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Autophagy as a basis for the health-promoting effects of vitamin D

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Autophagy is an evolutionarily conserved lysosomal self-digestion process essential for cellular homeostasis, differentiation and survival. As an adaptive response, it protects organisms against a wide range of pathologies, including cancer, infection, neurodegeneration, heart disease and ageing. Thus, compounds activating autophagy could have great potential in the prevention of common diseases. Interestingly, recent data link autophagy to two functions of the active form of vitamin D (VD): the induction of cancer cell death and the clearance of *Mycobacterium tuberculosis* in macrophages. Because VD deficiency is associated with many pathologies resembling those induced by defective autophagy, it is tempting to speculate that autophagy plays a more general role in the multiple health-promoting effects of VD.

VD and human health

The essential physiological effects of VD on calcium/phosphate homeostasis were established over 100 years ago when dietary VD deficiency was first recognized. Over the past two decades, more subtle studies employing the technologies of cellular and molecular biology as well as genetically modified mice have revealed that the active form of VD, 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$), functions as a pleiotropic hormone that controls gene expression in many different cell types in the human body, thereby regulating their proliferation, differentiation and survival [1–5]. The numerous health-promoting actions of 1α,25-$(OH)_2D_3$ in various experimental model systems together with accumulating epidemiological data support claims that 1α,25-$(OH)_2D_3$ and its synthetic analogs (hereafter referred to as VD compounds) are useful in the prevention or treatment of not only calcium-related disorders (*e.g.* rickets, osteomalacia, osteoporosis and hyperparathyroidism), but also a plethora of other diseases including psoriasis, certain types of cancers as well as infectious (*e.g.* tuberculosis and chronic hepatitis), cardiovascular and neurological diseases [6] (Table 1). Although many of the genes regulated by 1α,25-$(OH)_2D_3$ have been identified, the molecular bases of its health-promoting actions are only beginning to emerge. Interestingly, recent data have linked the activation of autophagy to the ability of 1α,25-$(OH)_2D_3$ to kill cancer cells and enhance the clearance of *M. tuberculosis* from cultured macrophages [7–12]. In this article, we will review this newly identified mechanism of action of 1α,25-$(OH)_2D_3$ and discuss its implications for the further development of VD- and autophagy-based therapies.

VD – a precursor of a multifunctional hormone

VD refers to a group of fat-soluble prohormones, the two major forms of which are ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3) [2,5]. Cholecalciferol is produced in response to UVB light (295–300 nm) from 7-dihydrocholesterol in the skin of most vertebrates including humans, whereas a similar photochemical reaction converts ergosterol to ergocalciferol in vertebrates, plants and fungi. Additionally, calciferols can be obtained via digestion from food (*e.g.* cod liver oil and fatty fish) and vitamin supplements. Calciferols produced in the skin or consumed in food are biologically inert and must undergo two hydroxylation reactions in the body to be converted to their active forms. First, enzymes with 25-hydroxylation activity in hepatocytes (*e.g.* CYP27A1) convert cholecalciferol to 25-hydroxyvitamin D_3 (25$(OH)D_3$ or calcidiol), which is further hydroxylated by 1α,25-$(OH)_2D_3$ 1α-hydroxylase (CYP27B1) to form 1α,25-dihydroxyvitamin D_3 (1α,25-$(OH)_2D_3$ or calcitriol), the active form of VD. Finally, the excess 1α,25-$(OH)2D_3$ can be inactivated by 24-hydroxylation mediated by 1α,25-$(OH)_2D_3$ 24-hydroxylase (CYP24A1). The final activation and inactivation occur mainly in the proximal tubules of the kidney, but can also take place in extra-renal sites.

Once released into circulation, 1α,25-$(OH)_2D_3$ binds to the VD-binding protein (VDBP) and is transported to the target tissues throughout the body in an endocrine fashion. When released from the VDBP, it diffuses through the cell membrane and binds with high affinity to the VD receptor (VDR) that is expressed in most tissues [2]. The ligand–receptor complex dimerizes with the retinoid X receptor, and the resulting heterodimer functions as a potent transcriptional regulator. 1,25-$(OH)_2D_3$ enhances or represses the expression of approximately 900 different genes [13].

