Vitamin D and lung cancer

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"Vitamin D regulates cytoplasmic signaling pathways that impact cellular differentiation and growth…"

An intriguing report on improved disease-free survival in patients undergoing surgery for lung cancer in the summer versus winter months piqued my interest as a thoracic oncologist several years ago. Furthermore, the same group demonstrated a statistically significant difference in the vitamin D levels in these patients and proposed this as a possible explanation for their observed phenomenon in a multivariate analysis model [1,2]. At the time, I was in collaboration with a group to ascertain the maximum tolerated dose of $1,25(OH)_2D_3$ (calcitriol, the active form of vitamin D) in dogs with cancer [3]. This was based on reports of the antiproliferative effects of $1,25(OH)_2D_3$ in cancer. Extrapolating dog pharmacology, we designed a Phase I/II study of $1,25(OH)_2D_3$ in combination with chemotherapy for advanced lung cancer; this trial is ongoing. Over the past few years, some interesting observations have been made regarding lung cancer and vitamin D. The following article will trace recent progress in our understanding of the potential role of vitamin D in lung cancer.

Vitamin D chemistry

Vitamin D is a lipid soluble secosteroid. There are two major forms of vitamin D, ergocalciferol (D_2) and cholecalciferol (D_3). D_2 can be acquired by diet of fortified milk products while D_3 is produced by UV B light isomerization of 7-dehydrocholestrol in the epidermis [4,5]. Isomerized vitamin D_3 enters the circulation bound to either vitamin D binding protein or to albumin to be transported to the liver. Upon reaching the liver, vitamin D_3 is converted to calcidiol (25-hydroxyvitamin D [$25(OH)D_3$]) by the 25-hydroxylase CYP27A1. $25(OH)D_3$ enters the systemic circulation and has a half-life of 12–19 days [6]. The serum levels of $25(OH)D_3$ are a reflection of overall vitamin D status in the body. Once $25(OH)D_3$ reaches the proximal tubules of the kidney, CYP27B1, a 1-αhydroxylase, converts calcidiol into $1,25(OH)_2D_3$ (calcitriol) the active form of vitamin D. $1,25(OH)_2D_3$ effects are mediated by binding to the vitamin D receptor (VDR). The human VDR protein is a 427 amino acid peptide that has a DNA-binding domain, a ligand-binding domain, and activating domains [7]. The VDR protein contains two zinc finger motifs that bind to the DNA [8], while the ligand-binding domain, located at the carboxyl terminus, changes conformation when $1,25(OH)_2D_3$ binds allowing interaction with transcription factors. Activated VDR forms a heterodimer with the retinoic acid X receptor, which translocates to the nucleus [9] and binds to vitamin D response element in the promoter region of target genes [8]. The serum half-life of $1,25(OH)_2D_3$ is 15 h [8]. Vitamin D is inactivated by the action of 24α-hydroxylase (CYP24A1). This enzyme catabolizes $25(OH)D_3$ to $24,25(OH)_2D_3$ and $1,25(OH)_2D_3$ to $1,24,25(OH)_3D_3$, respectively. These forms are water soluble and inactive.

When the dual origin of vitamin D (synthesis in the skin or dietary intake) was understood, oral vitamin D supplementation almost completely eradicated the endemic rickets. However, less severe

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vitamin D deficiency is still very common. It is estimated that more than 1 billion people worldwide have low serum levels of vitamin D. Several studies indicate that 40–70% of the elderly in the USA and in Europe are mildly or severely vitamin D deficient [6,10]. Vitamin D deficiency contributes to osteopenia, osteoporosis and increased risk of fractures in these individuals. In recent years, key activating and inactivating enzymes of vitamin D and VDR have been found in almost all mammalian cells, providing new insights for a paracrine role of vitamin D. Interestingly, phytoplankton and zooplankton (invertebrates) are known to produce vitamin D, suggesting a nonskeletal evolutionary role for vitamin D. Indeed, we now know that vitamin D has significant genomic as well as nongenomic effects in mammalian cells. Approximately 3% of the mouse and human genome is regulated via the vitamin D pathway, indicating a much broader role than initially presumed [11]. There is growing evidence that vitamin D might plays a role in the risk of many chronic illnesses including common cancers, myopathy, autoimmune disease, diabetes and the metabolic syndrome, infections and cardiovascular disease [12,13].

> "$1,25(OH)_2D_3$ also regulates cellular proliferation and apoptosis."

