Vitamin D as Radiosensitizer: A Review in Cell Line

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Abstract: *Introduction*: Vitamin D has been shown to have anti-cancer properties such as antioxidants, anti-proliferative, and cell differentiation. The property of vitamin D as an anticancer agent triggers researchers to find out whether vitamin D is useful as a radiosensitizer. Multiple studies have been carried out on cell lines in various types of cancer, but the benefits of vitamin D as a radiosensitizer still controversial. This paperwork aims to investigate the utilization of Vitamin D3 (Calcitriol) as radiosensitizer in various cell line through literature review.

Methods: A systematic search of available medical literature databases was performed on *in-vitro* studies with Vitamin D as a radiosensitizer in all types of cell lines. A total of 11 *in-vitro* studies were evaluated.

Results: Nine studies in this review showed a significant effect of Vitamin D as a radiosensitizer agent by promoting cytotoxic autophagy, increasing apoptosis, inhibiting of cell survival and proliferation, promoting gene in RelB inhibition, inducing senescene and necrosis. The two remaining studies showed no significant effect in the radiosensitizing mechanism of Vitamin D due to lack of evidence *in-vitro* settings.

Conclusion: Vitamin D have anticancer property and can be used as a radiosensitizer by imploring various mechanism pathways in various cell lines. Further research especially *in-vivo* settings need to be evaluated.

Keywords: Vitamin D, cancer, radiosensitizer, radiotherapy, cell line, review.

INTRODUCTION

Vitamin D is a micronutrient that has a major function in the metabolism of calcium and phosphate in the body [1]. Vitamin D is fat-soluble, and can be produced naturally in the body with the help of Ultraviolet isunlight and can also be obtained from food, such as fish oil, mushrooms, and Vitamin D-fortified foods such as milk and cereals, and supplementation [2].

So far, Vitamin D has always been associated with bone growth and development in humans. In recent studies, vitamin D has been shown to have anti-cancer properties such as antioxidants, anti-proliferative, and cell differentiation [3]. Vitamin D addition as supplementation is expected to help improve therapeutic response, one of which is radiation.

The property of vitamin D as an anticancer agent triggers researchers to find out whether vitamin D is useful as a radiosensitizer. Multiple studies have been carried out on cell lines in various types of cancer, but the benefits of vitamin D as a radiosensitizer are stillcontroversial due to some incoherent study results. This study aims to investigate the utilization of Vitamin D3 as a radiosensitizer in various cell lines through a review of the literature.

MATERIALS AND METHODS

We searched the Pubmed and Cochrane database for *in-vitro* research in the English language, published from April 2000 to May 2020. The keywords used were (("vitamin d" [MeSH Terms]) AND ((("radiotherapy" [MeSH Terms]) OR "radiation" [MeSH Terms]) OR "radiosensitization" [MeSH Terms])) AND (("cell line" [MeSH Terms]) OR "cancer" [MeSH Terms]). The articles chosen were judged based on their relevance and compatibility with the objectives of the exploratory review writing. The data collection flow is shown in Figure **1**.

In the beginning, 33 articles were found in accordance with the search terms, then the title and abstract were screened in accordance with the purpose of writing this article until 7 articles were finalized. Screening of the entire contents of the each article was done with inclusion criteria in the form of *in-vitro* studies on cancer cell lines. Based on the screening results, 9 articles were found that fit the inclusion criteria and the purpose of writing this literature review. The articles that were not selected were due to research not using cell lines. The author also added 6 articles articles after doing manual searches. Therefore, in the end, 11

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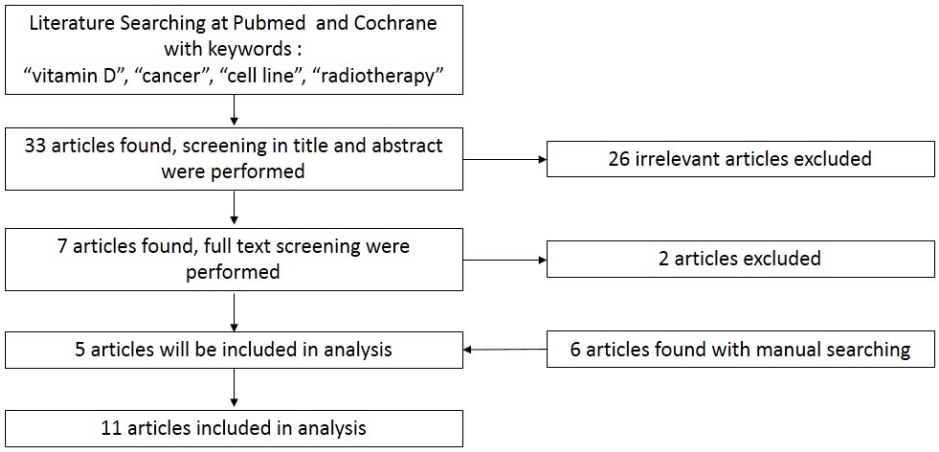

Figure 1: Search strategy diagram on Pubmed, Cochrane and search results.

articles were finalized to be included in this study: Dunlap *et al.* [4], Polar *et al.* [5], Xu *et al.* [6], Chaudry *et al.* [7], Wilson *et al.* [8], Sharma *et al.* [9], Mineva *et al.* [10], Podgorska *et al.* [11], Bristol *et al.* [12], DeMasters *et al.* [13], and Gavrilov *et al.* [14].

