



Article

In Vitro Investigations on the Antioxidant Effects of Vitamin D in a Panel of Cancer Cell Lines

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Abstract

Background: Oxidative stress plays a critical role in cancer initiation and progression. Although vitamin D exhibits multiple anti-tumorigenic properties, its antioxidant effects across different cancer types remain incompletely characterized. This study aimed to evaluate the antioxidant role of vitamin D in cancer. **Methods:** This *in vitro* study was conducted using breast (MCF-7, MDA-MB-231), colorectal (HCT-116, HT-29), and head and neck (Detroit-562, FaDu) cancer cell lines. Cells were treated with 1,25-dihydroxyvitamin D₃ (1 nM, 10 nM, and 100 nM) for 48 h. Reactive oxygen species (ROS) were quantified using the ROS-Glo™ H₂O₂ assay. Oxidative stress biomarkers were assessed by measuring 8-hydroxy-2'-deoxyguanosine (8-OHdG) using enzyme-linked immunosorbent assay (ELISA), while thiobarbituric acid reactive substances (TBARS) and protein carbonyls (PC) were measured using colorimetric assays. Xanthine oxidase (XOD) protein levels were determined by ELISA, whereas superoxide dismutase (SOD) and catalase (CAT) activity and/or expression were evaluated using colorimetric assays. **Results:** Vitamin D significantly reduced ROS levels in all investigated cell lines in a dose-dependent manner compared with control cells ($p < 0.05$). Levels of 8-OHdG and TBARS were significantly decreased across all cell lines, whereas reductions in PC and XOD were cell-type-dependent. Vitamin D significantly increased SOD activity and protein expression in all cell lines, while CAT activity was elevated in most cell lines. **Conclusions:** This study provides proof-of-concept evidence that vitamin D exerts dose-dependent and cell-specific antioxidant effects in cancer. These findings suggest potential antioxidant roles for vitamin D in the prevention or treatment of breast, colorectal, and head and neck cancers.

Keywords: cancer; oxidative stress; vitamin D; reactive oxygen species; antioxidant



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1. Introduction

Cancer remains one of the most severe and challenging diseases worldwide, affecting both industrialized and developing nations despite ongoing efforts to develop new preventive and therapeutic strategies [1]. Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) and antioxidants, has been implicated in various pathologies, including cardiovascular diseases, diabetes mellitus, neurological disorders, and cancer [2]. ROS can react with a wide range of biomolecules, including DNA, proteins, lipids, amino acids, carbohydrates, vitamins, and metals [3], potentially impairing their structure and function. Metabolites or compounds generated from such interactions can

serve as biomarkers of oxidative stress. These include lipid peroxidation products such as malondialdehyde (MDA), which is commonly measured using the thiobarbituric acid reactive substances (TBARS) assay [4]. DNA oxidation products, including 8-hydroxy-2'-deoxyguanosine (8-OHdG), are also widely used biomarkers of oxidative stress [5]. In addition, protein oxidation can be evaluated by measuring protein carbonyls (PC) [6]. Previous studies have reported elevated levels of TBARS in lung cancer [7] and ovarian cancer [8], increased 8-OHdG in head and neck cancer (HNC) [9], and higher PC levels in gastric cancer [10].

Oxidative stress contributes to multiple cancer hallmarks, including angiogenesis, invasiveness, stemness, and metastasis [11]. Accordingly, reducing oxidative stress with potent antioxidants has been explored as a strategy for cancer prevention [12].

Vitamin D, the precursor of 1,25-dihydroxyvitamin D ($1,25(\text{OH})_2\text{D}_3$), plays a key role in calcium homeostasis and bone health [13]. Beyond its skeletal functions, vitamin D deficiency has been linked to immune [14], endocrine [15], and cardiovascular disorders [16]. More recently, additional roles for vitamin D in cancer have been recognized. Vitamin D has been reported to induce apoptosis in prostate [17] and gastric cancers [18], promote differentiation in colon cancer [19], and inhibit angiogenesis, invasion, and metastasis in breast [20,21] and prostate cancers [22]. Additional studies have demonstrated anti-inflammatory effects in breast and ovarian cancers [20,23] and antiproliferative properties in breast cancer [24,25].

Emerging evidence suggests that vitamin D may protect against oxidative stress [26]. It has been shown to increase antioxidant enzyme levels, including superoxide dismutase (SOD) and catalase (CAT), in breast cancer [27] and bone cancer [28]. However, evidence regarding inhibitory effects of vitamin D on pro-oxidant enzymes in cancer remains limited. Previous studies indicate that vitamin D can reduce xanthine oxidase (XOD) levels in diabetes mellitus [29]. Vitamin D has also been reported to decrease oxidative stress biomarkers in cancer, such as 8-OHdG in colorectal cancer (CRC) [30] and TBARS in squamous cell carcinoma [31].

