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Review

Vitamin D: Potential in the Prevention and Treatment of Lung Cancer

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Abstract. *Vitamin D is a steroid hormone traditionally recognized for maintaining calcium and phosphorous homeostasis in the body. However, it is now widely accepted that it exerts several extraskeletal actions, including anti-tumorigenic and immunomodulatory effects in vitro and in vivo. There is now a huge interest in studying the modes of action of vitamin D in a wide range of infectious and chronic disease settings and its potential in cancer prevention and treatment is currently under detailed investigation. In relation to the lung, evidence from observational studies, animal models and in vitro cell culture suggest that vitamin D may play a beneficial role in pulmonary inflammation. In addition, an adequate vitamin D status may be important for lung cancer prevention. Furthermore, vitamin D or its analogs, alone or in combination with cytotoxics, have potential in the treatment of lung cancer. Vitamin D is converted to its active form locally in the lung, suggesting that it may play an important role in lung health. Here, we review the evidence from observational, clinical and experimental studies in relation to vitamin D and lung cancer. In addition, we discuss vitamin D resistance in lung tumors and the potential molecular mechanisms of vitamin D action in lung cancer cells.*

Vitamin D is unique among vitamins, as it can be synthesized in the body as long as there is adequate sun exposure. The precursor of vitamin D_3 (cholecalciferol) is produced from the action of UVB radiation of 7-dehydrocholesterol within the skin. Limited amounts can also be obtained from the diet, with the richest sources in oily fish and fish oils. In plants and yeast, vitamin D_2 (ergocalciferol) is produced from irradiation of ergosterol. Vitamin D precursors (D indicating D_2 and D_3) are transported to the liver bound to the vitamin D-binding protein (VDBP), where they are hydroxylated to 25-hydroxyvitamin D, (25(OH)D or calcidiol). This is the major circulating form of vitamin D and is used as a measure of an individual's vitamin D status. Vitamin D_2 is less efficient at maintaining circulating levels of 25(OH)D than vitamin D_3 (1, 2). 1,25-dihydroxyvitamin D ($1,25(OH)_2D$ or calcitriol), the most potent and biologically active form of vitamin D, is classically produced from further hydroxylation of 25(OH)D by the cytochrome 1α-hydroxylase enzyme CYP27B1 within the kidneys. Recently, however, numerous extrarenal tissues and several tumor tissues have been reported to express this enzyme and activate 25(OH)D locally, including the lung (3-5).

Vitamin D concentrations in blood and tissues are tightly regulated. Both 25(OH)D and $1,25(OH)_2D$ are metabolized to 24,25-dihydroxycholecalciferol ($24,25(OH)_2D$) by the 24-hydroxylase CYP24A1 enzyme. This metabolite also has biological activity (6). The *CYP24* gene is inducible by vitamin D, thereby providing negative feedback. Vitamin D deficiency is a worldwide pandemic (6, 7). Several sub-groups of the population are at risk of deficiency or low status, including children, pregnant women and people aged over 65 years. Older people have lower dermal synthesis of vitamin D and those residing in institutions often spend little time outdoors. There is also a seasonal and latitudinal gradient that affects vitamin D status. Non western immigrants living in countries of higher latitude with limited UVB radiation are at high risk of deficiency due to more skin pigmentation, which reduces vitamin D production (8). Other risk factors include the use of suncream and skin-covering clothes.

Vitamin D and Cancer

There has been a huge surge in interest in the potential of vitamin D in cancer prevention and therapy over the past decade, with extensive evidence of antitumorigenic effects from *in vitro* and *in vivo* studies. Vitamin D has been reported to have antiproliferative and pro-differentiative effects in

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various cancer cell lines, including those of breast, colon, prostate and skin (2, 9). In addition, it has been shown to induce apoptosis in some breast and colon cancer cells, but not in certain prostate cancer cells, suggesting differential effects in different types of cancer (9). Vitamin D also has many other properties which could be beneficial in cancer, including antiangiogenic, anti-inflammatory and antimetastatic actions (10). In addition, epidemiological evidence supports a beneficial role of vitamin D in the prevention and treatment of several types of cancer (11-15). There are few clinical studies on vitamin D metabolites and analogs alone or in combination with cytotoxic agents for cancer treatment. Most clinical trials have been carried out on prostate cancer, and as yet, a clear conclusion of the benefits of vitamin D has yet to be established (16). The remaining sections of this review will focus on the potential of vitamin D in lung cancer.