Most circulating 1α,25-$(OH)_2D_3$ is produced in the kidneys in response to parathyroid hormone (PTH) and controls the proper maintenance of calcium and phosphorus levels in the blood by acting in the gastrointestinal tract, kidney, bone and thyroid gland [2,5]. 1α,25-$(OH)_2D_3$ can also be produced in at least 10 extra-renal tissues including the immune system, skin, breast, prostate, brain, colon and endothelium [1,2,14], where it exerts multiple para- and autocrine effects, many of which are associated with the prevention of disease (Table 1). Extra-renal 1α,25-$(OH)_2D_3$

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Table 1. Actual and possible clinical uses of vitamin D and analogs

Disease	Suggested beneficial effects	Reference	Status[a]
Decalcifying skeletal diseases and diseases related to PTH	Increases intestinal absorption of Ca^{2+}	[2]	Approved
	Lowers PTH levels		
	Inhibits skeletal decalcification		
Cardiovascular diseases	Inhibition of atherosclerotic calcification	[64,65]	Phase I–IV
	Control of blood pressure via the regulation of the renin–angiotensin system		
	Inhibition of myocardial hypertrophy		
Infectious diseases	Induction of antimicrobial peptides	[13]	Approved
	Induction of cathelicidin and ß-defensin in keratinocytes		
	Enhanced clearance of *M. tuberculosis*		
Autoimmune diseases	Inhibition of IL-12, IFN-γ and TNFα	[13,66]	Phase I–IV
	Suppression of T helper lymphocytes (Th1)		
	Inhibition of B-lymphocyte differentiation		
	Activation of regulatory T lymphocytes and NK cells		
Diabetes Mellitus	Inhibition of autoimmune attack on pancreatic ß-cells	[67]	Phase I–IV
	Enhanced insulin production and sensitivity		
Psoriasis	Differentiation and growth inhibition of keratinocytes	[68,69]	Approved
Cancer	Differentiation	[20,70–73]	Phase I–IV
	Inhibition of growth and metastasis		
	Induction of cell death and sensitization to other therapies		
	Abnormal angiogenesis		
Pregnancy complications	Lowers the risk of gestational diabetes, premature birth and preeclampsia	[74,75]	Phase I–IV
	Prevents impaired developmental of the nervous system		
	Lowers the risk of caesarian section		
Neurological diseases	Lowers the risk of Alzheimer's disease, Parkinson's disease, multiple sclerosis, schizophrenia, seasonal affective disorder and premature ageing of the CNS	[76]	Phase I–IV

[a]Status of clinical development according to www.clinicaltrials.org and [77].

synthesis is not regulated by PTH and is not believed to contribute to the systemic effects of VD. Instead, it is controlled by local factors regulating the expression of VDR, CYP27B1 and CYP24A1, and by the level of circulating $25(OH)D_3$, which has to be present in excess to effectively trigger the extra-renal VD responses involved in the prevention of disease. It should be noted that the current recommendation for serum $25(OH)D_3$ level (>75 nM) is solely based on the levels needed for proper calcium homeostasis, whereas the levels needed to trigger health-promoting autocrine and paracrine functions are largely unknown and could be considerably higher.

Autophagy in disease prevention

Macroautophagy (in this review referred to as autophagy) is an evolutionary conserved catabolic process whereby cells degrade cytosolic macromolecules and organelles in the lysosome [15,16] (Figure 1). The housekeeping function of autophagy in the recycling of amino and fatty acids, sugars and nucleotides becomes especially essential for the maintenance of cellular energy levels and cell survival during periods of metabolic stress [17,18]. Furthermore, autophagy serves a protective function by removing damaged proteins and organelles as well as intracellular pathogens that are potentially dangerous to the cell. In line with this, autophagy has been assigned numerous health-promoting functions that share remarkable overlap with the extra-renal activities of VD compounds (*i.e.* tumor suppression, antimicrobial defense, longevity, inhibition of neurodegenerative diseases, cardiac hypertrophy and atherosclerosis as well as increased sensitivity to insulin) [15,16,19–21]. Finally, although VD compounds

