FUNDACIÓN
RAMÓN ARECES

Simposio Internacional
Vitamina D y cáncer: promesa o realidad

International Symposium
Vitamin D and cancer: promise or reality

Madrid, 28 y 29 de marzo de 2011
Madrid, March 28-29 , 2011

RESÚMENES / *ABSTRACTS*

1. **Luciano Adorini**
2. **Ana Aranda**
3. **Roger Bouillon**
4. **Moray Campbell**
5. **Carsten Carlberg**
6. **David Feldman**
7. **Edward Giovanucci**
8. **Michael Holick**
9. **Candace Johnson**
10. **María Jesús Larriba**
11. **Alberto Muñoz**
12. **Héctor G. Pálmer**
13. **Román Pérez-Fernández**
14. **Jörg Reichrath**
15. **Varda Rotter**
16. **Donald L Trump**
17. **JoEllen Welsh**

Immunomodulatory Effects of Vitamin D Receptor Agonists

Luciano Adorini

Vitamin D receptor (VDR) agonists modulate dendritic cell (DC) function, thus shaping T cell activation and development, but they also have direct effects on T and B lymphocytes. Lymphocytes, like macrophages and DCs, express the VDR and can synthesize 1,25(OH)2D3 able to exert autocrine and paracrine regulatory actions. VDR agonists primarily inhibit proinflammatory effector T cells like Th1 and Th17 cells, and promote the development of regulatory T (Treg) cells. These effects are partly due to direct T cell targeting, in addition to modulation of DC function favoring the induction of tolerogenic DCs. Thus, VDR agonists can target T cells both directly and indirectly, selectively inhibiting effector T cell subsets while promoting the development of Treg cells.

VDR agonists can inhibit cell growth, promote apoptosis, and induce differentiation of many cell types, in addition to inhibiting metastasis and angiogenesis, all desirable properties for a drug to control cancer. Based on sound epidemiological data supporting the vitamin D-cancer connection, the capacity of VDR agonists to inhibit cell growth and to promote cell differentiation has provided the foundations for extensive efforts aiming at the development of these hormones as anti-cancer agents, and proof of concept for their cancer-preventing and therapeutic properties is emerging.

However, from an immunological perspective, the immunomodulatory properties of VDR agonists are apparently just opposite to the main aims of cancer immunotherapy: boosting the immune response and breaking tumor-related tolerance. Indeed, among the pleiotropic activities of this class of drugs, some may not necessarily foster anti-tumor immune responses, and others may actually counteract them. Understanding these properties of the vitamin D system will help to identify VDR agonists with enhanced anti-proliferative/pro-differentiative and reduced immuno-modulatory activities as anti-cancer agents.

VOLVER / TO RETURN ↑

The Vitamin D receptor heterodimer

Ana Aranda

The vitamin D receptors (VDRs) normally act as ligand-inducible transcription factors by interacting, as heterodimers with the retinoid X receptor (RXR), with DNA response elements (VDREs) in target genes. It was assumed that RXR acts a silent partner of VDR with the only function of increasing affinity of the heterodimer for its DNA recognition site. However, our work demonstrates that the RXR ligand 9-cis-RA induces recruitment of coactivators by the DNA-bound heterodimer and potentiates vitamin D-dependent transcriptional responses. In fact, the presence of 9-cis-RA increases induction of cyp24 transcripts by vitamin D, and cooperates with this vitamin to produce differentiation of colon cancer cells and to cause senescence in keratinocytes. The RXR ligand also confers significant agonistic activity to a VDR ligand with very low agonistic activity and can even restore transcriptional activity of an AF-2 mutant VDR that causes hereditary rickets.

RXR also plays an important role on recruitment of corepressor complexes by VDR/RXR. Unlike other receptors, VDR/RXR recruits NCoR and SMRT strictly in a VDR agonist-dependent manner. Binding of an agonist to VDR allows its partner receptor, RXR, to bind the corepressors. The RXR ligand has an opposite effect and induces corepressor release from the heterodimer. The native heterodimer binds corepressors weakly, but deletion of the RXR AF-2 domain allows a strong agonist-dependent association with corepressors. Furthermore, corepressors depletion with siRNA enhances transcriptional responses to vitamin D, demonstrating that these proteins can function as physiological negative regulators of vitamin D-mediated transcription.

