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UV Radiation, Vitamin D and Human Health: An Unfolding Controversy

Ultraviolet Radiation, Vitamin D and Risk of Prostate Cancer and Other Diseases

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ABSTRACT

Most common diseases appear to result from complex, poorly understood interactions between genetic and environmental factors. Relatively few factors have been unequivocally linked with disease risk or outcome. Evidence from various studies using different experimental approaches has been interpreted as showing that, apart from its harmful effects on the pathogenesis of the common skin cancers, ultraviolet radiation (UVR) may exert a beneficial effect on development of various internal cancers and other pathologies. This concept is supported by parallel studies showing that hypovitaminosis D is linked with increased risk of various diseases including insulin resistance and multiple sclerosis. These findings suggest that, first, host factors such as skin pigmentation that affect UVR-induced synthesis of vitamin D and, second, polymorphism in genes that mediate the effectiveness of vitamin D action are susceptibility candidates for a variety of diseases. Collectively, these data suggest the hypothesis that, via effects on vitamin D synthesis, UVR exposure has beneficial effects on susceptibility and outcome to a variety of complex diseases. We describe evidence from studies in various diseases, but mainly from prostate cancer patients, that supports this hypothesis, but we emphasize that, although supportive data are available, the concept is unproven. Indeed, other explanations are possible. However, given the potentially important public health implications of the hypothesis and the potential for the development of novel therapeutic modalities, we believe the concept is worthy of further investigation.

INTRODUCTION

Humans are necessarily exposed to solar radiation and over time have developed phenotypes that mediate the potentially harmful and beneficial effects of exposure in different environments. Thus, it is believed that darkly pigmented humans evolved in Africa and 40 000–50 000 years ago migrated worldwide. In northern Europe, individuals with genetic variants that conferred skin with relatively little melanin and, therefore, low pigmentation were at an advantage, because they could more readily synthesize vitamin D in conditions of limited exposure to sunlight. However, individuals with light-colored skin are also more susceptible to the harmful effects of ultraviolet radiation (UVR), and over recent years public health agencies have emphasized these effects in an attempt to reduce the increasing frequency of skin cancer. In parallel, it has been proposed that indoor lifestyles have resulted in widespread hypovitaminosis D. These findings may have potentially important long-term clinical consequences (1). It is the purpose of this review to consider these issues.

HARMFUL EFFECTS OF UVR

The harmful effects of UVR are indisputable with much research showing that sunlight is the primary causative agent in the pathogenesis of basal cell carcinoma (BCC), squamous cell carcinoma (SCC) and malignant melanoma (2,3). BCC is the most common human cancer with predictions that one in three Caucasian Americans born after 1994 will develop at least one BCC during their lives (3). Although metastases are rare and mortality low, BCC can cause considerable morbidity and their treatment places a huge burden on health care agencies. SCC is three to four times less common than BCC in Caucasians and is rare in other populations (4). Malignant melanoma is less common than BCC and SCC, although its incidence is increasing rapidly (5).

Although UVR is a major causative factor for BCC and SCC, the importance of variation in patterns of exposure in terms of duration

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Abbreviations: BCC, basal cell carcinoma; BPH, benign prostatic hypertrophy; CI, confidence interval; DM, type 1 diabetes; LD, linkage disequilibrium; MC1R, melanocyte-stimulating hormone receptor; MS, multiple sclerosis; OR, odds ratio; PSA, prostate-specific antigen; RR, relative risk; SCC, squamous cell carcinoma; SNP, single-nucleotide polymorphism; TYR, tyrosinase; UVR, ultraviolet radiation; VDR, vitamin D receptor; VDRE, vitamin D response element.

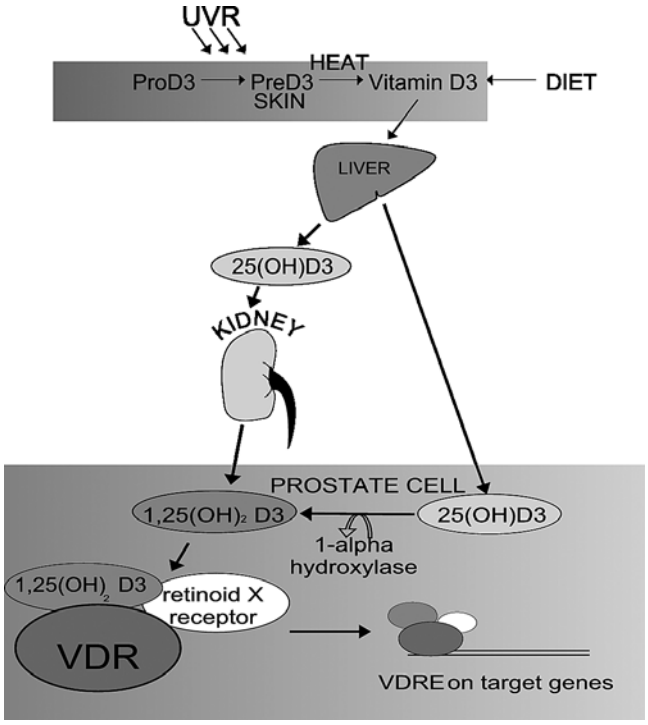

Figure 1. The prostate-specific metabolism of vitamin D. UVR, UV radiation; ProD3, provitamin D3; preD3, previtamin D3; 25(OH)D3, 25-hydroxyvitamin D3; 1,23(OH)D3, 1,25-dihydroxyvitamin D3; VDR, vitamin D receptor; VDRE, vitamin D response element.

