

VITAMIN D ANALOGS IN CANCER PREVENTION AND THERAPY

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Vitamin D Analogs in Cancer Prevention and Therapy

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Abstract. The Second International Symposium on "Vitamin D Analogs in Cancer Prevention and Therapy", was held in Lübeck, Germany, May 7-8, 2005. This meeting was specifically designed to summarize the latest developments in the epidemiology, molecular biology, metabolism, biological effects and clinical use of vitamin D analogs, leading to new concepts for their application in cancer prevention and therapy. Forty keynote lectures were presented at the Symposium and the following conclusions were summarized at a round-table discussion. **Cancer Prevention:** Evidence from epidemiological and laboratory investigations now convincingly demonstrate that vitamin D deficiency and insufficiency are under-recognized worldwide problems that are associated with several health problems, including the higher prevalence and unfavorable course of cancer. There is an urgent need for additional well-designed studies to define the optimal vitamin D status (25-hydroxyvitamin D serum level) and for campaigns to better inform the public and medical profession alike about the health risks related to vitamin D deficiency and insufficiency. **Cancer Therapy:** Knowledge of the underlying molecular mechanisms that mediate the antitumor effects of vitamin D analogs has expanded greatly during recent years and results from the first clinical trials indicate that such analogs hold promise for cancer therapy, most probably in combination with other agents. However, the era of vitamin D analogs in cancer therapy has just begun and efforts to perform well-designed clinical studies and to develop new analogs with fewer systemic side-effects have to continue.

The Second International Symposium, on "Vitamin D Analogs in Cancer Prevention and Therapy", that was

organized by the Klinik für Dermatologie, Venerologie und Allergologie, Universitätsklinikum des Saarlandes, Homburg/Saar and the Klinik für Frauenheilkunde und Geburtshilfe, Universitätsklinikum Schleswig-Holstein, Lübeck, Germany, was held in Lübeck, Germany on May 7-8, 2005. This meeting was specifically designed to summarize the latest developments in the field of vitamin D related to cancer. Experts in the field presented and discussed new findings in the epidemiology, molecular biology, synthesis and metabolism of vitamin D analogs, that have now led to promising concepts for the application of these compounds in cancer prevention and therapy and to the start of their clinical evaluation. Selected articles related to presentations at this symposium are published in this Special Issue of Anticancer Research (1-30). The most important findings and conclusions of this meeting can be summarized as follows:

Vitamin D analogs in cancer prevention. An increasing number of epidemiological and laboratory investigations have demonstrated an association between various types of cancer, including colon, prostate and breast cancer, with the (i) estimated exposure to solar UV-radiation, (ii) vitamin D intake or (iii) vitamin D deficiency or insufficiency (1-7). Although several of these studies have been hampered by methodological problems, including relatively few observation years or difficulties in correctly assessing the vitamin D status (exposure to solar UV-radiation, vitamin D intake or 25(OH)D measurements), a large number of epidemiological and laboratory investigations now clearly indicate a connection between vitamin D deficiency/ insufficiency and numerous diseases, including various types of cancer (1-7). It is recognized that the recent discovery of extrarenal 25(OH)D-1 α -hydroxylase activity in a broad variety of different tissues is of particular importance regarding the role of vitamin D in cancer prevention (1, 8, 9). The general consensus of the meeting was that further evidence has to be obtained from well-designed studies currently underway (randomized trials, prospective case control and cohort studies), using multivariate analysis of a large cohort population and

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sufficient observation time to clarify the role of vitamin D analogs in cancer prevention and to impute a causal relationship between lack of adequate vitamin D and cancer. In this context, it is particularly important for future investigations to measure plasma 25(OH)D levels from individuals, rather than speculating on possible vitamin D levels using geographical latitude as a parameter of possible exposure to solar UV-radiation.