These studies reveal that in VDR/RXR heterodimers allosteric communication triggered by the RXR ligand plays a previously unrecognized role on coactivators and corepressors recruitment and vitamin D signaling with important functional and therapeutic implications.

VOLVER / TO RETURN ↑

Vitamin D: current knowledge and future perspectives

Roger Bouillon

Ligand-activated Vitamin D receptor regulates about 3 % the mouse/human genome. It regulates calcium and bone homeostasis by increasing transepithelial calcium transport (intestine, kidney) and regulates the functions of bone and parathyroid cells. The vitamin D endocrine system can have both positive and negative effects on bone mass. Severe vitamin D deficiency or resistance causes rickets or osteomalacia and has been largely eliminated in the Western world by vitamin D supplementation early in life. Mild vitamin D deficiency can however also impair the structural integrity of bone and increase the risk of osteoporotic fractures The optimal dose of vitamin D should bring 25OHD levels above 20 ng/ml or 50 nmol/L and this can be achieved in most otherwise normal subjects by a daily intake between 400 and 800 IU/d

VDR-1,25$(OH)_2D_3$ also has major effects on many other target tissues as demonstrated in VDR KO mice and men. Indeed, based on detailed analysis of the phenotype of mice or men with VDR or other mutations affecting the vitamin D endocrine system, and also based on genetic, molecular and cell biology studies, there are good arguments that vitamin D is also important for the skin (total alopecia by VDR mutations), the immune system (macrophage defence system stimulated and acquired immune system tapered down by the vitamin D hormone), renal or cardiovascular system (high renin hypertension and cardiac hypertrophy in VDR- or CYP27B1-deficient animals). Moreover the vitamin D hormone has major effects on the cell cycle, cell proliferation and thus cancer. There are also good arguments to link the vitamin D endocrine system with other important effects including all components of the metabolic syndrome, brain and mental functions.
The preclinical data (genetic, molecular, cellular and animal studies) are thus clearly suggesting a broad spectrum of activities of vitamin D. In addition, a large number of observational studies in man also link a poor vitamin D status with major diseases of mankind, in line with the preclinical observations.

The major missing part of the overall picture is the proof of causality. The few controlled vitamin D supplementation studies have generated conflicting data. Therefore there is no (or not yet?) evidence that vitamin D supplementation can improve health beyond its beneficial effects on bone. This also implies that the definition of vitamin D deficiency can yet only be based on its effect on bone.

In conclusion: (1) There is great unanimity that mild vitamin D deficiency, as defined for optimal bone health is worldwide problem for at least 1 billion people. This deficiency could be easily eliminated. (2) Extra skeletal effects of vitamin D are a good working hypothesis (not more not less) and we need constructive and intense collaboration to provide adequate answers. Long after the discovery of vitamin D we still miss many essential answers but this no exception but the rule for all ligands of nuclear receptors.

VOLVER / TO RETURN ↑

Role of vitamin D in prostate cancer
Moray Campbell

It is now 30 years since initial reports demonstrated the anticancer actions of $1a,25(OH)_2D_3$ and following these studies, anti-proliferative effects were demonstrated against prostate cancer (CaP) cell lines, xenografts and transgenic CaP models. These findings have been complemented by epidemiological findings that support links between replete VDR signaling, growth restraint, and broad anticancer activities. Despite this tantalizing evidence, successful therapeutic exploitation of the VDR receptor in CaP has been mixed. A significant impediment remains the inability to predict accurately the extent CaP patients will respond to either chemoprevention or chemotherapy strategies centered on vitamin D compounds. To address this knowledge gap we have focused on dissecting epigenetic mechanisms that govern VDR function and revealed how these processes are distorted in CaP.