and intensity, differ. Thus, short, intense, skin-burning exposures particularly in childhood are thought to pose the greatest risk for BCC (and melanoma), whereas chronic exposure is an important factor in the pathogenesis of SCC. The relationship between exposure and malignant melanoma risk in adults is less clear. Other risk factors include individual level of skin pigmentation and propensity to burn on exposure to UVR (4). This complex phenotype can be assessed in Caucasians by the widely used Fitzpatrick scale: skin type 1 describes individuals who always burn on exposure and never tan, type 2 subjects usually burn and will tan with difficulty, type 3 subjects sometimes suffer a mild burn and have average tanning ability, whereas type 4 subjects rarely burn and easily tan (6). This classification system can be criticized, because there is no simple inverse relationship between burning and tanning. Thus, individuals who never burn do not necessarily tan easily. However, it is useful in that the ready identification of subjects with skin type 1 is important as they effectively cannot mount an adequate pigmentary response to UVR. Such individuals often may also have blue eyes, fair or red hair and excessive numbers of basal nevi and are at greatest risk of skin cancer (7). Worldwide, aggressive public health policies have attempted to reduce the incidence of these cancers by warning, particularly Caucasians, of the dangers of inappropriate levels or patterns of exposure.

BENEFICIAL EFFECTS OF UVR

Low levels of exposure to UVR are also deleterious. This was established with the realization that rickets was endemic in many urban areas in the early 1900s, because industrial pollution blocked passage of UVR to earth, leading to inadequate synthesis of vitamin D (8). Throughout the 20th century, it was believed that improvements in the urban environment had solved this problem, and currently clinical rickets is a rare finding. However, more recently, evidence of worldwide hypovitaminosis D has been reported. For example, low serum levels of 25-hydroxyvitamin D_3 have been found in Alaskan children, adult Americans of different ethnic backgrounds, elderly Italians and many others (9–12). This hypovitaminosis could result from various causes including sun avoidance, a largely indoor lifestyle, failure to eat vitamin D_3–rich foods (*e.g.* oily fish) or take vitamin supplements and the reduced ability of the skin of elderly people to synthesize vitamin D. In the United States, there appears to be a lower intake of vitamin D–fortified foods such as milk and cereals (13).

THE ACTIONS OF VITAMIN D

In most societies, UVR-mediated synthesis of vitamin D_3 in skin is the major means of obtaining this vitamin (9–12), although in some regions such as Japan and Southeast Asia, the relatively high consumption of oily fish provides a significant proportion of the required daily intake. Vitamin D is formed from 7-dehydrocholesterol after exposure of skin to UVR. 7-Dehydrocholesterol undergoes photolysis to generate thermolabile previtamin D_3 (14), which rearranges to form vitamin D_3. Skin has massive synthetic capacity, and if it receives adequate, regular exposure to UVR, vitamin D_3 can be endogenously produced with no dietary requirement. Thus, a total body exposure of 1 minimal erythemal dose is equivalent to an oral dose of 10 000–25 000 IU (daily requirement up to 1000 IU) (14,15). Vitamin D_3 has no biological activity and enters the circulation to be metabolized to 25-hydroxyvitamin D_3 in liver and to the most active metabolite, 1,25-dihydroxyvitamin D_3, predominantly in the kidney, although catalytic activity is found in other tissues (Fig. 1).

1,25-Dihydroxyvitamin D acts intracellularly via the nuclear vitamin D receptor (VDR) which is a member of the steroid receptor superfamily and is responsible for the regulation of transcription of a number of responsive genes. The natural ligand of VDR is the bioactive form of vitamin D, 1,25-dihydroxyvitamin D_3. 1,25-Dihydroxyvitamin D_3 diffuses passively into the cell to bind to the receptor causing a conformational change in the VDR allowing dimerization with the retinoid X receptor. Dimerization enables interaction with the vitamin D response element (VDRE) on target genes, initiating transcription (16). The best-recognized effect of 1,25-dihydroxyvitamin D_3 is in calcium homeostasis. The active form of vitamin D acts via the VDR in the small intestine to increase the dietary absorption of calcium and phosphate. In the event of reduced serum calcium, 1,25-dihydroxyvitamin D acts via VDR in osteoblasts to stimulate dissolution of mineralized bone. However, it is noteworthy that, apart from bone-related genes, it is estimated that about 200 genes (0.5% of the human genome) are targets for 1,25-dihydroxyvitamin D_3 (17). VDRE are also found on genes related to cellular differentiation and proliferation including p21, TGF-β2, fibronectin, urokinase plasminogen activator and β integrin. These gene products interact with and inhibit cyclin-dependent kinases, preventing uncontrolled progression through the cell cycle (18). VDRE have also been identified in the promoters of the insulin-like growth factor binding protein-3 and insulin receptor genes (19,20). 1,25-dihydroxyvitamin D may also act as a modulator of the immune system with the VDR being expressed in