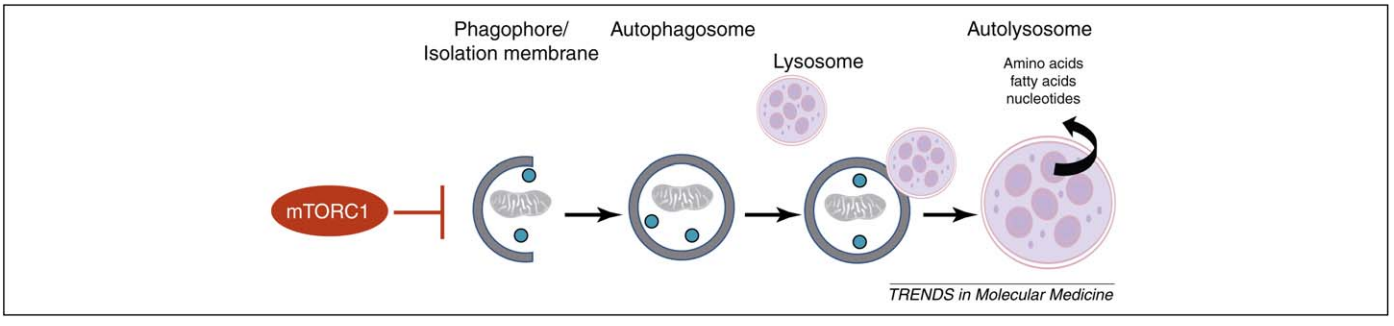

Figure 1. Schematic presentation of the autophagic process.
Autophagy is a catabolic process whereby cells degrade cytosolic proteins or organelles in the lysosome. When mTORC1 is inhibited, a flat cytosolic membrane cistern called a phagophore (or isolation membrane) forms. It wraps around cytoplasmic organelles and/or a portion of cytosol (mitochondria). The membrane elongates until the edges fuse, forming a double-membrane structure called an autophagosome. The autophagosome matures in a stepwise process, involving fusion with endolysosomal vesicles. The final degradation step takes place within the autolysosomes, where lysosomal hydrolases digest the luminal content of the autophagic vesicle to recycle breakdown products (nucleotides, fatty and amino acids) that are released and can be reused by the cell.

Table 2. Autophagy induction by VD compounds

Cells (cell line)	Treatment	Autophagy is inhibited by:	Outcome	[Ref.]
Human breast carcinoma (MCF-7)	1,α25-$(OH)_2D_3$ and EB1089 -100 nM; 72 h	siRNA-mediated depletion of CaMKK-ß, beclin 1 or Atg7 Ectopic expression of ER-localized Bcl-2 [a]Treatment with Bapta-AM, STO-609, 3MA and compound C	Autophagic cell death	[7,8]
Human head and neck squamous cell carcinoma (SCC25)	1,α25-$(OH)_2D_3$ -100 nM; 48 h	Cyclin-dependent kinase inhibitor $p19^{INK4D}$, whose expression is induced by 1,α25-$(OH)_2D_3$	Autophagic cell death	[9]
Human myeloid leukemia (HL-60)	1,α25-$(OH)_2D_3$ -5 nM; 72 h	siRNA-mediated depletion of beclin-1 or class III phosphatidyl-inositol 3-kinase catalytic subunit	Autophagic cell death	[11]
Human primary monocytes/ macrophages	1,α25-$(OH)_2D_3$ -0.1–20 nM; 24 h	siRNA-mediated depletion of cathelicidin	Enhanced clearance of *M. tuberculosis*	[12]
Human monocytes (THP-1)	1,α25-$(OH)_2D_3$ -20 nM; 24 h	siRNA-mediated depletion of beclin-1, Atg5 or cathelicidin [a]Treatment with Bapta-AM, STO-609, 3MA and compound C	ND[b]	[12]
Murine macrophages (Raw 264.7)	1,α25-$(OH)_2D_3$ -20 nM; 24 h	ND[b]	ND[b]	[12]

[a]Bapta-AM is chelates intracellular calcium; STO-609 inhibits CaMKKs; 3MA inhibits phosphatidyl-inositol 3-kinases; compound C inhibits AMPK.
[c]ND, not detected.

and autophagy induce primarily protective processes, both can paradoxically trigger the death of some cancer cells [22,23].

Recent data suggest that the striking similarities between the physiological and pathological functions of VD compounds and autophagy are not merely coincidental. As discussed in detail below, VD analogs are potent inducers of autophagy in different cell types, and autophagy is crucial for their cytotoxic activity towards cancer cells and the antimicrobial activity in macrophages infected with *M. tuberculosis* [7–9,11,12] (Table 2). It remains, however, to be studied whether autophagy plays a more general role in mediating the manifold activities of VD.