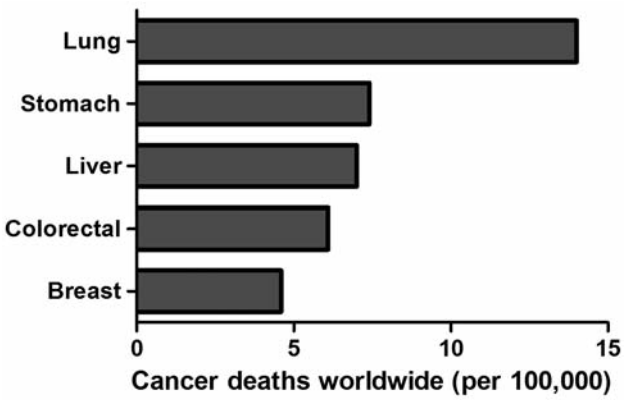

Figure 1. *Cancer mortality rates worldwide: the five leading causes (statistics provided by the World Health Organisation).*

Lung Cancer

Lung cancer is the leading cause of mortality from cancer worldwide (17), and is responsible for over twice the number of deaths than any other cancer (Figure 1). It has a poor prognosis, with only a 10% survival rate at 5 years (17). Incidence of lung cancer is highest in East Asia, but accounts for over 10% of new cases in most regions of Asia, all European regions and Northern America and the Caribbean (18). There are two main types of primary lung cancer, small cell and non-small cell. Small cell lung cancer is almost always associated with smoking. It is less common than non-small cell lung cancer, but is a highly aggressive form which can rapidly metastasize to other tissues. Due to its rapid growth, approximately 60% of patients initially present with metastatic disease. Non-small cell lung cancer accounts for approximately 85% of primary lung cancer cases. This type is subdivided into squamous cell carcinoma, adenocarcinoma and large cell carcinoma. Squamous cell carcinoma is the most common type of lung cancer, developing in the lining of the airways. It is also usually associated with smoking. Adenocarcinoma develops in the mucus-producing cells of the airway lining (19). Although non-small cell carcinoma is the less aggressive form of primary lung cancer, unfortunately, it is often asymptomatic in the early stages, remaining undiagnosed until it reaches an advanced stage, and therefore usually results in a poor prognosis (17). Depending on the stage of cancer, non-small cell lung cancer is most often treated with surgery with or without chemotherapy and radiotherapy. Surgery is generally not an option for small cell lung cancer and chemotherapy with or without radiotherapy is usually the best option (19). Chronic obstructive pulmonary disease (COPD) is a progressive chronic inflammatory lung disease that is characterized by irreversible airway obstruction and excessive mucus production resulting in breathing difficulties (20). Cigarette smoke induces production of reactive oxygen species which drives excessive inflammation, the production of proteases and growth factors, and airway remodeling in the pathogenesis of COPD (21-23). Since chronic inflammation is now recognized as an important central process in the pathogenesis of many types of cancer, it is perhaps not surprising that COPD is the single most important risk factor for the development of lung cancer after smoking exposure (21). Lung cancer is thought to be driven by DNA damage caused by excessive inflammation and lack of repair processes within the lung, resulting in genomic instability (21-23). Around 50-70% of patients with lung cancer suffer from COPD: however, a causal relationship between the two has not yet been established (24). Although smoking is the greatest risk factor for both of these diseases, only 10% of smokers develop lung cancer and 20-30% develop COPD (21). Turner *et al*. demonstrated that in a prospective cohort study of 448,600 people who had never smoked, lung cancer mortality was associated with history of COPD, suggesting an overlap in genetic predisposition for the two diseases (25). This suggests that the development of these diseases is complex, and involves interactions between the environment and host genetic susceptibility. Other risk factors for lung cancer development include exposure to asbestos, air pollution, radiation and second-hand cigarette smoke (26).

There is a huge requirement for improved medicines to help prevent and treat lung cancer. Evidence for the potential for vitamin D in this role shall be discussed below. In addition to the antiproliferative and pro-apoptotic properties of vitamin D that may directly affect tumor cells *in vitro* and *in vivo*, it also exerts numerous immunomodulatory effects (27, 28) which could be beneficial in both COPD and lung cancer. Since COPD is such a high risk factor for lung cancer development, preventing or treating COPD by inhibiting the underlying inflammation could help prevent or delay lung cancer progression.

