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REVIEW

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The role of vitamin D in head and neck cancer

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Abstract

Objective: Head and neck squamous cell carcinoma (HNSCC) describes a set of malignancies of the head and neck that continue to inflict considerable morbidity and mortality. Because HNSCC often presents at an advanced stage, patients frequently undergo intensive multi-modal therapy with an intent to cure. Vitamin D is a precursor to the biologically active hormone calcitriol which governs bone and calcium physiology that is obtained from diet and UV-B exposure. Vitamin D is known to have pleiotropic effects on health and disease. In this review, we examine the role of vitamin D in cancer with emphasis on HNSCC and discuss potential avenues for further research that might better elucidate the role of vitamin D in the management of HNSCC.

Review methods: A review of MEDLINE database indexed literature concerning the role and biology of vitamin D in HNSCC was conducted, with special consideration of recently published work and research involving immunobiology and HNSCC.

Conclusions: The available evidence suggests that vitamin D may play a role in protecting against HNSCC, particularly in persons who smoke, although conflicting and limited data exists. Promising initial work encourages the pursuit of further study.

Implications for practice: The significant morbidity and mortality that HNSCC brings warrants continued research in available and safe interventions that improve patient outcomes. With the rise of immunotherapy as an effective modality for treatment, continued research of vitamin D as an adjunct in the treatment of HNSCC is supported.

KEYWORDS

clinical review, head and neck squamous cell carcinoma, immunotherapy, micronutrient, vitamin D

1 | INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is a heterogenous disease entity that poses a significant clinical and surgical challenge. HNSCC can be defined by anatomical origin, etiology, molecular profile, and clinical behavior, with implications on treatment choice and prognosis. Newly diagnosed cancers of the head and neck in the US are estimated to number 65410 in 2019.[1] In this same period, an estimated 14 560 deaths from head and neck cancer will occur. Optimal care of these patients is delivered by a multidisciplinary team comprised of radiation oncologists, medical oncologists, and head and neck surgeons.[2] Between 60% and 70% of patients present with stage III or IV disease and frequently require intensive multi-modality treatment which carries significant morbidity.[2] As our understanding of the molecular mechanisms driving HNSCC have improved, so too have the range of treatments made available for these patients.

The role of micronutrients in the development and progression of cancer has been a subject of study for decades. This research has produced mixed results regarding the health benefits of micronutrient supplementation in those not already suffering from deficiency.[3,4] Of particular interest has been vitamin D, which in this review we refer to as encompassing the precursors to the biologically active hormone calcitriol unless otherwise indicated. Vitamin D acts in a pleiotropic manner in health and disease.[5] Traditionally considered as a key regulator of bone, calcium, and phosphate homeostasis, the sphere of influence of vitamin D has steadily grown with continued study. Evidence of cellular proliferation, angiogenesis, cellular metabolism, inflammatory cascades, and immunity as being sensitive to vitamin D status has been reported.[5,6] A combination of basic science, clinical, and epidemiological research has examined the mechanisms by which vitamin D exerts its biological effects attempted to identify those patients where vitamin D may have therapeutic benefit.

In this review, we describe the current understanding of the pathophysiology of HNSCC and the mechanisms by which vitamin D may act in HNSCC. Potential avenues for further research to better inform how to deploy vitamin D for the greatest therapeutic benefit are also explored.

2 | HEAD AND NECK SQUAMOUS CELL CARCINOMA AS A COMPLEX DISEASE ENTITY

The epithelium lining the aerodigestive tract is subject to a variety of carcinogenic insults. These insults notably include tobacco smoke, alcohol, and high-risk strains of human papilloma virus (HPV). Squamous cell carcinomas are the dominant histological neoplasm type arising in the head and neck, comprising upwards of 90% of all head and neck malignancies.[2,7] The inciting event of HNSCC is prognostically relevant. Patients bearing HPV-negative oropharyngeal tumors experience worse outcomes than patients with HPV-positive tumors.[8] As the rates of smoking have decreased across the Western world the typical demographic of the head and neck cancer patient has trended toward a younger, healthier individual with improved tolerance for treatment and better outcomes.[7]