pancreatic islet cells, activated T cells, circulating monocytes and others. 1,25-Dihydroxyvitamin D_3 suppresses synthesis of interleukin-12, interleukin-2, tumor necrosis factor-α and interferon-γ, thereby inhibiting Th-1 responses. It can also effect induction of T cell–mediated immune responses, perhaps explaining the apparent beneficial effects of vitamin D in various autoimmune diseases (21).

The recognition that 1,25-hydroxyvitamin D exerts pleiotropic effects in cells suggests that deficiency, perhaps even subclinical deficiency, of the vitamin will be associated with biochemical consequences. Indeed, although the clinical implications of these findings are still unclear, there is evidence that endemic hypovitaminosis D is responsible for a significant disease burden. Clearly, for many populations, low levels of exposure to UVR will be the cause of hypovitaminosis, and it is possible that risk of a variety of diseases is inversely associated with the pattern/intensity of exposure to UVR through a vitamin D–related mechanism. We propose to examine some of the evidence supporting this hypothesis using prostate cancer as a representative disease.

PROSTATE CANCER

Prostate cancer is an important disease to consider in the context of the UVR hypothesis. It is the most prevalent noncutaneous male cancer in the United States and the second leading cause of cancer in men. Worldwide, it is the third leading cause of cancer in men. In the developed world, one in nine men over 65 years of age will develop the disease. The most comprehensive statistical data on the disease has been collated in the United States by the National Cancer Institute (22). The data show an increase in incidence of the disease since 1975, but a marked increase from 1986 to 1995. This can be mostly attributed to the advent of prostate-specific antigen (PSA)-based screening. From 1986 to 1992, the incidence in white Americans increased from 86 in 100 000 to 179 in 100 000 men, but has since reduced to 110 per 100 000 men in 1995 and continues to fall further. The incidence in African Americans is much higher, rising from 124 in 100 000 in 1986 to 250 in 100 000 men in 1992 but reduced to 170 in 100 000 men in 1995. The observed increased risk is also seen in Jamaica (incidence of 305 in 100 000 men) (23), and Nigerian men have also been noted to have increased incidence (24).

The reasons why skin pigmentation is generally associated with high prostate cancer risk is not clear. Both environmental and genetic factors can be suggested. Other interesting geographical and racial trends in the disease have also been observed: Scandinavians have high incidences of prostate cancer compared with southern Europeans (25), and the incidence is observed to be the lowest in Asia, with China and Japan having reported incidences of 4 in 100 000, but Chinese and Japanese men in the United States have an increased risk of developing and dying from prostate cancer compared with those living in their native countries (their risk is, however, lower than that of white Americans) (26,27). Similar differences have been observed in Chinese, Jewish and Indian immigrant groups. However, studies have shown that part of the observed differences between incidence and mortality rates in different populations is attributable to differing detection methods for the disease, and real differences are not as great as raw data may suggest (28). However, there are still significant racial and geographical differences in the incidence of the disease. The reasons for these widely observed differences are not understood. Prostate cancer is a complex disease, and the development of prostate cancer is likely to depend on the interplay of many factors including age, race, family history, diet (29) and, as discussed here, environmental exposures.

UVR AND PROSTATE CANCER RISK

As early as 1936, evidence has existed that UVR is inversely related to cancer mortality rates (30). However, it was not until the latter part of the 20th century that attention focused on the potential anticancer effect of UVR exposure and vitamin D synthesis. In 1980, Garland *et al.* (31) showed a relationship between colon cancer mortality rates in the United States and UVB radiation, using an ecologic approach. The ecologic approach compares disease parameters with the extent of exposure to UVR in geographically defined areas. This was followed by further studies using the same approach to show an inverse relationship between UVR and breast, ovarian and prostate cancers. Hanchette and Schwartz (32) examined the geographic distribution of UVR and prostate cancer mortality in 3073 counties in the United States. UVR and mortality showed a significant inverse correlation with a marked North–South trend with rates lowest in the South.

The ecologic approach can clearly only describe associations and has been criticized. However, data from various studies support the concept that adequate exposure to UVR results in reduced risk of various diseases through a vitamin D–mediated mechanism. In the context of prostate cancer, we proposed to consider the hypothesis by determining whether, first, associations between various parameters of UVR exposure and disease risk can be established using a case-control approach. Such data would complement corresponding studies examining the link between risk and 25-hydroxyvitamin D levels. Second, because skin pigmentation mediates cutaneous vitamin D synthesis, the hypothesis would be supported by the finding that skin type is linked with prostate cancer risk. Third, genetic factors associated with pigmented skin type should be susceptibility markers for disease risk. We have tested for such associations in studies of prostate cancer cases and controls recruited in the midlands of England (33).