Induction of autophagy by VD compounds

Autophagy is induced by several forms of cellular stress including starvation, hypoxia and infection as well as numerous clinically relevant drugs used in therapy against cancer and other diseases [23]. The first indications that VD compounds activate autophagy came from studies on the molecular mechanism underlying the cancer cell-specific cytotoxic activity of 1α,25-$(OH)_2D_3$ and its synthetic analog EB1089 (seocalcitol), which was developed to diminish the effects of the natural hormone on calcium homeostasis while retaining or enhancing the growth-regulatory effects [24]. The treatment of breast cancer cells with 1α,25-$(OH)_2D_3$ or EB1089 results in an atypical cell death that is independent of the apoptotic proteases called caspases [25]. Instead, cytotoxicity depends on an increase in cytosolic calcium $[Ca^{2+}]_{cyt}$ and associates with an increase in lysosomal protease activity, a hallmark of autophagy induction [26,27]. More sophisticated methods for autophagy detection (*e.g.* transmission electron microscopy, demonstrating the accumulation of autophagosomes and autolysosomes and confocal microscopy, demonstrating the translocation of the autophagosomal protein LC-3/Atg8 to cytosolic dots as well as demonstrating an increase in the degradation of long-lived proteins) have now confirmed that EB1089 can trigger functional autophagy in breast cancer cells [7,8]. Importantly, 1α,25-$(OH)_2D_3$ also induces autophagy in cancer cells originating from other tissues (myeloid leukemia and head and neck squamous cell carcinoma) [9,11]. Furthermore, a physiologically relevant concentration of 1α,25-$(OH)_2D_3$ (100 pM) induces autophagy in primary monocytes and macrophages, indicating that the phenomenon is not restricted to cancer cells and suggesting that 1α,25-$(OH)_2D_3$ might stimulate autophagy under physiological conditions [12]. It remains, however, to be tested whether physiological 1α,25-$(OH)_2D_3$ concentrations also trigger autophagy in cancer cells. Alternatively, the expression of CYP27B1 in various cancer cells or in the surrounding tissue could increase the local 1α,25-$(OH)_2D_3$ concentration in cancer tissue to induce autophagy [14].

As shown in Figure 2 and Table 2, the signaling pathways connecting VD compounds to autophagy induction are similar in breast cancer cells and monocytes [7,12]. Autophagy induction in both cell types relies on an increase in $[Ca^{2+}]_{cyt}$, which could result from VDR-mediated changes in the expression levels of calcium-regulating proteins and the subsequent endoplasmic reticulum stress [28]. An increase in $[Ca^{2+}]_{cyt}$ activates Ca2+/calmodulin-dependent kinase kinase-ß (CaMKK-β), which is followed by the activation of AMP-activated kinase (AMPK) [7,12], a recently identified direct substrate of CaMKK-β [29,30], and a potent inducer of autophagy, for example in response to starvation and cytokines [31,32]. Correspondingly, both the activation of AMPK and autophagy by VD compounds require CaMKK-β activity [7,12]. AMPK activation induces autophagy via the inhibition of mammalian target of rapamycin complex 1 (mTORC1), the major gatekeeper of mammalian autophagy [33,34]. Autophagic processes downstream of mTORC1 depend on several evolutionarily conserved autophagy-related (Atg) proteins [35] (Figure 2). Accordingly, small interfering RNA (siRNA)-based depletion of essential Atg proteins (Atg5, Atg6/beclin 1 or Atg7) effectively inhibits autophagosome formation upon treatment with VD compounds in breast cancer cells, myeloid leukemia and monocytes [7,11,12].