A review of the literature reveals limited evidence regarding the effects of vitamin D on oxidative stress in breast, colorectal, and head and neck cancers. Accordingly, the aim of this *in vitro* study was to evaluate the impact of vitamin D on oxidative stress in a panel of cancer cell lines representing these cancer types. We hypothesized that vitamin D treatment would reduce oxidative stress in cancer cells by decreasing ROS (hydrogen peroxide, H_2O_2) and pro-oxidant enzyme levels (XOD) while enhancing antioxidant defenses (SOD, CAT). To test this hypothesis, we assessed ROS production, oxidative stress biomarkers (PC, TBARS, 8-OHdG), and the level/activity of key antioxidant and pro-oxidant enzymes.

2. Materials and Methods

2.1. Cell Lines and Chemicals

2.1.1. Cell Culture

The human breast cancer cell lines MCF-7 (ATCC[®] HTB-22[™]) and MDA-MB-231 (ATCC[®] HTB-26[™]), HNC cell lines Detroit-562 (ATCC[®] CCL-138[™]) and FaDu (ATCC[®] HTB-43[™]), and CRC cell lines HT-29 (ATCC[®] HTB-38[™]) and HCT-116 (ATCC[®] CCL-247[™]) were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). All cells, except FaDu, were cultured in RPMI-1640 medium (Euro-Clone SPA, Cat. No. ECB2000L, Pero, Milan, Italy) supplemented with 10% fetal bovine serum (FBS) (CAPRICORN Scientific GmbH, Cat. No. FBS-IH-11A, Ebsdorfergrund, Germany), 1% sodium pyruvate (Euro-Clone SPA, Cat. No. ECM0542D, Italy), and 1% L-glutamine (Euro-Clone SPA, Cat. No. ECB3000D, Italy). FaDu cells were cultured in minimum essential medium (MEM) (Euro-Clone SPA, Cat. No. ECB2071L, Italy) containing the same supplements, with the addition of 1% non-essential amino acids (Euro-Clone SPA, Cat.

No. NEAA-B, Italy). Cells at early passages (<10) were used for all experiments to ensure reproducibility and minimize phenotypic drift. Cultures were maintained at 37 °C in a humidified atmosphere with 5% CO₂ (S@FEGROW 188 PRO, Serial No. S2524, Euro-Clone).

2.1.2. Vitamin D Preparation

1,25(OH)₂D₃ was obtained from Sigma Chemical Co. (Sigma-Aldrich, Cat. No. D1530, CAS RN 32222-06-3, Burlington, MA, USA). It was dissolved in 95% ethanol (VWR Chemicals, Lutterworth, UK) to prepare a 10 µM stock solution. Working solutions at concentrations of 100 nM, 10 nM, and 1 nM were freshly prepared on the day of treatment by serial dilution of the stock in complete culture medium.

2.2. The Effect of Vitamin D Treatment on ROS Generation

The effect of 1,25(OH)₂D₃ on ROS production was evaluated using the ROS-Glo™ H₂O₂ assay (Promega, Madison, WI, USA, Cat. No. G8820) according to the manufacturer's instructions. Cells were seeded at a density of 8.5×10^4 cells per well in 200 µL of complete medium in 96-well white-walled plates (Greiner Bio-One, Cat. No. 650207, Kremsmünster, Austria). After 24 h, the medium was removed, and cells were treated with 200 µL of fresh medium containing 1,25(OH)₂D₃ at concentrations of 100 nM, 10 nM, or 1 nM. Medium with cells plus vehicle (ethanol used to dissolve vitamin D) served as the negative (baseline) control, while blank wells containing medium plus vehicle but no cells were included to determine background luminescence. Standard samples with known H₂O₂ concentrations provided in the kit were included to generate a calibration curve for quantification. After 42 h of treatment, 120 µL of medium was removed from each well, and 20 µL of H₂O₂ substrate solution was added to reach a final volume of 100 µL. Following an additional 6 h incubation, 100 µL of ROS-Glo™ Detection Solution was added. After incubation for 20 min at room temperature, luminescence was measured using a Synergy HTX multi-mode microplate reader (BioTek Instruments Inc., Winooski, VT, USA) at 528/20 nm. All conditions, including controls, were measured in three independent experiments, each performed in triplicate, and the average relative luminescence units (RLU) and standard deviation (SD) were calculated.