Vitamin D and Lung Cancer

In vitro studies. At a cellular level, most lung tumors are derived from lung epithelial cells that form an important barrier to the external environment. These cells are extremely resilient and can tolerate prolonged exposure to cancer promoting agents (such as cigarette smoke) and accumulation of genetic mutations before eventually becoming neoplastic. Several epithelial-derived lung cancer cell lines are commonly used for *in vitro* studies of lung cancer. Vitamin D exhibits antiproliferative properties in several lung cancer epithelial cell lines that express its receptor VDR. $1,25(OH)_2D_3$ significantly inhibited cell proliferation in the NCI-H82 and NCI-H209 small cell lung carcinoma and the EBC-1 and H520 non-small cell carcinoma cell lines (29, 30). Several analogs of vitamin D also exert antiproliferative effects in lung cancer cell lines (30, 31). For example, in NCI-H82 small cell carcinoma cells, 19-nor-22E demonstrated similar antiproliferative effects to $1,25(OH)_2D_3$. In addition, 1α OH pregnacalciferol, 19-nor-24 homo, and 19-nor-22E showed antiproliferative effects comparable to those of $1,25(OH)_2D_3$ in the NCI-H209 cell line. Satio *et al*. reported that a series of deuterated analogs of $1,25(OH)_2D_3$ were up to 284-fold more potent at inhibiting proliferation of H520 cells than the hormone itself (31). It is likely that the antiproliferative effects of $1,25(OH)_2D_3$ are mediated in part by stalling the cell cycle at the G_1/S checkpoint by increasing inhibitors and reducing activators of the cyclin-dependent kinase complexes which prevent DNA synthesis and cell growth (2, 32). Together, these studies demonstrate that similarly to other cancer cell lines, vitamin D and various analogs are capable of inhibiting lung cancer cell proliferation *in vitro*.

Vitamin D also exhibits numerous immunomodulatory properties, including inhibition of prostaglandins, proteases and pro-inflammatory cytokines. This is thought to occur through modulation of signalling pathways that include p38 mitogen activated protein kinase (MAPK) and nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) (10). Excessive inflammation is central to COPD and is a key underlying process in the progression of lung cancer; therefore agents that can reduce inflammation may be of benefit in lung cancer prevention and treatment. We have shown that $1,25(OH)_2D_3$ reduces interleukin-6 (IL-6) production in primary airway epithelial cells, but not in the lung cancer cell lines NCI-H292 and A549 (unpublished data). IL-6 is a key cytokine involved in the initiation and extension of the immune response and elevated levels have been implicated in both COPD and lung cancer (21). This suggests that by resisting vitamin D, lung cancer may also be driving inflammation. The anti-inflammatory properties of vitamin D in the lung have been reviewed in more detail elsewhere by Hughes and Norton (33).

In vivo studies. Several studies in animal models have examined the potential for vitamin D as a therapy for lung cancer. Most of these have investigated the effects of vitamin D on tumor volume and metastasis in different mouse models. One of the most widely used lung cancer models is the Lewis lung carcinoma (LLC), which involves transplantation of malignant tissue into mice which then progresses into a metastatic lung tumour. Several vitamin D studies have made use of this model. In early work, Maeda and co-workers found that $24,25(OH)_2D_3$ is biologically active in lung cancer and prolonged the survival rate of LLC mice, exhibiting both antimetastatic and analgesic effects (34). Young and co-workers demonstrated that $1,25(OH)_2D_3$ reduced tumor metastasis and recurrence and increased tumor immunity in the LLC model (35-38). In *VDR* knockout mice, circulating levels of vitamin D are increased due to overexpression of CYP27B1. More recently, Nakagawa and co-workers generated a fluorescent stable transfectant of the LLC to view metastasis and found that $1,25(OH)_2D_3$ and its analog 22-oxa-1α $25D_3$ significantly reduced growth and metastasis in *VDR* knockout mice compared to wild-type controls (39-41). They also found that both compounds reduced angiogenesis and invasiveness by inhibiting matrix metalloproteinase 9 (MMP9) and MMP2 expression (39).

Vitamin D has also been tested in combination with other treatments for lung cancer in the LLC model. Zhuravel *et al*. used a combination of LLC tumor-associated antigen with the murine beta defensin chemokine as a carrier for an experimental cancer vaccine (42). They found that this alone was not potent enough to significantly reduce tumor volume and metastasis; however, in combination with vitamin D_3, there was an elevated significant antimetastatic effect of vaccination, even without the chemokine carrier. In addition, Wietrzyk and co-workers found that two novel analogs of vitamin D, PRI-2202 (24R calcipotriol) and PRI-2205 (5,6-*trans* calcipotriol) were more effective in combination with cisplatin than cisplatin alone in the LLC model (43).

Chemical–induced mouse models of lung cancer have also been used in vitamin D studies. In A/J mice, tumors were induced with the carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). $1,25(OH)_2D_3$ supplementation resulted in lower tumor incidence, although it also induced toxicity in this model. This toxicity was mitigated by addition of retinoic acid, and the combination inhibited lung tumor development (44). In addition, Balb/c mice treated with urethane developed lung tumors that could be blocked by simultaneously introducing vitamin D to the diet (45). In spontaneous and experimental metastasis models of tumor-bearing mice, treatment with $1,25(OH)_2D_3$ inhibited pulmonary metastasis (46). In addition to interferon (IFN), combination treatment with $1,25(OH)_2D_3$ reduced tumor release of granulocyte macrophage colony-stimulating factor

(GMCSF) and increased the expression of tumor-infiltrating CD8 cells (35). Overall, in animal models of lung cancer, the evidence supports a role for vitamin D in lung cancer therapy.