In addition to the identified common pathways, 1α,25-$(OH)_2D_3$-triggered autophagy in monocytes depends on the LL-37 peptide derived from cathelicidin hCAP-18

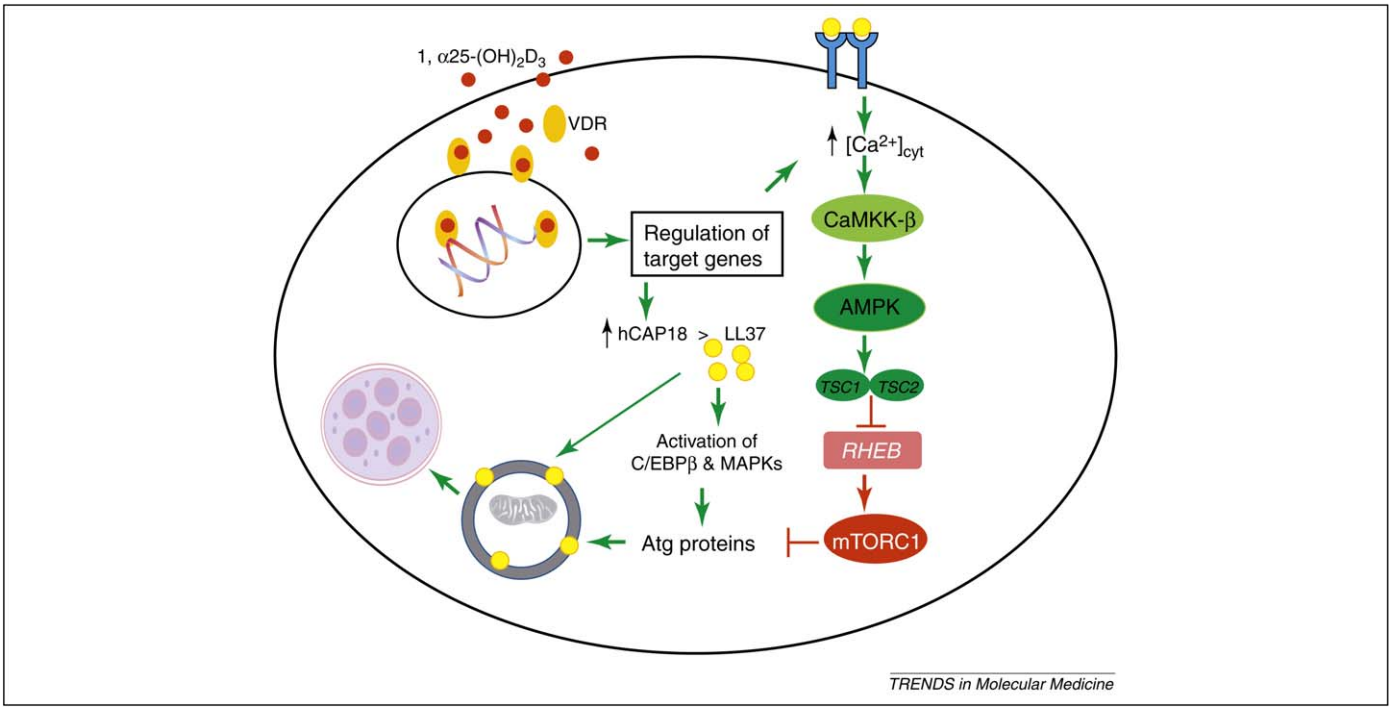

Figure 2. Schematic presentation of autophagy induced by 1α,25-$(OH)_2D_3$ or its analogs.
The active form of VD or its analogs (red circles) enter the cell and bind the VDR (orange ovals), which is a ligand-inducible transcription factor. The ligand–receptor complex translocates to the nucleus and regulates genes containing VD responsive elements. Among these are the antimicrobial protein cathelicidin (hCAP18) and other genes that increase cytoplasmic calcium levels ($[Ca^{2+}]_{cyt}$) by releasing them from the endoplasmatic reticulum or activating P2X(7) or other cell surface receptors regulating plasma membrane Ca^{2+} channels. Increased $[Ca^{2+}]_{cyt}$ activates the CaMKK-β–AMPK signaling pathway, leading to the inhibition of mTORC1 activity through the tuberous sclerosis complex (TSC1/TSC2) and RHEB. The inhibition of mTORC1 activates the Atg machinery needed for autophagy. hCAP18/LL-37 could also increase the expression of two ATG genes (*BECLIN1* and *ATG5*) and interact with autophagosomes to enhance their maturation to autolysosomes.