2.1 | Genetic character of head and neck squamous cell carcinoma

Genomic studies have identified molecular profiles that characterize HPV-negative vs HPV-positive HNSCC. The Cancer Genome Atlas (TCGA) profiled HPV-positive and HPV-negative HNSCC to identify patterns of shared or distinct gene expression.[9] Given the association of HPV-negative HNSCC with environmental carcinogens like tobacco smoke and alcohol, an enrichment of genes in the oxidative stress pathway, which includes the master transcriptional regulator of the cellular response to oxidative stress *NRF1*, were found to be selectively upregulated as compared to HPV-positive HNSCC.[9] In HPV-positive tumors activating mutations in *PIK3CA* were more common, though HPV-negative HNSCC also exhibited high rates of *PIK3CA* mutations as well.

Despite the diversity in the genetic alterations occurring in HPV-positive vs HPV-negative HNSCC, network analysis suggests common pathways are recurrently involved in both subtypes of HNSCC. Activation of the PI3K/Akt/mTOR pathway is a common occurrence in HNSCC, and heightened activity of this pathway is associated with more aggressive tumorigenesis and invasiveness.[10,11] Both HPV-negative and HPV-positive HNSCC also display activation of the NF-κB transcriptional program, which promotes cell survival, migration, and inflammation.[9,12]

2.2 | The immune system in head and neck squamous cell carcinoma

The elevation of the immune system to a significant actor in the development, progression, and treatment of cancer is a defining feature of modern cancer research. A growing literature examining the role of the immune system in HNSCC has been pursued at the preclinical and clinical levels. In order to persist and grow, HNSCC has been found to engage in several mechanisms to evade anti-tumorigenic immune responses.

Mutations in HLA genes responsible for antigen presentation are found in a subset of HNSCC at similar rates in HPV-positive and HPV-negative tumors (11% vs 7%, respectively).[9] Disruption of tumor antigen presentation via mutations in the antigen presentation machinery (APM) interferes with the ability of the immune system to mount an adaptive response to tumor cells. Laryngeal squamous cell carcinomas bearing HLA mutations and defective APM were found to correlate with reduced T cell infiltration into tumor stroma and worse prognosis.[13] Notably, laryngeal squamous cells carcinomas feature more frequent alterations in antigen presentation than do maxillary or tonsillar squamous cell carcinomas, though the underlying reasons for this subsite variability is unclear.[13]

Beyond immune evasion, HNSCC also acts to suppress immune reactivity using systemic and localized mechanisms. Patients with HNSCC experience a general state of immunosuppression with significantly decreased absolute T lymphocyte levels in circulation as compared to healthy controls, possibly secondary to increased circulating levels of immunosuppressive cytokines (TGF-β, IL-10).[14,15] Notably, as T cell counts decrease, the risk of disease recurrence following treatment has been reported to increase.[14] Another immunosuppressive mechanism used by HNSCC involves the upregulation of inhibitory immune checkpoint signaling to enforce immune cell anergy and tolerance to tumor antigens. T cells expressing the PD-1 coinhibitory receptor interact with tumor associated PD-L1 which triggers T cell exhaustion and relieves immune-mediated cancer suppression.[15] A study investigating tumor samples taken from HNSCC patients demonstrated a correlation between elevated tumoral PD-L1 expression and fewer tumor infiltrating T lymphocytes.[16] Exemplifying the importance of the immune-tumor interface in HNSCC is the observation that immunosuppressed patients are more likely to develop pre-malignant and malignant lesions of the head and neck.[17,18]

3 | VITAMIN D IN HEALTH AND DISEASE

Vitamin D is a fat-soluble vitamin obtainable by diet or UV-B exposure and has been the focus of research due to its pleiotropic effects on the maintenance of health and prevention of disease (Figure 1). Vitamin D is the precursor metabolite to the hormonally active calcitriol, or 1α,25-dihydroxyvitamin D. Vitamin D is well-known to have regulatory activity on bone, calcium, and phosphate homeostasis. Continued study, however, has illustrated that the scope of vitamin D activity is wider than previously appreciated, and vitamin D is now shown to be involved in regulation of the immune system, musculoskeletal system, cardiovascular system, and whole-body metabolism.[6,19]

3.1 | The biochemistry and physiology of vitamin D

The majority of circulating vitamin D is supplied by peripheral conversion of 7-dehydrocholesterol by UV-B radiation in the skin,