Non-malignant prostate epithelial cells, for example RWPE-1 cells, display a profound and rapid VDR-induced cell cycle arrest. Time resolved approaches on

established VDR target genes revealed the dynamics of epigenetic regulation and gene regulation. Specifically we measured the VDR and co-factor binding, combined with histone modifications on key gene promoters including CDKN1A (encodes $p21^{(waf1/cip1)}$). Multiple levels of regulatory control emerged, with rapid and highly dynamic co-repressor release and recapture associated with individual histone modification patterns at three VDR binding sites. The magnitude of these modifications was specific to each to the phase of the cell cycle, with only G_1 being most permissive for gene activation. In parallel, the VDR bound and activated MCM7 which encodes in an intron the miRNA, miR-106b that in turn represses $p21^{(waf1/cip1)}$ expression. We conclude that VDR binding site- and promoter-specific patterns of histone modifications combine with miRNA co-regulation to form VDR-regulated feed-forward loops, for example to control $p21^{(waf1/cip1)}$ expression and cell cycle arrest. In parallel, ChIP-Seq, mRNA and miRNA arrays were undertaken to define the wider networks that were VDR-regulated. From these data a number of other feed-forward loop motifs emerged.

Prostate cancer cells display disruption to the coordinated regulation of mRNA and miRNA and reveal mechanisms of resistance. Co-repressor proteins were elevated, and the ligand-activated recruitment seen in RWPE-1 cells was exaggerated in CaP cell lines, such that critical regulatory pathways were selectively attenuated. For example, the binding of NCOR1 was increased at the CDKN1A promoter and suppressed its regulation. The MCM-7 promoter was retained in a responsive state and hence miR-106b was strongly inducible. Again miRNA micro-array approaches in different isogenic CaP cells lines revealed disruption to VDR regulation of specific pathways, notably including the TGF-b signaling network. Finally key components of these relationships were examined in murine and human prostate tumors and revealed significant and specific correlations between VDR, co-repressors and key target miRNA.

Collectively these approaches suggest that VDR-regulated miRNA patterns are exquisitely specific barometers of underlying epigenetic set-points. Thus VDR regulation of miRNA, and their serum expression, has the potential to be exploited to predict efficacy of VDR-centric therapies in CaP patients.

VOLVER / TO RETURN ↑

Genome-wide shift in the locations of VDR chromatin occupancy

Carsten Carlberg.

A global understanding of the actions of the nuclear hormone 1a,25-dihydroxyvitamin D_3 (1a,25$(OH)_2D_3$) and its receptor, the transcription factor VDR, requires a genome-wide analysis of VDR binding sites. In THP-1 human monocytic leukemia cells we identified by ChIP-seq 2340 VDR binding locations genome-wide, of which 520 and 1171 occurred uniquely with and without a 40 min 1a,25$(OH)_2D_3$ treatment, respectively, while 649 were common. The usage of a *de novo* DR3-type response element (RE) was strongly associated with the ligand-responsiveness of VDR occupation, shifting from about 10% in peaks diminishing the most to about 90% in peaks growing the most upon ligand treatment. FAIRE-seq showed that open chromatin associates strongly with actively transcribed loci, but predicts inadequately VDR binding. A 4 h ligand treatment revealed 638 primary 1a,25$(OH)_2D_3$ target genes enriched in GO categories associated with immunity and signaling. Out of the 408 up-regulated 1a,25$(OH)_2D_3$ target genes, 72% showed VDR binding within 400 kb of their transcription start site (TSS), while this applied only for 43% of the 230 down-regulated genes. The VDR loci showed considerable variation in gene regulatory scenarios ranging from a single VDR location near the target gene TSS (for example, *SP100*) to very complex clusters of many VDR locations and target genes (for example, *THBD*, *CD93* and *GZF1*). In conclusion, ligand binding shifts the locations of VDR occupation to DR3-type REs that surround its primary target genes and occur in a large variety of regulatory constellations.

VOLVER / TO RETURN ↑

Actions of Vitamin D to Inhibit Inflammation and Estrogen Synthesis and Signaling in Breast Cancer

