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Chapter 7

Role of Ultraviolet-B Irradiance and Vitamin D in Reducing Risk of Cancer

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Abstract

The evidence that ultraviolet-B irradiance and vitamin D reduce the risk of cancer comes from many types of studies. The UVB-vitamin D-cancer hypothesis was developed based on finding an inverse correlation between annual sunlight doses and colon cancer mortality rates in the United States. Subsequent single-country geographical ecological studies extended the hypothesis to nearly 20 types of cancer. Observational studies have provided some support for the hypothesis, especially for breast and colorectal cancer. One problem with observational studies is that when nested case-control studies are derived from cohort studies, serum 25-hydroxyvitamin D levels at the time of enrollment are used. Since serum 25(OH)D levels change with time, studies with long follow-up times tend to underestimate the effect of vitamin D. Studies of cancer survival with respect to serum 25(OH)D level at time of diagnosis have provided good evidence of beneficial effects for a number of cancer types. Randomized controlled trials have not been conducted properly to test the hypothesis. Two of the most prominent studies used vitamin D and calcium supplementation, making it difficult to separate the effects of the two substances. The mechanisms whereby vitamin D reduces the risk of cancer incidence, progression and metastasis have been studied extensively. Such studies have investigated cell cycle control mechanisms and apoptosis, cellular stress, DNA damage and repair, proliferation and telomerase, alterations in cellular microenvironment promoting angiogenesis and metastasis, interactions with growth factors that mediate transformation, cell adhesion, invasion and metastasis, and inflammation. The scientific evidence regarding whether vitamin D can be considered a causal factor in reducing risk

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of cancer can be evaluated using Hill's criteria for causality in a biological system. The important criteria include strength of association, consistent findings in different populations, biological gradient, plausibility (e.g., mechanisms), experiment (e.g., randomized controlled trial), and analogy. The evidence to date largely satisfies these criteria for several types of cancer. Ongoing research will undoubtedly strengthen the evidence in the near future.

Introduction

In 1980, the brothers Cedric and Frank Garland proposed the hypothesis that solar ultraviolet-B (UVB) irradiance reduces the risk of cancer through production of vitamin D [1]. They based that hypothesis on inspection of the map of colon cancer mortality rates in the United States for 1974, noting an inverse correlation with annual solar radiation doses for this cancer. They followed that with one study finding that dietary vitamin D was inversely correlated with colorectal cancer risk [2], and another finding that serum 25-hydroxyvitamin D [25(OH)D] level was inversely correlated with incidence of colon cancer over an 8-year observational period [3]. They also found inverse correlations between annual sunlight doses and mortality rates for breast [4] and ovarian [5] cancer.

Many single-country or regional ecological studies have investigated the geographical correlation between indices of solar UVB doses and cancer incidence and/or mortality rates. Such studies include those from Australia [6-8], China [9-11], France [12], Japan [13, 14], Nordic countries [15], Spain [16], and the United States [17-20]. Several recent papers have reviewed these ecological studies [21-26].

Many of these studies included other cancer risk-modifying factors in the analysis. No factor other than vitamin D production has been proposed to explain how solar UVB might reduce the risk of cancer.

The ecological studies in the United States were supported by a prospective study of UV exposure and risk of cancer [27]. However, the UV index used in this study was 325-nm doses for July, a wavelength not in the UVB spectral region. Ozone does not absorb light at 325 nm, so variable ozone distributions over the United States result in a different geographical variation [28].

Ecological studies can serve as a guide for types of cancer against which vitamin D is probably protective. Table 1 summarizes the findings from several ecological studies. The findings for each cancer and country are ranked S (strong), M (medium), W (weak), or "No" on the basis of the relative findings for cancers in each study. Cancers with the strongest evidence (at least three S ratings) are breast, colon, esophageal, gastric, and rectal cancer. Cancers with moderate evidence (two S ratings or one S and one M rating) are bladder, cervical, endometrial, gallbladder, ovarian, and prostate cancer. Cancers with weak evidence (one S ranking) are laryngeal, liver, lung, pancreatic, and renal cancer.

Several more types of cancer have weaker evidence. However, some types of cancer with moderate or weak evidence may actually be strongly affected by vitamin D yet may not have significant inverse correlation with indices of solar UVB dose.

For example, a rare cancer, with few cases, can make the statistical analysis weak. Or a cancer may have other risk factors that make separating the effects of solar UVB difficult, such as lung cancer, for which no good index of smoking rates exists to use in such

studies (lung cancer mortality rates are often used as the index of the health effects of smoking [19]).

Table 1. Findings from ecological studies of solar ultraviolet-B indices and cancer incidence and mortality rates

Cancer	Australia [6]	State economic area [29]	France [12]	China* [10]	Occupation Nordic Countries [15]
Bladder		S		W	S
Brain					W
Breast	S	S	S	W	S
Cervical			S	S	
Colon	S	S	S	W	S
Endometrial		S	S		W
Esophageal		S	S	S	M
Gallbladder		S			S
Gastric	S	S		S	—
Hodgkin's lymphoma		W			
Laryngeal		M			S
Leukemia				M	
Liver				—	S
Lung		—	S	W	
Non-Hodgkin's lymphoma		M			
Oral					
Ovarian	S	S			
Pancreatic		W			S
Pharyngeal					M
Prostate		M	S		M
Rectal	S	S	S	W	S
Renal		M			S
Small intestinal					M
Thyroid		W			
Vulvar		M			

*Incidence and/or mortality rate, rural counties.
 S, strong; M, medium; W, weak; —, no effect.

Evidence That Ultraviolet-B Irradiance and Vitamin D Reduce the Risk of Cancer

Evidence that vitamin D affects cancer incidence and mortality rates comes from several types of studies. Observational studies include case-control and nested case-control studies derived from cohort studies, generally based on serum 25(OH)D levels. Other indices of

vitamin D are sometimes used, including dietary and supplement intake, natural and artificial UVB irradiance, occupation, and vitamin D allele polymorphisms. Intervention studies generally take the form of randomized, placebo-controlled trials.

Laboratory studies are conducted using cancer cells and vitamin D. Animal studies are also conducted. Each approach has strengths and weaknesses. In addition, results may differ when studies are repeated in different populations, different conditions, or different laboratories. The better the agreement between studies, the stronger the evidence becomes. Thus, better conclusions can be reached by considering all the evidence rather than any single study.

Case–Control Studies of 25(OH)D Level and Cancer Incidence Rates

Case–control studies of cancer incidence with respect to serum 25(OH)D levels have the advantage that no interval exists between drawing blood samples and diagnosis of cancer. Since serum 25(OH)D has a 4- to 6-week half-life, serum 25(OH)D levels can vary when UVB irradiance or oral vitamin D intake changes, or when a significant change in body mass occurs. Disease state might affect activities such as time spent outdoors, but since those who develop cancer generally are not aware that they have cancer until so diagnosed, for undiagnosed cancer to affect serum 25(OH)D levels seems unlikely. Further support for this hypothesis is found in comparing case–control and nested case–control study results for breast and colorectal cancer as a function of follow-up time after blood draw. For both cancers, the case–control results are in a linear relation to follow-up time [30].