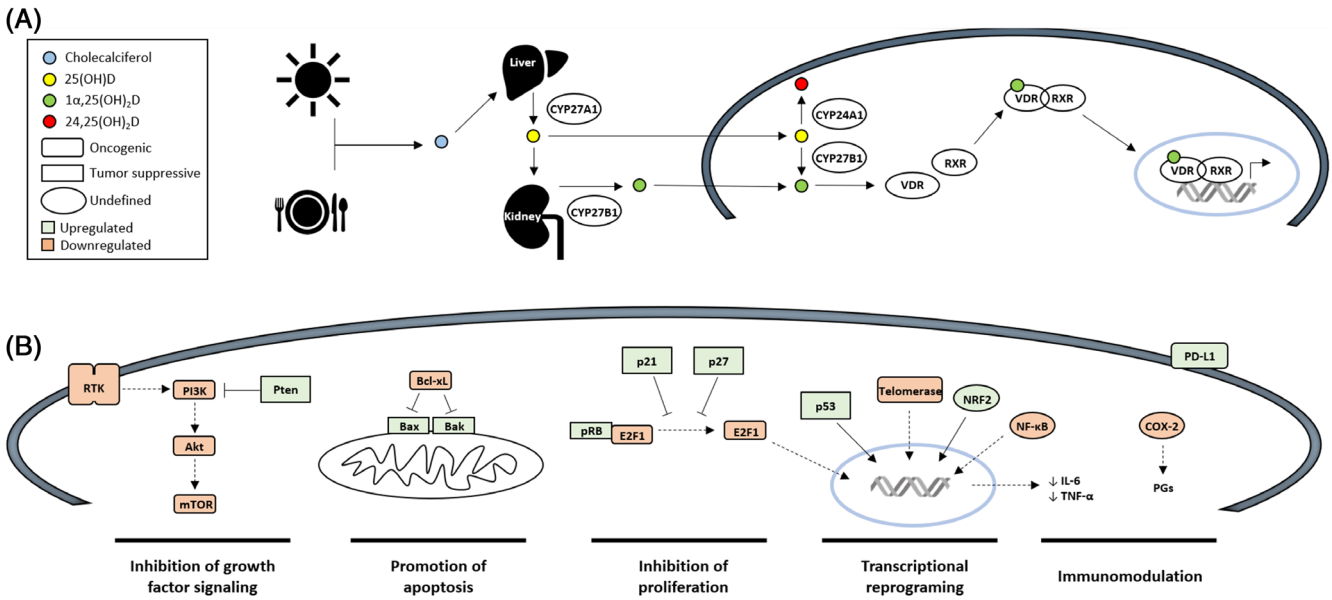

FIGURE 1 Vitamin D metabolism and activity in cancer. A, Cholecalciferol is obtained through either skin exposure to UV-B or diet. Cholecalciferol is converted to 25-hydroxyvitamin D [25(OH)D)]via CYP27A1 activity in the liver. 25(OH)D is converted to calcitriol (1α,25(OH)2D) via CYP27B1 activity primarily in the kidney. Calcitriol is the active form of vitamin D and is the primary ligand for the vitamin D receptor (VDR). Vitamin D-bound VDR heterodimerizes with RXR before translocating to the nucleus to engage VDR response elements and effect transcription. Vitamin D is metabolized for excretion by CYP24A1 which produces the inactive metabolite 24,25(OH)2D. B, Vitamin D via activation of VDR alters numerous cascades relevant to the pathophysiology of head and neck squamous cell carcinoma. Upregulation of the tumor suppressor Pten antagonizes PI3K-AKT–mTOR signaling downstream of receptor tyrosine kinase (RTK) activation by growth factors. The pro-apoptotic factors Bax and Bak are upregulated by vitamin D and prime cells for apoptosis, which is reinforced by the inhibition of the pro-survival Bcl-xL. Upregulation of p21 and p27 triggers inhibition of pRB phosphorylation and degradation by CDK complexes, thereby stabilizing the pRB-E2F1 complex and inhibiting E2F1-driven progression through the cell cycle. Vitamin D also antagonizes dedifferentiation and telomerase expression which limits malignancy. Upregulation of the master transcriptional regulator of the cellular antioxidant machinery, NRF2, promotes genomic stability and limits oxidative damage. NF-κB is inhibited from translocating to the nucleus by vitamin D thereby reducing transcription of pro-inflammatory cytokines including IL-6 and TNF-α. Vitamin D also downregulates prostaglandin (PG) synthesis via inhibition of cyclooxygenase-2 (COX-2). Lastly, the expression of the PD-L1 cell surface glycoprotein, which acts as a mediator of peripheral tolerance and attenuator of T cell activation, is up-regulated by VDR activation. Solid black arrows represent positive interactions, hatched black arrows represent actions inhibited downstream of vitamin D activity, and hammerhead arrows represent repressive interactions