David Feldman

Calcitriol (1,25-dihydroxyvitamin D_3), the hormonally active form of vitamin D, exerts antiproliferative, anti-inflammatory and proapoptotic effects in many malignant cells including breast cancer (BCa) cells, raising the possibility of its use in cancer therapy. Among the anti-inflammatory actions, we previously showed that calcitriol inhibits the prostaglandin (PG) pathway in cultured BCa cells by inhibiting COX-2 expression as well as other aspects of the PG signaling pathway. Since PGs are stimulators of BCa growth and aromatase expression, PGs provide a major stimulus to estrogen synthesis and signaling that drives BCa proliferation. Calcitriol inhibits this estrogenic driving force both by directly inhibiting aromatase expression and also by indirectly by inhibiting the PG pathway thus secondarily reducing aromatase by a separate mechanism. Calcitriol acts as a selective aromatase modulator (SAM), decreasing aromatase expression in human BCa cells and adipocytes while increasing it in human osteosarcoma cells. More recently, we examined the regulatory effects of calcitriol *in vivo* on PG synthesis, aromatase expression, estrogen signaling and tumor growth when used alone and in combination with aromatase inhibitors (AIs). In immunocompromised mice bearing MCF-7 xenografts, calcitriol and the AIs anastrozole and letrozole exhibited significant tumor inhibitory activities (> 50% shrinkage). Calcitriol also acted as a SAM *in vivo*, decreasing aromatase expression in xenograft tumors and the surrounding mammary adipose tissue (the major source of aromatase in the breast) while increasing aromatase expression in bone marrow cells. Cox-2 expression was also inhibited. We showed that calcitriol caused significant reductions in estrogen levels in BCa tumor and breast adipose tissue. In addition, calcitriol inhibited estrogen signaling by decreasing tumor ERlevels. Changes in tumor gene expression further demonstrated the suppressive effects of calcitriol on inflammatory and growth signaling pathways. We hypothesize that cumulatively these actions contribute to a beneficial effect in the treatment of BCa. When combined with an AI, the actions of calcitriol would enhance AI anti-proliferative activity and could potentially protect bone from estrogen deprivation.

VOLVER / TO RETURN ↑

The Epidemiology of Vitamin D and Cancer
Edward Giovannucci

As supported by laboratory investigations, vitamin D holds promise in the prevention and possible treatment for various cancers. From an epidemiologic perspective, the relation between vitamin D status and cancer risk has been investigated using a number of approaches to estimate vitamin D status. These approaches have included direct measures of circulating 25(OH)vitamin D levels, and surrogates or determinants of 25(OH)vitamin D, including region of residence, vitamin D intake, and sun exposure estimates. Because of the various strengths and limitations of the diverse types of study designs tend to differ, the evidence is considered strongest when different types of studies in diverse settings yield consistent results. Using various approaches, evidence consistently and strongly supports a role for vitamin D in lowering risk for colorectal cancer incidence. In meta-analyses, individuals in the high quartile or quintile of 25(OH)D had a 40 to 50 percent risk reduction of risk of colorectal cancer relative to those in the lowest group. The evidence using various approaches is not as consistent for other cancers, but tends to support a role for vitamin D in breast and ovarian cancer, though more study is needed. To date, most of the epidemiologic studies have examined vitamin D status in relation to risk of incident cancer, but emerging evidence suggests that vitamin D may be important for cancer progression and mortality. Cancer patients with low 25(OH)vitamin D prior or at the time of diagnosis appear to have an inferior prognosis relative to individuals with high 25(OH)vitamin D levels. Vitamin D does not appear to influence risk of developing prostate cancer, but better vitamin D status does appear associated with lower risk of dying from prostate cancer. Data from randomized clinical trials are sparse and while suggestive of a benefit of vitamin D, are not conclusive at this point. Further study is needed to establish the role of vitamin D in terms of when in the life span and on what stages of carcinogenesis vitamin D is relevant, the optimal levels required for benefits, and which cancer sites besides colorectal cancer are most affected.

VOLVER / TO RETURN ↑

Vitamin D: Anti-Proliferative Activity and the Cancer Connection
Michael F. Holick

The anti-proliferative activity of 1,25-dihydroxyvitamin D [1,25$(OH)_2$D] has been well documented in a variety of normal and cancer cell lines for more than 30 years. The one practical application has been the use of 1,25$(OH)_2D_3$ and its analogues for treating the hyperproliferative skin disorder psoriasis. It has been demonstrated that 1,25$(OH)_2D_3$ and its analogues are able to regulate a wide variety of genes that influence malignant cell proliferation and differentiation. In vivo mouse studies have suggested that vitamin D deficiency increases tumor growth of mouse colon cancer and human prostate cancer. The mechanism by which vitamin D status influences risk for many cancers is due to the local production of 1,25$(OH)_2$D which in turn controls a variety of genes to limit tumor cell proliferation. Manipulation of LNCaP cells by stabling transacting them with the one hydroxylase provides further evidence for the importance of the local production of 1,25$(OH)_2$D to regulate cell growth.