RESULTS

Type of Vitamin D used as Radiosensitizer

Four studies [6,8,10,14] used Calcitriol (Vitamin D3) only as Radiosensitizer. Other four studies [5,7,12,13] used analog of Vitamin D3 only. These Vitamin D3 analogs were mostly were EB 1089 and ILX 23-7553. Another type of vitamin D analog included was 19-nor-1-α,25(OH)2D2 in one of the studies (Table **1**).

Two studies [4,9] performed a comparison between both types of Vitamin D (Calcitriol and its analogs). Only one study [11] performed a comparison between Calcitriol and Calcidiol (Vitamin D2) as radiosensitizer agent (Table **1**).

Table 1: Type of Vitamin D and Cell Line used in Articles

Article/Year	Vitamin D type	Cell line
Dunlap *et al*., 2003	1. Calcitriol 2. Vitamin D3 analog: 19-nor-1 α,25(OH)2D2	Human prostate cell line (LNCaP)
Polar *et al*., 2003	1. Vitamin D3 analog: EB 1089 2. Vitamin D3 analog: ILX 23-7553	Human breast tumor cell line (p53 wild-type MCF-7)
Xu *et al*., 2007	Calcitriol	Human prostate carcinoma cell lines (LNCaP, PC-3 and DU-145)
Chaudry *et al*., 2001	Vitamin D3 analog: ILX-23-7553	Human breast tumor cell line (p53 wild-type MCF-7)
Wilson *et al*., 2011	Calcitriol	Breast tumor cell line (ZR-75)
Sharma *et al*., 2014	1. Calcitriol 2. Vitamin D analog: EB 1089	Non-small cell lung cancer/NSCLC cell line (A549 and H460)
Mineva *et al*., 2009	Calcitriol	Breast cell line: ERα-positive cell (MCF7 and ZR-75); ERα-negative cell (Hs578T and MDA-MB-231), NF639
Podgorska *et al*., 2018	1. Calcitriol 2. Calcidiol	Melanoma cell lines (human SKMEL-188, hamster BHM Ma, BHM Ab)
Bristol *et al*., 2012	Vitamin D3 analog: EB 1089	Human breast tumor cell line (p53 wild-type MCF-7)
DeMasters *et al*., 2006	Vitamin D3 analog: EB 1089	Human breast tumor cell line (p53 wild-type MCF-7)
Gavrilov *et al*., 2010	Calcitriol	Human prostate androgen-resistant cell line (DU145)

Cell Line

About half of these studies [5,7,8,10,12,13] used the breast cell line as an experiment. The common type of cell line used was p53 wild-type MCF-7. Another type of breast cell line which is uncommonly used was ZR-75 in [8]. Also, one study [10] focused on between ERα-positive cells (MCF7 and ZR-75) and ERα-negative cells (Hs578T and MDA-MB-231) (Table **1**).

Three studies [4,6,14] used human prostate cell lines, including LNCaP, PC-3, and DU-145, which is resistant to androgen. The two remaining studies [9,11] experimented with non-small cell lung cancer/NSCLC (A549 and H460) and melanoma cell line (human SKMEL-188, hamster BHM Ma, BHM Ab), respectively (Table **1**).

Radiation Type and Dose

Almost all studies use photon beam radiation. Only one study [11] used a low LET (Linear Energy Transfer) proton beam. Seven studies [4,6,7,9-11,14] performed radiation with single-dose irradiation. In the contrary, the remaining studies [5,8,12,13] performed fractionated irradiation (Table **2**).

Variable dosage were applied in these studies. In single-dose irradiation setting, the dose used starting from 0 Gy, and escalated into maximum of 6 Gy with interval of 2 Gy. In one study [14], the author only applied one dose with 4 Gy photon irradiation. On other hand, when it used fractionated irradiation, the conventional dose irradiation (2 Gy per fraction) was applied with 4-5 fractions. None of these studies performed hypofractination with > 2 Gy per fraction (Table **2**).

Research Arm and Outcome in Sensitizing Radiation

Most of these studies divided research arms with Vitamin D and irradiation only (with different dose; except in one study [14] that used single-dose of irradiation), and a combination of Vitamin D and irradiation, in cell line. Those research arms are then applied in two or more cell line types. Some studies included control (no intervention with Vitamin D nor irradiation) as their research arm.

Each of these eleven studies have a different outcome and parameter to be examined. Most of the studies perform cell viability measurements. In eight studies [4-9,12,13] Trypan blue staining is commonly used. Another way to assess cell viability is with ATP detection assay [10], Haemocytometer [11], and crystal violet staining [14] (Table **3**).

Like cell viability measurement, most examined critical parameters correlate with apoptotic cell death. Another type of cell death, i.e. autophagy, was also examined in these several studies. Senescene, cell morphology, DNA damage, and cell cycle distribution also become parameters in remaining studies.

