

The role of vitamin D in the pathogenesis of ocular diseases

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Abstract: The prevalence of vitamin D deficiency in the American and European population is estimated to be extremely high. Although fewer people today suffer from serious health problems related to calcium and phosphate metabolism resulting from vitamin D deficiency, there are more and more studies suggesting that calcitriol may play an important role in the pathogenesis of other diseases in virtually every body system. A growing body of research shows that through its ubiquitously expressed receptor, calcitriol displays potent anti-angiogenic and anti-inflammatory activity. This review summarizes recent discoveries regarding these non-classical effects of vitamin D and their clinical implications. Data collection focused on the prevention and treatment of ocular diseases as well as on the underlying mechanisms.

Key words: vitamin D, dihydroxycholecalciferol, calcitriol, vitamin D receptor, ophthalmology, angiogenesis, inflammation, retinopathy, retinoblastoma, age-related macular degeneration, corneal epithelium.

Background/introduction

Vitamin D is typically associated with calcium and phosphate metabolism but, since its first identification, there has been a number of publications reporting the involvement of vitamin D in a myriad of new functions. The growing interest in this substance has started with the discovery of the vitamin D receptor (VDR), its DNA-binding capabilities and vitamin D response elements (VDREs) — specific DNA binding

sites [1]. Current studies show that the active vitamin D metabolite ($1\alpha,25(\text{OH})_2\text{D}_3$) recognizes VDREs and alter DNA transcription. All of these actions are mediated by vitamin D receptors, heterodimerized with retinoic X receptors, which bind to the regulatory sites in target genes. Apart from at least eleven genes involved in bone and mineral homeostasis [2], VDRs and 1-hydroxylase enzyme have been discovered in multiple different tissues where $1,25(\text{OH})\text{D}$ influences locally the transcriptional output. It has been proven that vitamin D interacts directly with 0.8–5% of the total genome [3]. These new findings revealed that vitamin D controls multiple of biological processes including cellular growth, angiogenesis or even modulation of immune [4] and cardiovascular system [5], differentiation of keratinocytes [6], inhibition of proliferation of breast [7], colon [8] and prostate cancer cells [9]. The non-skeletal effects explain the wide variety of pathologic conditions that have been linked to vitamin D insufficiency.

The vitamin D metabolism pathway

Vitamin D can be either produced in the skin through two-step non-enzymatic process or obtained from the diet in a form of two biologically inactive precursors. The dietary sources include ergocalciferol (D₂) from plants and cholecalciferol (D₃) mostly from marine life. Both alimentary forms undergo the same activation pathways and their final effect/biological action on cells is almost equivalent. Nevertheless, the keratinocytes of the skin are the primary source of vitamin D for the body [6].

Sunlight-mediated photolysis of cutaneous 7-dehydrocholesterol begins when the epidermis is exposed, for a sufficient amount of time, to ultraviolet B radiation (280–320 nm). Irradiation of 7-DHC breaks the ring of precursor sterol to form pre-D₃ and then, due to temperature dependent rearrangement, it is converted to actual D₃ (Fig. 1).

Before vitamin D becomes the active hormone ($1\alpha,25(\text{OH})_2\text{D}_3$), it undergoes metabolic activation by hydroxylases — forms of cytochrome P450. The first step is conducted mostly in the liver by CYP2R1 resulting in 25-hydroxylated vitamin D. Moreover, there is some research indicating that other hydroxylases may be involved such as mitochondrial CYP27A1 or microsomal CYP2D11, CYP2D25, CYP2J2/3, and CYP3A4. The synthesis of $25(\text{OH})\text{D}$ also occur, to some extent, locally because of the expression of CYP2R1 in many tissues [10]. It may be the reason why in patients with liver failure vitamin-D production is maintained. Even so, the hepatic CYP2R1 remains the main site of its 25-hydroxylation in a healthy body.

In the circulation, $25(\text{OH})\text{D}$ is bound to vitamin D binding protein (DBP). In order for the next hydroxylation to take place, complexes of $25(\text{OH})\text{D}$ and DBP need to be reabsorbed from the glomerular filtrate at the proximal tubule of the kidney.

This uptake DBP is facilitated by endocytic receptor — megalin in conjunction with cubilin [11]. Then, lysosomal enzymes inside the cell degrade DBP, making 25(OH)D available for further enzymatic modifications. In clinical practice, both free and total serum level of 25(OH)D is used to assess vitamin D status. It has significantly longer half-life and reaches higher plasma concentrations than 1,25(OH)₂D.

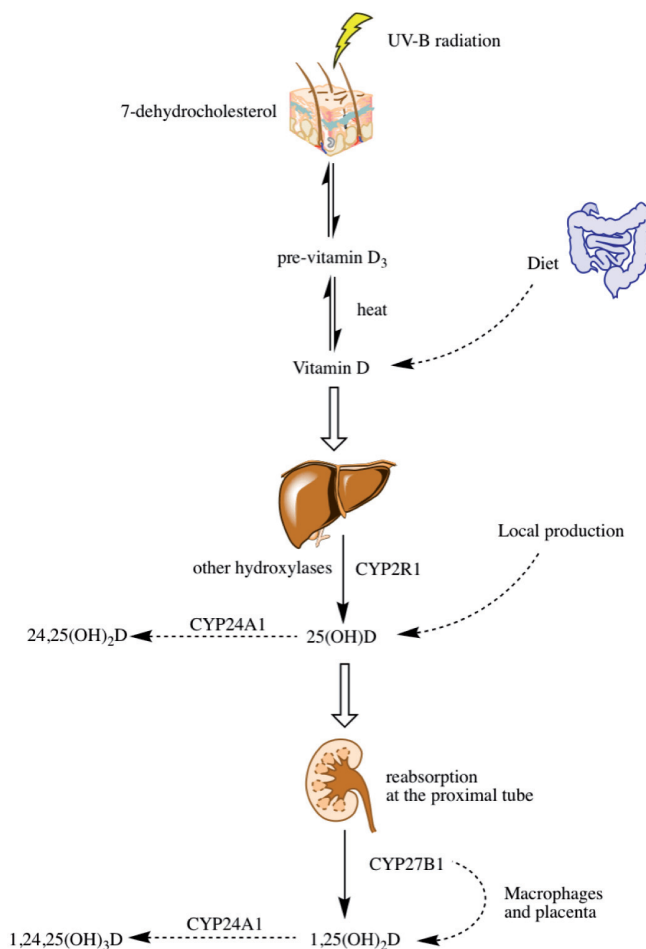


Fig. 1. Vitamin D production.