VOLVER / TO RETURN ↑

Anti-Angiogenic and Anti-Metastatic Effects of Vitamin D
Candace S. Johnson

Studies have demonstrated that calcitriol (vitamin D or 1,25 dihydroxycholecalciferol) has significant anti-tumor activity *in vitro* and *in vivo* in a number of tumor model systems. Angiogenesis is an essential requirement for growth and metastasis of solid neoplasms. Calcitriol can inhibit angiogenesis *in vivo* through direct effects on tumor vasculature. We developed a model for isolation of endothelial cells freshly from tumors and demonstrate that CYP24

becomes epigenetically silenced selectively in tumor-derived endothelial cells (TDEC). TDEC are distinct from endothelial cells isolated from normal tissues and from Matrigel plugs (MDEC). In TDEC, calcitriol induces G_0/G_1 arrest, modulates p27 and p21, and induces apoptotic cell death and decreases P-Erk and P-Akt. In contrast, endothelial cells isolated from normal tissues and MDEC are unresponsive despite intact signaling through the VDR. In TDEC, which is sensitive to calcitriol, the CYP24 promoter is hypermethylated in two CpG island regions located at the 5'end, which may contribute to gene silencing of CYP24. The extent of methylation in these two regions is significantly less in MDEC. Methylation of the CYP24 promoter as measured by methylation-specific PCR was significantly increased in endothelial cells isolated by expression microdissection from human prostate tumors. Calcitriol treatment also resulted in altered morphology and behavior of tumor cells. Numerous actin stress fibers were observed throughout the cytoplasm following calcitriol treatment. Calcitriol reduced adhesion to laminin, fibronecton and the expression of the laminin receptors integrin α6 and ß4 and promoted adhesion to collagen I, II & V. Calcitriol inhibited motility by the wound healing/migration assay and suppressed invasion of by the Matrigel-based invasion assay and in situ zymography assay, with decreased expression of MMP-2 and MMP-9. Calcitriol promoted the activation of focal adhesion kinase (FAK) and enhanced the expression of E-cadherin and R-cadherin. In vivo, calcitriol significantly inhibited the development of pulmonary tumor formation, as assessed by MRI analysis and colony counts. These data suggest that epigenetic silencing of CYP24 modulates cellular responses to calcitriol and that calcitriol suppresses metastasis, possibly through the promotion of E-cadherin-mediated cell-cell adhesion and the inhibition of cell motility and invasion.

VOLVER / TO RETURN ↑

Mechanisms of resistance to vitamin D in colon cancer

María Jesús Larriba.

Numerous epidemiological data suggest a protective and therapeutic effect of vitamin D against colon cancer. In line with this, the most active metabolite of vitamin D (1a,25-dihydroxyvitamin D3, 1,25(OH)2D3) has antiproliferative, prodifferentiation and pro-apoptotic effects on cultured human colon cancer cells and antitumoral action in colon cancer mouse models. However, cancer cells can develop resistance to 1,25(OH)2D3 action due to aberrant expression of the enzymes responsible for itssynthesis (CYP27B1) and catabolism (CYP24A1), to the downregulation of vitamin D receptor (VDR), or to the deregulation of transcription corepressors that modulate VDR action (SMRT, NCoR).

We have shown that the transcription factors Snail1 and Snail2, known as inducers of epithelialmesenchymal transition, repress VDR expression and block 1,25(OH)2D3 action in colon cancer cells. Moreover, Snail1 and Snail2 expression is upregulated in human colorectal tumors and inversely correlates with that of VDR. Our data suggest that high levels of Snail1 and Snail2 are probably responsible for VDR downregulation in colon cancer and may generate resistance to treatments with 1,25(OH)2D3 or its analogs. These results may contribute to the improvement of protocols for the clinical use of vitamin D compounds, as they indicate that advanced colon cancer patients overexpressing Snail1 and/or Snail2 are not suitable candidates for this therapy.