Observational studies. Several factors may influence the production of vitamin D precursor within the skin, and hence vitamin D status, including season and latitude (47-49). Associations between these factors and risk of lung cancer have been reported. In some countries, for example the UK and in Norway, there is a significant gradient in UVB exposure from north to south and a better lung cancer survival rate in patients with higher exposure (48, 49). In the UK, Mavroeidi *et al*. found a significant difference in vitamin D status between north and south and a significant seasonal variation (47). In relation to season, in a cohort of just over 45,000 Norwegian patients, Porojnicu *et al*. found that male lung cancer patients younger than 50 years old had a 15% reduced risk of dying from the disease within 18 months when diagnosed in the summer/autumn *vs*. winter/spring months (48). In addition, a meta-analysis with data from 111 countries studied the relationship between latitude, UVB irradiance and the incidence of lung cancer (50). Latitude was positively associated with lung cancer incidence rates in both men and women. There was also an independent association of higher lung cancer incidence with lower UVB irradiance (50). These results suggest that lower production of vitamin D may be a risk factor for lung cancer.

Some studies have also shown a direct association between lung cancer incidence and vitamin D status. For example, Pilz *et al*. demonstrated that increased circulating 25(OH)D was associated with improved survival in lung cancer patients (51). In addition, in a Finnish prospective cohort of 6937 subjects, 25(OH)D status was inversely associated with lung cancer incidence in women and those under 50 years old, although there was no association in men or older people (52). Furthermore, in a US study of 456 patients with early-stage non-small cell lung cancer, Zhou *et al*. found that those individuals with higher intakes of vitamin D and whose surgery occurred during the summer months, had improved survival rates and a greater recurrence-free survival (53, 54). However, in a nested case-control study of 500 male smokers with lung cancer and 500 matched controls from the alpha tocopherol beta carotene study, although an inverse association of 25(OH)D and lung cancer risk was found in those whose blood was collected in winter *vs*. summer months, there was no overall association between the risk of disease and serum 25(OH)D (55). Furthermore, Freedman *et al*. found no association between 25(OH)D status and total or lung cancer mortality in 16,818 subjects from the third National Health and Nutrition Examination Survey (56). On further analysis with longer follow-up, they even found an increased risk of lung cancer mortality in men with higher circulating 25(OH)D levels (57). In conclusion, data from epidemiological studies of the benefit of vitamin D in lung cancer is conflicting. Future studies should focus on large cohorts in smokers and non-smokers, with detailed information on vitamin D pathway genotypes, dietary intakes, supplements and amount of time spent outdoors.

Clinical trials. Vitamin D has been reported to have multiple beneficial effects in several cancer types *in vitro* and/or *in vivo*, including breast, ovarian, blood, pancreatic, liver, colorectal and prostate cancer (11). Several trials have been carried out, mainly investigating the potential of vitamin D in the treatment of prostate cancer. Although high-dose $1,25(OH)_2D_3$ used in combination with paclitaxel is safe in prostate cancer patients, it was not effective in all studies (11, 16). Further clinical studies are currently underway investigating $1,25(OH)_2D_3$ with cytotoxics in prostate cancer and vitamin D analogs and metabolites in colon, breast, and oral cancer. The potential of vitamin D in the prevention and treatment of other lung diseases such as COPD, tuberculosis and cystic fibrosis is also underway.

Despite supporting evidence from *in vitro* and *in vivo* studies for a beneficial role of vitamin D in lung cancer therapy, there have been very few clinical studies using vitamin D or its analogs for prevention or treatment, either alone or in combination with chemotherapeutic drugs. This may be partly due to concerns about toxicity of vitamin D in an extremely fragile and vulnerable population or lack of knowledge regarding its mechanism of action in different genotypes. Early studies using 2 μg/day of $1,25(OH)_2D_3$ for 12 weeks in the treatment of patients with myelodysplastic syndrome demonstrated no benefit in disease and even induced hypercalcemia, suggesting vitamin D was toxic to the patients (58). However, in a study of 36 patients, Muindi *et al*. reported that supplementation resulting in high concentrations of plasma $1,25(OH)_2D_3$ (600-1440 pg/ml), in combination with paclitaxel, a chemotherapeutic agent for lung cancer patients, showed no dose-limiting toxicity (59). Although this study has shown that high-dose $1,25(OH)_2D_3$ can be safely administered, there is only limited data available on the maximum tolerated dose and dose-limiting cytotoxicity for different types of cancer (16). In addition, there are issues about the absorption of vitamin D and whether certain preparations are capable of achieving the doses required (16, 59). More research is required in this area with appropriately designed trials to investigate the effect of vitamin D in different types of cancer, including the lung. Currently, Ramnath and co-workers are carrying out a phase I/II clinical trial assessing the maximum tolerated dose and dose-limiting toxicities of $1,25(OH)_2D_3$ with cisplatin/docetaxel in advanced non-small cell lung cancer patients and assessing the response rates. In addition, as a secondary outcome, they are correlating systemic exposure

to $1,25(OH)_2D_3$ with polymorphisms in the CYP24 enzyme, which breaks down vitamin D, on systemic changes on specific coding regions of the gene associated with low vitamin D breakdown (60). With the limited studies in this area, it will be some time before we know for certain whether or not vitamin D treatment in different lung cancer subtypes may be beneficial in the long term.