with a smaller fraction being supplied by diet. Whether supplied by diet or by UV-B conversion, vitamin D is transported to the liver where it is metabolized by vitamin D 25-hydroxylase, CYP2R1 or CYP27A1, to produce 25-hydroxyvitamin D (25-OHD), otherwise known as calcidiol. Calcidiol is the predominant form of circulating vitamin D and is the typical measure used to clinically assess vitamin D status.[19] Calcidiol is further metabolized in the kidney by CYP27B1, otherwise known as 1α-hydroxylase, to produce calcitriol, or 1α,25-dihydroxyvitamin D, which is the most biologically active vitamin D metabolite.[20] Circulating calcitriol distributes to target tissues where it binds and activates its target receptor, the steroid receptor family member vitamin D receptor (VDR).[21] VDR is the major effector of vitamin D at the cellular level. Calcitriol binding to VDR promotes dimerization of cytosolic VDR with retinoid-X-receptor (RXR), thereby allowing for nuclear translocation and binding to VDR elements upstream of target genes and driving changes in the transcriptional activity of the cell.[21] A key negative regulator of vitamin D activity, and itself a transcriptional target of VDR, is CYP24A1 which mediates 24-hydroxylation of calcitriol to promote inactivation and excretion.[20]

Despite uncertainty regarding what cut-offs to use to define vitamin D deficiency,[22-24] epidemiologic studies have reported that geographic regions with lower sunlight exposure were inversely correlated with cancer incidence and mortality.[25,26] Population wide studies linking lower circulating vitamin D levels to higher incidence of breast, colorectal, gastric, and prostate cancer bolstered the idea of vitamin D status as a protective factor in carcinogenesis.[6,27-29]

3.2 | Vitamin D and cancer

Several mechanisms by which vitamin D may protect against carcinogenesis have been proposed (Figure 1). Vitamin D has been shown to elicit anti-proliferative effects, inhibit survival signals and promote apoptosis, limit DNA damage secondary to reactive oxygen species generation, and dampen inflammatory activity.[30] As an anti-proliferative agent, vitamin D increases the expression of CDK inhibitors p21 and p27, thereby stabilizing the E2F-pRB complex and antagonizing cell cycle progression.[31] Breast cancer cells cultured in vitro with vitamin D were demonstrated to have upregulated p53 expression.[32] Vitamin D also antagonizes the PI3K/AKT/mTOR pathway via upregulation of the tumor suppressor PTEN.[33] In terms of cell survival, cells are primed for apoptosis by vitamin D by the upregulation of the pro-apoptotic factors Bax, Bak, and Bad, and the simultaneous inhibition of the anti-apoptotic factors Bcl-2 and Bcl-XL.[34]

Reactive oxygen species (ROS) are produced under normal physiological conditions and may act as secondary messengers mediating the balance between cell survival and death. Cells subject to reactive oxygen stress upregulate antioxidant mechanisms to limit damage to cellular macromolecules, including DNA. Several lines of evidence suggest vitamin D acts to upregulate antioxidant pathways and limit oxidative damage to DNA. A small clinical trial found that those patients taking supplementary vitamin D demonstrated less oxidative DNA damage in colorectal mucosa than those patients taking placebo.[35] In agreement with this finding, mice lacking VDR have been reported to endure higher levels of oxidative DNA damage in their colorectal mucosa.[36] Mechanistically, the master transcriptional regulator of the cellular antioxidant response, NRF2, is found to be a transcriptional target of VDR which may explain the connection between vitamin D action and protection against oxidative stress.[6,37] This response is coincident with an upregulation in the expression of reductive scavengers (eg, thioredoxin which mediates the reduction of disulfide bonds, and superoxide dismutase 2 which reduces mitochondrial ROS into hydrogen peroxide and diatomic oxygen) as well as upregulation of the pentose phosphate pathway and generation of the NADPH required for neutralization of ROS.[38,39] These data suggest that vitamin D may play a role in determining the sensitivity of tissues to oxidative stress on the path to malignancy.

