Kip2}$. As recognized by the authors, it is difficult to extrapolate the results of cellular and animal models to humans; however, their findings uncover new regulatory circuits which might be important in a range of degenerative diseases that are reciprocally affected by VitD/VDR and the RAS, and that therefore might benefit from combined VitD supplementation and RAS inactivation, such as cardiovascular and renal disease, obesity, and diabetes.

CONFLICT OF INTEREST

The author reports no relationships that could be construed as a conflict of interest.

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REFERENCES

[1] Touyz, RM and Schiffrin, EL. Signal transduction mechanisms mediating the physiological and pathophysiological actions of angiotensin II in vascular smooth muscle cells. Pharmacol Rev 2000;52:639-72.

[2] Crowley, SD and Coffman, TM. Recent advances involving the renin-angiotensin system. Exp Cell Res 2012;318:1049-56.

[3] Wang, TJ, Pencina, MJ, Booth, SL, Jacques, PF, Ingelsson, E, Lanier, K, et al. Vitamin D deficiency and risk of cardiovascular disease. Circulation 2008;117:503-11.

[4] Goltzman, D, Hendy, GN and White, JH. Vitamin D and its receptor during late development. Biochim Biophys Acta 2014;(in press).

[5] Ferder, M, Inserra, F, Manucha, W and Ferder, L. The world pandemic of vitamin D deficiency could possibly be explained by cellular inflammatory response activity induced by the renin-angiotensin system. Am J Physiol Cell Physiol 2013;304:C1027-39.

[6] Burgess, ED, Hawkins, RG and Watanabe, M. Interaction of 1,25-dihydroxyvitamin D and plasma renin activity in high renin essential hypertension. Am J Hypertens 1990;3:903-5.

[7] Kristal-Boneh, E, Froom, P, Harari, G and Ribak, J. Association of calcitriol and blood pressure in normotensive men. Hypertension 1997;30:1289-94.

[8] Lind, L, Hanni, A, Lithell, H, Hvarfner, A, Sorensen, OH and Ljunghall, S. Vitamin D is related to blood pressure and other cardiovascular risk factors in middle-aged men. Am J Hypertens 1995;8:894-901.

[9] Lind, L, Wengle, B, Wide, L and Ljunghall, S. Reduction of blood pressure during long-term treatment with active vitamin D (alphacalcidol) is dependent on plasma renin activity and calcium status. A double-blind, placebo-controlled study. Am J Hypertens 1989;2:20-5.

[10] Pfeifer, M, Begerow, B, Minne, HW, Nachtigall, D and Hansen, C. Effects of a short-term vitamin D(3) and calcium supplementation on blood pressure and parathyroid hormone levels in elderly women. J Clin Endocrinol Metab 2001;86:1633-7.

[11] Yuan, W, Pan, W, Kong, J, Zheng, W, Szeto, FL, Wong, KE, et al. 1,25-dihydroxyvitamin D3 suppresses renin gene transcription by blocking the activity of the cyclic AMP response element in the renin gene promoter. J Biol Chem 2007;282:29821-30.

[12] Li, YC, Kong, J, Wei, M, Chen, ZF, Liu, SQ and Cao, LP. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. J Clin Invest 2002;110:229-38.

[13] Valcheva, P, Cardus, A, Panizo, S, Parisi, E, Bozic, M, Lopez Novoa, JM, et al. Lack of vitamin D receptor causes stress-induced premature senescence in vascular smooth muscle cells through enhanced local angiotensin-II signals. Atherosclerosis 2014;235:247-55.

[14] Fuster, JJ, Diez, J and Andres, V. Telomere dysfunction in hypertension. J Hypertens 2007;25:2185-92.

[15] Kunieda, T, Minamino, T, Nishi, J, Tateno, K, Oyama, T, Katsuno, T, et al. Angiotensin II induces premature senescence of vascular smooth muscle cells and accelerates the development of atherosclerosis via a p21-dependent pathway. Circulation 2006;114:953-60.