The further enzymatic process occurs in the kidney where 25(OH)D is hydroxylated into the biologically active 1,25-dihydroxyvitamin D. This metabolite has the highest affinity for the nuclear VDR, thereby making it the most active form of vitamin D. The second hydroxylation is catalyzed by the mitochondrial enzyme CYP27B1. Its renal activity is crucial for maintaining the optimal level of circulating 1,25(OH)₂D, although

1-hydroxylase is expressed widely in non-renal cells as well. Regarding the extra-renal CYP27B1 expression, of particular significance are disease-activated macrophages and placenta which are the only sites, so far as is known, capable of providing enough hormone to raise the serum level of vitamin D [12].

24-hydroxylase enzyme (CYP24A1 gene) is responsible for the ensuing step in vitamin D metabolism. This enzyme inactivates both 25(OH)D and 1,25(OH)₂D through its multi-catalytic activity. It can be induced by 1,25(OH)₂D, and hence auto-regulates its circulating concentration. As a result, CYP24A1 defects may cause severe hypercalcemia [13].

In addition, there are reports suggesting that the product of this inactivation 24,25(OH)₂D has significant activity, and influences processes such as cartilage and osteoblasts maturation [14, 15].

Regulation of plasma 1,25(OH)₂D concentration

Production of 1,25(OH)₂D is tightly regulated by the action of specific hormones on the expression of CYP27B1 and CYP24. As mentioned above, the role of these genes is to, respectively, activate and inactivate vitamin D metabolites, and thus they maintain calcium and phosphate homeostasis.

In hypocalcemic state, parathyroid hormone(PTH) induces expression of CYP27B1 in the renal proximal tubular cells, thereby stimulating 1-hydroxylation of 25(OH)D. At the same time, PTH inhibits CYP24 — vitamin D inactivator. As a result, the production of 1,25(OH)₂D increases, leading to a rise in the serum level of calcium.

On the contrary, fibroblast growth factor (FGF 23) suppress vitamin D hormone production in the kidney. FGF-23, which requires an obligatory co-receptor Klotho, is a protein produced mostly by osteocytes and osteoblasts. Bony secretion of this hormone is stimulated by phosphate, PTH and 1,25(OH)₂D [16]. There is a negative feedback loop since it functions as a down-regulator of renal 1-hydroxylation by suppression of CYP27B1. Additionally, both FGF23 and 1,25-dihydroxyvitamin D up-regulate CYP24 expression.

As indicated earlier, 1,25(OH)₂D is a strong activator of CYP24 gene transcription. But to fully understand vitamin D feedback circuit, it is important to mention that this hormone also suppresses CYP27B1 expression, as well as, PTH production and secretion. Moreover, it acts as the FGF23 stimulator. Several additional factors also regulate renal CYP27B1 activity including calcitonin, insulin, growth hormone and prolactin [17].

1,25-dihydroxyvitamin D acts as a ligand for the vitamin D receptor in target cells. VDR is a DNA-binding transcriptional factor, which in active (liganded) state, heterodimerizes with retinoic X receptor. Subsequently, the signal transducing

complex of $1,25(\text{OH})_2\text{D}$, VDR and RXR is able to migrate into the nucleus where recognizes specific DNA sites and influences gene transcription. DNA sequences of vitamin-D regulated genes are called vitamin D response elements (VDRE). After binding to the VDRE, heterodimer recruits complexes of co-regulators. These various regulating proteins complexing to the liganded VDR-RXR determine the cell or tissue specificity of the response. They act as inducers or repressors of genomic activities through chromatin remodeling, histone modifications and recruiting RNA polymerase II [18].

There is evidence that vitamin D activity includes also a number of rapid actions that usually last only a short time. As the effect at the genome level needs hours to occur, these actions are considered to be “non-genomic”. However, VDR has been recognized to be necessary for this non-genomic pathway to function [19] and the final result of many of these non-transcriptional responses is, in fact, an alteration of gene output.

These non-classical actions are recognized to be responsible for the regulation of transmembrane transport of calcium and chloride as well as stimulation of intracellular signaling pathways through triggering the activation of signaling molecules, the rapid generation of second messengers and the activation of protein kinases [20]. $1,25(\text{OH})_2\text{D}$ induce these rapid actions through binding to the membrane receptors. The scientists identified a specific binding protein for $1,25(\text{OH})_2\text{D}$ named membrane-associated rapid response steroid (MARRS) [21]. The protein is also known as Pdia3, ERp60, ERp57 or Grp58. It is located within caveolae which are small, ‘cup-shaped’ invaginations of the plasma membrane.

On the other hand, it was demonstrated that $1,25(\text{OH})_2\text{D}$ can initiate rapid responses via the classic VDR, which is typically associated with traditional genomic responses. The vitamin D receptor, which is normally found in the nucleus, is also present in caveolae-enriched plasma membranes [22]. It is suggested that these VDR-mediated rapid responses can be generated through binding of $1,25(\text{OH})_2\text{D}$ to an alternative ligand binding pocket [23].

Both Pdia3 and VDR are complexed to caveolin-1, the caveolae marker protein, which seems to be an essential scaffold to activate downstream mediators — different for each of the receptors. The Pdia3 binds phospholipase A2 activation protein (PLAA) while VDR is coupled to c-Src [24].

Eye-specific effects

The latest research on metabolism and action of vitamin D revealed that it acts more extensively than merely regulation of calcium and phosphate homeostasis. Due to VDR expression in non-classic tissues, $1,25(\text{OH})_2\text{D}$ displays local activity regulating many additional cell-specific biological processes. Using immunochemistry scientists

identified VDR in the intestinal epithelium, renal tubules, parathyroid gland, keratinocytes, mammary epithelium, pancreas, pituitary gland, skeleton, immune system, and germ tissues [25]. Furthermore, vitamin D can be activated locally due to the extra-renal expression of CYP27B1 which has been discovered in a wide variety of tissues including respiratory [26], prostate, colonic epithelial cells, as well as, osteoblasts and macrophages [27].

Most importantly, for this review, recent studies revealed the presence of VDR, along with 1-hydroxylase, in the eye [28]. RT-PCR of ocular cells has found strong expression of VDR in primary human scleral fibroblasts, corneal endothelial cells, adult retinal pigment epithelial cells and non-pigmented ciliary body epithelial cells. Further examination of mRNA expression, combined with immunofluorescent staining, detected in these cells, not only the receptor but also the enzymes required in vitamin D metabolism [29]. It may suggest that vitamin D function as a paracrine/autocrine regulator. This hypothesis has recently been supported by experimental results. Cultures of retinal and corneal cells treated with 25(OH)D have demonstrated ability to convert it into active 1,25(OH)₂D [29]. A separate study presented 25(OH)D synthesis in corneal limbal cells from exogenous 7-dehydrocholesterol after being exposed to the low doses of UV-B radiation [30]. Above-mentioned findings indicate that vitamin D may be produced locally following sunlight exposure.