The interplay between the immune system and cancer is complex and evolves over the course of transformation.[40] Evidence has emerged that vitamin D acts as an immune regulator limiting inflammation.[41] Immune cells of all lineages, to varying degrees, express VDR at some point in their maturation.[41] Several mechanisms by which vitamin D limits inflammation have been reported. Vitamin D inhibits prostaglandin synthesis by repressing expression of the cyclooxygenase COX2. An inverse correlation between VDR and COX2 expression has been noted in malignant breast, ovarian and prostate cancer.[42,43] When activated, VDR is also capable of inhibiting the nuclear translocation of NF-κB and the transcription of TNF-α and IL-6 mRNA.[44,45] Interestingly, the expression of the PD-L1 cell surface glycoprotein, which acts as a mediator of peripheral tolerance and attenuator of T cell activation, is up-regulated by VDR activation.[46] While the above data propose mechanisms by which vitamin D can limit the chronic inflammatory milieu that promotes cancer development, it should be considered that over-suppression of local immune responses downstream of heightened vitamin D activity via upregulation of tolerogenic factors (PD-L1) is also a possibility. Indeed, in some thyroid and kidney tumors the levels of CYP27B1 and local vitamin D production are increased, which may act to promote an immune tolerant environment for tumor growth.[47,48]

VDR is widely expressed in human tissues and in different cancers. This widespread expression suggests that vitamin D may be capable of exerting influence directly on VDR-positive cancer cells. As an example, tumor differentiation status is reported to be correlated with VDR expression. More aggressively dedifferentiated breast, ovarian, and prostate cancers were correlated with reduced expression of VDR.[49-52] Patients with non-small cell lung carcinomas bearing higher levels of VDR expression and nuclear localization had improved overall survival when compared to tumors with minimal VDR.[53] Local regulation of vitamin D metabolism is also sensitive to tumor progression. Downregulation of CYP27B1 and local production of calcitriol has been reported in cancers of the colon, prostate, and skin.[6,54-56] Conversely, elevated expression of CYP24A1, which corresponds to local vitamin D inactivation, is associated with more advanced cancers of the colon, lung, and breast.[6,57,58] These data suggest that cancer cells arising from a variety of tissues reduce their sensitivity to vitamin

D by reducing VDR expression, while also reducing local availability of active hormone by altering vitamin D metabolism. To what extent vitamin D can influence cancer cell biology would ultimately be dependent on the above factors.

Preclinical work has suggested that vitamin D is also involved in the cellular response to cancer therapies. Breast cancer cell lines treated in vitro with radiation that exhibited treatment resistance and persistent survival were sensitized to radiotherapy when it was co-administered with vitamin D.[59] Vitamin D treatment was also sufficient to sensitize non-small cell lung cancer cell lines to radiotherapy in vitro.[60] Mice bearing breast tumor xenografts were similarly sensitized to radiotherapy and exhibited reduced local tumor growth when co-treated with a vitamin D analogue.[61] The above studies suggest that alterations in autophagy underlies the sensitization of tumor cells to radiotherapy by vitamin D, although evidence of the clinical utility of vitamin D as a radiosensitizer remains outstanding.