Our first study used a case-control approach and was based on a comparison of parameters of acute and chronic exposure in 210 prostate cancer cases and 155 controls with benign prostatic hypertrophy (BPH) (33). All of the men were northern European Caucasians resident in North Staffordshire, England (latitude, 53.01° N) and attending urology clinics in the University Hospital of North Staffordshire. BPH patients acted as the comparison group (controls), because the disease is common and establishing this diagnosis largely excludes the possibility of concurrent prostate cancer (34). Exposure was assessed using a variety of parameters of acute and chronic exposure to UVR derived from a validated questionnaire (33,35). Of particular interest were the proportions of cancer and BPH patients in each quartile of exposure assessed as mean weeks of exposure during life. Comparison of the odds of having prostate cancer, between the lowest and highest quartiles, resulted in a significant odds ratio (OR) (3.03). Thus, 18.7% of BPH but 29.0% of cancer cases were in the lowest quartile group. Other parameters of exposure were also linked with reduced risk; high sunbathing score, regular foreign holidays (average weeks abroad/year) and presence of childhood sun burning were protective. Furthermore, within the case group, cumulative exposure was associated with age at diagnosis; men with the lowest quartile of exposure appeared to develop prostate

cancer earlier (median age at diagnosis, 67.7 years) than all other patients (median age at diagnosis, 72.1 years) ($P = 0.006$; hazards ratio, 1.52) (33).

Although these findings support the UVR hypothesis, they were derived from a small, exploratory study with the possibility that observed associations are spurious. Clearly, these initial findings needed confirmation in new patient groups. Accordingly, a further 212 prostate cancer and 135 BPH patients were recruited during August 2001 to April 2002 from Caucasians resident in North Staffordshire attending urology clinics in the University Hospital of North Staffordshire, with a view to reinvestigating the observed associations between UVR and prostate cancer risk. Again, it was observed that childhood sun burning, foreign holidays, adult sunbathing and low exposure are predictors for prostate cancer risk (36), indicating the robustness of the original study. Indeed, data from the second study confirmed that men with the lowest quartile of exposure (average, <1.9 h/day) have an about three-fold greater risk of prostate cancer than men in the highest quartile. Similarly, low levels of sunbathing were linked with a 5.33-fold greater risk of prostate cancer than levels in the highest quartile. Intermediate exposure or sunbathing conferred less protection, suggesting a graded effect. Replication of the original findings provides useful support for the UVR hypothesis.

Interestingly, a recent study from the United States (37) observed findings consistent with those of Bodiwala *et al*. (36). Residential sunlight exposure was associated with a decreased risk of prostate cancer. A total of 153 prostate cancer cases was identified from a cohort of 3414 male Caucasians who were examined in 1971–1975 and followed up regularly until 1992. Exposure to UVR in prostate cancer cases and men without the cancer was assessed by questionnaire. Sun exposure variables were calculated from region of residence and state of longest residence amongst others. Each state was assigned an average solar radiation level. State of longest residence in the South (relative risk [RR], 0.62; 95% confidence interval [CI], 0.40–0.95) and high solar radiation in state of birth (RR, 0.49; 95% CI, 0.30–0.79) were associated with a reduction in prostate cancer risk.

These findings suggest that the effect observed in English Caucasians from North Staffordshire is not a geographically localized effect and that UVR exposure may have a protective role in prostate cancer etiology in populations with differing genetic backgrounds and baseline UVR exposures and also in men living at latitudes that allow continual exposure (33). Thus, although corresponding data are not available for central England, studies in Edmonton, Canada, which is on similar latitude to North Staffordshire (52° N), showed that vitamin D synthesis ceased by mid-October and did not resume until mid-April. By contrast, in Los Angeles (34° N) and Puerto Rico (18° N), vitamin D synthesis continued all year (38).

The semiquantitative nature of questionnaire-derived exposure data makes definition of an adequate level/duration of exposure difficult. Data from the prostate cancer studies (33,36) suggest that increased risk is linked with particularly low levels of exposure. Thus, it may be possible on the basis of more precise data to define exposures that allow adequate synthesis of vitamin D without risking skin cancer development. Interestingly, at least in sunny locations, relatively little exposure is needed to ensure adequate vitamin synthesis. Holick (14,38) has suggested that exposure to UVR for 25% of the time it takes for light pinkness to develop on the skin is sufficient. Indeed, Holick and Jenkins (39) have presented tables that define their assessments of safe and effective exposure times for adequate vitamin D synthesis for subjects with skin types 1–4 (Caucasians) and pigmented individuals with skin types 5 and 6 at different times during the day and in temperate and tropical climates. Thus, subjects with skin type 1 living at latitude 0–23° (Jamaica) require 1–5 min during June to August between 11:00 A.M. and 3:00 P.M., whereas those at latitude 50–75° (Stockholm) require 5–10 min. Corresponding times in subjects with skin types 5 and 6 were 15–20 and 30–40 min, respectively.