Findings from case–control studies of breast cancer incidence with respect to serum 25(OH)D can be used to develop a serum 25(OH)D level–breast cancer incidence relation. Five such studies have been conducted, from Germany [31, 32], Mexico [33], the United Kingdom [33], and the United States [34]. Figure 1 presents a graphical meta-analysis of breast cancer incidence rates with respect to serum 25(OH)D level measured near time of cancer diagnosis. The values for serum 25(OH)D were taken as the midpoint of the serum 25(OH)D quantiles or an appropriate value for the first and last quantile. The values for the odds ratio (OR) were based on values from two papers by Abbas and colleagues [31, 32], using those values as given, then multiplying the ORs from other studies until the power series regression fit to the data resulted in the fits overlapped well with the Abbas fits. The reason for doing this is that each study has different ranges for the quartiles. As the graph shows, the OR changes rapidly from 20 to 50 nmol/l, then slowly thereafter. Also, the 95% confidence intervals (CIs) overlap from 30 to 125 nmol/l. However, in the study of postmenopausal women [31], the ORs for all 25(OH)D quantiles >30 nmol/l were significantly lower than for <30 nmol/l, and for premenopausal women [32], the ORs for 25(OH)D >45 nmol/l were significantly lower than for <30 nmol/l.

One case–control study examined ovarian cancer prevalence with respect to serum 25(OH)D level [36]. It was based on 7273 subjects from the National Health and Nutrition Examination Surveys (NHANES). The adjusted odds ratio for low serum 25(OH)D level for those with ovarian cancer was 3.92 (95% CI, 1.11–13.85, $p = 0.03$). Since this study was based on prevalence rather than incidence rates, the possibility of reverse causality cannot be dismissed.

A study relating long-term UVB irradiance to risk of ovarian cancer was based on comparing self-reported history of cataract with prevalence of primary ovarian carcinoma [37]: “A history of cataract, reported by 14% of cases and 17% of controls, was significantly associated with a reduced ovarian carcinoma risk (OR = 0.6; 95% CI, 0.4–0.8; $P = 0.002$).”

Some concerns have been raised about case–control studies of cancer incidence in general, such as the possibility of “reverse causation,” that is, that the disease state might affect the biological factor of interest [38], and the possibility that a bias may exist in selection of controls. As to the first concern, most women do not know they have breast cancer until it is diagnosed, often the result of mammography, so the existence of breast cancer would not affect sun exposure or oral vitamin D intake. I know of no evidence that having cancer lowers serum 25(OH)D levels through any mechanism related directly to tumor effects. Also, for both breast and colorectal cancer, the ORs for case–control studies lie on the linear regression lines for nested case–control studies from cohort studies plotted versus years of follow-up [39]. As to the concern about bias in selection of controls, the selection procedures described in the papers seem to be unbiased. The fact that all five studies from four countries reported similar results further supports the validity of the findings.

Nested Case–Control Studies of 25(OH)D Level and Cancer Incidence Rates

Most studies of cancer incidence rates with respect to serum 25(OH)D level are nested case–control studies from cohort studies. Cohort studies typically enroll thousands of people and take blood samples at time of enrollment. The cohorts are followed up for many years and health outcomes are noted. Although dietary factors are often recorded every 2 years or so, additional blood samples are generally not taken. As a result, cancer incidence may occur many years after blood draw. As shown in a graph in [40], the correlation coefficients for serum 25(OH)D levels taken in the same population decrease with follow-up time, dropping to 0.4 at 14 years. Serum 25(OH)D levels change both with season and with time as people age, change sun exposure habits, diet, weight, and so on.

Breast cancer is somewhat unusual in that it is a very fast-growing cancer. For example, globally, breast cancer is more frequently diagnosed in spring and fall [41]. The authors suggested that vitamin D reduces growth of breast cancer in summer, whereas melatonin does so in winter. Thus, seasonal variations in solar UVB and 25(OH)D levels [42] can render long follow-up studies of breast cancer incidence rates unreliable.

Colorectal cancer, however, appears to be a slow-growing cancer, so long follow-ups are not a major concern. In a graphical meta-analysis, the linear regression fit to the relative risk of colorectal cancer with respect to follow-up period changed from 0.43 at year 0 to 0.68 at year 14 [39]. By comparison, for breast cancer, the corresponding values were 0.49 and 0.87 for a maximum follow-up time of 7 years.

One cohort study that has received considerable attention is the Vitamin D Pooling Project [43]. This study combined results from two studies from China, one from Finland, and seven from the United States. The median follow-up time was 9.6 years. The only significant finding was an increased rate of pancreatic cancer for those with serum 25(OH)D > 100 nmol/l. The figures indicate that the 95% CIs of the ORs for serum 25(OH)D > 100 nmol/l were from about 0.3 to 0.8. These values are too large to make effects of less than 30%–80%

visible. As noted in a comment on this paper, both the long follow-up time and the small number of cases with serum 25(OH)D level > 100 nmol/l contributed to the findings.

Several cohort studies have analyzed lymphoma. The problem with cohort studies is apparent in a study from the Alpha-Tocopherol Beta-Carotene Cancer Prevention Study (ATBCPS) cohort (1985–2002) of 29,133 Finnish male smokers (aged 50–69 years): “Cases diagnosed less than 7 years from the baseline showed an inverse association (OR for highest vs. lowest tertile = 0.43; 95% CI: 0.23, 0.83; p for trend = 0.01), but not later diagnoses (OR = 1.52; 95% CI: 0.82, 2.80; p for trend = 0.17)” [44]. The finding for less than 7 years is consistent with ecological studies [26]. The finding for more than 7 years is not.

Several cohort studies have analyzed pancreatic cancer incidence rates with respect to serum 25(OH)D levels. Studies in the ATBCPS cohort found a direct correlation between serum 25(OH)D level and incidence of pancreatic cancer [45]. The study had up to a 16.7-year follow-up period, which probably affected the findings. Also, the participants of this study were male smokers. Another cohort study in the United States found that “vitamin D concentrations were not associated with pancreatic cancer overall (highest versus lowest quintile, >82.3 versus <45.9 nmol/L: OR, 1.45; 95% CI, 0.66–3.15; P trend = 0.49). However, positive associations were observed among subjects with low estimated annual residential solar UVB exposure, but not among those with moderate to high annual exposure (P interaction = 0.015)” [46]. In this study, the follow-up period was as long as 11.7 years, and about half the participants were current or former smokers.

Another nested case–control study was reported from five Harvard cohorts [47]. The median follow-up time varied from 14.1 to 25.3 years. Current and former smokers made up 55%–60% of the participants. “Participants in quintiles two through five had multivariable-adjusted ORs (95% confidence intervals) of 0.79 (0.56–1.10), 0.75 (0.53–1.06), 0.68 (0.48–0.97), and 0.67 (0.46–0.97; P (trend) = 0.03), respectively, compared with the bottom quintile. Compared with those with insufficient levels [25(OH)D, <50 nmol/L], ORs were 0.75 (0.58–0.98) for subjects with relative insufficiency [25(OH)D, 50 to <75 nmol/L] and 0.71 (0.52–0.97) for those with sufficient levels [25(OH)D, ≥ 75 nmol/L]. No increased risk was noted in subjects with 25(OH)D ≥ 100 nmol/L, as suggested in a prior study. In subgroup analyses, ORs for the top versus bottom quartile of 25(OH)D were 0.72 (0.48–1.08) for women, 0.73 (0.40–1.31) for men, and 0.73 (0.51–1.03) for whites.”