DISCUSSION

Radiosensitization Effectiveness of Vitamin D in Cell Line

From all eleven articles in this review, almost all of these studies prove that Vitamin D has significant effect as a radiosensitizer. In Polar *et al.* [5] and Chaudry *et al.* [7] study, they observed that a combination of Vitamin D and irradiation would bring a threefold greater decline in viable cell numbers if treated with

Table 2: Radiation Type and Dose

Article/Year	Radiation type	Radiation Delivery	Dose
Dunlap *et al*., 2003	Photon	Single dose	0 - 4 Gy
Polar *et al*., 2003	Photon	Fractionated	5 x 2 Gy
Xu *et al*., 2007	Photon	Single dose	0.5-6 Gy.
Chaudry *et al*., 2001	Photon	Single dose	0 - 5 Gy
Wilson *et al*., 2011	Photon	Fractionated	4 x 2 Gy
Sharma *et al*., 2014	Photon	Single dose	0 - 6 Gy
Mineva *et al*., 2009	Photon	Single dose	0 - 6 Gy
Podgorska *et al*., 2018	Low LET Proton	Single dose	0 - 5 Gy
Bristol *et al*., 2012	Photon	Fractionated	5 x 2 Gy
DeMasters *et al*., 2006	Photon	Fractionated	5 x 2 Gy
Gavrilov *et al*., 2010	Photon	Single dose	4 Gy

Table 3: Research Arm and Outcome

Article/Year	Arm Study	Examined Parameters	Outcome and Conclusion
Dunlap *et al*., 2003	1. Control 2. IR: 1 Gy; 2 Gy; 4 Gy 3. Vit D + IR: 0 Gy; 1 Gy; 2 Gy; 4 Gy	Apoptosis assay (fragmented nuclei and PI-positive cells) Cell proliferation and viability with Trypan blue staining	Calcitriol potentiated IR-induced apoptosis of LNCaP cells Calcitriol and 19-nor-1a,25-(OH)2D2 showed synergistic inhibition of growth of LNCaP cells at radiobiologically relevant doses of IR (1–2 Gy). At higher doses of IR, the combination of Vitamin D and IR resulted in moderate antagonism.
Polar *et al*., 2003	1. IR: 5x2 Gy 2. ILX 23-7553 3. ILX 23-7553 + IR 5x2 Gy	Cell viability with Trypan blue staining TUNEL assay for DNA fragmentation to assess apoptotic cell death	1. IR reduce viable cell by 72 ± 3.1% 2. ILX 23-7553 reduce viable cell by 62 ± 4.8% 3. ILX 23-7553 + IR 5x2 Gy reduce viable cell by 93.2 ± 0.7% Approximately threefold greater decline in viable cell numbers in cells treated with ILX 23-7553 and IR as compared to cells treated with IR alone in MCF-7 breast cancer cells
Xu *et al*., 2007	1. LNCaP + IR 0 Gy; 0.5 Gy; 1 Gy; 2 Gy; 3 Gy; 6 Gy 2. PC-3 + IR 0 Gy; 0.5 Gy; 1 Gy; 2 Gy; 3 Gy; 6 Gy 3. DU-145 + IR 0 Gy; 0.5 Gy; 1 Gy; 2 Gy; 3 Gy; 6 Gy	Cell viability with Trypan blue staining NF-κB Binding Assay with ELISA SOD activity gels to quantify MnSOD activity	Calcitriol induce radiosensitivity of LNCaP and PC-3 cell line at dose 0.5 to 3.0 Gy. Calcitriol with the VDR significantly enhances radiosensitivity of prostate cancer cells at clinically relevant radiation doses.
Chaudry *et al*., 2001	1. IR 2. ILX-23-7553 3. ILX-23-7553 + IR 0 Gy; 0.5 Gy; 1 Gy; 2.5 Gy; 5 Gy	Cell viability with Trypan blue staining Clonogenic analysis with colony count Apoptotic cell death quantification with TUNEL method Cell morphology	1. IR reduce viable cell by 75% 2. ILX 23-755 reduce viable cell by 56% 3. ILX 23-7553 + reduce viable cell by 93% ILX 23-7553 enhances the effects of irradiation in MCF-7 breast tumor cells by decreasing viable cell numbers, reducing clonogenic survival and inducing apoptotic cell deaths
Wilson *et al*., 2011	1. IR 4x2 Gy 2. Calcitriol 3. Calcitriol + IR 4x2 Gy	Cell viability with Trypan blue staining Clonogenic analysis with colony count Detection and quantification of autophagic cells by staining with Acridine Orange	Combination of calcitriol with radiation resulted in a reduction of viable cells that was followed by growth arrest in the residual surviving cell population In contrast, radiation alone appeared to inhibit cell growth without producing an actual reduction in viable cell number When IR combined with Calcitriol, there was a pronounced decrease in clonogenicity compared to IR alone at all doses.
Sharma *et al*., 2014	1. A549 + IR 0 Gy; 2 Gy; 4 Gy; 6 Gy 2. H460 + IR 0 Gy; 2 Gy; 4 Gy; 6 Gy	Clonogenic survival by quantify viable cell number with Trypan Blue DNA damage by measuring phosphorylated H2AFX/gH2AX GLB staining indicates senescenes Autophagy assessment by Autophagic protein (SQSTM 1 and LC3-II), Vesicle formation, GFP-LC	In A549 and H460, both Calcitriol and EB 1089 prolonged the growth arrest induced by radiation alone and suppressed proliferative recovery, which translated to a significant reduction in clonogenic survival. In H838 or H358 NSCLC cells, which lack VDR/vitamin D receptor or functional TP53, respectively, Calcitriol failed to modify the extent of radiation-induced growth arrest or suppress proliferative recovery post-irradiation
Mineva *et al*., 2009	1. MCF7 + IR 0 Gy; 1 Gy; 2 Gy; 3 Gy; 4 Gy; 6 Gy 2. ZR-75 + IR 0 Gy; 1 Gy; 2 Gy; 3 Gy; 4 Gy; 6 Gy 3. Hs578T + IR 0 Gy; 1 Gy; 2 Gy; 3 Gy; 4 Gy; 6 Gy 4. MDA-MB-231 + IR 0 Gy; 1 Gy; 2 Gy; 3 Gy; 4 Gy; 6 Gy	Cell viability by ATP detection assay Quantitation of protein expression via immunoblot analysis and antibodies against RelB NF-κB, pro-survival factors (Survivin, Bcl-2) and MnSOD	Treatment of Hs578T and Her-2/neu-driven NF639 cells Calcitriol decreased RelB/RELB gene expression and levels of pro-survival targets Survivin, MnSOD and Bcl-2, while increasing their sensitivity to γ-irradiation.