Tumor Resistance to Vitamin D

Since vitamin D has many antitumorigenic properties, it is perhaps not surprising that some types of cancer develop resistance by modulating the expression of important members of the vitamin D metabolism pathway, including CYP27B1, CYP24A1 and the VDR.

CYP27B1. In the lung, normal airway epithelial cells and alveolar macrophages express CYP27B1, converting 25(OH)D to its more active form (5, 61). In addition, dendritic cells and lymphocytes also express this enzyme (27). These cell types play a central role in pulmonary inflammation and the pathogenesis of lung cancer. As airway epithelial cells can be chronically exposed to cigarette smoke, mutations in these cells can result in carcinogenesis. In contrast to normal airway epithelial cells, some small cell and non-small cell cancer cell lines have been shown to express very low CYP27B1 or none at all (5, 62). Indeed, Mawer *et al*. found that only 1 in 16 human small cell lung cancer cell lines tested was able to synthesize $1,25(OH)_2D_3$ (62). This suggests that lung cancer cells may inhibit CYP27B1 expression, and hence formation of active $1,25(OH)_2D_3$, in order to prevent its antiproliferative effects. In contrast, increased CYP27B1 expression has been reported in alveolar macrophages from patients with lung cancer, with highest expression being found in more advanced stages of lung cancer (61). This may be a means by which the tumor evades the immune system by modulating cytokine production and suppressing immune cell function by increasing vitamin D, or it may be the body's response to increase $1,25(OH)_2D_3$ in order to activate its antiproliferative and anticancer properties.

CYP24A1. Both 25(OH)D and $1,25(OH)_2D$ are tightly regulated and induce the expression of their own metabolizing enzyme 24-hydroxylase, CYP24A1. Another mechanism by which lung cancer can resist the action of vitamin D is by up-regulating this enzyme. Several studies have demonstrated that CYP24A1 is overexpressed in lung tumor tissue compared with normal lung tissue (60). For example, Parise and co-workers reported that CYP24A1 was expressed in 10 out of 18 primary lung tumor tissues but only 1 out of 11 normal non neoplastic lung tissue specimens (63). Similarly, Anderson *et al*. showed an increased mRNA expression of *CYP24A1* in lung tumors *vs*. normal lung tissue (64), and more recently, Chen and co-workers carried out a study on samples from 89 lung adenocarcinoma patients and 10 non-neoplastic controls and demonstrated that CYP24A1 expression was 8-50 fold higher in the tumor tissue (65). It was more highly expressed in poorly differentiated cancer and patient survival was lower in those with higher expression, suggesting that CYP24A1 is an independent prognostic marker of survival in lung cancer (65).

Several cell lines derived from lung tumor tissue highly express CYP24A1, including 201T (adenocarcinoma), 128-88T (squamous cell carcinoma) and A549 (adenocarcinoma). However, it is not basally expressed in primary small airway epithelial cells or the non-tumorigenic bronchial epithelial cell line BEAS2b [(63), and unpublished work from our laboratory]. We have shown that primary human small airway epithelial cells, transformed bronchial epithelial cells (16HBE14o-) and the mucoepidemoid carcinoma cell line NCI-H292 express CYP24A1 at much lower levels than A549 cells and that CYP24A1 expression is inducible in all except the A549 cells, suggesting that A549 cells are resistant to the effects of vitamin D. Based on the information to date, it would seem prudent to examine the expression of CYP24A1 in lung cancer patients before commencing treatment with vitamin D to determine whether patients would be sensitive to vitamin D treatment, unless used in combination with a CYP24 inhibitor.