Reinhold Vieth has proposed that large-amplitude seasonal variations in serum 25(OH)D levels at high latitudes may explain why serum 25(OH)D levels directly correlated with pancreatic cancer incidence rates in the Finnish study and the portion of the US study from dark regions [48]. Although this hypothesis is on its face reasonable, that it would apply to pancreatic cancer but no other cancer seems strange. Although a paper from Finland reports a U-shaped relation between serum 25(OH)D level and incidence of prostate cancer, on the basis of a 15- to 17-year follow-up period [49], a meta-analysis of serum 25(OH)D level and prostate cancer incidence finds no relation in general [30].

I propose an explanation for the different findings having to do with relative amounts of vitamin D derived from oral intake and solar UVB. The regions where the direct correlations with serum 25(OH)D level were found—Finland and the less sunny portion of the United States—may be where people derive more vitamin D from oral intake than from solar UVB. Vitamin D from oral intake may vary more with years of follow-up than vitamin D from solar UVB. In support of this hypothesis, a nested case–control study from the Harvard Health Professionals Follow-up Study of males measured serum 25(OH)D levels in about 1000 of

the cohort and then used those data in a regression analysis with respect to skin pigmentation, leisure time spent outdoors, and geographical location to estimate serum 25(OH)D levels in the other 49,000 participants [50]. Significant inverse correlations were found between the estimated serum 25(OH)D levels and six types of cancer, and insignificant inverse correlations for another six.

The findings for those 12 types of cancer were in general agreement with results from single-country ecological studies based on solar UVB indices [26].

Bladder Cancer

Several nested case-control studies have analyzed bladder cancer incidence or mortality rate with respect to serum 25(OH)D levels or vitamin D intake. The first one compared those with >389-IU/d vitamin D intake with those with <389 IU/d; ever smokers in New Hampshire had an odds ratio (OR) of 0.82 (95% CI, 0.54–1.24), whereas never smokers had OR = 1.04 (95% CI, 0.050–2.15) [51].

For the same comparison of vitamin D intake, those ≥ 63 years had an OR of 0.65 (95% CI, 0.40–1.08), whereas those <63 years had an OR of 1.55 (95% CI, 0.88–2.74). Although the amount of vitamin D consumed is not a reliable indicator of serum 25(OH)D level, the findings are nonetheless interesting, especially in light of a subsequent study [51].

A nested case-control study of male smokers in Finland with about a 12-year follow-up period found an inverse correlation between serum 25(OH)D level at time of enrollment and incidence of bladder cancer: “After multivariable adjustment, we found that lower 25(OH)D was associated with a statistically significantly increased risk of bladder cancer (versus ≥ 50 nmol/L; <25 nmol/L: OR, 1.73; 95% CI, 1.03–2.91; 25 to <37.5 nmol/L: OR, 1.81; 95% CI, 1.05–3.14; 37.5 to <50 nmol/L: OR, 1.76; 95% CI, 1.02–3.02; P trend=0.04)” [52]. However, another nested case-control study in the United States with a 13-year follow up failed to find a significant correlation between serum 25(OH)D level and incidence of bladder cancer [53]. Eighty-one percent of the cases were male, and 74% were present or former smokers.

The fourth study was based on mortality rates in Denmark. The cohort was 9791 white individuals from the Copenhagen City Heart Study monitored for up to 28 years [54]. Approximately 80% of the participants were ever smokers. The hazard ratio for bladder cancer death comparing serum 25(OH)D >20 ng/ml vs. <5 ng/ml was 1.28 (95% CI, 1.06–1.54).

These four studies suggest a beneficial effect of vitamin D in reducing risk of bladder cancer, especially among smokers. However, the long follow-up times used may have affected the findings [30, 40].

Ovarian Cancer

Several nested case-control studies have analyzed risk of ovarian cancer with respect to serum 25(OH)D level. A meta-analysis of 10 of these studies published between 2007 and 2010 found a relative risk of for low versus high serum 25(OH)D level of 0.83 (95% CI, 0.63–1.08, $p = 0.17$) [55].

None of the studies found a significant inverse correlation.

One study not included in the meta-analysis was conducted in Finland. For high versus low serum 25(OH)D level, the OR was 0.57 (95% CI, 0.26–1.24, $p_{\text{trend}} = 0.07$) [56].

However, comparing serum 25(OH)D level >75 nmol/l to <75 nmol/l gave an OR of 0.32 (95% CI, 0.12–0.91, $p = 0.03$).

Another study not included was from the Vitamin D Pooling Project [42]. This study had 516 cases, followed up for a median time until cancer diagnosis of 5.9 years. No statistically significant differences occurred from OR = 1 for all six serum 25(OH)D quartiles.

Prostate Cancer

In general, little to no correlation exists between serum 25(OH)D level and prostate cancer incidence. Four meta-analyses form the basis for this statement [39, 57-59]. However, evidence indicates that low serum 25(OH)D level is associated with risk of aggressive prostate cancer. In a study reported from the United Kingdom, “in case-only analyses, we used unconditional logistic regression to quantify associations of total 25(OH)D with stage (advanced vs. localized) and Gleason grade (high-grade (≥ 7) vs. low-grade (< 7)). Predetermined categories of total 25(OH)D were defined as: high: ≥ 30 ng/mL; adequate: 20- < 30 ng/mL; insufficient: 12- < 20 ng/mL; deficient: < 12 ng/mL. Fractional polynomials were used to investigate the existence of any U-shaped relationship. We included 1,447 prostate cancer cases (153 advanced, 469 high-grade) and 1,449 healthy controls. There was evidence that men deficient in vitamin D had a 2-fold increased risk of advanced versus localized cancer (OR for deficient vs. adequate total 25(OH)D=2.33, 95% CI: 1.26, 4.28) and high-grade versus low-grade cancer (OR for deficient vs. adequate total 25(OH)D=1.78, 95% CI: 1.15, 2.77).” [60].

In summary, nested case-control studies offer some support to the hypothesis that vitamin D reduces the risk of cancer. The evidence is strongest for colorectal cancer, moderately strong for pancreatic cancer, plausible for bladder cancer for present and former smokers, weak but likely for ovarian cancer, and weak for prostate cancer. In my opinion, too little evidence from such studies for other types of cancer exists to decide whether vitamin D plays a role.

Cancer Survival with Respect to Serum 25(OH)D Level

Studies examining survival with respect to serum 25(OH)D level at time of diagnosis are also useful in evaluating the effect of vitamin D on cancer risk. Vitamin D affects all stages of cancer: initiation, progression, and metastasis. Depending on the stage of cancer when diagnosed, vitamin D may have been able to play a role in one to three stages. Since many other factors are involved in cancer initiation, the effects of vitamin D are probably diluted. However, for survival or death from cancer, progression and metastasis play important roles, and vitamin D probably has less competition in these stages.