The research on VDR involves the retinal vasculature as well. It was shown that in retinal pericytes VDR is strongly expressed compared to endothelial cells and that calcitriol in vitro amplifies the level of this expression [31].

Measurable concentrations of vitamin D metabolites have been also detected in tear fluid as well as in aqueous and vitreous humor [30]. In addition, there is an evidence, obtained from a mouse study, for the presence of megalin and cubilin in the lacrimal and Harderian glands which are responsible for the production of tear fluid [32]. This complex of receptors, required for the internalization of 25(OH)D-DBP in kidneys, may also be involved in the process of vitamin D secretion into tear fluid. Moreover, chemiluminescent immunoassay allowed to measure the 25-hydroxyvitamin D concentrations in human tears and it was found to be even higher than corresponding serum levels of 25(OH)D [33].

In aqueous humor, calcitriol was not merely measured but also identified as potential factor able to modulate both aqueous humor production and outflow. Microarray analysis of rat and mouse DNA led to the discovery that 1,25(OH)₂D induces changes in the expression of the genes involved in the regulation of intraocular pressure [34]. Following studies showed that topical administration of vitamin D analogs significantly lower IOP in nonhuman primates [34]. However, changes in the aqueous humor formation or outflow were not observed.

Effects of vitamin D on corneal function

Proper functioning of the cornea is necessary for good vision since it is an outer, transparent, protective layer of the eye that refracts light. Corneal cells express all the enzymes necessary for the metabolism of vitamin D therefore they are able to produce the active form of vitamin D from the non-hydroxylated metabolite [35]. Local production suggests the local activity of calcitriol in the cornea. Studies involving vitamin D therapy have shown that it may affect the function of the corneal epithelium. Yin *et al.* reported that $1,25(\text{OH})_2\text{D}$ and $25(\text{OH})\text{D}$ cause an increase in transepithelial resistance in human corneal epithelial cells which reflects the integrity of tight junctions [28]. Additionally, they observed decrease in inulin permeability (a marker of paracellular transport) and elevated levels of occludin. Experiments in mice with inactivated VDR gene confirmed these findings and showed that in these animals the healing of the corneal epithelium is impaired [36]. This indicates the important role of vitamin D in maintaining the integrity of the cornea. Further studies on the positive effects of vitamin D on the barrier function of the corneal epithelium revealed that $1,25(\text{OH})_2\text{D}$ stimulates the migration of human corneal epithelial cells [37]. Interestingly, $24,25$ -dihydroxyvitamin D has the same effect on the migration of these cells and also increases their proliferation [37]. However, experiments on mouse models *in vivo* and *ex vivo* have shown that topical calcitriol administration delays or completely inhibits wound healing in the cornea [38, 39]. It may be the result of the calcitriol inhibitory effect on corneal epithelial proliferation [37].

Anti-angiogenic properties

Research conducted in order to investigate $1,25(\text{OH})_2\text{D}$ effect on angiogenesis have proven that it acts as a potent inhibitor of the angiogenic processes *in vitro* and *in vivo* [40]. The question then arose whether calcitriol has the same effect in the eye. In the experiment performed using the mouse model of oxygen-induced ischemic retinopathy, $1,25$ -dihydroxyvitamin D demonstrated the ability to inhibit retinal neoangiogenesis in a dose-dependent manner [41]. Although *in vitro* studies revealed that it suppresses endothelial cell capillary morphogenesis, calcitriol was shown to have no influence on endothelial cell proliferation or migration at the concentrations observed to be minimally cytotoxic. Nevertheless, it inhibits the proliferation of pericytes in culture [31] what is consistent with the mentioned above VDR expression in these cells.

Further research, conducted recently by Jamali *et al.*, was focused on analyzing the mechanism of action by which vitamin D affects angiogenesis in the eye. Since VDR is associated with both genomic and rapid pathways it was hypothesized that it may play a significant role in the retinal vascular development and its inhibition by calcitriol.

First of all, it was discovered that the lack of VDR expression in mice, resulting in an increase in pericytes, impairs neovascularization but only at a late stage [31]. Secondly, the vitamin D receptor-deficient mice were presented to be resistant to the anti-angiogenic properties of $1,25(\text{OH})_2\text{D}$ during oxygen-induced retinopathy [31]. These findings may suggest that VDR expression is relevant for the physiologic maturation of blood vessels and, what is more, for vitamin D anti-angiogenic activity in pathological conditions.

In support of this hypothesis is evidence acquired from experiments on zebrafish which are an acclaimed model for studying embryonic vascular development. Calcitriol and 8 other VDR agonists were identified as inhibitors of developmental angiogenesis in zebrafish larvae eye [42]. This anti-angiogenic effect of VDR agonists correlated with increased expression of dre-miR-21, which is one of the micro-RNAs involved in the regulation of cell proliferation, migration, and apoptosis. Surprisingly, a recent study demonstrated that miR-21 is responsible for neovascularization and retinal inflammation in a mouse model of diabetic retinopathy through down-regulation of PPAR α [43]. These results are in agreement with experiments which identified miR-21 as a promoter of pathological angiogenesis in the ischemic retina [44]. Activation of miR-21 has been shown to be dependent on the STAT3 transcription factor and results in significantly decreased expression of the tissue inhibitor of the metalloproteinase gene (TIMP3). However, there is also evidence of miR-21 anti-angiogenic activity since it suppressed pathological vascular development in a mouse model of choroidal neovascularization [45].

Topical administration of $1,25(\text{OH})_2\text{D}$ has a mild anti-angiogenic effect on corneal vessel formation as well. Scientists induced angiogenesis by sutures placed in the center of mouse cornea. The neovascularization was suppressed in mouse cornea treated with vitamin D but only at the highest concentrations used in this experiment [46].

Anti-inflammatory properties

It is now known that vitamin D is a significant modulator of innate and adaptive immune system, through VDR that is present in immune cells, and thus play an important role in the regulation of inflammatory responses. As CYP27B1 is expressed in T cells, B cells and antigen presenting cells, vitamin D can be activated locally and act in an autocrine manner. Moreover, both VDR and 1-hydroxylase expression in macrophages increase after Toll-like receptors activation by pathogens [47].