VOLVER / TO RETURN ↑

Mechanism of action of vitamin D in colon cancer

Alberto Muñoz

Numerous epidemiological and preclinical data suggest a protective and perhaps therapeutic effect of vitamin D against colon cancer. Our results shown that the most active vitamin D metabolite 1a,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) inhibits proliferation, induces E-cadherin, and promotes differentiation of human colon cancer cells. In addition, $1,25(OH)_2D_3$ antagonizes the Wnt/b-catenin signalling, which is aberrantly activated in most human colon cancers, by at least three mechanisms: a) promoting VDR binding to b-catenin, thus reducing the formation of b-catenin/TCF4 complexes and their transcriptional activity, b) inducing the nuclear export of b-catenin, and c) inducing the expression of DICKKOPF (DKK)-1, a Wnt inhibitor. In addition, recent data from others suggest another mechanism of (indirect) Wnt/signaling inhibition: the repression in macrophages of interleukin-1b, which activates b-catenin transcriptional activity in colon cancer cells.

Our data show that the gene regulatory activity of $1,25(OH)_2D_3$ requires the activation of a rapid non-genomic signalling pathway that includes the entry of Ca^{2+} from the external medium and the posterior activation of the RhoA GTPase and the kinases ROCK, p38MAPK and MSK. This pathway is also necessary for the antagonism of the Wnt/b-catenin signalling and the inhibition of cell proliferation. $1,25(OH)_2D_3$ induces CST5 gene encoding the protease inhibitor Cystatin D, which behaves as a candidate tumor suppressor gene, and represses SPROUTY (SPRY)-2, which regulates signalling from tyrosine kinase receptors and behaves as an oncogene in colon cancer. The regulation of CDH1/E-cadherin, DKK-1, CST5/cystatin D and SPRY-2 must contribute to the protective action of $1,25(OH)_2D_3$ against colorectal cancer. To examine the role of $1,25(OH)_2D_3$/VDR in colon tumorigenesis in vivo, we have generated mice expressing a mutated Apc gene and lack VDR (Apc^{min} $Vdr^{-/-}$). These animals develop similar number of spontaneous intestinal tumors than the Apc^{min} $Vdr^{+/+}$ or Apc^{min} $Vdr^{+/-}$, but an increased tumoral load, indicating a role of $1,25(OH)_2D_3$/VDR in ameliorating tumor growth. Recently, we have identified novel nuclear proteins regulated by $1,25(OH)_2D_3$ in colon cancer cells by means of comparative proteomic studies. Remarkably, several regulated proteins belong to the spliceosome, suggesting that the splicing process, which is frequently altered in cancer, is controlled by $1,25(OH)_2D_3$.

VOLVER / TO RETURN ↑

The vitamin D receptor is a Wnt effector that controls hair follicle differentiation and specifies tumor type in adult epidermis

Héctor G. Pálmer

We have investigated how Wnt and vitamin D receptor signals regulate epidermal differentiation. Many epidermal genes induced by b-catenin, including the stem cell marker keratin 15, contain vitamin D response elements (VDREs) and several are induced independently of TCF/Lef. VDR is required for b-catenin induced hair follicle formation in adult epidermis, and the vitamin D analog EB1089 synergises with b-catenin to stimulate hair differentiation. Human trichofolliculomas (hair follicle tumours) are characterized by high nuclear b-catenin and VDR, whereas infiltrative basal cell carcinomas (BCCs) have high b-catenin and low VDR levels. In mice, EB1089 prevents b-catenin induced trichofolliculomas, while in the absence of VDR, b-catenin induces tumours resembling BCCs. We conclude that VDR is a TCF/Lef-independent transcriptional effector of the Wnt pathway and that vitamin D analogues have therapeutic potential in tumors with inappropriate activation of Wnt signalling.