(Table 3). Continued.

Article/Year	Arm Study	Examined Parameters	Outcome and Conclusion
Podgorska *et al*., 2018	1. SKMEL-188 + IR 0 Gy; 1 Gy; 3 Gy; 5 Gy 2. BHM Ma + IR 0 Gy; 1 Gy; 3 Gy; 5 Gy 3. BHM Ab + IR 0 Gy; 1 Gy; 3 Gy; 5 Gy	Cell count by Haemocytometer	Calcitriol inhibited human melanoma proliferation at 10 nM Calcidiol inhibited proliferation of hamster lines at 10 and 100 nM doses. Treatment with either Calcitriol or Calidiol radiosensitized melanoma cells to low doses of proton beam radiation
Bristol *et al*., 2012	1. IR 5x2 Gy 2. EB 1089 + IR 5x2 Gy	Cell viability with Trypan blue staining Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling assay for apoptosis Detection of autophagic cells by staining with acridine orange.	Calcitriol promoted autophagy in irradiated MCF-7 cells, sensitized the cells to radiation and suppressed the proliferative recovery that occurs after radiation alone. Calcitriol enhanced radiosensitivity and promoted autophagy in MCF-7 cells that overexpress Her-2/neu as well as in p53 mutant Hs578t breast tumor cells. Calcitriol failed to alter radiosensitivity or promote autophagy in the BT474 breast tumor cell line with low-level expression of the VDR.
DeMasters *et al*., 2006	1. IR 5x2 Gy 2. EB 1089 + IR 5x2 Gy	Cell viability with Trypan blue staining Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling assay for apoptosis Alkaline Unwinding Assay to assess bulk damage to DNA β-Galactosidase Histochemical Staining for assess senescence Detection of Autophagic Cells by Staining with Acridine Orange	EB 1089 failed to increase the extent of radiation-induced DNA damage or to attenuate the rate of DNA repair. MCF-7 cells expressing caspase-3 showed significant apoptosis with radiation alone as well as with EB 1089 followed by radiation.
Gavrilov *et al*., 2010	1. Calcitriol 2. Calcitriol + IR 4 Gy	Cell proliferation with crystal violet assay Cell-cycle distribution analysis with flow cytometric Apoptosis measurement with the sub-G1 (<2N ploidy) fraction in cell-cycle histograms	Four (4 Gy) irradiation in DU145 cells pretreated with a combination of 1mM VPA and 100nM Calcitriol efficiently suppressed (87.9%) DU145 cell proliferation. IR after combined pretreatment resulted in increased DNA double-strand breaks compared with non-treated cells the increase was 58.1% in pretreated cells and 11.8% in non-pretreated cells ($p < 0.002$).

Vitamin D or analog and IR as compared to cells treated with IR alone in MCF-7 breast cancer cells.

In concordance with two previous studies, Dunlap *et al.* [4] reported that Calcitriol and 19-nor-1a,25-(OH)2D2 showed synergistic inhibition of growth of LNCaP cells at radiobiologically relevant doses of irradiation (1–2 Gy), but this could not be proved in higher dose of irradiation. This study also will give a significant effect because irradiation dose 1-2 Gy per fraction is commonly used in the clinical settings. Xu *et al.* [6] also reported that Calcitriol induces radiosensitivity of LNCaP and PC-3 cell line at dose 0.5 to 3.0 Gy which is also commonly used in clinical settings in prostate cancer irradiation. Other studies [8,11,13,14] also shared similar results that irradiation combined with Vitamin D is associate with decreasing cancer cell viability.

On other hands, two studies could not prove the sensitizing effect of Vitamin D in combination with irradiation. DeMasters *et al.* [13] showed EB 1089, a vitamin D analog, failed to increase radiation-induced DNA damage or to decrease the rate of DNA repair. Whereas MCF-7 cells expressing caspase-3 showed significant apoptosis with radiation alone as well as with EB 1089 followed by radiation. DeMasters concluded that there was no outcome difference between using either radiation alone vs radiation + Vitamin D analog. In Sharma *et al.* [9] study, the combination treatment of radiation and Vitamin D did not increase either apoptosis or necrosis. They also found that the combination of Vitamin D and irradiation primarily extended growth inhibition and suppression of proliferation instead of cell killing.

Proposed Mechanism and Pathways of Vitamin D as Radiosensitizer

Some studies explicitly explain the mechanism of action in Vitamin D as a radiosensitizer, and in others,

the mechanism can be found in the results and discussion section.

a. Promotes Various form of Autophagy

Several studies [8,9,11,12] shared a similar proposed mechanism of action in Vitamin D: autophagy. Autophagy is a cell condition when cytoplasmic proteins and cellular organelles are enveloped in autophagosomes and degraded by fusion with lysosomes for amino acid and energy recycling [15]. When apoptosis is defective, autophagy can become alternative of cellular suicide pathway [16,17].