Vitamin D receptor (VDR). The actions of $1,25(OH)_2D_3$ are mainly elicited by binding to the VDR which then heterodimerizes with the retinoid X receptor (RXR) and binds to vitamin D-response elements in the regulatory regions of over 200 genes, including several that are involved in cell growth and differentiation; therefore many of the antiproliferative effects of vitamin D are VDR dependent (6). VDR is expressed in most cell types, although levels of its expression vary widely. Vitamin D can also elicit non-genomic rapid responses which are thought to be mediated in part by binding to the newly described 1,25D-3-membrane-associated-rapid-response-steroid-binding-protein receptor (1,25MARRS) (66). Expression of this receptor in breast cancer cells interferes with antiproliferative actions of $1,25(OH)_2D_3$ (67, 68). Further studies are required to determine if blocking this receptor might enhance the antitumor effects of $1,25(OH)_2D_3$. In addition, nuclear VDR is functional independently of vitamin D treatment and has other naturally occurring ligands, including lithocholic acid, curcumin and polyunsaturated fatty acids. These are low affinity agonists which have been shown to reduce the risk of cancer (69). Srinvanan *et al*. have shown that high nuclear VDR expression was independently associated with better overall survival in a small cohort of 73 patients with non-small cell lung cancer (70).

Several cancer cell types express VDR, including lung tumors. Kaiser *et al*. found that over 50% of primary non-small cell lung tumors were positive for VDR, with squamous cell and adenocarcinoma showing the greatest VDR expression (71). However, Anderson *et al*. showed a decrease in *VDR* mRNA expression in lung tumors compared to non-neoplastic tissue in a study of 20 lung tumor and control samples (64).

The *VDR* is a highly polymorphic gene, and several polymorphisms have been found to modulate ligand binding or transcriptional activity of the receptor. The presence of the 'T' allele at the *TaqI* site correlates with increased transcriptional activity, mRNA stability and high serum levels of $1,25(OH)_2D_3$ (72). In a Turkish case-control study with 137 patients and 156 controls, Dogan and co-workers found that TT homozygotes for the *TaqI* polymorphism were at higher risk of lung cancer than tt homozygotes or heterozygotes, and men with TT who smoked were more likely to develop lung cancer, suggesting that the presence of this allele is a risk factor for lung cancer.

Polymorphisms of G>A are located within the *CDX2*-binding site of the *VDR* promoter and the A allele is associated with increased VDR transcriptional activity (73). Polymorphisms of C>T at the *Fok1* site are located at the *VDR* translational start site and the C allele has greater efficiency at exerting $1,25(OH)_2D_3$ effects than the T allele (73). In addition, the BsmI C>T polymorphism is at the *VDR* 3' end and the T allele is associated with increased *VDR* mRNA expression and increased levels of $1,25(OH)_2D_3$ *than* the C allele. Zhou and co-workers studied the effects of VDR polymorphisms of *CDX2, FokI* and *BsmI* in 373 patients with early- and 294 with later stage non-small cell lung cancer (73, 74). In early-stage patients, G/A and A/A genotypes of the *CDX2* polymorphism were associated with better overall and recurrence-free survival than those with G/G genotypes among squamous cell carcinoma patients (73). In addition, there was a combined protective effect of the A-C-T haplotype of *CDX2-FokI-BsmI* polymorphism on survival of squamous cell carcinoma patients in this study. In late-stage patients, the T allele of *FokI* and the G-T-C *CDX2-FokI-BsmI* haplotype were both associated with worse survival (74).

In conclusion, it is clear that there are numerous factors within the vitamin D pathway which can be dysregulated in lung cancer (Figure 2). It will be critical to completely characterize expression of all of these and polymorphisms in order to determine whether or not individual lung tumors will be responsive to vitamin D treatment.

Effects of Vitamin D on Signaling Pathways in Lung Cancer

Vitamin D has been shown to modulate various cellular signaling pathways in cancer cell lines, including epidermal growth factor receptor (EGFR) expression and signaling in colon and breast cancer and MAPK signaling in breast and blood cancer [reviewed in (11)].

Numerous genes that regulate cell growth and differentiation have been found to be mutated in lung cancer and several of these oncogenes are modulated by $1,25(OH)_2D_3$ in various types of cancer. The (EGFR), a tyrosine kinase receptor, is one of the most commonly mutated proteins found in non-small cell lung cancer, resulting in overexpression of this receptor. EGFR activates signaling pathways that result in proliferation, angiogenesis, invasion and inhibition of apoptosis, all processes that are central to tumorigenesis (17). Interestingly, the EGFR-selective tyrosine kinase inhibitors erlotinib and gefitinib have been reported to be of benefit in the treatment of some subtypes of non small cell lung cancer that harbor somatic mutations in the tyrosine kinase domain of the EGFR; these are mostly adenocarcinomas (75). The VDR has been shown to directly inhibit EGFR transcriptional activity in breast cancer cells, which is augmented on $1,25(OH)_2D_3$ treatment (76). $1,25(OH)_2D_3$ also suppresses mitogenic signaling through EGFR in ovarian cancer cells and induces cell cycle arrest (77). In addition, it potentiates growth arrest induced by tyrosine kinase inhibitors in an EGFR-overexpressing epidermoid carcinoma cell line, and in non-antiproliferative doses combined with erlotinib, it was found to inhibit parathyroid hyperplasia (78). This suggests that, as with vitamin D resistance, found in hyperparathyroidism, low-dose $1,25(OH)_2D_3$ in combination with an EGFR inhibitor may also be able to elicit growth arrest in lung cancer cells resistant to vitamin D. Although there are no reports to date on vitamin D affecting EGFR in lung cancer cells, these studies suggest it may be useful to determine if EGFR is targeted by vitamin D in lung cancer.