VOLVER / TO RETURN ↑

The Pit-1 transcription factor and vitamin D in breast cancer

Román Pérez-Fernández

The Pit-1 transcription factor, a member of the POU domain factor family (Pit-1, Oct-1, and Unc-86), plays a key role in cell differentiation during organogenesis of the anterior pituitary in mammals and is a transcriptional activator for pituitary gene transcription. However, Pit-1 is also expressed in non-pituitary cell lines and tissues, such as the mammary gland. In these extrapituitary tissues, it has been suggested that Pit-1 could be related to cell proliferation and tumorigenesis. Specifically in breast, we demonstrate that Pit-1 presents higher expression in tumors than in normal breast and regulates the expression of two breast cancer-

related hormones, GH and PRL. In addition, we show that Pit-1 induces profound phenotypic changes in proteins involved in cell proliferation, apoptosis, and invasion. We found that overexpression of Pit-1 in SCID mice induced tumor growth, and promoted metastasis in lung. In patients with invasive ductal carcinoma of the breast and node-positive tumor, high expression of Pit-1 was significantly and independently associated with the occurrence of distant metastasis.

In contrast to the pro-tumoral effects of Pit-1 on breast, the anti-proliferative and pro-apoptotic actions of 1,25(OH)2D3 in the mammary gland have been widely demonstrated. Data from an earlier study of our laboratory showed that administration of $1,25(OH)_2D_3$ to MCF-7 cells has a suppressive effect on both Pit-1 mRNA and protein levels, through direct binding of the vitamin D receptor (VDR) to the Pit-1 promoter. On the other hand, Pit-1, by cooperating with other transcription factors such as CBP, increases VDR expression at the transcription stage, suggesting a negative feedback between VDR and Pit1, in which Pit1 stimulates VDR transcription while $1,25(OH)_2D_3$ inhibits Pit1 gene transcription.

These relationships between VDR and Pit1 could be involved in the physiological and/or pathological regulation in breast cells. Thus, we might speculate that the balance between the transcription factor Pit1 (which stimulates cell proliferation, and reduces apoptosis) and $1,25(OH)_2D_3$/VDR (which inhibits proliferation, and induces differentiation and apoptosis) could become altered in some circumstances, with Pit1 prevailing and leading to cell transformation. In cases of breast cancer cells with Pit-1-overexpresion, targeted therapies using low-calcemic vitamin D analogs could be an alternative strategy for breast cancer treatment.

VOLVER / TO RETURN ↑

Role of vitamin D in human skin cancers
Jörg Reichrath

Increasing evidence indicates that vitamin D deficiency and distinct functional polymorphisms across the 105 kb vitamin D receptor (VDR) gene are associated with various types of cancer. Cancer-associated VDR genotypes were shown to be common in all racial groups, having a minor allele frequency >10% and on average may double the risk of cancer. Moreover, it has been shown that many cancer cell lines respond to the antiproliferative and pro-differentiating effects of $1,25(OH)_2D_3$, the biologically active vitamin D metabolite, *in vitro*. Consequently, $1,25(OH)_2D_3$ and analogues represent promising compounds for cancer treatment. In recent years, we have investigated expression and function of key components of the vitamin D endocrine system (VDR, CYP27A1, CYP27B1, CYP24A1) in cutaneous malignancies, including malignant melanoma (MM), basal cell carcinoma (BCC) and cutaneous squamous cell carcinoma (SCC). Moreover, we have looked at the biological effects of $1,25(OH)_2D_3$, in skin cancer cell lines *in vitro*. This presentation summarizes these findings and gives an overview of our present understanding of the role of vitamin D in human skin cancers.

VOLVER / TO RETURN ↑

High dose vitamin D analogues or cholecalciferol: role in cancer therapy and prevention
Donald L. Trump

Considerable evidence supports the hypothesis that high dose calcitriol as well as other vitamin D analogues (e.g. paricalcitol, inecalcitol) have direct and indirect antitumor activity and potentiate the anticancer effects of most classes of chemotherapeutic drugs (e.g. taxanes, anthracyclines, topoisomerases inhibitors, antimetabolites). Low serum 25(OH) vitamin D3 [25D3] levels are associated with higher cancer incidence, mortality and overall morbidity. Our own studies in acute