[16] Wang, M, Monticone, RE and Lakatta, EG. Arterial aging: a journey into subclinical arterial disease. Curr Opin Nephrol Hypertens 2010;19:201-7.

[17] Fukuda, N, Satoh, C, Hu, WY, Soma, M, Kubo, A, Kishioka, H, et al. Production of angiotensin II by homogeneous cultures of vascular smooth muscle cells from spontaneously hypertensive rats. Arterioscler Thromb Vasc Biol 1999;19:1210-7.

[18] Hetman, M, Perschl, A, Saftig, P, Von Figura, K and Peters, C. Mouse cathepsin D gene: molecular organization, characterization of the promoter, and chromosomal localization. DNA Cell Biol 1994;13:419-27.

[19] Muñoz-Espín, D and Serrano, M. Cellular senescence: from physiology to pathology. Nat Rev Mol Cell Biol 2014;15:482-96.

[20] Szeto, FL, Reardon, CA, Yoon, D, Wang, Y, Wong, KE, Chen, Y, et al. Vitamin D receptor signaling inhibits atherosclerosis in mice. Mol Endocrinol 2012;26:1091-101.

[21] Tesio, M and Trumpp, A. Breaking the cell cycle of HSCs by p57 and friends. Cell Stem Cell 2011;9:187-92.

[22] Andrés, V and Castro, C. Antiproliferative strategies for the treatment of vascular proliferative disease. Curr Vasc Pharmacol 2003;1:85-98.

[23] Braun-Dullaeus, RC, Mann, MJ, Ziegler, A, von der Leyen, HE and Dzau, VJ. A novel role for the cyclin-dependent kinase inhibitor p27(Kip1) in angiotensin II-stimulated vascular smooth muscle cell hypertrophy. J Clin Invest 1999;104:815-23.

[24] Servant, MJ, Coulombe, P, Turgeon, B and Meloche, S. Differential regulation of p27(Kip1) expression by mitogenic and hypertrophic factors: Involvement of transcriptional and posttranscriptional mechanisms. J Cell Biol 2000;148:543-56.

[25] Chiang, KC and Chen, TC. Vitamin D for the prevention and treatment of pancreatic cancer. World J Gastroenterol 2009;15:3349-54.

[26] Johnson, CS, Muindi, JR, Hershberger, PA and Trump, DL. The antitumor efficacy of calcitriol: preclinical studies. Anticancer Res 2006;26:2543-9.

[27] Herbert, KE, Mistry, Y, Hastings, R, Poolman, T, Niklason, L and Williams, B. Angiotensin II-mediated oxidative DNA damage accelerates cellular senescence in cultured human vascular smooth muscle cells via telomere-dependent and independent pathways. Circ Res 2008;102:201-8.