Antitumor effects of vitamin D analogs in vitro. Many presentations were related to the antitumor effects of 1,25-dihydroxyvitamin D (1,25(OH)₂D, calcitriol) and its analogs (11-27). Recent relevant laboratory investigations, analyzing the molecular mechanisms that underly genomic and nongenomic vitamin D signaling pathways and their importance in terms of the antitumor effects of vitamin D analogs, were discussed. It was shown that a multitude of independent molecular events, including effects on cell proliferation, differentiation, apoptosis and DNA repair, are related to the antitumor effects of vitamin D. The molecular mechanisms are complex and those of the very early events have still to be clarified. Several papers focused on vitamin D receptor (VDR)-mediated genomic effects, particularly the impact on the chromatin organization of vitamin D target genes (4), on the recruitment of cofactor proteins to the VDR (e.g., DRIP-complex, WINAC, SKIP, WSTF, PGC-1 α) (4, 11), on the interaction of VDR with components of the basal transcription apparatus (e.g., TFIIB, TFIID) (11) and on the regulation of VDR expression by other molecules, (e.g., the transcription factor SNAIL) (12). The molecular mechanisms involving VDR include effects on cell cycle regulation, with G1 arrest being particularly relevant. Several mitogen-activated protein (MAP) kinase isoforms, as well as cyclin-dependent kinases and cyclins, are of importance for mediating the responses to 1,25(OH)₂D and its analogs, resulting in the involvement of other signaling pathways (various growth factors, including fibroblast growth factor 8 and insulin-like growth factors), adhesion molecules, retinoblastoma/pocket proteins, E2F transcription factors, oncogenes including c-fos, and multiple proteins involved in DNA replication and repair. There was general consensus that additional studies are needed to elucidate the precise mode of action and the underlying molecular mechanisms mediating the antitumor effects of vitamin D analogs. A better understanding of these mechanisms would certainly help to identify which other signal transduction pathways are promising targets for synergism in blocking cell cycle progression and angiogenesis, in inducing apoptosis or cell death and, finally, for the prevention, inhibition or cure of primary or secondary tumor progression. Several tumors were shown to be resistant to the antitumor effects of vitamin D analogs and able to escape VDR-mediated cell

cycle control. Here again, better insights into the synthesis, metabolism and molecular biology of these analogs could help identify possible strategic options for cancer prevention and therapy.

New strategies for the use and development of vitamin D compounds, that are effective in the treatment of malignancies and reveal few calcemic or other side-effects.

Dosing technique: The use of vitamin D analogs at therapeutically-efficient doses has been limited in cancer treatment by systemic side-effects, most importantly hypercalcuria and/or hypercalcemia. It has been demonstrated that the dosing technique is of particular importance in the systemic administration of 1,25(OH)₂D, and that weekly dosing of 1,25(OH)₂D allowed substantial dose escalation without dose-limiting toxicities (27).

Multiple protein-protein and protein-DNA interactions of the VDR: The identification and characterization of different signaling pathways for vitamin D have resulted in promising new strategies for the synthesis of vitamin D analogs to exert selective vitamin D activity (10, 12). These pathways are determined by the multiple protein-protein and protein-DNA interactions of the VDR that involve chromatin organization. A better understanding of the molecular events that determine VDR-mediated transcription (dimeric or multimeric complexes of the VDR with other nuclear partner proteins in addition to the structural variety of the known 1,25(OH)₂D response elements, i.e., direct repeats, palindromes, inverted palindromes), may enable the synthesis and characterization of new analogs of 1,25(OH)₂D that selectively activate distinct vitamin D signaling pathways. Altered VDR-analog conformations may produce heterogeneous induction of target genes that could lead to the activation of cascades of genes which differ for various analogs, a strategy possibly identifying compounds that reveal potent antiproliferative effects without inducing calcemic side-effects at clinically-relevant doses.