The evidence suggesting a relationship between extent of exposure, skin type and vitamin D synthesis raises the possibility that measures of skin pigmentation such as skin type will mediate prostate cancer risk. Thus, by competing for UVR, the melanin content of skin will influence vitamin D synthesis. Mortality from prostate cancer demonstrates marked variation by ethnic group (22,32,40) with African Americans having up to twice the mortality of Caucasians. Bodiwala *et al*. (41), studying an English Caucasian population, showed that cancer cases with sun-sensitive skin (type 1) had significantly reduced exposure per year ($P = 0.014$) and sunbathing scores ($P < 0.0001$) than those with skin type 4. This finding, expected from studies in skin cancer patients (42), demonstrates the complex interrelationship between exposure and host characteristics. In a further analysis, Bodiwala *et al*. (41) used recursive partitioning analysis to select the exposure parameters and host characteristics that best stratified prostate cancer from BPH patients. They showed that, in men with low exposure, assessed by sunbathing score, skin type 1 conferred protection against prostate cancer compared with types 2–4 (OR, 4.78; 95% CI, 3.01–8.25). This effect was significant but less evident in men with intermediate levels of exposure and was not found in men with high levels of exposure. These data indicate that skin type exerts a role in susceptibility to prostate cancer but that this effect is evident only in individuals with low levels of exposure (1).

It is interesting to note that evidence exists for an inverse relationship between UVR exposure, 25-hydroxyvitamin D levels and risk of various diseases other than cancers, including autoimmune diseases such as type 1 diabetes (DM), multiple sclerosis (MS) and arthritis. Staples *et al*. (43) in Australia demonstrated an inverse relationship between latitude and ambient levels of UVR and type 1 DM prevalence. Higher sun exposure in childhood and early adolescence is associated with a reduced risk of MS (44), and there is suggestive geographic variation for the prevalence of rheumatoid arthritis (45).

VITAMIN D AND PROSTATE CANCER

There is considerable *in vitro* and *in vivo* evidence for wide-ranging anticancer effects of 1,25-hydroxyvitamin D both in many tissues. 1,25-Dihydroxyvitamin D_3 (the active form of vitamin D) has prodifferentiation and antiproliferative effects in cancer cell lines and nonmalignant cells including prostate cancer cells. Miller *et al*. (46) showed exposing the LNCaP prostate cancer cell line to 1,25-dihydroxyvitamin D_3 stimulated their differentiation. More recently, 1,25-dihydroxyvitamin D_3, along with the vitamin D analog EB1089, has been shown to inhibit prostate cancer metastasis *in vivo* (47). 1,25-Dihydroxyvitamin D_3 has also been shown to inhibit telomerase expression (48), cause apoptosis (49), induce cell cycle arrest at G_1/G_0, inhibit tumor cell invasiveness (50) and suppress tumor angiogenesis (51). Furthermore, the VDR is expressed in many cells including prostate breast and colon (52). It has therefore been postulated that the observed inverse relation-

ship between UVR exposure and cancer risk can be explained by the antiproliferative effects of 1,25-dihydroxyvitamin D_3. UVR exposure increases circulating levels of 25-hydroxyvitamin D_3. However, the levels of circulating 1,25-dihydroxyvitamin D_3 are tightly controlled by the kidney in response to serum calcium, phosphate and parathyroid hormone levels. Recent demonstration that prostate cells synthesize 1,25-dihydroxyvitamin D_3 from 25-hydroxyvitamin D_3 with their own 25-hydroxyvitamin D-1α-hydroxylase (53) and that prostate 25-hydroxyvitamin D-1α-hydroxylase is not influenced by parathyroid hormone and calcium (54), provides a mechanism whereby UVR-induced increases in intracellular 1,25-dihydroxyvitamin D_3 could exert antiproliferative effects in prostate tissue. Other tissues including skin and colon cells have intrinsic 25-hydroxyvitamin D-1α-hydroxylase activity (55,56), allowing local regulation of the active form of vitamin D in these tissues. However, evidence has shown that prostate cancer cells have a greatly reduced activity of the 25-hydroxyvitamin D-1α-hydroxylase and are therefore resistant to the indirect antitumor effects of 25-dihydroxyvitamin D_3 (57), implying that loss of 1α-hydroxylase activity in prostate cancer cells may be a key event in prostate carcinogenesis.