In vivo study has shown that $1,25(\text{OH})_2\text{D}$ is able to inhibit corneal inflammation by suppressing Langerhans cells migration into cornea [46]. In vitro calcitriol inhibits the production of IL-1 α , IL-1 β and IL-8 [48]. When researchers induced inflammation in human corneal epithelial cells by a TLR3 agonist, simultaneous

treatment with vitamin D protected against excessive production of pro-inflammatory mediators such as IL-1 β , IL-6, IL-8, TNF α , and CCL20 [35]. However, this effect is observed only after co-treatment lasting longer than 6 hours or in cells pretreated for 24 hours [49]. A more thorough analysis of the mechanism of this anti-inflammatory response revealed an increase in I κ B α (nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha) protein levels [49]. Similar results were obtained from experiments on the rat dry eye model induced by topical administration of benzalkonium chloride, in which 1,25(OH) $_2$ D attenuated inflammation [50]. In addition, studies of dry eye using human corneal epithelial cells under hyperosmotic conditions showed that calcitriol suppress also the expression of other chemokines (MIP1A and MIP1B) as well as activation of NF- κ B [50]. In agreement with above mentioned findings regarding I κ B α , it appears that vitamin D influences NF- κ B pathway. Moreover, activation of NF- κ B is induced by TLR-signaling and it has been found that calcitriol down-regulates TLR3, TLR4 and TLR7 expression [50].

Likewise, vitamin D down-regulated the expression of the pro-inflammatory mediators (IL-1 β and IL-8) in the culture of human corneal epithelial cells colonized with *Pseudomonas Auerginosa* [51]. Notably, corneal cells are more effective in killing bacteria when treated with vitamin D [35]. This beneficial effect on the surface of the eye may be due to the increased production of the antimicrobial peptide LL-37 [35]. By means of immunochemistry, the authors of another experiment demonstrated that the fungal pathogen (*Fusarium solani*) increases the expression of VDR in corneal epithelial cells [52] and it is already known that the fungal antigens increase the expression of TLR2 together with TLR4 and dectin-1 in these cells [53, 54]. Interestingly, treatment with Toll-like receptor 2 antibody inhibited this up-regulated expression suggesting the presence of TLR2-dependent VDR activation pathway. Vitamin D also appears to influence the efficacy of medication used to treat fungal keratitis. Calcitriol enhances the immunosuppressive function of cyclosporine, during *A.fumigatus* infection, in human corneal cells, while VDR inhibitor weakens it [55].

The vast number of reports indicate that vitamin D may influence the development and progression of autoimmune diseases [56]. It seems to be mainly the result of its inhibitory effect on the differentiation of Th17 and Th1 cells [57]. In a model of autoimmune uveitis, calcitriol appears to suppress Th17 response by affecting lineage commitment and production of IL-17 [58]. This ability of vitamin D to prevent and even partially reverse abnormal immune response may be also due to its impact on the function of dendritic cells [57].

The anti-inflammatory effect of vitamin D was also observed in the retina. According to Lee *et al.*, subcutaneous injections of calcitriol significantly reduce the number of activated macrophages and attenuate chronic inflammation in the mouse retina [59]. In the meantime, intraperitoneal administration of 1,25(OH) $_2$ D has been shown to reduce oxidative stress and inflammation in the lacrimal

glands of radioiodine treated rats [60]. However, these results were based solely on histopathological and biochemical changes, regardless of their effect on the function of the lacrimal glands.

Diabetic Retinopathy

It can be hypothesized that due to its anti-angiogenic and anti-inflammatory activity, vitamin D protects against the development of diabetic retinopathy. In 2012, a group of scientist found that $1,25(\text{OH})_2\text{D}$ is able to inhibit VEGF and TGF- β expression in the retinal tissues of diabetic rats [61]. Interestingly, recent findings in this field have reported that in a rat model of diabetic retinopathy, calcitriol has a protective effect on the retinal structure, i.e. the number of cells in the ganglion cell layer and retinal thickness [62]. In addition, treatment with calcitriol was found to inhibit retinal cells apoptosis and decrease retinal vascular permeability. This is the result of reducing the production of reactive oxygen species, thus blocking the TXNIP/NLRP3 pathway.

Age-related macular degeneration

Although the exact pathogenesis of AMD remains unclear, inflammation, oxidative stress, and neoangiogenesis are thought to underlie this disease [63]. Therefore, it has been suggested that vitamin D activity is a factor that can significantly affect these pathogenic steps. As we know, VDR and the enzymes involved in the vitamin D metabolism are expressed both in the retina and choroid. It allows $1,25(\text{OH})_2\text{D}$ to act directly in the eye. In the mouse cone cells treated with H_2O_2 in order to induce oxidative stress, vitamin D has been shown to increase cell viability and reduce the production of reactive oxygen species [64]. In addition, it up-regulated the expression of antioxidant enzymes such as catalase, superoxide dismutase, and glutathione peroxidase. Mice injected subcutaneously with vitamin D for 6 weeks showed not only a reduction in retinitis but also a lower accumulation of beta-amyloid, which is a component of AMD-associated deposits [59]. This activity together with its ability to inhibit inflammation and angiogenesis present vitamin D as a potential protection against the development of AMD, but further investigation is necessary to confirm this.

Retinoblastoma

There is compelling evidence for the role of vitamin D in cancer growth and development. It has been found to have anticancer activity through regulation of apoptosis, angiogenesis, cell differentiation, proliferation, and migration [65]. As a result, *in vitro* and *in vivo* calcitriol, as well as its analogs, inhibit the growth of

various malignant tumors such as breast, colon or prostate cancer [66]. Some analogs are currently undergoing clinical trials as anti-cancer drugs [67].

Vitamin D receptor expression was detected in a human retinoblastoma cell line (Y-79), tumors from the transgenic mouse retinoblastoma model (LH beta-Tag tumors) and ultimately in human retinoblastoma samples [68]. The effect of calcitriol on these cells was then investigated. It was shown that *in vitro* calcitriol inhibits the growth of Y-79 retinoblastoma cells [68, 69]. Subsequently, *in vivo* experiments were conducted. In athymic nude mice injected with Y-79 cells (xenograft model) and LH beta-Tag mice (transgenic model) treatment with calcitriol or ergocalciferol led to a significant reduction in tumor growth [68]. However, the doses required for this effect appeared to be highly toxic for the animals due to hypercalcemia. There is a retrospective study suggesting that the co-administration of calcitriol with a saline solution reduces its toxicity, and hence mortality of animal models [70]. It is based mainly on the result of the experiment, in which it was proven that the combination therapy with cisplatin and calcitriol is highly effective in the treatment of retinoblastoma xenograft model [71]. For 5 weeks, the authors of this study administered saline in addition to calcitriol with cisplatin or calcitriol alone without any deaths in any of the groups. Another approach to reducing the mortality caused by vitamin D treatment is focused on its analogs with less hypercalcemic activity. Synthetic analogs of calcitriol such as 1,25-dihydroxy-16-ene-23-yne-Vitamin D₃ (16,23-D₃) and 1 α -hydroxyvitamin D₂ (1 α -OH-D₂) were tested. It has been proven that they inhibit the growth of relatively small tumors with less toxicity than calcitriol or ergocalciferol [68, 72, 73]. However, effective doses in the treatment of large tumors continued to cause hypercalcemia and increased mortality as a final complication [74]. In addition, 16,23-D₃ proved ineffective during prolonged use *i.e.* 15 weeks [74]. Following experiments investigated the effectiveness of another analog 2-methylene-19-nor-(20S)-1-hydroxybishomopregnacalciferol (2MbisP). Finally, 2MbisP has been shown to inhibit the growth of retinoblastoma without causing hypercalcemia or increased mortality [75].