myeloid leukemia (AML) indicate that low 25(OH) D3 serum levels are an independent factor prognostic for time to treatment failure and survival. Preclinical studies in prostate and carcinogen-induced lung cancer models indicate that vitamin D deficiency promotes tumor development & growth. These data suggest that cancer prevention as well as reduced morbidity in cancer patients might be achieved by cholecalciferol supplementation. Phase III studies of high dose calcitriol have been conducted, but did not demonstrate improved response or survival in men with castration resistant prostate cancer (CRPC). However, serious flaws in study design and dose selection compromise these trials. We are completing a phase II trial of high dose oral calcitriol + ketoconazole in CRPC based on preclinical evidence that inhibition of calcitriol catabolism through the CYP24 inhibitory properties of ketoconazole will potentiate ketoconazole antitumor activity in this setting. Ongoing preclinical and clinical studies with calcitriol and other analogues continue to support the hypothesis that vitamin D analogues may have an important clinical role in cancer. We have recently completed a study of four different daily doses of cholecalciferol (4000, 6000, 8000 or 10,000 IU) in 137 men with prostate cancer with either localized or advanced disease. 25D3, serum & urine calcium, PTH levels & toxicity were assessed at 1, 3 & 6 month. Among 117 patients analyzed toxicity was negligible. No clinically significant changes in serum or 24hr urine calcium occurred. 25D3 levels achieved were proportionate to dose.

Median 25D3 (ng/ml)

	Baseline	1mo	3mo	6mo
4000IU	24.7	35.5	49.3	56.4
6000IU	27.8	51.9	63.7	68.3
8000IU	27.8	41.3	55.5	63.9
10,000IU	25.4	52.2	72.8	84.1

We have also developed a plan for rapid 25(OH) D3 repletion and will open a study in AML within 6 months. High dose calcitriol and other analogues as well as aggressive D3 replacement regimens are well tolerated among cancer patients and merit careful clinical evaluation as approaches for cancer prevention and therapy.

VOLVER / TO RETURN ↑

Role of vitamin D in breast cancer

JoEllen Welsh

Although vitamin D receptor (VDR) expression is retained in the majority of human breast cancers, it is unclear whether 1,25-dihydroxyvitamin D (1,25D) regulates similar target genes and pathways in normal tissue and established tumors.

Our recent studies have utilized genomic profiling of both normal and transformed mammary tissues in human and mouse models to identify common and distinct VDR targets. In mouse experiments, we assessed genomic profiles of glandular tissue and established tumors from MMTV-neu mice fed deficient, adequate or high dietary cholecalciferol (D3). Our result indicate that the profile of genes altered by dietary D3 in mammary glands from MMTV-neu mice was distinctly different from that altered in established MMTV-neu tumors. Using a 2 fold-change cutoff, twelve genes were identified as common targets in tumor and glands, however, the direction and/or magnitude of dietary D3 regulation often differed.

The most highly regulated genes in non-transformed glands included those related to energy metabolism [Ucp1, Fabp4, Lep, Pparg] and immune regulation [Defb1, Irf4, Irf7, Ifi44]. Of the top highly regulated genes in normal tissue, only Defnb1 was also regulated by dietary D3 in tumors. In complementary studies, the vitamin D pathway was evaluated in human mammary epithelial (HME) cells as a function of transformation. Genes regulated by 1,25D in HME cells included those involved in differentiation/apoptosis (ITGB3, CDH1, BMP4/6, ID1/2, BIRC3), immunity (CD14, TLR2/4, TREM1, CSF1, IL-8) and metabolism (GLUL, SLC1A1, GPT2, G6PD). The regulation of a subset of these target genes by 1,25D was found to be abrogated upon transformation of HME cells with known oncogenes. Comparison of gene profiles between HME cells and the breast cancer cell line MCF7 revealed only six common targets (AKR1C2, CLMN, CYP24A1, PMEPA1, SERPINB1, TIMP3), which were all up-regulated in response to 1,25D.

Follow-up studies are focused on clarifying the impact of vitamin D signaling on tissue inflammation and cell metabolism in relation to tumor progression.

Studies in the mammary gland of VDR null mice have demonstrated age-related increases in inflammation and decreases in tissue adiposity. Cellular studies have confirmed that 1,25D modulates both synthesis and secretion of multiple cytokines and anti-microbial peptides from HME cells.

Collectively, these studies indicate that the vitamin D pathway modulates innate immunity and metabolic pathways in both human and murine mammary cells, although the majority of the target genes are species-specific. We hypothesize that in addition to known VDR targets involved in cell differentiation and survival, newly identified VDR target genes involved in immune responses and energy metabolism contribute to its tumor suppressive actions.

VOLVER / TO RETURN ↑