(hCAP18/LL-37), a VDR-regulated antimicrobial protein [12]. In this model system, hCAP18/LL-37 is suggested to be essential not only for autophagosome formation, but also for the fusion of autophagosomes with phagosomes and for the upregulation of genes encoding Atg5 and Atg6/beclin 1. It should, however, be noted that the two latter events can also be consequences of autophagy induction and, thereby, would depend on hCAP18/LL-37 only indirectly. It remains to be determined how hCAP18/LL-37 connects to the calcium-dependent signaling cascade controlling mTORC1 activity [33]. In other model systems, hCAP18/LL-37 induces an increase in $[Ca^{2+}]_{cyt}$ by activating calcium mobilizing cell surface receptors such as the P2X(7) purinergic receptor that functions as an ATP-gated ion channel [36]. In this context, it is interesting to note that the ATP-mediated activation of purinergic receptors in cancer cells is sufficient for autophagy induction via a calcium-dependent signaling cascade indistinguishable from that activated by VD compounds [7], and that akin to the 1α,25-$(OH)_2D_3$-induced clearance of *M. tuberculosis*, the activation of P2X(7) augments the autophagy-dependent elimination of *Toxoplasma gondii*-containing phagosomes from infected macrophages [37]. Thus, P2X(7) and other purinergic receptors emerge as obvious candidates for molecules linking 1α,25-$(OH)_2D_3$-induced hCAP18/LL-37 expression to calcium-dependent autophagy. Notably, hCAP18, which is required for 1α,25-$(OH)_2D_3$-induced calcium mobilization in monocytes, is one of the most commonly induced VDR target genes in different cell types, including several cancer cell lines [38]. Its role in 1α,25-$(OH)_2D_3$-induced autophagy in cancer cells remains, however, to be investigated.

Taken together, VD compounds trigger autophagy in monocytes and cancer cells via noticeably similar CaMKK-ß- and AMPK-dependent signaling cascades that are initiated by a slow increase in $[Ca^{2+}]_{cyt}$ (Figure 2). To evaluate the importance of this pathway for the multiple effects of VD, it will be of utmost importance to extend these studies to other 1α,25-$(OH)_2D_3$-responsive cell types and to living organisms.

VD and autophagy in cancer

In 1941, Apperly published the first epidemiological study reporting that people living in northern states of the USA have higher cancer mortality compared with similar populations living in the southern states where the exposure to solar radiation is higher [39]. The pattern was strikingly similar to the rickets epidemic in Europe and North America in the 18th and 19th centuries, and this report led to further investigations of the connection between exposure to sunlight, serum levels of 25$(OH)D_3$ and morbidity and mortality from cancer. Several studies with different designs, populations and parameters have now shown that serum levels of 25$(OH)D_3$ are inversely related to the overall morbidity and mortality of cancer [6,40,41]. Furthermore, accumulating epidemiological data support the hypothesis that 1α,25-$(OH)_2D_3$ plays a preventive role in cancer [42,43]. Some VD analogs with reduced calcemic properties have reached clinical trials for the treatment of cancer, but the results from monotherapy trials have been

somewhat disappointing and limited by hypercalcemia and hypercalciuria [5]. However, better responses have been reported in trials where a VD compound is combined with other chemotherapeutic drugs such as docetaxel or cisplatin [43].

Studies in mice deficient for VDR strongly support the tumor-suppressive role of 1α,25-$(OH)_2D_3$; VDR participates in the negative growth control of the normal mammary gland and the disruption of VDR signaling associates with abnormal ductal morphologic features, increasing the incidence of preneoplastic lesions and an accelerating mammary tumor development [44]. Notably, the preneoplastic and neoplastic mammary pathologies of VDR-deficient mice are similar to those observed in autophagy-defective mice heterozygous for the *Becn1* gene, which encodes Atg6/beclin 1 [45,46]. Importantly, the tumor-suppressive functions of 1α,25-$(OH)_2D_3$ are not limited to breast and the list of cancer cell lines that express VDR is extensive [5].

The role of autophagy in the anticancer effects of 1α,25-$(OH)_2D_3$

The antitumor effects of 1α,25-$(OH)_2D_3$ and its analogs have been linked mainly to the inhibition of proliferation by blocking the cell cycle at G1 phase, the induction of differentiation and cell death, the regulation of genes involved in metastasis and angiogenesis as well as the activation of the innate immunity [5,42]. The newly discovered connection between 1α,25-$(OH)_2D_3$ and autophagy makes it tempting to speculate that the induction of autophagy also plays a key role in the tumor-suppressive effects of 1α,25-$(OH)_2D_3$. Although accumulating evidence suggests that autophagy effectively suppresses tumorigenesis, the mechanisms underlying this effect are still debated and could involve the removal of damaged organelles and subsequent reductions in oxidative stress, mutation rate and genomic instability [23,47]. Akin to 1α,25-$(OH)_2D_3$, autophagy can also activate cell death and induce G1 arrest in cancer cells.