1,25(OH)₂D and its analogs exert anti-neoplastic activity by inducing tumor cell apoptosis without affecting cell proliferation either in xenograft or transgenic models. Based on immunostaining for p53 in the region of cell death, the activation of apoptosis by vitamin D analogs seems to be mediated by the tumor suppressor protein p53 [68, 76]. Consistent with the fact that the p53 suppressor gene regulates the expression of both anti-apoptotic Bcl-2 protein and pro-apoptotic Bax [77], calcitriol has been shown to increase Bax levels and reduce Bcl-2 in retinoblastoma cells [69]. Various authors have reported that programmed cell death induced by vitamin-D analogs is also associated with an increase in p21 protein levels [68, 76]. The cyclin-dependent kinase inhibitor p21 may promote as well as inhibit apoptosis, either through p53-dependent or p53-independent pathways [78], and therefore the

exact role of this protein requires more detailed studies. Inhibition of angiogenesis appears to be a secondary mechanism responsible for this anti-tumor effect [79].

Conclusions

Numerous epidemiological studies have linked vitamin D deficiency with ocular diseases such as myopia, age-related macular degeneration, diabetic retinopathy, glaucoma, retinoblastoma, and uveitis, but the underlying mechanism is not yet fully understood. However, recently discovered anti-angiogenic, anti-inflammatory, and anti-neoplastic properties of calcitriol have shed some light on its role in the pathogenesis of ocular diseases. Vitamin D, through ubiquitously expressed receptors, appears to act as a protective factor in the eye, where its local production and activation is possible due to the presence of required enzymes.

Conflict of interest

None declared.

References

1. Pike J.W.: The Vitamin D Receptor: Discovery, Structure, and Function. *Adv Organ Biol.* 1998; 5 (C): 213–241. doi: 10.1016/S1569-2590(08)60114-7.
2. Haussler M.R., Jurutka P.W., Mizwicki M., Norman A.W.: Vitamin D receptor (VDR)-mediated actions of 1 α ,25(OH) 2vitamin D 3: Genomic and non-genomic mechanisms. *Best Pract Res Clin Endocrinol Metab.* 2011; 25 (4): 543–559. doi: 10.1016/j.beem.2011.05.010.
3. Bouillon R., Carmeliet G., Verlinden L., et al.: Vitamin D and human health: lessons from vitamin D receptor null mice. *Endocr Rev.* 2008; 29 (6): 726–776. doi: 10.1210/er.2008-0004.
4. Etten E. van, Mathieu C.: Immunoregulation by 1,25-dihydroxyvitamin D₃: Basic concepts. *J Steroid Biochem Mol Biol.* 2005; 97 (1–2): 93–101. doi: 10.1016/j.jsbmb.2005.06.002.
5. Li Y.C.: Vitamin D regulation of the renin-angiotensin system. *J Cell Biochem.* 2003; 88 (2): 327–331. doi: 10.1002/jcb.10343.
6. Bikle D.D.: Vitamin D metabolism and function in the skin. *Mol Cell Endocrinol.* 2011; 347 (1–2): 80–89. doi: 10.1016/j.mce.2011.05.017.
7. Swami S., Raghavachari N., Muller U.R., Bao Y.P., Feldman D.: Vitamin D Growth Inhibition of Breast Cancer Cells: Gene Expression Patterns Assessed by cDNA Microarray. *Breast Cancer Res Treat.* 2003; 80 (1): 49–62. doi: 10.1023/A: 1024487118457.
8. Pálmer H.G., González-Sancho J.M., Espada J., et al.: Vitamin D(3) promotes the differentiation of colon carcinoma cells by the induction of E-cadherin and the inhibition of beta-catenin signaling. *J Cell Biol.* 2001; 154 (2): 369–387. <http://www.ncbi.nlm.nih.gov/pubmed/11470825>. Accessed March 28, 2018.
9. Zhao X.-Y., Peehl D.M., Navone N.M., Feldman D.: 1 α ,25-Dihydroxyvitamin D₃ Inhibits Prostate Cancer Cell Growth by Androgen-Dependent and Androgen-Independent Mechanisms. *Endocrinology.* 2000; 141 (7): 2548–2556. doi: 10.1210/endo.141.7.7549.
10. Tissue expression of CYP2R1 — Summary — The Human Protein Atlas. <http://www.proteinatlas.org/ENSG00000186104-CYP2R1/tissue>. Accessed March 22, 2018.