2.3. Effect of Vitamin D Treatment on Protein Levels

2.3.1. Cells Treatment with Vitamin D

MCF-7, MDA-MB-231, HCT-116, Detroit-562, and FaDu cell lines were seeded into 6-well plates (SPL Life Sciences, Cat. No. 30006, Pocheon, Republic of Korea) at 5×10^5 cells per well in 2 mL of medium. HT-29 cells were seeded at 6×10^5 cells per well. After 24 h, cells were treated in triplicate with 1,25(OH)₂D₃ at 100 nM, 10 nM, or 1 nM. Vehicle controls contained ethanol (20 µL of 95% ethanol per 1.98 mL medium). After 48 h of treatment, supernatants were collected, and cells were detached using a cell culture scraper (SPL Life Sciences, Cat. No. 90030) and harvested with phosphate-buffered saline (PBS) (Biowest, Cat. No. L0615-500, Nuaille, France). Cells were then centrifuged at 1500 rpm for 5 min (DLAB Centrifuge, DMO412S, Cat. No. 9034002222, DLAB Scientific Co., Ltd., Beijing, China), PBS was removed, and cell pellets were stored for protein analyses.

2.3.2. Protein Extraction

Cell pellets were lysed using ice-cold lysis buffer prepared by gently mixing 4 mL of Radioimmunoprecipitation Assay (RIPA) buffer (Sigma) with 20 µL of protease inhibitor cocktail (Sigma, P8849) and 20 µL of phosphatase inhibitor cocktail (Sigma, P5726). The lysis buffer was added to each pellet, and samples were sonicated using an Ultra X Cordless Ultrasonic Endo Activation device (Ultra X Dental, Cat. No. SKU: 6280001, Jena, Germany). Lysates

were then centrifuged at 15,000 rpm for 10 min at 4 °C (DLAB Centrifuge, DMO412S, Cat. No. 9034002222). Supernatants were collected, divided into aliquots, and stored at −80 °C.

2.3.3. Total Protein Measurement

Total protein of cell lysates was quantified using the DC Protein Assay reagent S (Bio-Rad, Cat. Nos. 500-0111, 500-0112, 500-0116, Hercules, CA, USA) in 96-well plates (Greiner Bio-One, Cat. No. 650207) according to the manufacturer's instructions. After incubation for 15 min at room temperature (20–25 °C), absorbance was measured at 750 nm using a microplate reader (BioTek Instruments Inc., SN 1402047, USA). A standard curve was generated using known protein concentrations, and the total protein concentration of each sample was calculated accordingly.

2.4. Assay Optimization and Sample Matrix Selection

Prior to final measurements, all oxidative stress and oxidant/antioxidant assays were performed in both cell lysates and conditioned to identify the most suitable sample type for each marker. PC, TBARS, and CAT activity showed robust and reproducible signals in lysates, whereas 8-OHdG, SOD, and XOD levels were more clearly detectable in conditioned medium. This selection was guided by assay performance and biological interpretability, reflecting intracellular oxidative stress and extracellular release of relevant markers. All subsequent analyses were performed using the sample type that provided the most consistent and biologically informative data.

2.5. Assessment of Oxidative Stress Biomarkers

2.5.1. Measurement of PC for Protein Oxidation

Protein oxidative damage was assessed by measuring PC levels in cell lysates using the Protein Carbonyl Colorimetric Assay Kit (MyBioSource, Cat. No. MBS2548472, San Diego, CA, USA), following the manufacturer's instructions. Cells were lysed, and 100 µL of lysate was transferred to a sample tube and reacted with 400 µL of 2,4-dinitrophenylhydrazine (DNPH) to derivatize carbonyl groups. For background correction, a reagent control tube containing 100 µL of lysate and 400 µL of Reagent 4 was included to account for non-specific absorbance. Additionally, a blank tube containing all reagents but no lysate was measured to correct for reagent background. Both sample and control tubes were incubated at 37 °C in the dark for 30 min, followed by the addition of 500 µL of Reagent 5. After centrifugation at $13,780 \times g$ for 10 min at 4 °C, the supernatant was discarded, and the precipitate was washed four times with 1 mL of anhydrous ethanol–ethyl acetate mixture to remove excess reagents. The precipitate was then solubilized in 1.25 mL of Reagent 6, incubated at 37 °C for 15 min, and centrifuged again at $13,780 \times g$ for 15 min at 4 °C. The optical density (OD) of the supernatant was measured at 370 nm using a microplate reader (BioTek Instruments Inc.). Protein concentrations were determined using the BIO-RAD protein assay kit (Cat. Nos. 500-0111, 500-0112, 500-0116), and PC content was calculated according to the manufacturer's formula:

$$PC \text{ (nmol/mg protein)} = 0.227 \times \Delta A / C_{PR}$$
where $\Delta A = OD_{\text{sample}} - OD_{\text{control}}$ (after blank subtraction) and C_{PR} is the protein concentration in mg/mL. Vehicle-treated cell lysates were used as negative (baseline) controls. No biological positive control was included, as the kit does not provide an oxidized protein standard. All measurements were performed in three independent experiments, each with triplicate samples, and the mean $\pm$ SD was calculated for each condition.