In fact, ecological studies that examined both cancer incidence and mortality rates generally found stronger inverse correlations between indices of solar UVB doses with mortality rates [11, 20].

Several reports describe outcomes of cancer patients with respect to serum 25(OH)D levels at time of diagnosis. These studies investigate up to three outcomes: cancer-specific survival, overall survival, and relapse-free survival or related nonlethal cancer outcome. An excellent review of such prospective epidemiological studies is given in the open-access paper by Pilz [61]. Table 2 presents the findings of the papers reviewed in that study.

Table 2. Effects of serum 25(OH)D level on cancer survival rates

Cancer	Country	Info	25(OH)D level	Follow-up period (years)	Adjusted overall survival [HR (95% CI)]	Adjusted cancer-specific survival [HR (95% CI)]	Adjusted relapse-free survival [HR (95% CI)]	References
Breast	Germany		<35 nmol/l vs. ≥55 nmol/l	Median 5.8	1.55 (1.00–2.39)		(Distant disease) 2.09 (1.29–3.41)	[62]
	Norway		>81 nmol/l vs. <46 nmol/l	For those still alive: 9	0.37 (0.21–0.67, $p_{\text{trend}} < 0.01$)	0.41 (0.22–0.78, $p_{\text{trend}} = 0.01$)		[63]
	Canada		<50 nmol/l vs. >72 nmol/l	Mean 11.6	1.60 (0.96–2.64)		1.71 (1.02–2.86)	[64]
Colorectal	US		32 ng/ml vs. 24.5 ng/ml		0.62 (0.42–0.93, $p_{\text{trend}} = 0.002$)	0.50 (0.26–0.95, $p_{\text{trend}} = 0.02$)		[65]
	Europe		Increase of 25 nmol/l	Mean 6.1	0.67 (0.50–0.88, $p_{\text{trend}} < 0.01$)	0.69 (0.50–0.93, $p_{\text{trend}} = 0.02$)		[33]
	Norway	Colon	>81 nmol/l vs. <46 nmol/l	For those still alive: 9	0.40 (0.10–1.60, $p_{\text{trend}} = 0.23$)	0.37 (0.08–1.81, $p_{\text{trend}} = 0.27$)		[63]
	Japan		16–36 ng/ml vs. 11–15 ng/ml		0.16 (0.04–0.63, $p = 0.009$)			[66]
			?	For those still alive at end: median 2.7	0.91 (0.84–0.99, $p = 0.03$)	0.98 (0.89–1.08, $p = 0.67$)	1.02 (0.96–1.10, $p = 0.50$)	[66]
	US	Stage IV	27–75 ng/ml vs. 2.3–13.1 ng/ml		0.94 (0.72–1.23, $p_{\text{trend}} = 0.55$)			[67]
Gastric	China		<50 nmol/l vs. ≥50 nmol/l	5	0.59 (0.37–0.91, $p = 0.02$)*			[68]
Head and neck	Canada		91 nmol/l vs. 37 nmol/l		0.85 (0.57–1.28, $p_{\text{trend}} = 0.65$)		0.72 (0.40–1.30, $p_{\text{trend}} = 0.23$)	[69]
Leukemia	US		<10 ng/ml vs >25 ng/ml	Median 2.8	1.2 (0.7–2.1)	1.2 (0.2–4.1)		[70]
								[70]
Lung	US			Median 6	0.74 (0.50–1.10, $p_{\text{trend}} = 0.07$)		0.92 (0.64–1.33, $p_{\text{trend}} = 0.37$)	[71]

Table 2. (Continued)

Cancer	Country	Info	25(OH)D level	Follow-up period (years)	Adjusted overall survival [HR (95% CI)]	Adjusted cancer-specific survival [HR (95% CI)]	Adjusted relapse-free survival [HR (95% CI)]	References
	Norway		>81 nmol/l vs. <46 nmol/l	For those still alive: 9	0.19 (0.12–0.30, $p_{\text{trend}} < 0.01$)	0.18 (0.11–0.29, $p_{\text{trend}} < 0.01$)		[63]
Diffuse large B-cell lymphoma	US		<25 ng/ml vs. >25 ng/ml	Median 2.9	1.99 (1.27–3.13, $p = 0.003$)*	2.16 (1.33–3.61, $p = 0.002$)	1.41 (0.98–2.04, $p = .07$)	[72]
T-cell lymphoma			"		2.38 (1.04–5.41, $p = 0.04$)	2.26 (0.99–5.17, $p = 0.05$)	1.94 (1.04–3.61, $p = 0.04$)	
Mantle cell lymphoma			"		1.35 (0.53–3.39, $p = 0.53$)	1.35 (0.53–3.39, $p = 0.53$)	1.07 (0.71–1.62, $p = 0.78$)	
Follicular lymphoma			"		1.52 (0.60–3.88, $p = 0.38$)	0.90 (0.23–3.49, $p = 0.88$)	1.07 (0.71–1.62, $p = 0.75$)	
Chronic lymphocytic leukemia	US	Discovery cohort	<25 ng/ml vs. >25 ng/ml	Median 3	2.39 (1.21–4.70, $p = 0.01$)		1.66 (1.16–2.37, $p = 0.005$)	[73]
		Confirmation cohort	"	Median 9.9	1.63 (0.99–2.69, $p = 0.06$)		1.59 (0.99–2.56, $p = 0.05$)	
		Both cohorts	"				1.47 (1.11–1.96, $p = 0.008$)	
Lymphoma	Norway		>81 nmol/l vs. <46 nmol/l	For those still alive: 9	0.33 (0.16–0.69, $p_{\text{trend}} < 0.01$)	0.38 (0.17–0.83, $p_{\text{trend}} = 0.02$)		[63]
Melanoma	UK		Increase of 20 nmol/l	Median 4.7			0.79 (0.64–0.96)	[74]
Prostate	Norway							[63]
	US		17 ng/ml vs. 40 ng/ml	10	1.10 (0.87–1.39)	1.31 (0.86–1.99)		[75]
Upper aerodigestive tract	Austria		>10 ng/ml vs. <10 ng/ml	Mean time, 428, 386 days	RR = 0.89 (0.83–0.97, $p = 0.006$)		RR = 0.85 (0.75–0.96, $p = 0.01$)	[76]

*Poorer survival.

For breast cancer, the studies from Germany and Norway found significant inverse correlations between serum 25(OH)D level at time of diagnosis and overall survival, and cancer-specific survival or relapse-free survival [62, 63]. The study from Canada found a significant inverse correlation for relapse-free survival and a marginally insignificant inverse correlation for overall survival [64].

For colorectal cancer, studies in the United States [65] and Europe [33] found significant inverse correlations for overall and cancer-specific survival with respect to serum 25(OH)D, and the study from Japan found a significant inverse correlation for overall survival [66]. A study from Norway did not find a significant correlation for either overall or cancer-specific survival, and a study of those with stage IV colorectal cancer did not find a significant correlation for overall survival [67].

For gastric cancer, a study found an inverse correlation between serum 25(OH)D level and gastric cancer survival [68]. The adjusted relative risk given indicates a direct correlation, but the text indicates that it was an inverse correlation.

No significant correlation was apparent between serum 25(OH)D level and head and neck cancer survival [69] or leukemia survival [70].