Numerous *in vitro* studies have shown that 1α,25-$(OH)_2D_3$ and its analogs trigger cell death in various cancer cell lines [25,48,49]. Cell death induced by VD is independent of caspases [25,50] and associates with autophagy [8,9,11]. Autophagy usually exerts a cytoprotective function in stressed cells; however, in EB1089-treated breast cancer cells, the enhancement of the autophagic response by ectopic expression of *Becn1* increases cell death, whereas the inhibition of autophagy with 3-methyladenine (3-MA) decreases cell death [8]. Consistent with these observations, the 1α,25-$(OH)_2D_3$-induced death of human myeloid leukemia cells is inhibited by cotreatment with bafilomycin A, an effective inhibitor of the autophagic flux [11]. Importantly, 1α,25-$(OH)_2D_3$-treated primary monocytes do not show any signs of cell death even though their autophagy response is similar to that observed in cancer cells [12]. Thus, it is tempting to speculate that 1α,25-$(OH)_2D_3$-induced autophagic cell death could be specific for cancer cells; if true, this would represent a new cancer-specific treatment. There is no experimental evidence that physiological concentrations of 1α,25-$(OH)_2D_3$ trigger autophagic cell death in cancer cells; however, the ability of 1α,25-$(OH)_2D_3$ to trigger AMPK-dependent autophagy (without cell death) in normal cells could contribute to the effects of VD compounds on cancer morbidity by reducing oxidative stress-induced mutations or by other as yet unknown mechanisms. Similarly, metformin, an AMPK activator used in diabetes treatment, induces autophagy *in vitro* and decreases the risk of developing prostate cancer in men [51,52]. Thus, further investigations on the ability of the AMPK–autophagy pathway to suppress tumor formation are warranted.

Most human cancers have multiple defects in apoptotic signaling pathways [53]. Thus, the ability of VD compounds to trigger caspase-independent autophagic cell death could prove therapeutically useful in the treatment of apoptosis-defective, highly chemoresistant cancers. Another clinically relevant observation is the successful outcome from clinical trials of 1α,25-$(OH)_2D_3$ and its analogs in combination with microtubule-disturbing and DNA damaging drugs [42], both of which induce lysosomal cell death in transformed cells [54,55]. One way autophagy could sensitize cells to lysosomal permeabilization by increasing the volume and activity of the lysosomal compartment [27], a hallmark of autophagy induction and lysosomal destabilization [8,55,56]. By inducing cytoprotective autophagy, VD compounds could, however, also protect tumor cells against cell death triggered by nutrient deprivation and hypoxia in the tumor environment [23].

In spite of multiple similarities in the regulation of tumorigenesis and tumor growth by VD signaling and autophagy induction, the ability of VD compounds to trigger autophagy-dependent cell death in cancer cells is so far the only experimental evidence directly linking autophagy to the tumor-suppressive functions of VD compounds. To address the putative role of autophagy in tumor suppression, extensive *in vivo* studies on animals with tissue-specific changes in autophagy or VDR signaling in combination with treatment with VD compounds or other VDR-independent effectors of autophagy are required.

VD, autophagy and *M. tuberculosis* infection

The use of VD in the treatment of tuberculosis has a long history (reviewed in [13]). The beneficial effects of cod liver oil in the treatment of tuberculosis were recognized as early as 1849, and a half a century later Niels Finsen received a Nobel Prize for his discovery of UV light as an effective therapy for lupus vulgaris, a cutaneous form of tuberculosis. After the discovery of vitamins D_2 and D_3, VD-based therapies were widely used in the treatment of tuberculosis until more effective therapies were introduced in the middle of the last century. In the 1980 s, 1α,25-$(OH)_2D_3$ was shown to inhibit the growth of *M. tuberculosis* in culture [57] and, more recently, to enhance innate immunity by inducing the expression of genes encoding antimicrobial peptides, such as hCAP18/LL-37 [58]. $25(OH)D_3$ circulating in the blood actively stimulates the production and secretion of hCAP18/LL-37 when immune and epithelial cells in the respiratory and gastrointestinal tract meet bacteria. *M. tuberculosis* activates this mechanism via Toll-like receptors by upregulating CYP27B1 and thereby the local synthesis of 1α,25-$(OH)_2D_3$ [58]. hCAP18/LL-37 demonstrates an antimicrobial activity