Studies have attempted to ascertain a relationship between serum 25-hydroxyvitamin D concentrations and prostate cancer risk with variable results. Braun *et al.* (58) and Nomura *et al.* (59) showed no relationship between serum 25-hydroxyvitamin D levels and cancer risk in populations in Maryland and Hawaii. Thus, in a nested case-control study, a single blood specimen was obtained from 3737 Japanese American men examined from 1967 to 1970. After a surveillance period of over 23 years, 136 cases of prostate cancer were identified and serum concentrations of 25-hydroxyvitamin D, 1,25-dihydroxyvitamin D, calcium, phosphorus and parathyroid hormone were measured and compared with those in sera from 136 matched controls. Odds ratios for prostate cancer, for the highest quartiles relative to the lowest, were 0.8 (95% CI, 0.4–1.8) for 25-hydroxyvitamin D and 1.0 (95% CI, 0.5–2.1) for 1,25-dihydroxyvitamin D (59). However, Corder *et al.* (60) in the United States showed a significant ($P < 0.002$) reduction in mean serum 1,25-dihydroxyvitamin D_3 in 90 black and 91 white prostate cancer cases with respect to age-matched controls. A series of reports in Finnish subjects have also provided supporting evidence. For example, in a nested case-control study based on a 13 year follow-up of about 19 000 middle-aged men, 149 prostate cancer cases were identified. Serum levels of 25-hydroxyvitamin D in cases and controls were measured at entry. Men with 25-hydroxyvitamin D concentrations below the median had an adjusted relative risk of 1.7 compared with men with levels above the median. Prostate cancer risk was highest among younger men (<52 years) at entry and low serum 25-hydroxyvitamin D (adjusted OR, 3.5). The mean age at diagnosis of the patients with 25-hydroxyvitamin D concentration above the median was 1.8 years higher than that of patients with 25-hydroxyvitamin D below the median (63.1 *versus* 61.3 years). Ahonen *et al.* (61) concluded that low levels of serum 25-hydroxyvitamin D are associated with an increased risk of prostate cancer especially before the andropause. In a further recent study from this group, a U-shaped risk profile was found, with both low and high levels of 25-hydroxyvitamin D_3 being associated with elevated cancer risk in 622 cases and 1451 controls (62). This group suggested that the results may be explained by 24-hydroxylation of active metabolites in the target tissues in response to elevated 25-hydroxyvitamin D_3, thereby reducing the levels of 1,25-hydroxyvitamin D_3, although there is no evidence to support this view. Given the methodology of such studies whereby 25-hydroxyvitamin D levels are measured on one occasion, the results do not preclude an important role for vitamin D in the prevention of development of malignancy. The etiological steps in tumorigenesis are likely to develop over a long period of time and point measurement of 25-hydroxyvitamin D levels may not provide an accurate representation of long-term vitamin D adequacy.

ROLE OF GENETIC POLYMORPHISMS IN DETERMINING PROSTATE CANCER RISK

The identification of single-nucleotide polymorphisms (SNP) and gaining an understanding of their potential role in disease causation is an area of intense investigation (63). Many studies have attempted to identify polymorphic genes associated with sporadic prostate cancer risk (64,65). Generally, candidate genes have been selected on the basis of their perceived roles in the pathogenesis of the cancer. The finding that 25-hydroxyvitamin D and skin type is associated with prostate cancer risk presents a rationale for further candidates.

Pigmentation-related gene polymorphism and prostate cancer risk

Pigmentation plays an important role in the degree of vitamin D synthesis by the skin in response to UVR. Pigmentation is determined by melanin synthesis, and the rate-limiting step in this process is catalysed by the tyrosinase (TYR) enzyme, which is regulated by the melanocyte-stimulating hormone acting through the melanocyte-stimulating hormone receptor (MC1R). Mutations of TYR have been associated with albinism and MC1R with red hair color. Both TYR and MC1R genes are polymorphic (66–69). For example, over 30 variant MC1R alleles have been identified in Caucasian and Asian populations (66). Similarly, TYR is highly allelic, with more than 90 alleles identified (67). The first studies investigating the possibility that polymorphism in these genes was implicated in susceptibility to internal cancers focused on prostate cancer (70) and investigated the A→C change in exon 1 (codon 192) of TYR. Although this change has not been shown to have functional consequences, the A1 and A2 alleles have similar frequencies (0.44 and 0.56 in cases) and are useful markers. Five allelic sites in the *MC1R* were assessed for associations with prostate cancer. Polymorphisms in both TYR (codon 192 variants) and MC1R were associated with prostate cancer risk (70). MC1R Val92/Val92 was associated with increased risk of bone metastases (OR, 4.30; $P = 0.011$) and TYR A1A2 was associated with a reduced risk of metastases (OR, 0.41; $P = 0.033$).

In MS, a disease also potentially linked to UVR exposure (44), preliminary studies have associated the MC1R His294 alleles with increased risk of MS (OR, 2.21) (71).

VDR polymorphism and prostate cancer risk

1,25-Dihydroxyvitamin D_3 and its receptor are central to the hypothesis that prostate cancer risk is mediated by ultraviolet radiation exposure, the VDR is therefore an obvious focus for study. Since 1992, investigations into the potential role of VDR polymorphism in vitamin D-related disease have been ongoing. The VDR gene, located in chromosome 12q13 is large (>100 kb), has a number of documented polymorphisms (Fig. 2). Many

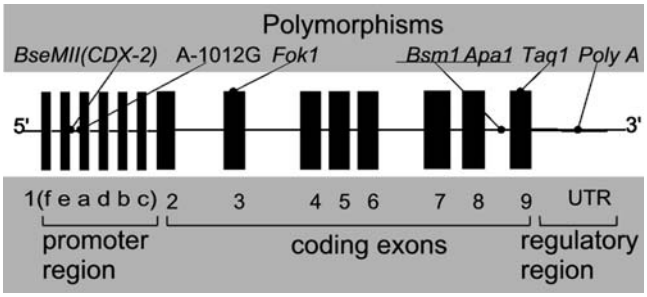

Figure 2. The vitamin D receptor gene with studied polymorphisms highlighted. UTR, Untranslated region.