Antiproliferative effects of $1,25(OH)_2D_3$ in cancer

Calcitriol has been shown to inhibit the proliferation of human cancer cells. Vitamin D regulates cytoplasmic signaling pathways that impact cellular differentiation and growth through proteins such as Ras and MAPK, protein lipase A, prostaglandins, cyclic AMP, protein kinase A, and phosphatidyl inositol 3 kinase [14]. It is proposed that $1,25(OH)_2D_3$ inhibits cyclin D or induces cyclin dependent kinase (CDK) inhibitors that will result in retinoblastoma gene product remaining hypophosphorylated, reducing the activity of the E2F transcription factor [15]. $1,25(OH)_2D_3$ also regulates cellular proliferation and apoptosis. Wang *et al.* demonstrated that upon treating tumor HL-60 leukemia cells and U937 myelomonocytic cells with increasing doses of $1,25(OH)_2D_3$, there was induction of G_1 CDK inhibitors, $p27^{Kip1}$ and $p21^{Waf1/Cip1}$ [16]. Therefore, the cells remain arrested in G_1 phase of the cell cycle. McGuire *et al.* observed in murine squamous cell carcinoma (SCC) cells that $1,25(OH)_2D_3$ induced caspase-dependent cleavage of MEK leading to apoptosis [17].

In addition to causing cell cycle arrest (G_0/G_1) by stimulating $p27^{Kip1}$ [18] and down-regulating $p21^{Waf1/Cip1}$ [19], $1,25(OH)_2D_3$ significantly enhances the *in vitro* and *in vivo* antitumor efficacy of chemotherapy agents including the platinum analogues and taxanes [20,21]. $1,25(OH)_2D_3$ can induce cleavage of caspase 3, PARP and the MAPK in a caspase-dependent manner, leading to apoptosis [17,19,22]. The expression and phosphorylation of Akt, a kinase that regulates a second cell survival pathway, is also inhibited after treatment with $1,25(OH)_2D_3$. The differentiation properties of $1,25(OH)_2D_3$ mediated through transcriptional activation of the CDK inhibitor p21, have been demonstrated in leukemic cells [23]. The effects of vitamin D on multiple signal transduction pathways operational in cancer cells are reviewed by Deeb *et al.* [14].

Lung cancer & vitamin D

Epidemiological studies

Lung cancer is the leading cause of cancer related mortality in the world, killing over 120,000 people annually in the USA alone [24]. Observational studies have shown that the incidence of lung cancer in certain populations were related to serum vitamin D levels. A Finnish prospective study analyzed data on serum vitamin D levels and cases of lung cancer for 6937 men and women [25]. An association between the serum level of $1,25(OH)_2D_3$ and lung cancer risk was observed for the highest versus lowest tertile with an odds ratio 0.72. In the USA, Zhou *et al.* demonstrated that improved survival in early stage lung cancer is associated with higher circulating levels of $1,25(OH)_2D_3$ [1,2]. They observed a 26% improved survival for patients whose $1,25(OH)_2D_3$ levels were >21.6 ng/ml compared with those whose levels were <10.2 ng/ml. This group also noted associations between VDR polymorphisms and poor survival among lung SCC [26].

> "Reciprocal changes that involve an increase in CYP27B1 mRNA and a decrease in CYP24A1 mRNA may play a pivotal role in maintaining the local tissue level of $1,25(OH)_2D_3$ to be antiproliferative to lung cancer cells."

Preclinical studies

In vitro and *in vivo* studies have demonstrated the antiproliferative effects of $1,25(OH)_2D_3$ in lung cancer. Higashimoto *et al.* reported that $1,25(OH)_2D_3$ inhibited the growth of lung cancer cell lines [27]. This effect was mediated by VDR, and affected cell cycle regulation in SCC [28]. $1,25(OH)_2D_3$ has also been shown to inhibit lung tumor growth and lung metastases in mouse models [29]. Owing to the high number of blood vessels in the lungs, circulating tumor cells easily metastasize there and have proven to be difficult to treat with chemotherapy. Nakagawa *et al.* demonstrated using a Lewis Lung Carcinoma cells: green fluorescent protein (GFP) construct in a murine model that $1,25(OH)_2D_3$ strongly inhibited metastatic growth in the lung of VDR null mice [30]. In parallel *in vitro* experiments using Lewis Lung Carcinoma cells, it was noted that VEGF mRNA, an indicator of angiogenesis, was suppressed following treatment with $1,25(OH)_2D_3$ at 24 h [30]. The data suggests that $1,25(OH)_2D_3$ directly reduces tumor metastatic growth in lung cancer cells and motivates our current treatment trial.

Differential expression of CYP27B1 & CYP24A1 in normal lung versus cancer

There are three integral components of the vitamin D pathway, the VDR, CYP27B1 (the enzyme that converts inactive $25(OH)D_3$ to its active form ($1,25[OH]_2D_3$) and CYP24A1 (the enzyme that catabolizes $1,25[OH]_2D_3$ to $1,24,25[OH]_3D_3$). Normal tracheobronchial cells have high levels of 1-α-hydroxylase (CYP27B1) enzyme that leads to increased local production of $1,25(OH)_2D_3$ and low levels of CYP24A1 that leads to

increased breakdown. This is in contrast to lung cancer cells that show higher CYP24A1 expression and low to absent CYP27B1 [31]. Reciprocal changes that involve an increase in CYP27B1 mRNA and a decrease in CYP24A1 mRNA may play a pivotal role in maintaining the local tissue level of $1,25(OH)_2D_3$ to be antiproliferative to lung cancer cells.