11. Kaseda R., Hosojima M., Sato H., Saito A.: Role of Megalin and Cubilin in the Metabolism of Vitamin D₃. *Ther Apher Dial.* 2011; 15: 14–17. doi: 10.1111/j.1744-9987.2011.00920.x.
12. Adams J.S., Hewison M.: Extrarenal expression of the 25-hydroxyvitamin D-1-hydroxylase. *Arch Biochem Biophys.* 2012; 523 (1): 95–102. doi: 10.1016/j.abb.2012.02.016.
13. Schlingmann K.P., Kaufmann M., Weber S., et al.: Mutations in *CYP24A1* and Idiopathic Infantile Hypercalcemia. *N Engl J Med.* 2011; 365 (5): 410–421. doi: 10.1056/NEJMoa1103864.
14. Boyan B.D., Sylvia V.L., Dean D.D., Schwartz Z.: 24,25-(OH)₂D₃ regulates cartilage and bone via autocrine and endocrine mechanisms. *Steroids.* 66 (3–5): 363–374. <http://www.ncbi.nlm.nih.gov/pubmed/11179745>. Accessed May 23, 2018.
15. Curtis K.M., Aenlle K.K., Roos B.A., Howard G.A.: 24R,25-dihydroxyvitamin D₃ promotes the osteoblastic differentiation of human mesenchymal stem cells. *Mol Endocrinol.* 2014; 28 (5): 644–658. doi: 10.1210/me.2013-1241.
16. Erben R.G.: Update on FGF23 and Klotho signaling. *Mol Cell Endocrinol.* 2016; 432: 56–65. doi: 10.1016/J.MCE.2016.05.008.
17. Shinki T., Ueno Y., DeLuca H.F., Suda T.: Calcitonin is a major regulator for the expression of renal 25-hydroxyvitamin D₃-1 α -hydroxylase gene in normocalcemic rats. *Proc Natl Acad Sci U S A.* 1999; 96 (14): 8253–8258. <http://www.ncbi.nlm.nih.gov/pubmed/10393981>. Accessed April 30, 2018.
18. Pike J.W., Christakos S.: Biology and Mechanisms of Action of the Vitamin D Hormone. *Endocrinol Metab Clin North Am.* 2017; 46 (4): 815–843. doi: 10.1016/j.ecl.2017.07.001.
19. Buitrago C., Vazquez G., De Boland A.R., Boland R.: The Vitamin D Receptor Mediates Rapid Changes in Muscle Protein Tyrosine Phosphorylation Induced by 1,25(OH)₂D₃. *Biochem Biophys Res Commun.* 2001; 289 (5): 1150–1156. doi: 10.1006/BBRC.2001.6072.
20. Hii C.S., Ferrante A.: The Non-Genomic Actions of Vitamin D. *Nutrients.* 2016; 8 (3): 135. doi: 10.3390/nu8030135.
21. Nemere I., Dormanen M.C., Hammond M.W., Okamura W.H., Norman A.W.: Identification of a specific binding protein for 1 α ,25-dihydroxyvitamin D₃ in basal-lateral membranes of chick intestinal epithelium and relationship to transcaltachia. *J Biol Chem.* 1994; 269 (38): 23750–23756. <http://www.ncbi.nlm.nih.gov/pubmed/8089147>. Accessed March 29, 2018.
22. Huhtakangas J.A., Olivera C.J., Bishop J.E., Zanello L.P., Norman A.W.: The Vitamin D Receptor Is Present in Caveolae-Enriched Plasma Membranes and Binds 1 α ,25(OH)₂ — Vitamin D₃ *in Vivo* and *in Vitro*. *Mol Endocrinol.* 2004; 18 (11): 2660–2671. doi: 10.1210/me.2004-0116.
23. Mizwicki M.T., Keidel D., Bula C.M., et al.: Identification of an alternative ligand-binding pocket in the nuclear vitamin D receptor and its functional importance in 1 α ,25(OH)₂-vitamin D₃ signaling. *Proc Natl Acad Sci U S A.* 2004; 101 (35): 12876–12881. doi: 10.1073/pnas.0403606101.
24. Chen J., Doroudi M., Cheung J., Grozier A.L., Schwartz Z., Boyan B.D.: Plasma membrane Pdia3 and VDR interact to elicit rapid responses to 1 α ,25(OH)₂D₃. *Cell Signal.* 2013; 25 (12): 2362–2373. doi: 10.1016/j.cellsig.2013.07.020.
25. Wang Y., Zhu J., DeLuca H.F.: Where is the vitamin D receptor? *Arch Biochem Biophys.* 2012; 523 (1): 123–133. doi: 10.1016/j.abb.2012.04.001.
26. Hansdottir S., Monick M.M., Hinde S.L., Lovan N., Look D.C., Hunninghake G.W.: Respiratory epithelial cells convert inactive vitamin D to its active form: potential effects on host defense. *J Immunol.* 2008; 181 (10): 7090–7099. <http://www.ncbi.nlm.nih.gov/pubmed/18981129>. Accessed March 28, 2018.
27. Bikle D.D.: Extrarenal Synthesis of 1,25-Dihydroxyvitamin D and Its Health Implications. In: Holick M. eds. *Vitamin D. Nutrition and Health.* Humana Press 2010; 277–295. doi:10.1007/978-1-60327-303-9_12.
28. Yin Z., Pintea V., Lin Y., Hammock B.D., Watsky M.A.: Vitamin D enhances corneal epithelial barrier function. *Invest Ophthalmol Vis Sci.* 2011; 52 (10): 7359–7364. doi: 10.1167/iovs.11-7605.
29. Alsalem J.A., Patel D., Susarla R., et al.: Characterization of Vitamin D Production by Human Ocular Barrier Cells. *Investig Ophthalmology Vis Sci.* 2014; 55 (4): 2140. doi: 10.1167/iovs.13-13019.