Studies reporting correlative data that suggest patients with lower circulating levels of vitamin D were at higher risk of developing cancer or succumbing to their cancer, particularly colorectal cancer, provided the rationale for the launch of prospective trials.[62-64] Data from clinical trials examining whether vitamin D supplementation has any effect on lowering cancer risk have been conflicting, however. A nationwide, placebo controlled, randomized trial found no effect on either the incidence of invasive cancer of any type, or death from cancer in those receiving daily high dose vitamin D supplementation compared to placebo.[65] Notably, the authors reported a median age at enrollment of 67 years with a follow-up of 5.3 years and limited cancer subsite analysis. Other smaller studies with shorter follow-ups were similarly non-significant.[66,67] Alternatively, a pooled study of 17 cohorts found that decreasing levels of pre-diagnostic vitamin D levels significantly increased risk of colorectal cancer among women, with a trend toward increased risk in men.[68] A recent systematic review and meta-analysis similarly found reductions in circulating vitamin D increased risk of cancer incidence and mortality in a dose dependent fashion, although no further subsite analysis was made.[69] With regards to vitamin D dietary intake, another systematic review found increased intake to be associated with a lower risk of mortality from any cause among cancer patients.[70]

Ultimately, discerning to what degree, if any, vitamin D is able to influence the course of cancer will require prolonged follow-up reflecting the long gestation of cancer with an emphasis on distinguishing between cancers of different anatomical and histological type.

4 | THE ROLE OF VITAMIN D IN HEAD AND NECK SQUAMOUS CELL CARCINOMA

The mechanisms by which vitamin D acts in cancer have been explored in preclinical models of HNSCC. Treatment of an oral squamous cell carcinoma cell line with increasing concentrations of vitamin D as a single agent reduced cell proliferation in vitro.[71] Several cell cycle checkpoint inhibitors have been reported to be upregulated in HNSCC in response to vitamin D treatment, including p21, p18, and p27.[72-74] Upregulation of telomerase is a common feature in HNSCC, and vitamin D treatment has been shown to antagonize *TERT* expression in vitro.[75] Other actions of vitamin D using in vitro models of HNSCC include the promotion of cellular differentiation, promotion of genomic integrity by upregulating DNA damage response pathways, and inhibition of invasive and metastatic activity.[75-77] These results were observed using both calcitriol and synthetic calcitriol analogues of increased potency in hypopharyngeal and oral squamous cell carcinoma cell lines. Using a hamster buccal pouch model of carcinogenesis, vitamin D was shown to protect against carcinogen-induced buccal squamous cell carcinoma.[78] This study demonstrated that only one of 10 hamsters treated with intraperitoneal injections of vitamin D developed a histologically confirmed neoplasm after carcinogen exposure, as compared to seven of 10 hamsters developing neoplasms when treated with vehicle control. The above combination of in vitro and in vivo pre-clinical data suggests a protective relationship exists between the action of vitamin D and HNSCC onset and progression.

Genetic sequence variants in vitamin D metabolism pathway genes have been shown to influence the risk and prognosis of HNSCC. Patients with HNSCC bearing the VDR Fok*I* T/T genotype, which is a genetic variant thought to reduce the transcriptional activity of the activated VDR complex, experienced shorter progression-free survival even after adjustment for age, smoking status, and cancer stage.[79,80] Genetic variants in other vitamin D metabolism pathway genes, including vitamin D binding protein, CYP2R1, and CYP24A1, were found to correlate to circulating vitamin D levels and overall survival in HNSCC patients.[81,82] These data suggest that intrinsic differences in vitamin D metabolism may be prognostic indicators, although larger studies that integrate genomic data with clinical correlates would be required to establish this relationship.

In the course of the development of frank malignancy, distinct histopathological precancerous lesions can be identified. In the case of oral squamous cell carcinoma, these precancerous lesions can be identified as simple hyperplasia or squamous intraepithelial carcinoma, representing increasing degrees of local tissue derangement. A study examining VDR expression in precancerous and invasive oral squamous cell carcinoma found that all lesions expressed higher levels of VDR when compared to healthy controls,[83] a finding that has been reported in subsequent work.[84] Interestingly, early precancerous lesions expressed VDR at higher levels than later precancerous lesions or invasive carcinoma, suggesting that the degree of VDR expression is sensitive to the degree of malignant derangement of affected tissues.[83] Consistent with previous work, vitamin D deficiency was observed in all patients, and no correlation between circulating vitamin D levels and tumor VDR expression was observed. It remains unclear, however, what may be driving the increased VDR expression seen in oral precancerous and invasive disease, or to what degree these cells may be responsive to vitamin D supplementation as an anti-neoplastic agent.