In Bristol *et al.* [12] study, they confirmed the radiosensitizing mechanism with autophagy based on evidence of autophagic vesicle formation, RFP-LC3 redistribution and punctuation, and degradation of the p62 protein. RFP-LC3 is indicative of LC3 association with the autophagosomal membrane [18], and p62 protein is associated with starved cell [19]. Calcitriol promoted autophagy in irradiated MCF-7 cells, sensitized the cells to radiation and suppressed the proliferative recovery that occurs after radiation alone. The limitation in this study is that in BT474 cells, calcitriol failed to alter radiosensitivity or promote autophagy due to the lack of VDR in those cell lines.

Sharma *et al.* [9] explained that the mechanism of sensitization was not associated with increased DNA damage, decreased DNA repair, or an increase in apoptosis, necrosis, or senescence. Instead, sensitization appeared to be a consequence of the conversion of the cytoprotective autophagy induced by radiation alone to the cytostatic form of autophagy in Vitamin D and radiation.

Wilson *et al.* [8] observed that at 72 hours post-irradiation, there was about a 4-fold increase in the percent of AVOs (acidic vacuolar organelles) in cells that received radiation treatment alone and an approximately 5-fold increase by the combination treatment. Time course data also suggest that autophagy is initiated earlier in cells treated with Vitamin D + radiation and autophagy is sustained to a higher extent treatment compared to radiation treatment alone.

b. Increase Apoptosis

Dunlap *et al.* [4] explained that Vitamin D can potentiate radiation-induced apoptosis. In LNCaP cells, Vitamin D or radiation alone did not promote substantial apoptosis, as is evident from the absence of cells with fragmented and/or condensed nuclei in this representative field. However, the combination of radiation and Vitamin D resulted in a significant increase in the percentage of cells with apoptotic morphology (fragmented nuclei and PI-positive cells). Similar to Dunlap, Polar *et al.* [5] and Chaudry *et al.* [7] observed that when cells were given ILX 23-7553, an analog of vitamin D, followed by fractionated radiation, DNA fragmentation that is indicative of apoptotic cell death increases as compared to cells that were given ILX 23-7553 alone. The mechanism basis for apoptosis induction is still not clear enough.

A bit different from two previous studies, Sharma *et al.* [9] showed that Vitamin D and radiation may have a little role in apoptosis. Sharma detected that the primary responses observed either with radiation alone or radiation and Vitamin D appeared to be growth arrest, but it was possible that some of the cell population run into apoptosis. There was minimal induction of apoptosis by radiation and radiation and Calcitriol or EB 1089 treatments in A549 and H460 cell lines and this was confirmed by the lack of PARP (poly ADP ribose polymerase) cleavage.

Only Podgorska *et al.* [11] mentioned that in melanoma cell line combined with low LET proton beam radiotherapy, it seems Vitamin D works by increasing reactive oxygen species (ROS) production, inhibiting cell cycle and apoptotic signaling.

c. Inhibition of Cell Survival and Proliferation Promoting Gene

Mineva *et al.* [10] proposed that Vitamin D promotes the sensitivity of breast cancer cells to irradiation with inhibition of RelB. ReIB is one of NF-κB, a family of dimeric transcription factors that promote cell survival, proliferation and invasive phenotype via induction of the BCL2 gene [20,21]. Treating breast cancer cell line NF639 cells with Calcitriol decreased RelB/RELB gene expression and levels of pro-survival targets Survivin, MnSOD and Bcl-2, while increasing their sensitivity to irradiation.

The study of Xu *et al.* [6] is the predecessor of Mineva *et al.* [10] study. Xu *et al.* concluded that biological levels of Calcitriol effectively enhance radiation sensitivity of prostate cancer cells at a level 10-fold lower than the amount added to the media. The mechanism of sensitivity observed correlates with NF-κB. Cancer cells usually express high levels of NF-κB compared with normal cells [22]. Then, NF-κB transactivation is induced by radiation as a therapeutic agent [23]. Activation of NF-κB then induces MnSOD, a

primary antioxidant enzyme that removes superoxide radicals from mitochondria [24]. Calcitriol transcriptionally represses RelB genes through interaction with VDR that specifically binds to VDRE located in the RelB promoter regions [25,26]. The results from the present study further show that Calcitriol represses IR-induced RelB transcription leading to suppression of RelB-mediated radioprotection.

d. Inducing Cell Cycle Arrest

The study of Gavrilov *et al.* [14] explained that a combination of VPA (Valproic Acid) and $1,25(OH)_2D_3$ enhances IR-induced cell-cycle S-phase arrest through the Chk pathway. DNA damage checkpoint kinase Chk2 showed a significant increase in Chk2 activation in pretreated prostate cancer cells compared to radiation alone. Chk2 kinase is a key component of the ATM pathway. Activation of this pathway and phosphorylation of Chk2 induces a transient blockade of DNA replication and S-phase cell-cycle arrest [27].

e. Inducing Senescence

Wilson *et al.* [8] mentioned that after administration of calcitriol and radiation, residual surviving cells are in a state of senescence based on cell morphology and β-Galactosidase staining. To further confirm β-Galactosidase activity 5-dodecanoyl aminofluorescein di-β-D-galactopyranoside (C12FDG), a fluorogenic substrate for βgal activity [28] was analyzed by FACS analysis, and fluorescence intensity was measured. For both experimental conditions, senescence is most pronounced at 144 hours post-treatment.