variants were initially discovered as restriction fragment length polymorphisms and have unknown functional consequences. More recently, sequence analysis has enabled other polymorphic sites to be determined and importantly gives potential for additional study into their potential functional implications. The polymorphisms within the VDR gene can be classified into three groups by their position in the gene: the promoter region, the coding region and the regulatory region (72). Three linkage disequilibrium (LD) blocks termed A, B and C (73) have been identified in the VDR gene and are reported to demonstrate intra- but little inter-haplotype block LD (73). The SNP within the LD block B region including *Bsm1, Apa1, Taq1* and the polyA, have been most extensively studied (74,75). These polymorphisms appear to have no functional consequences. Later, the *Fok1* polymorphism was identified in exon 2 (76), between LD blocks B and C giving rise to a T→C nucleotide substitution. It is of particular interest given that it results in an alteration in the start codon, the C allele resulting in a protein that is three amino acids longer than that coded by the T allele. This is the only polymorphism in the VDR, resulting in a change in the protein sequence. In spite of intensive efforts to establish whether prostate cancer risk is associated with polymorphism in the VDR gene, results have been inconsistent between groups and much work still needs to be done. The TT allele of the *Taq1* polymorphism has been shown to confer a 2.5-fold increased risk of metastatic disease and a 5.4-fold increased risk of poorly differentiated disease in a group of 115 patients and 133 controls of Japanese descent (77). Oakley-Girvan *et al.* (78) assessed the *Fok1, Taq1, Bsm1, Apa1* and polyA sites in African Americans and whites. No relationship between prostate cancer risk and any of the alleles was observed in the white population. However, in the African American population, prostate cancer was associated with homozygosity for the F allele at the *Fok1* site (OR, 1.9; 95% CI, 1.0–3.3); the findings are at the limits of significance. However, our studies showed an increased risk with the ff genotype of the *Fok1* allele, of metastatic disease but not with advanced stage or grade (79). These discrepant results may be a result of poor study power, but the possibility exists that SNP may have altered effects among different populations and racial groups. A recently published meta-analysis (65) has assessed data from 17 studies investigating the association of the *Taq1*, polyA, *Bsm1* and *Fok1* polymorphisms with cancer risk. The individual studies did not involve all the of the above polymorphisms, and therefore numbers varied from 1870 patients to 2843 controls for the *Taq1* site to 540 patients and 870 controls for the polyA repeat. The analysis did not show any statistically significant differences in prostate cancer risk. No assessment was made of the effects of polymorphisms on the clinical progression of prostate cancer.

More recently, polymorphisms have been identified in the promoter region (LD block C) of the VDR that appear to be situated at transcription factor binding sites and therefore may have functional implications. In 2001, an SNP was identified in the exon 1e promoter region termed the Cdx2 polymorphism (80). The polymorphism is located in the binding site of the Cdx2 protein, a transcription factor that regulates VDR gene expression in the small intestine and other tissues. A further G/A polymorphism in the exon 1a promoter region was identified in 2004 (81). The A allele lies within the core sequence of a likely GATA-3 binding site. This polymorphism is associated with altered susceptibility and prognosis in malignant melanoma, and our own studies have shown a relationship between this polymorphism and prostate cancer risk (our unpublished data).

It has been suggested (82) that the consequences of VDR polymorphisms may be more evident in patients stratified by the extent of UVR exposure, this being a surrogate for vitamin D status. We have also assessed the importance of UVR exposure by stratifying patients by cumulative exposure/skin type and associated factors using a validated questionnaire. The *Fok1* ff allele was associated with increased risk of prostate cancer in men with UVR exposure above the median (OR, 2.91). Relative to Cdx2 GG, GA and AA genotypes were associated with increased risk of prostate cancer (OR, 2.11 and 2.02, respectively) in men with UVR exposure above the median (83).

The role of VDR polymorphisms in other internal cancers has been investigated. Studies of breast cancer have shown interesting results. A British study comprising 313 patients with breast cancer and 410 controls showed an increased risk of breast cancer with the bb *vs* BB genotype of the *Bsm1* polymorphism (OR, 1.79; 95% CI, 1.12–2.86), and 70% of commonly used breast cancer cell lines were found to have the ''at risk'' bb genotype. No relationship was seen between the *Fok1* genotype and cancer risk (84). However, contradictory results were seen in an American study of 143 cases and 300 controls of Latino descent. In this group, the BB genotype of *Bsm1* was associated with increased risk compared to Bb and bb (OR, 1.6; 95% CI, 1.1–2.5; and OR, 2.2; 95% CI, 1.0–4.7, respectively). The polyA short allele was also associated with breast cancer risk (85).