For lung cancer, a study in the United States found nonsignificant inverse correlations with respect to serum 25(OH)D level for overall survival and relapse-free survival [71]. A study in Norway found significant inverse correlations with respect to serum 25(OH)D level and overall and cancer-specific survival [63].

For lymphoma, a study in the United States found significant inverse correlations with respect to serum 25(OH)D level for several outcomes for diffuse large B-cell lymphoma, T-cell lymphoma, and chronic lymphocytic leukemia, but not mantle cell lymphoma or follicular lymphoma [72].

Significant inverse correlations between serum 25(OH)D level and survival with chronic lymphocytic leukemia was also found [73]. A study in Norway found significant inverse correlation with respect to serum 25(OH)D level for lymphoma [63].

For melanoma, a study in the United Kingdom found a significant inverse correlation between serum 25(OH)D level and melanoma recurrence [74].

A study in the United States did not find a significant correlation between serum 25(OH)D level and survival after diagnosis of prostate cancer [75].

A study in Austria found significant inverse correlations between serum 25(OH)D levels and overall survival and cancer recurrence for upper aerodigestive tract cancer [76].

In summary, studies found increased survival rates with respect to serum 25(OH)D levels at time of diagnosis for breast, colorectal, gastric, lung, and upper aerodigestive cancers; several types of lymphoma; and melanoma. No significant increases in survival rates with respect to serum 25(OH)D level were found for head and neck cancer, leukemia, and prostate cancer.

The findings for survival with respect to serum 25(OH)D level are in reasonable agreement with findings from ecological studies (Table 1) and observational studies for incidence rates. Breast and colorectal cancer have strong support for a beneficial vitamin D effect, lung cancer and lymphoma have moderate support, whereas leukemia and prostate cancer have weak support.

As mentioned in the discussion of cancer incidence studies, a major problem with prospective studies is that a single serum 25(OH)D level from time of enrollment is used as the index of vitamin D status [30, 53]. The change in serum 25(OH)D level after cancer

diagnosis is probably greater than for before such diagnosis since lifestyle is more likely to change.

Thus, studies of survival after cancer diagnosis with respect to serum 25(OH)D level should be considered useful but not definitive.

Racial Disparities in Cancer Survival

Differences in cancer survival rates between African Americans (AAs) and white Americans (WAs) can be used as an indirect method to estimate the role of vitamin D in reducing risk of death after diagnosis. AAs have mean serum 25(OH)D levels near 16 ng/ml, whereas WAs have mean serum 25(OH)D levels near 26 ng/ml [77]. The differences are due largely to differences in skin pigmentation and production rates of vitamin D in solar UVB. A large body of literature examines differences in survival rates for AAs and WAs. Various factors such as differences in socioeconomic status (SES), cancer stage at time of diagnosis, and treatment—which this analysis considers primary explanatory factors—have accounted for many of these differences. A recent review tabulated results of these studies [78]. The journal literature indicates that disparities exist for 13 types of cancer after consideration of SES, stage at diagnosis, and treatment: bladder, breast, colon, endometrial, lung, ovarian, pancreatic, prostate, rectal, testicular, and vaginal cancer; Hodgkin's lymphoma; and melanoma [78]. The differences in survival rates not accounted for by SES, stage at diagnosis, and treatment ranged from 0 to 50%, with a median value near 25%, which is similar to the difference in incidence rates for breast and colorectal cancer for 16 and 26 ng/ml [39]. Solar UVB doses and/or serum 25(OH)D concentrations have been reported inversely correlated with incidence and/or mortality rates for all these cancers. Findings from these studies might be more indicative of vitamin D effects than prospective studies based on single serum 25(OH)D level measurements since these studies involve more cases, and differences in serum 25(OH)D levels between the two ethnic groups have been found repeatedly.

Randomized Controlled Trials

Few randomized controlled trials (RCTs) have examined cancer incidence with respect to vitamin D supplementation. Two used calcium or calcium plus vitamin D [79, 80], whereas one used vitamin D3, calcium, vitamin D3 plus calcium, or placebo [81].

In the Women's Health Initiative (WHI) study, women were given 400 IU/d vitamin D3 and 1500 mg/d calcium (CaD), 1500 mg/d calcium, or a placebo [79]. No effect of vitamin D supplementation was found for colorectal cancer incidence rate for all participants. However, in 15,646 women (43%) not taking personal calcium or vitamin D supplements at randomization, CaD significantly decreased the risk of total, breast, and invasive breast cancers by 14%–20% and nonsignificantly reduced the risk of colorectal cancer by 17%. In women taking personal calcium or vitamin D supplements, CaD did not alter cancer risk (HR: 1.06–1.26). [82].

In the randomized, placebo-controlled trial of vitamin D3 and/or calcium (RECORD trial) [81], inclusion criteria included fragility fracture within the past 10 years, being aged at least 70 years, and not taking more than 200 IU/d vitamin D or 500 mg/d calcium. Participants were randomly allocated to daily vitamin D3 (800 IU), calcium (1000 mg), both, or placebo for 24–62 months, with a follow-up of 3 years after intervention. For those taking vitamin D, the hazard ratio for cancer incidence was 1.26 (95% CI, 0.73–3.26) and, for cancer mortality rate, 0.61 (95% CI, 0.37–1.30).

Since the subjects had suffered a fragility fracture in the past 10 years and were not taking more than 200 IU/d vitamin D, they were probably vitamin D deficient for many years.

As Lappe and Heaney [83] noted, conducting good RCTs of vitamin D for cancer prevention is difficult. The steps for a high-quality RCT include starting with an understanding of the serum 25(OH)D level–cancer incidence relation, enrolling people who are at the low end of the relation, giving them enough vitamin D to raise their serum 25(OH)D level to the upper end, and measuring serum 25(OH)D levels. Vitamin D RCTs have rarely followed these steps. Use of RCTs for cancer prevention related to dietary factors has been challenged [84]. Given the history of RCTs of vitamin D for cancer prevention, as well as the very high cost and time involved, it seems unlikely that vitamin D RCTs will provide strong evidence anytime soon that vitamin D reduces risk of cancer.

Occupation in Nordic Countries

Occupation can be associated with serum 25(OH)D levels, with outdoor occupation leading to higher serum 25(OH)D levels than indoor occupations. However, a simple classification of indoor–outdoor occupations does not capture the variations in serum 25(OH)D level because of variations in time spent in the sun. A recent paper developed a way to categorize occupation by solar UVB dose on the basis of cancer incidence data from the five Nordic countries: Denmark, Finland, Iceland, Norway, and Sweden. The study covers the 15 million people aged 30–64 years in the 1960, 1970, 1980/1981, and/or 1990 censuses in Denmark, Finland, Iceland, Norway, and Sweden, and the 2.8 million incident cancer cases diagnosed in these people in a follow-up until about 2005. [85]. The study included 54 occupation groups. Both lip cancer and melanoma are cancers with increased risk from solar UV. However, smoking is also a risk factor for lip cancer, and UVA (320–400 nm) is a more important risk factor for melanoma than is UVB. The study determined that incidence rates for lip cancer less lung cancer was a good index of integrated solar UVB dose [15]. This index was weakly inversely correlated with both melanoma and nonmelanoma skin cancer (NMSC) incidence rates. Since most NMSC is basal cell carcinoma, and since it shares many risk factors with melanoma, this finding is acceptable. Lung cancer standardized incidence ratios were used as the index of the effects of smoking. For men, the UVB index was significantly inversely correlated with 14 types of internal cancer: bladder, breast, colon, gallbladder, kidney, laryngeal, liver, lung, oral, pancreatic, pharyngeal, prostate, rectal, and small intestine cancer. For women, the same UVB index was inversely correlated with bladder, breast, and colon cancer. Developing an independent UVB index for women was not possible, probably because they are likely to wear lipstick, which blocks UVB radiation. This paper has open access, so the findings, including graphs, are available online.