A common feature of HNSCC is tumor-driven immune dysfunction which produces an inflammatory environment conducive to

malignancy.[85] Mounting evidence suggests that the efficacy of the immune response mounted against HNSCC is sensitive to vitamin D levels. Immunosuppressive immature dendritic cells are defective at antigen presentation and accumulate in the tumor stroma and serum of HNSCC patients.[86,87] Treatment of HNSCC patients with vitamin D prior to surgical resection reduced the intra-tumoral levels of immature dendritic cell precursors, while increasing the levels of mature dendritic cell infiltrates into tumor stroma.[88] Infiltration of both activated $CD4^+CD69^+$ T cells and regulatory $Foxp3^+CD4^+$ T cells into HNSCC tumor tissue has been reported to confer prognostic advantage.[89] In those HNSCC patients with higher circulating levels of vitamin D, the levels of $CD4^+$ T cell infiltrates in tumoral and peri-tumoral stroma was elevated and associated with longer overall survival.[90] Also reported was higher levels of cytotoxic $CD8^+$ T cells, natural killer cells, and M1 macrophages in HNSCC tumor tissue in patients with higher vitamin D levels, suggesting vitamin D status may influence the immunological composition of the tumor microenvironment with some consequence on patient outcomes.[90] An additional burden experienced by patients with HNSCC is malnutrition both before and after treatment.[91] Malnutrition with consequent muscle wasting and weight loss is exacerbated by the development of treatment-related mucositis. Patients with HNSCC who developed treatment-associated mucositis were significantly more likely to be vitamin D deficient at a baseline and after treatment than those patients who did not develop mucositis.[91] A trend toward increased muscle wasting was also observed in patients deficient in vitamin D.[91] Limited data suggests that in vitamin D deficient cancer patients experiencing mucocutaneous toxicity supplementation with vitamin D improved mucocutaneous integrity, although further study is required to investigate whether this applies in HNSCC.[92] Lastly, a report using a pre-treatment food intake questionnaire found that those HNSCC patients with the lowest reported dietary vitamin D intake had increased risk of recurrence of their cancer as compared to patients with high levels of dietary vitamin D.[93]

4.1 | Population studies of head and neck squamous cell carcinoma and vitamin D

A number of retrospective studies have been conducted investigating vitamin D status and HNSCC. A small Danish study identified 38 HNSCC cases and reported no significant association between vitamin D levels and HNSCC incidence.[94] A larger Finnish study similarly found no association between serum vitamin D levels and risk of developing HNSCC among 348 incident cases.[95] Importantly, no analysis was made in this study regarding overall survival and baseline vitamin D status in those diagnosed with HNSCC. The Copenhagen City Heart Study (CCHS) was a prospective cohort study following 9791 patients with a median follow-up time of 21 years with endpoints consisting of onset of a tobacco-related cancer, emigration, or death.[96] The authors found that those patients with lower circulating vitamin D levels at baseline were at higher risk of developing tobacco related cancers (eg, cancers of the lung, bladder, head and neck) but carried no change in risk of developing non-tobacco related cancers.

The largest study to date examining a link between vitamin D status and HNSCC incidence and mortality has been the European Prospective Investigation into Cancer and Nutrition (EPIC) study; this effort collected blood samples, questionnaire data, and demographic information from over 380 000 patients between 1992 and 2000 across 10 European nations.[97] By identifying those patients who later developed HNSCC by either querying population-based cancer registries or by active follow-up, the authors collected the original blood samples to measure circulating vitamin D levels, often years before diagnosis.[98] The authors identified 350 eligible cases of HNSCC and employed an LC-MS/MS based approach for quantification of circulating vitamin D from collected blood samples. They found that a doubling in circulating vitamin D levels corresponded to an adjusted risk reduction of developing HNSCC of 30%.[98] Those patients with 25 nmol/L of circulating vitamin D were also 1.72 times more likely to die from any cause than those patients with a level of 50 nmol/L. Interestingly, as circulating levels of vitamin D began to exceed 50 nmol/L, an increase in risk of mortality from HNSCC became apparent, suggesting a U-shaped response curve to vitamin D levels may exist in HNSCC with very low or very high levels of vitamin D being deleterious. Subsite analysis revealed that vitamin D status exerted the most significant effects on the development of cancers of the larynx and hypopharynx. A doubling of circulating vitamin D levels corresponded to an adjusted risk decrease of developing cancer of the larynx or hypopharynx of 58%.[98] Of note, the protective effect of vitamin D against HNSCC was limited to former or current smokers (comprising 78% of cases), which was similar to the observed conclusion in the CCHS study.[96] Never smokers experienced similar risk of HNSCC no matter their vitamin D levels, suggesting the protective effects of vitamin D against HNSCC is modified by tobacco exposure.