Selective activity of vitamin D compounds via co-stimulation with synergistic drugs: Several *in vitro* and *in vivo* studies showed that the biological effects of 1,25(OH)₂D can be selectively modulated by combination with various other drugs (23, 26, 27). To obtain synergistic effects on cell proliferation and differentiation, combination therapy of 1,25(OH)₂D with ligands of the nuclear VDR partner proteins, most importantly 9-cis retinoic acid, the naturally occurring ligand for the retinoid-X receptors (RXR- α , β , γ) may prove successful and possibly reduce the systemic side-effects associated with 1,25(OH)₂D treatment. Another strategy to increase efficacy and to decrease toxicity is to use a combination of agents that act by different mechanisms, at lower doses than those used therapeutically when the agents are administered individually. Glucocorticoids have been shown to potentiate the antitumor effect of 1,25(OH)₂D and to

decrease 1,25(OH)₂D-induced hypercalcemia (26). Other interesting candidates for combination therapy with vitamin D analogs include chemotherapeutics such as cisplatin or paclitaxel, RXR-ligands, distinct cytokines, as well as modulators of chromatin organization such as histone deacetylation inhibitors.

Tissue-selective potentiation of vitamin D activity via inhibition of vitamin D-metabolizing enzymes: The 1,25(OH)₂D levels are tightly controlled by its synthesis via 1 α -hydroxylase and its catabolism through hydroxylations mediated by specific cytochrome P-450 enzymes, such as 24-hydroxylase (8, 9, 14-17, 26, 30). Tissue-specific metabolism may allow the tissue-selective activity of vitamin D compounds, as has been demonstrated for other steroid hormone systems. Inhibition of 1,25(OH)₂D-catabolizing hydroxylases slows down catabolism and results in increased levels of 1,25(OH)₂D. Consequently, it has been shown that vitamin D activity in various target tissues can be potentiated by cytochrome P-450 enzyme-inhibiting drugs such as ketoconazole. Therefore, the expression of such cytochrome P-450 enzymes in target tissues deserves systematic analysis, with several presented papers being related to this topic. Combination therapy of vitamin D analogs with inhibitors of vitamin D-metabolizing enzymes may potentiate the biological effects of 1,25(OH)₂D on cell proliferation and differentiation in target tissues that strongly express the 24-hydroxylase for 1,25(OH)₂D without inducing substantial calcemic effects.

Clinical studies with vitamin D analogs in cancer. Several presentations were related to the safety and efficacy of vitamin D analogs in the treatment of various malignancies (26, 27). These clinical studies demonstrated that these analogs show promise for cancer therapy, at least for palliative treatment, in synergistic combination with cisplatin, docetaxel or other compounds (26, 27).

New vitamin D analogs. Selected presentations focused on the synthesis, metabolism and biological activity of new vitamin D analogs, including methyl-introduced A-ring analogs of 1,25(OH)₂D and 2-alkyl-1 α ,25-dihydroxy-19-norvitamin D₃ (28, 29). These newly-synthesized analogs differ from the mother compound 1 α ,25-dihydroxyvitamin D₃ in their intracellular metabolism, genomic or non-genomic actions, pharmacokinetics, interaction with the vitamin D-binding protein (DBP), or the VDR. The data presented indicated that new vitamin D analogs may be promising agents for cancer treatment.

Conclusion

New findings in epidemiology, molecular biology and cell physiology have substantially increased our understanding of the mechanisms that exert the biological effects of

vitamin D analogs in target tissues. Convincingly, it has been demonstrated that vitamin D deficiency and insufficiency are under-recognized worldwide problems, associated with the higher prevalence and unfavorable course of cancer. Additionally, new strategies for the clinical application of vitamin D analogs with selective anticancer activity, that reveal no or few systemic side-effects, have been introduced. Several new approaches, that may enable the application of vitamin D analogs for the treatment of various malignancies, were discussed at this symposium. Some of these new concepts are based on recent laboratory results demonstrating that certain vitamin D analogs differ in their intracellular metabolism, genomic or non-genomic actions, pharmacokinetics, interaction with the DBP, or the VDR. Currently, additional clinical studies are needed to exploit the therapeutic potential of the broad variety of newly-synthesized vitamin D analogs in cancer treatment, with and without other compounds including chemotherapeutics.

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