Work

Related to outdoor occupation is night-shift work. Those who work night shifts often have higher cancer rates. For many years, the leading hypothesis was that by disturbing the circadian rhythm, night-shift workers had increased cancer risk due to lower production of melatonin [86].

This hypothesis made sense because, for example, blind women have a lower risk of breast cancer than do sighted women [87]. Also, global breast cancer incidence rates are higher in spring and fall than in summer or winter [40]. The authors hypothesized that vitamin D reduces risk of breast cancer in summer, whereas melatonin reduces risk of breast cancer in winter. However, two recent studies presented findings that led to questioning the melatonin hypothesis. The first reported that nurses with rotating night shifts had lower risk of NMSC and melanoma: “Working 10 years or more on rotating night shifts was associated with a 14% decreased risk of skin cancer compared with never working night shifts (age-standardized incidence rate: 976 per 100,000 person-years (PY) vs 1070 per 100,000 PY, respectively; adjusted hazard ratios = 0.86, 95% confidence interval = 0.81 to 0.92, $P(\text{trend}) < 0.001$). This association was strongest for cutaneous melanoma; working 10 years or more of rotating night shifts was associated with 44% decreased risk of melanoma, after adjustment for melanoma risk factors (age-standardized incidence rate: 20 per 100,000 PY vs 35 per 100,000 PY, respectively; adjusted hazard ratios = 0.56, 95% confidence interval = 0.36 to 0.87, $P(\text{trend}) = 0.005$)” [88]. The authors hypothesized that melatonin reduced risk of skin cancer.

The second study was a population-based case-control study conducted on men in Montreal, Quebec, Canada, between 1979 and 1985. “Compared with men who never worked at night, the adjusted odds ratios among men who ever worked at night were 1.76 (95% confidence interval (CI): 1.25, 2.47) for lung cancer, 2.03 (95% CI: 1.43, 2.89) for colon cancer, 1.74 (95% CI: 1.22, 2.49) for bladder cancer, 2.77 (95% CI: 1.96, 3.92) for prostate cancer, 2.09 (95% CI: 1.40, 3.14) for rectal cancer, 2.27 (95% CI: 1.24, 4.15) for pancreatic cancer, and 2.31 (95% CI: 1.48, 3.61) for non-Hodgkin's lymphoma. Equivocal evidence or no evidence was observed for cancers of the stomach (odds ratio (OR) = 1.34, 95% CI: 0.85, 2.10), kidney (OR = 1.42, 95% CI: 0.86, 2.35), and esophagus (OR = 1.51, 95% CI: 0.80, 2.84) and for melanoma (OR = 1.04, 95% CI: 0.49, 2.22)” [89]. In my letter to the editor, I pointed out that most of these cancers were vitamin D-sensitive cancers, so a more likely hypothesis was that night-shift workers slept during the day and so got less solar UVB irradiance than non-night-shift workers and thus had lower serum 25(OH)D levels [90].

Vitamin D Mechanisms in Cancer Biology

Cancer has several causative factors at the molecular level that promote or potentiate cellular transformation, malignancy, and metastasis, including the following:

- 1 Elevated cell proliferation rates due to elevated telomerase levels or activity
- 2 Elevated proliferation due to impaired cell cycle control mechanisms and failure of aberrant cells to destroy themselves through apoptosis
- 3 Cellular stress leading to DNA damage, mutations, and impaired DNA repair

- 4 Alterations in the microenvironment of the cell (e.g., hypoxia) that promote cell transformation, impair cellular adhesion, promote metastatic cellular homing, and promote angiogenesis
- 5 Increased proliferation (compared with differentiation) stimuli such as those from mitogenic growth factors
- 6 Immune modulation associated with increased inflammatory responses in the microenvironment of the transformed cell

We review below the evidence that highlights how vitamin D and its analogues can protect against or block cellular transformation and metastasis associated with cancer. Vitamin D mediates these effects in normal and malignant cells through molecular mechanisms involving the genomic action of the vitamin D receptor (VDR).

Proliferation and Telomerase

Cancer cells, and hyperproliferating stem cells, often exhibit high levels of telomerase activity [91]. Telomerase activity ensures that tumor cells can proliferate eternally as a consequence of preserving their telomeres (chromosome ends). Telomerase consists of several components, namely, telomerase reverse transcriptase (TERT), which contains the enzymatic subunit; telomerase RNA (TR or TERC); and dyskerin (DKC1) [92]. Recent studies by Kasiappan and colleagues demonstrated that treating human ovarian cancer cell lines and ovarian tumors with vitamin D decreased TERT activity [93]. This downregulation of TERT occurred via inducing microRNA-498 mRNA expression in a vitamin D response element-dependent manner.

Treating ovarian cancer cells (OVCAR3 cells) with vitamin D induces apoptosis through destabilizing and degrading hTERT mRNA. This destabilization of TERT and suppression of telomerase activity significantly suppressed growth in these cells [94]. In prostate cancer cell lines, treatment with vitamin D and retinoic acid significantly reduced hTERT mRNA expression by 30% within 6 hours in PC3 prostate cancer cells and gradually reduced such expression within 48 hours in LNCaP prostate cancer cells. In both cell lines, combined vitamin D–retinoic acid treatment significantly decreased telomerase activity in cancer cell lines compared with controls within 48 hours. Significant reduction in telomerase activity did not occur in cells treated with either vitamin D or retinoic acid alone [95]. In human myeloid leukemia cells (HL-60), treatment with an analogue of vitamin D, namely, 1,25(OH)₂-16-ene-5,6-*trans*-D₃, for 4 days inhibited telomerase activity [96].

Cell Cycle Control Mechanisms and Apoptosis

Treating BGC-823 gastric cancer cells with vitamin D induced significant tumor-suppressive effects by activating apoptosis in a caspase 3-dependent manner. This was accompanied by increased expression of BAX and decreased levels and activity of mitogen-activated extracellular signal-regulated kinases ERK1/2 (also known as MAPK 3/1) and AKT (also known as PKB or PI3K) [97].

Vitamin D also inhibits proliferation in human head and neck squamous cell carcinoma cells (SCC2), via cell cycle arrest at the G_0 - G_1 phase [98]. Moreover, Akutsu and colleagues showed that vitamin D can upregulate *GADD45 α* , a growth-arrest DNA damage repair factor, at both the mRNA and protein levels. This effect was accompanied by an induction of cyclin-dependent kinase inhibitor p21 mRNA, but no change in protein levels was observed.