The insights gleaned from the EPIC study are valuable given its prospective nature following patients through HNSCC diagnosis and death. An important limitation, however, was the reliance on a single measure was taken at the outset of the study, with no other vitamin D measures recorded at any point throughout the study. This leaves open the question as to how, if at all, circulating vitamin D levels changed throughout the course of disease. Taken together, the above studies suggest that, for a select group of patients, the disease course of HNSCC may be influenced by vitamin D status.

5 | FUTURE INVESTIGATION

The use of epidemiologic data to draw conclusions about nutrition and health has its challenges.[24,99] Careful consideration of the limitations of such data is necessary to prevent overly broad interpretation. When translating epidemiological research to molecular mechanisms, plausibility and consistency with the existing literature are important. Epidemiologic data is best leveraged when testable hypotheses can be generated and scrutinized in controlled settings. The present literature detailing vitamin D and HNSCC, while limited at present, provides

some suggestion of meaningful interaction. Further study informed by existing epidemiologic, basic, and clinical science remains necessary.

At the molecular level, vitamin D is reported to restrict some of the mechanisms driving HNSCC. Activation of the PI3K signaling cascade is a common feature of HNSCC, and vitamin D is reported to upregulate negative regulators of PI3K including PTEN. To what degree that vitamin D might affect PI3K signaling in HNSCC is not yet known.[9,11,33] Similarly, the NF-κB transcriptional program is often engaged in HNSCC, but has been reported as being negatively regulated downstream of VDR activation.[9,12,44,45] Whether this regulatory relationship is relevant in HNSCC models remains to be investigated.

A notable finding arising from prospective studies measuring baseline circulating vitamin D and HNSCC incidence was that smokers and former smokers were most likely to benefit from higher vitamin D levels.[96,98] Several potential mechanisms may explain why this is the case. Vitamin D has been reported to increase the expression of NRF2 and the anti-oxidant machinery in cells.[6,37] The ROS generated by tobacco smoke inhalation is partly responsible for its carcinogenicity, and the improvement in ROS scavenging downstream of VDR activation may limit the severity of this oncogenic insult in people at risk of developing HNSCC. Moreover, tobacco smoke is rife with carcinogenic compounds that directly exert mutagenic stress on DNA, while mice lacking VDR have been reported to be more sensitive to carcinogen-induced tumorigenesis.[100,101] These data suggest that further examination of vitamin D as a protector of genomic integrity against tobacco smoke is worthwhile to better understand how vitamin D may limit the development and progression of HNSCC.

Leveraging immunotherapy in the treatment of HNSCC has become a priority in light of promising clinical trial results.[102] Patients with HNSCC and low circulating vitamin D had fewer immune cell infiltrates and impaired cytotoxic activity against tumor cells as compared to patients with higher vitamin D levels.[90] Moreover, patients with HNSCC treated pre-operatively with vitamin D were found to have greater T cell infiltration into tumor stroma and decreased recurrence rates following surgery and adjuvant therapy.[103] In light of the ability of vitamin D to regulate the expression of immune checkpoint receptors on tumor cells[46] and the degree of immune infiltration into tumor stroma,[90] exploration of vitamin D as a predictor of immunotherapy success or as an agent used to bolster immunotherapy efficacy are intriguing avenues for further research.

6 | CONCLUSION

A significant fraction of patients with HNSCC present with significantly reduced circulating vitamin D levels compared to their healthy counterparts.[104] A ubiquitous micronutrient with pleiotropic functions in health and disease, vitamin D is a potentially actionable target for improving outcomes in HNSCC. The intersection between vitamin D biology and novel immunotherapies in HNSCC remains understudied. Promising preliminary work describing the relation between vitamin D and HNSCC encourages further investigation into how best to leverage this essential nutrient in our approach to treatment.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

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