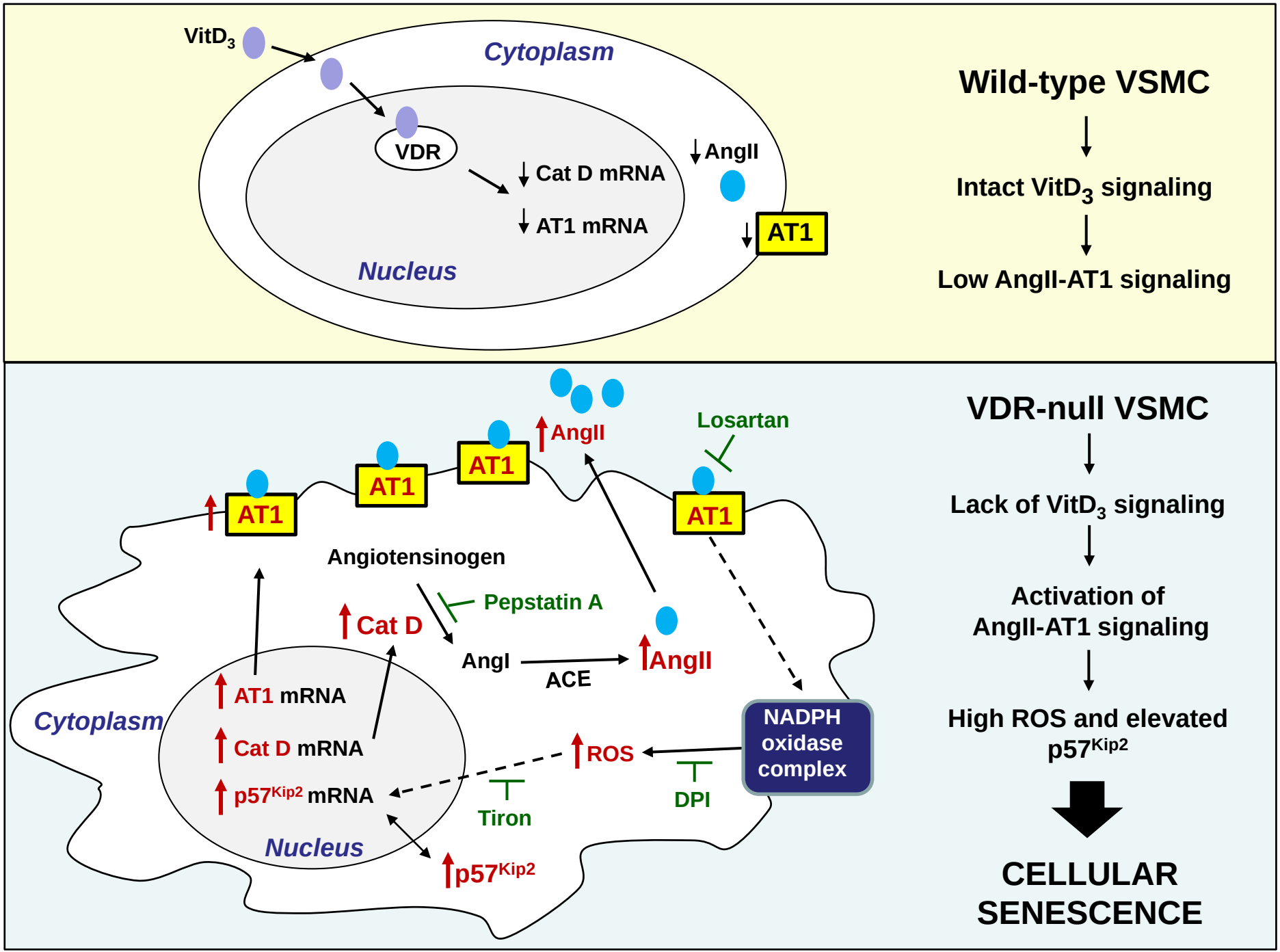
[28] Dixon, KM, Sequeira, VB, Camp, AJ and Mason, RS. Vitamin D-fence. Photochem Photobiol Sci 2010;9:564-70.

[29] Nair-Shalliker, V, Armstrong, BK and Fenech, M. Does vitamin D protect against DNA damage? Mutat Res 2012;733:50-7.

FIGURE LEGEND

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.

FIGURE 1


VitD3
Cytoplasm
VDR
Cat D mRNA
AT1 mRNA
AngII
AT1
Nucleus
Wild-type VSMC
Intact VitD3 signaling
Low AngII-AT1 signaling
AT1
AT1
AT1
AngII
Losartan
AT1
Angiotensinogen
Pepstatin A
Cat D
AngI
ACE
AngII
AT1 mRNA
Cat D mRNA
p57Kip2 mRNA
Cytoplasm
Nucleus
ROS
NADPH oxidase complex
Tiron
DPI
p57Kip2
VDR-null VSMC
Lack of VitD3 signaling
Activation of AngII-AT1 signaling
High ROS and elevated p57Kip2
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