30. Lin Y., Ubels J.L., Schotanus M.P., et al.: Enhancement of vitamin D metabolites in the eye following vitamin D3 supplementation and UV-B irradiation. *Curr Eye Res.* 2012; 37 (10): 871–878. doi: 10.3109/02713683.2012.688235.
31. Jamali N., Wang S., Darjatmoko S.R., Sorenson C.M., Sheibani N.: Vitamin D receptor expression is essential during retinal vascular development and attenuation of neovascularization by 1,25(OH)2D3. *PLoS One.* 2017; 12 (12): e0190131. doi:10.1371/journal.pone.0190131.
32. Lu X., Elizondo R.A., Nielsen R., et al.: Vitamin D in Tear Fluid. *Invest Ophthalmol Vis Sci.* 2015; 56 (10): 5880–5887. doi: 10.1167/iovs.15-17177.
33. Sethu S., Shetty R., Deshpande K., et al.: Correlation between tear fluid and serum vitamin D levels. *Eye Vis.* 2016; 3 (1): 22. doi: 10.1186/s40662-016-0053-7.
34. Kutuzova G.D., Gabelt B.T., Kiland J.A., Hennes-Beann E.A., Kaufman P.L., DeLuca H.F.: 1 α ,25-Dihydroxyvitamin D3 and its analog, 2-methylene-19-nor-(20S)-1 α ,25-dihydroxyvitamin D3 (2MD), suppress intraocular pressure in non-human primates. *Arch Biochem Biophys.* 2012; 518 (1): 53–60. doi: 10.1016/j.ABB.2011.10.022.
35. Reins R.Y., Baidouri H., McDermott A.M.: Vitamin D Activation and Function in Human Corneal Epithelial Cells During TLR-Induced Inflammation. *Invest Ophthalmol Vis Sci.* 2015; 56 (13): 7715–7727. doi: 10.1167/iovs.15-17768.
36. Elizondo R.A., Yin Z., Lu X., Watsky M.A.: Effect of Vitamin D Receptor Knockout on Cornea Epithelium Wound Healing and Tight Junctions. 2014; 55 (8). doi: 10.1167/iovs.13-13553.
37. Lu X., Chen Z., Mylarapu N., Watsky M.A.: Effects of 1,25 and 24,25 Vitamin D on Corneal Epithelial Proliferation, Migration and Vitamin D Metabolizing and Catabolizing Enzymes. *Sci Rep.* 2017; 7 (1): 16951. doi: 10.1038/s41598-017-16698-3.
38. Reins R.Y., Hanlon S.D., Magadi S., McDermott A.M.: Effects of Topically Applied Vitamin D during Corneal Wound Healing. Ljubimov A.V., ed. *PLoS One.* 2016; 11 (4): e0152889. doi: 10.1371/journal.pone.0152889.
39. Sel S., Trau S., Paulsen F., Kalinski T., Stangl G.I., Nass N.: 1,25-dihydroxyvitamin D3 inhibits corneal wound healing in an ex-vivo mouse model. *Graefes Arch Clin Exp Ophthalmol.* 2016; 254 (4): 717–724. doi: 10.1007/s00417-016-3267-4.
40. Mantell D.J., Owens P.E., Bundred N.J., Mawer E.B., Canfield A.E.: 1 α ,25-dihydroxyvitamin D(3) inhibits angiogenesis in vitro and in vivo. *Circ Res.* 2000; 87 (3): 214–220. doi: 10.1161/01.RES.87.3.214.
41. Albert D.M., Scheef E.A., Wang S., et al.: Calcitriol Is a Potent Inhibitor of Retinal Neovascularization. *Investig Ophthalmology Vis Sci.* 2007; 48 (5): 2327. doi: 10.1167/iovs.06-1210.
42. Merrigan S.L., Kennedy B.N.: Vitamin D receptor agonists regulate ocular developmental angiogenesis and modulate expression of dre-miR-21 and VEGF. *Br J Pharmacol.* 2017; 174 (16): 2636–2651. doi: 10.1111/bph.13875.
43. Chen Q., Qiu F., Zhou K., et al.: Pathogenic Role of microRNA-21 in Diabetic Retinopathy Through Downregulation of PPAR α . *Diabetes.* 2017; 66 (6): 1671–1682. doi: 10.2337/db16-1246.
44. Gutsaeva D.R., Thounaojam M., Rajpurohit S., et al.: STAT3-mediated activation of miR-21 is involved in down-regulation of TIMP3 and neovascularization in the ischemic retina. *Oncotarget.* 2017; 8 (61): 103568–103580. doi: 10.18632/oncotarget.21592.
45. Sabatel C., Malvaux L., Bovy N., et al.: MicroRNA-21 exhibits antiangiogenic function by targeting RhoB expression in endothelial cells. *PLoS One.* 2011; 6 (2): e16979. doi: 10.1371/journal.pone.0016979.
46. Suzuki T., Sano Y., Kinoshita S.: Effects of 1 α ,25-dihydroxyvitamin D3 on Langerhans cell migration and corneal neovascularization in mice. *Invest Ophthalmol Vis Sci.* 2000; 41 (1): 154–158. <http://www.ncbi.nlm.nih.gov/pubmed/10634615>. Accessed May 1, 2018.
47. Liu P.T., Stenger S., Li H., et al.: Toll-Like Receptor Triggering of a Vitamin D-Mediated Human Antimicrobial Response. *Science* (80–). 2006; 311 (5768): 1770–1773. doi: 10.1126/science.1123933.

48. Suzuki T, Sano Y, Sotozono C, Kinoshita S: Regulatory effects of 1 α ,25-dihydroxyvitamin D(3) on cytokine production by human corneal epithelial cells. *Curr Eye Res.* 2000; 20 (2): 127–130. <http://www.ncbi.nlm.nih.gov/pubmed/10617914>. Accessed May 27, 2018.
49. Reins R.Y, Mesmar F, Williams C, McDermott A.M.: Vitamin D Induces Global Gene Transcription in Human Corneal Epithelial Cells: Implications for Corneal Inflammation. *Investig Ophthalmology Vis Sci.* 2016; 57 (6): 2689. doi: 10.1167/iovs.16-19237.
50. Zhang J, Dai Y, Wu D, Xu J: Calcitriol, the Active Metabolite of Vitamin D₃, Inhibits Dry Eye Related Corneal Inflammation *In Vivo* and *In Vitro*. *Ocul Immunol Inflamm.* October 2017: 1–9. doi: 10.1080/09273948.2017.1372486.
51. Xue M.-L., Zhu H., Thakur A., Willcox M.: 1 α ,25-Dihydroxyvitamin D₃ inhibits pro-inflammatory cytokine and chemokine expression in human corneal epithelial cells colonized with *Pseudomonas aeruginosa*. *Immunol Cell Biol.* 2002; 80 (4): 340–345. doi: 10.1046/j.1440-1711.80.august.1.x.
52. Cong L., Xia Y.-P., Zhao G.-Q., et al.: Expression of vitamin D receptor and cathelicidin in human corneal epithelium cells during fusarium solani infection. *Int J Ophthalmol.* 2015; 8 (5): 866–871. doi: 10.3980/j.issn.2222-3959.2015.05.03.
53. Che C.-Y., Li C., Gao A., et al.: Dectin-1 expression at early period of *Aspergillus fumigatus* infection in rat's corneal epithelium. *Int J Ophthalmol.* 2013; 6 (1): 30–33. doi: 10.3980/j.issn.2222-3959.2013.01.06.
54. Zhao J., Wu X., Yu F.-X.: Activation of Toll-like receptors 2 and 4 in *Aspergillus fumigatus* keratitis. *Innate Immun.* 2009; 15 (3): 155–168. doi: 10.1177/1753425908101521.
55. Xia Y., Zhao G., Lin J., et al.: 1,25(OH)₂D₃ and VDR Signaling Pathways Regulate the Inhibition of Dectin-1 Caused by Cyclosporine A in Response to *Aspergillus Fumigatus* in Human Corneal Epithelial Cells. Slominski A.T., ed. *PLoS One.* 2016; 11 (10): e0164717. doi: 10.1371/journal.pone.0164717.
56. Prietl B., Treiber G., Pieber T.R., Amrein K.: Vitamin D and immune function. *Nutrients.* 2013; 5 (7): 2502–2521. doi: 10.3390/nu5072502.
57. Tian Y., Wang C., Ye Z., Xiao X., Kijlstra A., Yang P.: Effect of 1,25-Dihydroxyvitamin D₃ on Th17 and Th1 Response in Patients with Behçet's Disease. *Investig Ophthalmology Vis Sci.* 2012; 53 (10): 6434. doi: 10.1167/iovs.12-10398.
58. Tang J., Zhou R., Luger D., et al.: Calcitriol suppresses antiretinal autoimmunity through inhibitory effects on the Th17 effector response. *J Immunol.* 2009; 182 (8): 4624–4632. doi: 10.4049/jimmunol.0801543.
59. Lee V., Rekhi E., Hoh Kam J., Jeffery G.: Vitamin D rejuvenates aging eyes by reducing inflammation, clearing amyloid beta and improving visual function. *Neurobiol Aging.* 2012; 33 (10): 2382–2389. doi: 10.1016/j.neurobiolaging.2011.12.002.
60. Eksioglu U., Atilgan H.I., Yakin M., et al.: Antioxidant Effects of Vitamin D on Lacrimal Glands against High Dose Radioiodine-Associated Damage in an Animal Model. *Cutan Ocul Toxicol.* July 2018: 1–21. doi: 10.1080/15569527.2018.1498507.
61. Ren Z., Li W., Zhao Q., Ma L., Zhu J.: The impact of 1,25-dihydroxy vitamin D₃ on the expressions of vascular endothelial growth factor and transforming growth factor- β 1 in the retinas of rats with diabetes. *Diabetes Res Clin Pract.* 2012; 98 (3): 474–480. doi: 10.1016/j.diabres.2012.09.028.
62. Lu L., Lu Q., Chen W., Li J., Li C., Zheng Z.: Vitamin D₃ Protects against Diabetic Retinopathy by Inhibiting High-Glucose-Induced Activation of the ROS/TXNIP/NLRP3 Inflammasome Pathway. *J Diabetes Res.* 2018; 2018: 8193523. doi: 10.1155/2018/8193523.
63. Yonekawa Y., Miller J.W., Kim I.K.: Age-Related Macular Degeneration: Advances in Management and Diagnosis. *J Clin Med.* 2015; 4 (2): 343–359. doi: 10.3390/jcm4020343.
64. Tohari A.M., Zhou X., Shu X.: Protection against oxidative stress by vitamin D in cone cells. *Cell Biochem Funct.* 2016; 34 (2): 82–94. doi: 10.1002/cbf.3167.
65. Christakos S., Dhawan P., Verstuyf A., Verlinden L., Carmeliet G.: Vitamin D: Metabolism, Molecular Mechanism of Action, and Pleiotropic Effects. *Physiol Rev.* 2016; 96 (1): 365–408. doi: 10.1152/physrev.00014.2015.