VDR expression in lung cancer

The antiproliferative effects of $1,25(OH)_2D_3$ is mediated by ligand binding to the VDR. VDR expression is ubiquitous and there is data to suggest that higher nuclear VDR expression in lung cancer correlates with improved survival [32]. This may relate to increased genomic effects mediated by nuclear VDR on cell cycle related genes that lead to apoptosis, but this is yet to be confirmed in lung cancer. There is also data to suggest that VDR expression is higher in well differentiated SCC compared with normal or dysplastic bronchial epithelium [33]. This finding is intriguing and worthy of further study to elucidate the relationship between the differentiation status of lung cancer and vitamin D.

CYP24A1 expression in lung cancer

Our group recently reported on the prognostic significance of CYP24A1 expression in lung adenocarcinoma [34]. We corroborated earlier reports regarding increased expression of CYP24A1 in lung adenocarcinoma [35–37]. We found that the tumors that had a higher CYP24A1 expression were more poorly differentiated as well as associated with poor survival. In a parallel *in vitro* experiment, we demonstrated that lung cancer cell lines with high CYP24A1 expression had a poorer response to the antiproliferative effects of $1,25(OH)_2D_3$ as compared with those with lower levels of CYP24A1 mRNA. Earlier to our observations, Parise *et al.* confirmed that CYP24A1 expression was indeed highly expressed in lung cancer as compared with nontumorigenic normal bronchial epithelium [35]. Analysis of NSCLC cell cultures revealed time-dependent loss of $1,25(OH)_2D_3$ coincident with the appearance of CYP24A1-generated metabolites. Specific inhibition of CYP24A1 slowed the loss of $1,25(OH)_2D_3$ and increased the $1,25(OH)_2D_3$ half-life. These data suggest that increased CYP24A1 expression in lung tumors restricts $1,25(OH)_2D_3$ antitumor activity.

The reason for increased CYP24A1 expression in lung tumors is unclear. One of the possible causes of elevated CYP24A1 expression might be gene amplification. Several studies have already identified gain of 20q13 (the gene locus of human CYP24A1) in a variety of malignancies [38,39] including colorectal carcinoma [40]. Our own data (unpublished) indicates that overexpression of CYP24A1 mRNA in some cases was associated with increase in gene copy number, however; this was present only in a small percentage of patients, suggesting other mechanism(s) for increased CYP24A1 transcription. Interestingly, we have recently observed that smoking-related epigenetic changes may indeed alter the expression of CYP24A1 enzyme and we are studying this phenomenon more thoroughly. The role of vitamin D as a therapy in lung cancer may be hampered by tumor-specific inactivation. Our observations show a high level expression of CYP24A1 in subsets of lung cancers and demonstrate an inverse relationship between high CYP24A1 expression and antiproliferative activity of vitamin D [34]. These observations highlight not only the importance of choosing the right molecular target but also understanding gene expression in individual tumors.

> "We found that the tumors that had a higher CYP24A1 expression were more poorly differentiated as well as associated with poor survival."

Conclusion & future directions

Lung cancer is a highly prevalent, highly lethal cancer. Current treatments are inadequate, possibly owing to the complex molecular heterogeneity and a 'one-target-fits-all' approach. Therapies with impact on multiple pathways, targeted to individual patients based on tumor-specific biomarkers offer a new approach for this devastating disease. For many reasons, vitamin D is an ideal therapeutic agent for secondary prevention either by itself or in combination with a CYP24A1 inhibitor. Vitamin D affects a broad range of cellular pathways. The net effect of vitamin D treatment of malignant cells is differentiation and decreased proliferation. Low serum levels of vitamin D are associated with the development of lung cancer. *In vitro*, vitamin D is synergistic with chemotherapy for lung cancer. The antiproliferative effects of vitamin D involve arrest in the G1/S phase of the cell cycle. The eventual role of this property may be as a chemopreventive agent to prevent a recurrence after surgery. It is now apparent that autocrine and paracrine regulation of vitamin D in tumor tissue is differentially regulated. There is a difference in the expression of key metabolizing enzymes in tumor versus normal tissue. It may be required to increase local $1,25(OH)_2D_3$ levels by stimulating CYP27B1 in the normal tissue or prevent breakdown of $1,25(OH)_2D_3$ in tumors by blocking CYP24A1. In patients with high CYP24A1 expression, it may be necessary to use a specific CYP24A1 inhibitor in combination with $1,25(OH)_2D_3$ treatment. Alternatively, one could study vitamin D analogs that are not substrates for the CYP24A1 enzyme. Additional studies are crucial for designing clinical trials to maximize the benefit from exogenous vitamin D. Further investigations are required to completely characterize the vitamin D/CYP450 system in lung cancer as one size does not fit all.

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