Several well characterised signalling pathways lie downstream of EGFR, including the RAS/RAF/mitogen activated protein kinase kinase (MEK)/extracellular signal-regulated kinase (ERK) pathway and the phosphatidyl-inosititide 3-kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) pathway (Figure 3), and $1,25(OH)_2D_3$ has been shown to modulate some of the key mediators involved. The RAS genes encode guanosine triphosphate (GTP)-binding proteins that act early in the EGFR signaling pathway. *KRAS* is the most commonly mutated RAS gene in human cancer and mutation of this gene is one of the most important steps in lung carcinogenesis (79). $1,25(OH)_2D_3$ has been reported to modulate RAS activity in leukemia cells (80).

PI3K is also an important signaling effector of EGFR. The main catalytic subunit of PI3K is p110 which is encoded by *PIK3CA*. Mutations in *PI3KCA*, resulting in overexpression, are also very common in lung cancer (17). Wang *et al*. showed that vitamin D_3 disassembles the AKT-

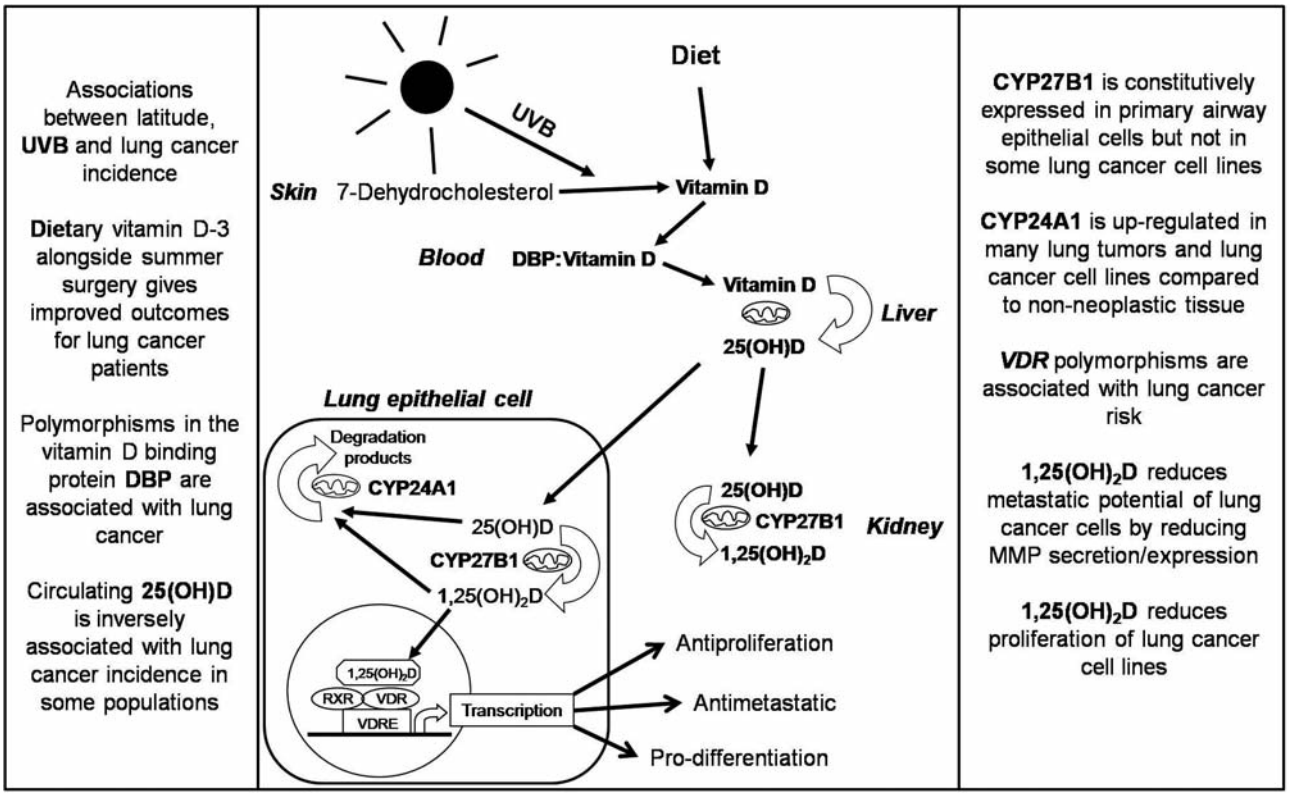

Figure 2. *Summary of the associations between lung cancer and the vitamin D pathway.*

RAF1 complex and down-regulates AKT in leukemia cells, thereby activating the MEK/ERK pathway, leading to cell differentiation (81). Interestingly, $1,25(OH)2D_3$ and PI3K/AKT inhibitors synergistically induce growth arrest in prostate cancer cells (82). However, VDR overexpression is independently associated with *KRAS*- and *PI3KCA*- activating mutations in colorectal cancer, suggesting that the presence of these mutations may interfere with vitamin D as a chemopreventive or chemotherapeutic agent (83).