The five proposed mechanisms of action in Vitamin D as radiosensitizer can be summarized with the figure below (Figure **2**).

Does Vitamin D as an Antioxidant Suppresses Radiation Effectivity?

The antioxidative property of Vitamin D was later discovered compared to Vitamin A, C, and E by scientists. Vitamin D as antioxidants works through several mechanisms: 1) Increasing production of superoxide dismutase 1 and 2 (SOD1 and SOD2) in prostate epithelial cells (PECs) and in androgen-sensitive prostate cancer cells (LNCaP) [29]; 2) Induced expression of thioredoxin reductase 1 (TXNRD1) [30]; 3) Increase of glucose-6-phosphate dehydrogenase (G6PDH) expression [31]; 4) Induction of nuclear factor erythroid-derived 2-like 2 (NFE2L2) transcription factor that controls the gene expression of several enzymes of the antioxidants systems such as glutathione peroxidase (GPX) 3, heme oxygenase 1 (HMOX-1), and aldo-keto reductase 1C2(AKR1C2) [30].

Figure 2: Proposed Mechanism of Action in Vitamin D as radiosensitizer.

The author's main concern in this review is whether Vitamin D should be used concurrently with radiation due to contradictive action: radiation works through generating ROS (reactive oxygen species), and antioxidants works by suppressing ROS formation. Whether antioxidants alter antitumor effects during radiotherapy are still unclear. There might be a possibility that potent antioxidant supplementation could reduce the therapeutic effects of radiotherapy or chemotherapy.

Chandel and Tuveson *et al.* [32] study in Yasueda *et al.* [33] review demonstrated that antioxidants do not prevent cancer and may accelerate tumor development by targeting ROS in the cell. Yasueda also mentioned that in melatonin and vitamin C, higher doses or stronger antioxidants might protect not only normal cells from ROS-generating therapies but might also protect cancer cells themselves by helping them proliferate. A negative effect was also reported by Meyer *et al.* [34]. Meyer's study in HNC patients who were undergoing radiotherapy and who also smoked indicated that α-tocopherol and β-carotene supplementation with radiotherapy significantly increased recurrence and mortality in patients who smoked during radiation therapy, although not in non-smoking patients.

On the other hand, Moss [35] stated that dietary antioxidants do not interfere with the beneficial effects of radiotherapy. It is possible that the careful use of antioxidants may enhance therapeutic results. It is also known that a higher dose of antioxidants can become a pro-oxidant in cancer cells [36]. Thus, high-dose antioxidants might strengthen the effects of ROS-generating therapies including radiotherapy [33]. In lower-dose antioxidant supplementation, it may protect normal cells and reduce the toxicity of radiation and chemotherapy [34]. In Cancer Treatment Centers of America (CTCA) study [37], concomitant daily supplements treatment does not affect or control radiation therapy-mediated tumor response and its recurrence rates in localized prostate cancer patients who underwent 72 Gy dose of radiation. Neither the magnitude of the response or its durability for at least 2 years isnegatively affected by antioxidant supplements. Similar things also come from Finnish Clinical Trial [38]. The trial concluded that antioxidant treatment, in combination with chemotherapy and irradiation, prolonged the survival time of patients with small-cell lung cancer compared to most published combination treatment regimens alone.

The limitation of two previous reviews (Moss [35] and Yasueda [33]) is that both only mentioned Vitamin A, C, and E as antioxidants, but neither of two reviews mentioned Vitamin D also having as antioxidants properties. Thus, the final conclusion, whether Vitamin D due to contradictive resultss can be considered as an anti-oxidant or not, cannot be drawn. Despite the controversy, the authors believe that Vitamin D has its own role in radiosensitizing agents. Further research about Vitamin D supplementation along with radiotherapy with a large setting, well-defined population, and precise methods must be encouraged to conclude this never-ending controversy.

CONCLUSION

Current scientific research and its evidences suggest that Vitamin D have anticancer properties and can be used as radiosensitizer by imploring various mechanism pathways like promoting autophagy, increasing apoptosis, inhibiting cell survival and proliferation promoting gene, inducing cell cycle arrest and senescence in various cell line. Further research especially in in-vivo settings need to be evaluated. Randomized Clinical Trial (RCT) is encouraged to be performed to prove Vitamin D as a potent radiosensitizer agent in a clinical setting.

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ETHICAL CONSIDERATION

Ethical issues (including plagiarism, data fabrication, etc) have been completely observed by the authors.

CONFLICT OF INTEREST

The author(s) declared that there is no conflict of interest in this review.

REFERENCES

[1] International Agency for Research on Cancer. Vitamin D and cancer. International Agency for Research on Cancer 2008. 449.

[2] Bouillon R, de Groot L, Jameson J. Vitamin D: From Photosynthesis, Metabolism, and Action to Clinical Applications. New South Wales, Australia: Saunders 2001. 1010-1028.

[3] Feldman D, Leeuwen J, Munoz A, Driel M. Vitamin D. 4th ed. Vol. 2. Elsevier 2018.