The findings of these studies indicate that complex interactions exist between UVR and genes encoding for skin type, VDR and other associated genes. This effect may be more general because studies in MS patients showed VDR ff was associated with reduced risk of the disease, particularly in males (71). However, recent studies in patients with type 1 diabetes failed to show an association between risk and VDR variants (86). Overall, such studies suggest a role for skin type and ethnicity in determining cancer risk, and indeed the lack of reproducible results between studies may be a result of the genetic differences between the populations studied. However, because some studies lack statistical power and require replication, further work is needed to elucidate the putative role of VDR and indeed whether such effects are UVR mediated.

IMPLICATIONS FOR PREVENTION AND TREATMENT

The findings that UVR is associated with reduced risk of prostate, breast, colon and other cancers and that 1,25-hydroxyvitamin D has wide-ranging prodifferentiation and antiproliferative effects on prostate and other cancer cells has important implications for both the prevention and treatment of cancer.

Although sun burning and excessive tanning do have significant adverse consequences for skin cancer risk and skin aging, moderate sensible exposure to the sun to prevent hypovitaminosis, particularly for persons with deeply pigmented skin, may be beneficial. Presently, there is much current interest into the potential role of vitamin D and vitamin D analogs as novel anticancer agents. Phase I and II trials of 1,25-dihydroxyvitamin D_3 alone or in combination with carboplatin, taxanes or dexamethasone for both androgen-dependent and -independent prostate cancer indicate the regimens are feasible (87). Clinical responses have been observed using 1,25-dihydroxyvitamin D_3 in combination with dexamethasone in androgen-independent prostate cancer (88), and potentiation of the antitumor effects of docetaxel have also been observed (88). Although trials are continuing, the current evidence suggests that vitamin D may have a role as an anticancer agent of the future.

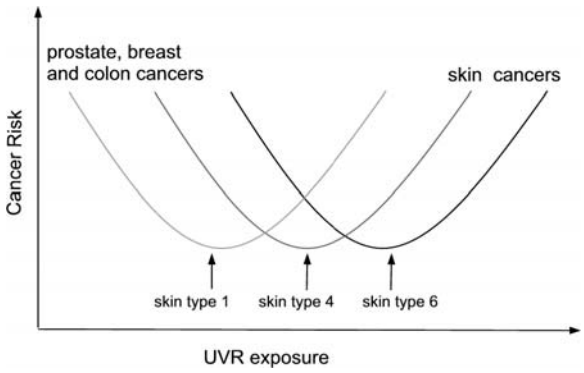

Figure 3. Graph depicting the proposed U-shaped risk of cancer with reference to UVR exposure. The three curves depict the differential risks for skin type1 (skin that does not tan), skin type 4 (skin that tans easily and does not burn) and skin type 6 (highly pigmented skin).

SUMMARY

A large volume of data exists on the antiproliferative and prodifferentiation effects of 1,25-hydroxyvitamin D_3 acting via the intracellular VDR *in vitro* on many cell types, including prostate cells. It cannot be argued that 1,25-hydroxyvitamin D does not have an important regulatory role on many intracellular mechanisms relating to cell cycle control. This evidence forms the backbone of the vitamin D–prostate cancer hypothesis. Ecologic and case control studies showing an association between UVR exposure, age at presentation and susceptibility to prostate cancer (32,33,36) are supportive; however, these have only been performed in certain populations and ethnic groups. The associations between serum levels of 25-hydroxyvitamin D_3 and prostate cancer risk do add to the hypothesis but are not conclusive, and we are far from being able to define a safe level of serum 25-hydroxyvitamin D from a cancer perspective. Current reference ranges for serum 25-hydroxyvitamin D levels are based on maintenance of bone health and prevention of rickets, and these may have to be modified in light of current and future findings. Molecular epidemiology data investigating the role of genetic polymorphism of candidate genes are promising, but few reported significant associations have been replicated (65). Reasons given include population stratification, disease heterogeneity, variable UV exposure, small sample sizes and failure to exclude chance as the reason for the finding of significance. Clearly, further efforts are needed to confirm or refute current findings and to establish the true effect of polymorphic variants in disease risk.

The effects of UVR acting via 25-hydroxyvitamin D is not exclusive to prostate cancer; ecologic studies have shown an inverse relationship between UVR and breast, colon and other cancers. UVR exposure is also strongly correlated to reduced incidence of MS (44) and is associated with a reduced prevalence of DM (43) and arthritis (45), and there are associations with decreased risk of hypertension and individuals living at lower latitudes (89).

Clearly, further work has to be done to confirm the hypothesis given the potential implications for public health advice, especially for people with deeply pigmented skin and low exposures to sunlight who may be at increased risk of prostate cancer and other diseases as a result of their actions (Fig. 3). In our view, it is too early to advocate deliberate exposure (such as increased sunbathing) as a public health approach, because such a message may be misconstrued and have an adverse impact on efforts to reduce the incidence of skin cancer. The issue does warrant further consideration and investigation, as does the role of vitamin D and vitamin D analogs, which hold the promise of being potentially important as preventative agents and as anticancer drugs of the future (90).

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