66. Feldman D., Krishnan A.V., Swami S., Giovannucci E., Feldman B.J.: The role of vitamin D in reducing cancer risk and progression. *Nat Rev Cancer*. 2014; 14 (5): 342–357. doi: 10.1038/nrc3691.
67. Duffy M.J., Murray A., Synnott N.C., O'Donovan N., Crown J.: Vitamin D analogues: Potential use in cancer treatment. *Crit Rev Oncol Hematol*. 2017; 112: 190–197. doi: 10.1016/j.critrevonc.2017.02.015.
68. Albert D.M., Nickells R.W., Gamm D.M., et al.: Vitamin D analogs, a new treatment for retinoblastoma: The first Ellsworth Lecture. *Ophthalmic Genet*. 2002; 23 (3): 137–156. <http://www.ncbi.nlm.nih.gov/pubmed/12324873>. Accessed May 15, 2018.
69. Wagner N., Wagner K.-D., Schley G., Badiali L., Theres H., Scholz H.: 1,25-dihydroxyvitamin D3-induced apoptosis of retinoblastoma cells is associated with reciprocal changes of Bcl-2 and bax. *Exp Eye Res*. 2003; 77 (1): 1–9. <http://www.ncbi.nlm.nih.gov/pubmed/12823982>. Accessed May 16, 2018.
70. Azari A.A., Kanavi M.R., Darjatmoko S.R., et al.: Hydration with saline decreases toxicity of mice injected with calcitriol in preclinical studies. *J Environ Pathol Toxicol Oncol*. 2013; 32 (3): 241–244. <http://www.ncbi.nlm.nih.gov/pubmed/24266410>. Accessed May 16, 2018.
71. Kulkarni A.D., van Ginkel P.R., Darjatmoko S.R., Lindstrom M.J., Albert D.M.: Use of combination therapy with cisplatin and calcitriol in the treatment of Y-79 human retinoblastoma xenograft model. *Br J Ophthalmol*. 2009; 93 (8): 1105–1108. doi: 10.1136/bjo.2008.152843.
72. Grostern R.J., Bryar P.J., Zimbric M.L., et al.: Toxicity and dose-response studies of 1alpha-hydroxyvitamin D2 in a retinoblastoma xenograft model. *Arch Ophthalmol (Chicago, Ill 1960)*. 2002; 120 (5): 607–612. <http://www.ncbi.nlm.nih.gov/pubmed/12003610>. Accessed May 17, 2018.
73. Wilkerson C.L., Darjatmoko S.R., Lindstrom M.J., Albert D.M.: Toxicity and dose-response studies of 1,25-(OH)₂-16-ene-23-yne vitamin D3 in transgenic mice. *Clin Cancer Res*. 1998; 4 (9): 2253–2256. <http://www.ncbi.nlm.nih.gov/pubmed/9748146>. Accessed May 17, 2018.
74. Lokken D.M., Kumar A., Strugnell S.A., et al.: Effectiveness of Vitamin D Analogues in Treating Large Tumors and During Prolonged Use in Murine Retinoblastoma Models. *Arch Ophthalmol*. 2004; 122 (9): 1357. doi: 10.1001/archoph.122.9.1357.
75. Albert D.M., Plum L.A., Yang W., et al.: Responsiveness of human retinoblastoma and neuroblastoma models to a non-calcemic 19-nor Vitamin D analog. *J Steroid Biochem Mol Biol*. 2005; 97 (1–2): 165–172. doi: 10.1016/j.jsbmb.2005.06.019.
76. Audo I., Darjatmoko S.R., Schlamp C.L., et al.: Vitamin D Analogues Increase p53, p21, and Apoptosis in a Xenograft Model of Human Retinoblastoma. *Investig Ophthalmology Vis Sci*. 2003; 44 (10): 4192. doi: 10.1167/iovs.02-1198.
77. Miyashita T., Krajewski S., Krajewska M., et al.: Tumor suppressor p53 is a regulator of bcl-2 and bax gene expression in vitro and in vivo. *Oncogene*. 1994; 9 (6): 1799–1805. <http://www.ncbi.nlm.nih.gov/pubmed/8183579>. Accessed May 22, 2018.
78. Abbas T., Dutta A.: p21 in cancer: intricate networks and multiple activities. *Nat Rev Cancer*. 2009; 9 (6): 400–414. doi: 10.1038/nrc2657.
79. Shokravi M.T., Marcus D.M., Alroy J., Egan K., Saornil M.A., Albert D.M.: Vitamin D inhibits angiogenesis in transgenic murine retinoblastoma. *Invest Ophthalmol Vis Sci*. 1995; 36 (1): 83–87. <http://www.ncbi.nlm.nih.gov/pubmed/7529753>. Accessed May 17, 2018.