[4] Dunlap N, Schwartz GG, Eads D, Cramer SD, Sherk AB, John V, *et al*. 1α,25-Dihydroxyvitamin D3 (calcitriol) and its analogue, 19-nor-1α,25(OH)2D2, potentiate the effects of ionising radiation on human prostate cancer cells. British Journal of Cancer 2003; 89(4). https://doi.org/10.1038/sj.bjc.6601161

[5] Polar MK, Gennings C, Park M, Gupta MS, Gewirtz DA. Effect of the vitamin D3 analog ILX 23-7553 on apoptosis and sensitivity to fractionated radiation in breast tumor cells and normal human fibroblasts. Cancer Chemotherapy and Pharmacology 2003; 51(5). https://doi.org/10.1007/s00280-003-0606-z

[6] Xu Y, Fang F, st. Clair DK, Josson S, Sompol P, Spasojevic I, *et al*. Suppression of RelB-mediated manganese superoxide dismutase expression reveals a primary mechanism for radiosensitization effect of 1 ,25-dihydroxyvitamin D3 in prostate cancer cells. Molecular Cancer Therapeutics 2007; 6(7). https://doi.org/10.1158/1535-7163.MCT-06-0700

[7] Chaudhry M, Sundaram S, Gennings C, Carter H, Gewirtz DA. The vitamin D 3 analog, ILX-23-7553, enhances the response to Adriamycin and irradiation in MCF-7 breast tumor cells. Cancer Chemotherapy and Pharmacology 2001; 47(5). https://doi.org/10.1007/s002800000251

[8] Wilson EN, Bristol ML, Di X, Maltese WA, Koterba K, Beckman MJ, *et al*. A Switch Between Cytoprotective and Cytotoxic Autophagy in the Radiosensitization of Breast Tumor Cells by Chloroquine and Vitamin D. Hormones and Cancer 2011; 2(5). https://doi.org/10.1007/s12672-011-0081-7

[9] Sharma K, Goehe RW, Di X, Hicks MA, Torti S v, Torti FM, *et al*. A novel cytostatic form of autophagy in sensitization of non-small cell lung cancer cells to radiation by vitamin D and the vitamin D analog, EB 1089. Autophagy 2014; 10(12). https://doi.org/10.4161/15548627.2014.993283

[10] Mineva ND, Wang X, Yang S, Ying H, Xiao Z-XJ, Holick MF, *et al*. Inhibition of RelB by 1,25-dihydroxyvitamin D_3 promotes sensitivity of breast cancer cells to radiation. Journal of Cellular Physiology 2009; 220(3). https://doi.org/10.1002/jcp.21765

[11] Podgorska E, Drzal A, Matuszak Z, Swakon J, Slominski A, Elas M, *et al*. Calcitriol and Calcidiol Can Sensitize Melanoma Cells to Low-LET Proton Beam Irradiation. International Journal of Molecular Sciences 2018; 19(8). https://doi.org/10.3390/ijms19082236

[12] Bristol ML, Di X, Beckman MJ, Wilson EN, Henderson SC, Maiti A, *et al*. Dual functions of autophagy in the response of breast tumor cells to radiation. Autophagy 2012; 8(5). https://doi.org/10.4161/auto.19313

[13] DeMasters G, Di X, Newsham I, Shiu R, Gewirtz DA. Potentiation of radiation sensitivity in breast tumor cells by the vitamin D_3 analogue, EB 1089, through promotion of autophagy and interference with proliferative recovery. Molecular Cancer Therapeutics 2006; 5(11). https://doi.org/10.1158/1535-7163.MCT-06-0316

[14] Gavrilov V, Leibovich Y, Ariad S, Lavrenkov K, Shany S. A combined pretreatment of 1,25-dihydroxyvitamin D3 and sodium valproate enhances the damaging effect of ionizing radiation on prostate cancer cells. The Journal of Steroid Biochemistry and Molecular Biology 2010; 121(1-2). https://doi.org/10.1016/j.jsbmb.2010.03.004

[15] Chen N, Karantza-Wadsworth V. Role and regulation of autophagy in cancer. Biochimica et Biophysica Acta (BBA) - Molecular Cell Research 2009; 1793(9). https://doi.org/10.1016/j.bbamcr.2008.12.013

[16] Maiuri MC, Zalckvar E, Kimchi A, Kroemer G. Self-eating and self-killing: crosstalk between autophagy and apoptosis. Nature Reviews Molecular Cell Biology 2007; 8(9). https://doi.org/10.1038/nrm2239

[17] Gorka M, Daniewski WM, Gajkowska B, Lusakowska E, Godlewski MM, Motyl T. Autophagy is the dominant type of programmed cell death in breast cancer MCF-7 cells exposed to AGS 115 and EFDAC, new sesquiterpene analogs of paclitaxel. Anti-Cancer Drugs 2005; 16(7). https://doi.org/10.1097/01.cad.0000171514.50310.85

[18] Kirisako T, Ichimura Y, Okada H, Kabeya Y, Mizushima N, Yoshimori T, *et al*. The Reversible Modification Regulates the Membrane-Binding State of Apg8/Aut7 Essential for Autophagy and the Cytoplasm to Vacuole Targeting Pathway. Journal of Cell Biology 2000; 151(2). https://doi.org/10.1083/jcb.151.2.263

[19] Nedelsky NB, Todd PK, Taylor JP. Autophagy and the ubiquitin-proteasome system: Collaborators in neuroprotection. Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease 2008; 1782(12). https://doi.org/10.1016/j.bbadis.2008.10.002