Both vitamin D and its analogue MART-10 can arrest the cell cycle at the G_0 - G_1 transition phase in MCF-1 breast cancer cell lines. This inhibition was mediated concurrently with increased expression of BAX/BCL proapoptotic proteins and initiation of apoptosis via mitochondrial release of cytochrome C. Moreover, MART-10 treatment caused no hypercalcemic side effects, indicating that such analogues could be good agents to prevent cancer progression without causing undesired harmful side effects [99].

In adenoma and carcinoma colorectal cell lines (SW620, PC/JW, and HT29), treatment with vitamin D or its analogue EB1089 induced apoptosis and arrested the cell cycle at G_1 phase. Activation of apoptotic responses to EB1089 treatment correlated with increased protein levels of Bak (a member of the *BCL-2* gene family). In all three cell lines tested, the antiproliferative proapoptotic mechanisms were reported to be independent of p53 [98]. These findings suggested that vitamin D and EB1089 could act as effective therapeutic agents for the treatment colorectal cancers. In contrast, in rat glioma C6.9 cell lines, vitamin D responses do involve upregulation of *p53* and *GADD45* genes and the induction of apoptosis via DNA fragmentation [100]. In prostate cancer cell lines, vitamin D treatment arrested cell cycle progression at G_0 - G_1 phase and inhibited cyclin-dependent kinase 2 activity [101]. All these in vitro studies performed on different cell types, representing different degrees of cell malignancy, collectively support the role of vitamin D in blocking cellular hyperproliferation, triggering cell cycle arrest, and promoting apoptosis in transformed cells.

Cellular Stress, DNA Damage and Repair

Gene mutations and chromosomal breaks can cause malignant cell transformation. Such DNA aberrations could result from damage due to cellular stress (e.g., oxidative damage by reactive oxygen species, UV irradiation, and chemical mutagens) or improper repair of such chromosomal damage. Recent evidence suggests that vitamin D may help protect against tumorigenesis through either elevating antioxidant defense mechanisms against damaging cellular species or promoting transcription of genes involved in DNA repair needed to restore chromosomal integrity [102]. For example, studies by De Haes and colleagues have shown that treating primary human keratinocytes with vitamin D analogues can decrease the DNA-damaging effects of UVB [103]. Banakar and colleagues demonstrated that vitamin D restored antioxidant enzymes in chemically induced carcinogenesis in rat hepatocytes [104]. Ting and colleagues showed in a mouse model of chemical carcinogenesis that vitamin D treatment increased the expression and activity of VDR and in turn increased the gene expression of downstream targets ATM and RAD50, which are crucial for cells to repair double-stranded DNA breaks after DNA damage [105].

Alterations in Cellular Microenvironment (E.G., Hypoxia) Promoting Angiogenesis and Metastasis

Hypoxia and oxidative stress act as potentiating factors for cancer progression. DNA damage and loss of DNA-repair ability are often observed after oxidative stress [106, 107]. Hypoxia promotes angiogenesis in a hypoxia-inducible factor 1 α (HIF-1 α)–dependent manner and promotes expansion of tumor size and metastatic invasion [108].

Treatment of several cell lines such as SW-480-ADH, LNCaP (a prostate cancer cell line), and MCF-7 with vitamin D activates signaling cascades that promote antioxidant responses, induce mRNA expression of superoxide dismutase (in prostate epithelial cells), and downregulate glutathione levels through upregulating glucose-6-phosphate dehydrogenase expression [109-111].

Studies in several other cancer cell lines confirm that vitamin D inhibits angiogenesis. Adding vitamin D to the highly aggressive androgen-insensitive prostate cancer cell line CL-1 inhibits proliferation of these cells in both normoxic and hypoxic conditions.

Similar inhibition has been reported in studies performed using LNCaP and in SW-480 colon cancer cell lines, as well as in the MCF-7 breast cancer cell line. This inhibition is accompanied by a decreased secretion of vascular endothelial growth factor (VEGF) essential for blood vessel formation. Also, vitamin D downregulates other essential genes involved in angiogenesis, namely, endothelin 1 (ET-1) and glucose transporter 1 (Glut-1). This molecular effect is mediated via significant downregulation of HIF-1 α transcription and translation [112].

More recently, Chung and colleagues confirmed that VDR plays an important role in regulating HIF-1 α expression and its effect on downstream genes involved in angiogenesis. Loss of VDR in tumor-derived endothelial cells from VDR-knockout mice increased levels of HIF-1 α , VEGF, angiopoietin 1, and platelet-derived growth factor. Moreover, mice lacking VDR exhibited enlarged blood vessels perfusing tumor lesions. Collectively, these findings clearly implicate loss of VDR-mediated signaling in tumor angiogenesis [113].

Interactions with Growth Factors That Mediate Transformation, Cell Adhesion, Invasion, and Metastasis

Estrogen is a key mitogen in breast tumor progression. Aromatase, an enzyme encoded by the gene CYP19A1, catalyzes estrogen production and thus plays an important role in the malignancy of breast tissues. Lundqvist and colleagues demonstrated that treating breast cancer cells with vitamin D analogue EB1089 can decrease both aromatase mRNA and protein levels and inhibit aromatase-activated estrogen-dependent proliferation and cell growth of breast cancer cells. This repression was mediated by the transcription-regulatory effect of VDR, Williams syndrome transcription factor (WSTF), and vitamin D receptor interacting repressor (VDIR) on the CYP19A1 gene [114].

Therefore, if vitamin D analogues can effectively block estrogen production through downregulating aromatase expression and activity, such analogues can be effectively used as a chemopreventive agent to protect against estrogen-dependent forms of breast tumors.

Signaling cross-talk has also been reported between vitamin D and androgens. Treating prostate cancer cells with vitamin D showed that the androgens have potent growth-inhibitory

effects. However, this protective effect cannot be mediated without androgen receptors, indicating a signaling interdependency, or cross-talk, between both hormones to promote the protective outcomes of vitamin D treatment [101].

Vitamin D triggers its antitumor activity in human renal cell carcinoma by interfering with the Sonic Hedgehog signaling cascade. Studies in mice implanted with xenografts of renal cell carcinoma cells indicate that vitamin D strongly inhibits tumor development and growth by inhibiting Sonic Hedgehog signaling molecules [115].

Vitamin D can also affect the expression and function of insulin-like growth factors (IGFs) and their binding proteins. The balance between mitogenic IGF levels and availability of their sequestering binding proteins has been studied in several cancers [116]. In prostate cancer, for example, IGFBP3 protein levels decrease upon progression of cellular transformation from a benign state to malignancy [117]. Gene array analysis of LNCaP human prostate cancer cell lines shows that IGFBP3 is upregulated upon vitamin D treatment. This in turn sequesters and reduces the availability of IGF-I levels that would otherwise cause tumor expansion [118, 119].

In malignant and metastatic MCF10CA breast cancer cells, IGFBP3 transcripts are upregulated after treatment with Gemini, an analogue of vitamin D [120]. Swami and colleagues showed that IGFBP5 gene expression was induced in MCF-7 breast cancer cell lines treated with vitamin D [121].