against both Gram-positive and Gram-negative bacteria as well as several viruses and fungi; mice deficient for the gene encoding hCAP18 and humans with a deficient production of hCAP18/LL-37 are more vulnerable to infections of the epithelial surfaces [13,59].

The ability of macrophages to kill intracellular pathogens is pivotal to the outcome of microbial infections. In tuberculosis, *M. tuberculosis* resides in phagosomes and evades host antimicrobial mechanisms by blocking phagosome maturation and fusion with the lysosome [60]. Ultimately, the host must overcome this evasion strategy to destroy the pathogen. Accumulating evidence suggests that this occurs via the autophagic degradation of bacteria-containing phagosomes and the subsequent killing of the bacteria in autolysosomes [61–63]. Interestingly, a recent paper links the 1α,25-$(OH)_2D_3$- and autophagy-controlled antimycobacterial defense-pathways. This *in vitro* study demonstrates for the first time that at a physiologically relevant concentration, 1α,25-$(OH)_2D_3$ triggers the destruction of *M. tuberculosis* by activating autophagy in infected macrophages [12]. Yuk and co-workers showed that blocking 1α,25-$(OH)_2D_3$-triggered autophagy with pharmacological inhibitors or siRNA against hCAP18 increases the number of bacteria in infected macrophages *in vitro*. These data are consistent with the hypothesis that autophagy is part of the mechanism of action by which 1α,25-$(OH)_2D_3$ mediates its antimicrobial effect against tuberculosis. In support of the essential role of autophagy in the defense against tuberculosis, other autophagy-inducing stimuli such as rapamycin and starvation trigger the autophagosomal engulfment of bacteria-containing phagosomes and enhance the clearance of *M. tuberculosis* in infected macrophages in an autophagy-dependent manner [61,62]. Interestingly, starvation- and rapamycin-induced autophagy and antimycobacterial effects do not depend on hCAP18/LL-37 that is essential for the 1α,25-$(OH)_2D_3$-induced autophagy-dependent clearance of *M. tuberculosis* [57]. These data support the hypotheses that hCAP18/LL-37 is essential for the induction of autophagy upon 1α,25-$(OH)_2D_3$ treatment rather than for the fusion of autophagosomes with bacteria-containing phagosomes, and that autophagy exerts antimycobacterial effects independent of the initiating stimulus.

Concluding remarks

Recent data link autophagy to two of the beneficial effects of VD: the induction of cancer cell death and the clearance of *M. tuberculosis*. This opens the possibility that autophagy could be a general mediator of the health-promoting effects of 1α,25-$(OH)_2D_3$. Accordingly, there is a striking overlap among the diseases promoted by VD deficiency and defective autophagy. The new data linking the two health-promoting pathways open an interesting research field that could lead to new options for the treatment and prevention of many common diseases. Although none of the previously known autophagy-activating drugs such as rapamycin are suitable for long-term treatment, the "natural" compound VD can be obtained from both diet and sun exposure without side effects, and thereby its effects on diseases linked to defective autophagy should be carefully investigated.

Several VD compounds are already in clinical trials for the treatment of cancer. New data revealing strong autophagy induction and the enlargement of the lysosomal compartment by VD compounds are likely to guide the design of future trials towards more successful combination therapies, for example with lysosome-targeting drugs. In the case of the treatment of microbial infections, the new data underlying the importance of the 1α,25-$(OH)_2D_3$-induced autophagy-dependent clearance of *M. tuberculosis* should stimulate research on the beneficial effects of 1α,25-$(OH)_2D_3$ in other microbial infections where the autophagosomal clearance of the microbe-containing phagosomes is essential for the clearance of the pathogen. Finally, the link between VD and autophagy will hopefully stimulate experiments to test the role of defective autophagy in other pathologies related to VD deficiency, and these studies could ultimately lead to the prevention of or new treatments for several common diseases.

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