[20] Ghosh S, May MJ, Kopp EB. NF-κB and rel proteins: Evolutionarily Conserved Mediators of Immune Responses. Annual Review of Immunology 1998; 16(1). https://doi.org/10.1146/annurev.immunol.16.1.225

[21] Sonenshein GE. Rel/NF-κB transcription factors and the control of apoptosis. Seminars in Cancer Biology 1997; 8(2). https://doi.org/10.1006/scbi.1997.0062

[22] Karin M. Nuclear factor-κB in cancer development and progression. Nature 2006; 441(7092). https://doi.org/10.1038/nature04870

[23] Magné N, Toillon R-A, Bottero V, Didelot C, Houtte P van, Gérard J-P, *et al*. NF-κB modulation and ionizing radiation: mechanisms and future directions for cancer treatment. Cancer Letters 2006; 231(2). https://doi.org/10.1016/j.canlet.2005.01.022

[24] Xu Y, Kiningham KK, Devalaraja MN, Yeh C-C, Majima H, Kasarskis EJ, *et al*. An Intronic NF-kappaB Element Is Essential for Induction of the Human Manganese Superoxide Dismutase Gene by Tumor Necrosis Factor-alpha and Interleukin-1beta. DNA and Cell Biology 1999; 18(9). https://doi.org/10.1089/104454999314999

[25] Dong X, Craig T, Xing N, Bachman LA, Paya C v., Weih F, *et al*. Direct Transcriptional Regulation of RelB by 1α,25-Dihydroxyvitamin D_3 and Its Analogs. Journal of Biological Chemistry 2003; 278(49). https://doi.org/10.1074/jbc.M308448200

[26] Dong X, Lutz W, Schroeder TM, Bachman LA, Westendorf JJ, Kumar R, *et al*. Regulation of relB in dendritic cells by means of modulated association of vitamin D receptor and histone deacetylase 3 with the promoter. Proceedings of the National Academy of Sciences 2005; 102(44). https://doi.org/10.1073/pnas.0506516102

[27] Falck J, Mailand N, Syljuåsen RG, Bartek J, Lukas J. The ATM-Chk2-Cdc25A checkpoint pathway guards against radioresistant DNA synthesis. Nature 2001; 410(6830). https://doi.org/10.1038/35071124

[28] Debacq-Chainiaux F, Erusalimsky JD, Campisi J, Toussaint O. Protocols to detect senescence-associated beta-galactosidase (SA-βgal) activity, a biomarker of senescent cells in culture and *in vivo*. Nature Protocols 2009; 4(12). https://doi.org/10.1038/nprot.2009.191

[29] Lambert JR, Kelly JA, Shim M, Huffer WE, Nordeen SK, Baek SJ, *et al*. Prostate derived factor in human prostate cancer cells: Gene induction by vitamin D via a p53-dependent mechanism and inhibition of prostate cancer cell growth. Journal of Cellular Physiology 2006; 208(3). https://doi.org/10.1002/jcp.20692

[30] Kovalenko PL, Zhang Z, Cui M, Clinton SK, Fleet JC. 1,25 dihydroxyvitamin D-mediated orchestration of anticancer, transcript-level effects in the immortalized, non-transformed prostate epithelial cell line, RWPE1. BMC Genomics 2010; 11(1). https://doi.org/10.1186/1471-2164-11-26

[31] Bao B-Y, Ting H-J, Hsu J-W, Lee Y-F. Protective role of 1α, 25-dihydroxyvitamin D3 against oxidative stress in nonmalignant human prostate epithelial cells. International Journal of Cancer 2008; 122(12). https://doi.org/10.1002/ijc.23460

[32] Chandel NS, Tuveson DA. The Promise and Perils of Antioxidants for Cancer Patients. New England Journal of Medicine 2014; 371(2). https://doi.org/10.1056/NEJMcibr1405701

[33] Yasueda A, Urushima H, Ito T. Efficacy and Interaction of Antioxidant Supplements as Adjuvant Therapy in Cancer Treatment. Integrative Cancer Therapies 2016; 15(1). https://doi.org/10.1177/1534735415610427

[34] Meyer F, Bairati I, Fortin A, Gélinas M, Nabid A, Brochet F, *et al*. Interaction between antioxidant vitamin supplementation and cigarette smoking during radiation therapy in relation to long-term effects on recurrence and mortality: A randomized trial among head and neck cancer patients. International Journal of Cancer 2007; 122(7). https://doi.org/10.1002/ijc.23200

[35] Moss RW. Do Antioxidants Interfere With Radiation Therapy for Cancer? Integrative Cancer Therapies 2007; 6(3). https://doi.org/10.1177/1534735407305655

[36] Wilson MK, Baguley BC, Wall C, Jameson MB, Findlay MP. Review of high-dose intravenous vitamin C as an anticancer agent. Asia-Pacific Journal of Clinical Oncology 2014; 10(1). https://doi.org/10.1111/ajco.12173

[37] Cain L, Flynn J, Kelly D. Effect of complementary alternative medical (CAM) therapy on tumor response, control and recurrence in prostate cancer patients (PCpts) treated with radiation therapy (RT). Journal of Clinical Oncology 2007; 25: 15585-undefined. https://doi.org/10.1200/jco.2007.25.18_suppl.15585

[38] Jaakkola K, Lähteenmäki P, Laakso J, Harju E, Tykkä H, Mahlberg K. Treatment with antioxidant and other nutrients in combination with chemotherapy and irradiation in patients with small-cell lung cancer. Anticancer Research 1992; 12: 599-606.

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