Liver kinase B1 (LKB1) and regulated in development and DNA damage responses 1 (REDD1) are negative regulators of mTOR, a serine threonine kinase that lies downstream of PI3K/AKT and signals cell proliferation (84). Loss of function mutations of *LKB1* are common in lung cancer, resulting in overexpression of mTOR and subsequent cell growth. REDD1 has been shown to inhibit the invasiveness of lung cancer cell lines (85) and $1,25(OH)_2D_3$ has recently been found to enhance REDD1 expression, suppressing mTOR function in bone cells (86). Further investigation is required into whether this mechanism exists in other cell types.

In summary, vitamin D targets several cellular molecules in cancer, including EGFR and downstream members of its

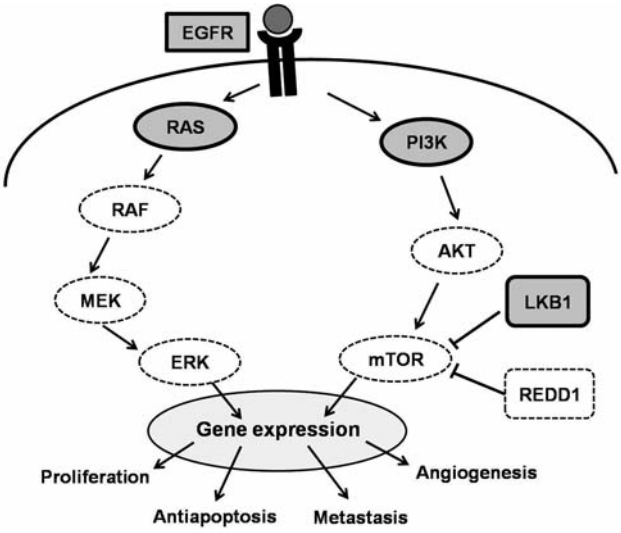

Figure 3. *The EGFR pathway (17). Mutations of EGFR, RAS, PI3K and LKB1 (highlighted) are common in lung cancer, with both the RAS-RAF-MEK-ERK and PI3K-AKT-mTOR pathways converging on expression of genes important for tumorigenesis including proliferation, apoptosis, metastasis and angiogenesis. Vitamin D has been shown to modulate members of these pathways.*

intracellular signaling pathways that lead to tumor growth and metastasis. It remains to be seen whether this pathway is an important target of $1,25(OH)_2D_3$ in lung cancer.

Conclusion and Future Directions

Lung cancer is the most common cause of cancer-related deaths worldwide. The survival rate is poor, due to late diagnosis, the inherent resistance of tumors and lack of effective treatments. Evidence from *in vitro* and *in vivo* studies on proliferation and tumorigenesis suggest that vitamin D may have potential in the prevention and treatment of lung cancer. Although some observational studies have found no association between vitamin D status and lung cancer risk, some subgroups of the population appear to benefit from adequate status. Further studies are required to evaluate the impact of low vitamin D status on lung cancer incidence and mortality. $1,25(OH)_2D_3$ regulates numerous genes involved in proliferation and tumorigeneisis through direct VDR binding and modulation of intracellular signaling pathways. However, little is known about its effects on these pathways in lung cancer cells and whether mutation of the genes involved will impact on the actions of $1,25(OH)_2D_3$. Further work is required to clarify this. In addition, the extent of dysregulation of the vitamin D pathways with *CYP27B1, CYP24A1* and *VDR* polymorphisms within individual lung tumors will be key to completely define subsets of lung cancer where vitamin D treatment may be beneficial. The optimum concentration of circulating 25(OH)D required for extraskeletal actions of vitamin D and the maximum tolerated dose are still in dispute (87-90), suggesting that defining optimal levels of vitamin D to prevent or treat cancer is still a distant goal. Many clinical trials have used suboptimal concentrations of vitamin D and have therefore not accurately determined the effects of vitamin D in cancer (16). In terms of lung cancer, there are limited clinical trials to date. However, clinical trials are now underway in this area, including investigations into the maximum tolerated dose, and the results of these are eagerly awaited. In the meantime, achieving an adequate vitamin D status should be a goal for everyone and may prove to be beneficial in the prevention of lung cancer.

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