Scientists investigating the effect of using combination chemotherapy of vitamin D and melatonin (a hormone with antioxidant and DNA-protective roles) found that either singularly or synergistically, both agents inhibit cell growth of MCF-7 breast cancer cells after a 6-day treatment. This growth arrest was accompanied by activation of the transforming growth factor β 1 (TGF β -1) pathways, leading to increased TGF β -1, Smad4, and phosphorylated Smad3 levels associated with triggering apoptosis. The effect of vitamin D, alone or in combination, significantly decreased AKT phosphorylation and murine double-minute proto-oncogene (MDM2) levels and hence increased the p53/MDM2 ratio [122].

Yang and colleagues also demonstrated in malignant and nonmalignant breast cancer cells that significant cross-talk exists between TGF β -1 and vitamin D to downregulate the mitogenic signals mediated by the PI3K pathway in a VDR-dependent manner [123].

Studies in colon cancer cell line Caco-2 show that vitamin D is necessary to initiate TGF β -1 apoptotic effects. Unlike how it affects normal epithelial cells, TGF β -1 cannot inhibit cell growth in Caco-2 (and other colon cancer-derived cell lines such as SW-480). However, this resistance is abolished upon treatment of Caco-2 cell lines with vitamin D [124]. In these cells, vitamin D upregulates IGF-II receptors and increases expression of TGF β -1 itself. Also, the sensitization of cells to apoptosis by vitamin D treatment in otherwise apoptosis-resistant cell lines indicates that vitamin D could be a novel therapeutic tool for treating resistant refractory tumors.

Furthermore, vitamin D interacts with TGF β -SMAD1 signaling and blocks transcriptional expression of cell cycle proteins and inhibits the action of cyclins D1, D2, D3, and E. Vitamin D also inhibits epidermal growth factor signaling and its Ras signaling pathways [125-127].

Cell surface markers involved in cellular adhesion and homing to secondary site are often involved in cancer metastasis and the associated poor prognosis. For example, elevated expression levels of CD44, a transmembrane glycoprotein, indicate an increased metastatic

invasive phenotype in breast cancer cells and a poor disease prognosis [128]. In recent studies, Young So and colleagues showed that treating MCF10DCIS basal-like human breast cancer cells with vitamin D analogue Gemini (also known as BXL0124) significantly decreased expression of CD44 transmembrane protein [129]. This reduction in CD44 was associated with decreased mRNA and protein expression of Signal Transducer and Activator of Transcription 3 (STAT3), decreased Janus kinase 2 activation of STAT3, and reduced CD44–STAT3–JNK2 complexes required for cellular adhesion and homing [130, 131].

Moreover, treating MCF10DCIS with Gemini reduced mRNA expression levels of STAT3-regulated downstream genes [132-134], such as the metalloproteinases (MMPs) MMP-9, MMP-2, and urokinase-type plasminogen activator (uPA) [131]. Recent studies by Meehansan and colleagues showed that Calcipotriol, a synthetic analogue of vitamin D, can suppress signaling cascades associated with tumor necrosis factor α -activated inflammatory responses. Treating human squamous cell carcinoma cell line (DJM cells) with Calcipotriol inhibited phosphorylation of ERK and p38 production, thus switching off gene expression of MMP-9 and MMP-13 at the mRNA and decreasing their protein levels in a dose-dependent manner [135]. MMPs are implicated in extracellular matrix degradation associated with malignant transformation and tumor invasion [136]. The above marked suppressive effects of vitamin D treatment on gene expression, protein levels, and activity of several invasive proteases involved in cellular homing and invasive metastasis strengthen the argument to support using vitamin D to protect against the recurrence or metastasis of secondary-site malignancy.

Wnt signaling is implicated in the pathogenesis of several cancers [137-140]. Wnt acts through the nuclear localization of β -catenin, which forms activation complexes with transcription factors TCF1 and TCF4 downstream. β -Catenin can then activate several genes involved in tumor growth, metastasis, or angiogenesis. Wnt plays a role in tumorigenesis by upregulating genes involved in cellular proliferation such as cyclin D, c-myc, and c-Jun; MMP-7 [141]; and limb, bud, and heart (LBH) transcription factor. Wnt also promotes angiogenesis through upregulating endothelin 1, VEGF, and interleukin 8 (IL-8) [142-145]. By contrast, Wnt downregulates E-cadherin normally required for cellular adhesion [146]. This downregulation is a plausible explanation for the metastatic transformation observed in Wnt-activated tumor cells [96, 147].

Several studies performed using breast, colorectal, and prostate cancer cells document the antitumor abilities of vitamin D in antagonizing Wnt's oncogenic signaling action. Vitamin D treatment downregulates several of the above tumorigenesis-promoting genes [141-145] via VDR– β -catenin interactions that suppress Wnt-activated target gene expression [111, 121, 148-152]. Moreover, vitamin D upregulates the Wnt antagonist DKK-1, thus suppressing Wnt-initiated cellular transformation [152, 153].

Inflammation and Cancer

In macrophages, vitamin D treatment blocks production of IL-1 β and inflammation associated with colon carcinoma progression. This inactivation of IL-1 β suppresses inflammation and Wnt signaling activation in colon cancer epithelial cells [154]. Further studies in breast and prostate cancer cells confirm the inflammation-suppressive

effects of vitamin D. Several groups have shown that vitamin D can suppress IL-1 β , IL-6, and IL-17 [152] as well as NF- κ B responsible for inflammation associated with malignant tumors [147, 155, 156].

Autophagy is important in preventing tumor progression in vivo [157, 158]. Vitamin D regulates cancer-associated autophagy in several cell lines [158-160]. Vitamin D-induced autophagy is postulated to exert its beneficial effects by attenuating inflammation associated with tumorigenesis [145]. Further mechanistic details are reviewed in [145].

Causality Using Hill's Criteria

The scientific way to evaluate whether an agent is causally linked to an outcome in a biological system is to apply Hill's criteria for causality in a biological system [161]. The criteria important for vitamin D include strength of association, consistent findings in different populations, temporality, biological gradient (dose-response relation), plausibility (e.g., mechanisms), experiment (e.g., RCTs), and analogy. Others added "study designs, statistical tests, bias, confounding, and measurement issues" [162]. The medical system criterion, satisfaction of an RCT, is just one of the Hill criteria.

Hill's criteria for causality have been evaluated for cancer. In a review in 2009, "results for breast and colorectal cancer satisfy the criteria best, but there is also good evidence that other cancers do as well, including bladder, esophageal, gallbladder, gastric, ovarian, rectal, renal and uterine corpus cancer, as well as Hodgkin's and non-Hodgkin's lymphoma. Several cancers have mixed findings with respect to UVB and/or vitamin D, including pancreatic and prostate cancer and melanoma" [163]. Another review for breast cancer found the criteria largely satisfied [164]. Given the additional evidence since 2009, two more types of cancer seem likely to satisfy Hill's criteria for causality with respect to vitamin D: pancreatic and vulvar cancer. The evidence is nonexistent for prostate cancer incidence, although vitamin D deficiency seems to be a risk factor for aggressive prostate cancer [60].

Since the medical system uses RCTs to establish causality, it probably will not accept that vitamin D reduces risk of cancer for some time.

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