2.5.2. Measurements of TBARS for Lipid Oxidative Damage

Lipid peroxidation was assessed by measuring TBARS in cell lysates using the TBARS Microplate Assay Kit (MyBioSource, Cat. No. MBS8305390), following the manufacturer's

instructions. The dye reagent was pre-warmed to 37 °C prior to use. A standard curve was prepared by mixing 150 µL of the top standard with 150 µL of assay buffer, followed by serial dilutions. Absorbance of the standards was measured at 535 nm, and the standard curve was plotted to determine TBARS concentrations in samples.

For sample measurement, 150 µL of cell lysate was mixed with 150 µL of the dye reagent in a microcentrifuge tube and incubated in a boiling water bath for 10 min to facilitate the reaction. After cooling, the supernatant was collected and transferred to a 96-well microplate. A reagent blank containing 150 µL of assay buffer plus 150 µL of dye reagent without lysate was included to correct for background absorbance. Absorbance was measured at 535 nm, and TBARS concentrations were calculated using the standard curve. Vehicle-treated cell lysates were used as negative (baseline) controls. A biological positive control (lysate treated with a known oxidant) was not included, as the kit does not provide one. All measurements were performed in three independent experiments, each with triplicate samples, and results are expressed as mean $\pm$ SD.

2.5.3. Measurement of 8-OHdG for DNA Oxidative Damage

DNA oxidative damage was assessed by measuring 8-OHdG in conditioned cell culture medium using the DNA Damage Competitive ELISA Kit (MyBioSource, Cat. No. MBS808265), following the manufacturer's instructions. Conditioned medium samples (50 µL) were added to ELISA wells along with the kit reagents. A reagent blank containing all reagents but no sample was included to correct for background absorbance. Vehicle-treated conditioned medium was used as a negative (baseline) control. A zero standard (0 ng/mL) was also included. The enzymatic substrate reaction at 20–25 °C produced a blue-colored solution, and OD was measured at 450 nm using a microplate reader (BioTek Instruments Inc., SN 1402047, USA). 8-OHdG concentrations were calculated by comparing sample absorbances to the standard curve plotted in Microsoft Excel. No biological positive control (e.g., medium from cells treated with a known oxidizing agent) was included, as the kit does not provide one. All measurements were performed in three independent experiments, each with triplicate samples, and data are presented as mean $\pm$ SD.

2.6. Assessment of Oxidative Stress-Related Enzymes

2.6.1. Measurement of XOD Levels

XOD levels in conditioned medium were measured using the Human XOD ELISA Kit (MyBioSource, Cat. No. MBS2533395) according to the manufacturer's instructions. A blank tube containing only Reference Standard & Sample Diluent was included to correct for background absorbance. Standards and samples (100 µL each) were added to ELISA wells in triplicate. The plate was incubated for 90 min at 37 °C, followed by sequential addition of biotinylated detection antibody, HRP conjugate, substrate reagent, and stop solution, with wash steps according to the protocol. OD was measured at 450 nm using a microplate reader (BioTek Instruments Inc., SN, USA). Sample absorbances were corrected by subtracting the blank OD, and XOD concentrations were calculated using the standard curve in Microsoft Excel. Vehicle-treated conditioned medium was used as negative (baseline) control. No biological positive control (e.g., medium from cells treated with a known XOD inducer) was included. All measurements were performed in three independent experiments, each with triplicate samples, and data are presented as mean $\pm$ SD.

2.6.2. Measurement of SOD Levels and Activity

SOD levels and activity in conditioned medium were measured using the colorimetric SOD Assay Kit (Sigma-Aldrich, Cat. No. CS0009) according to the manufacturer's instructions. Serial dilutions of the SOD standard were prepared to generate a standard curve. A blank (B) containing only dilution buffer without sample or enzyme was included

to correct for background absorbance. Vehicle-treated conditioned medium was used as negative (baseline) control. A No SOD control (NS) was included to determine the maximal absorbance in the absence of SOD.

For the assay, 20 μL of sample was added to each well, followed by 160 μL of a working solution (a water-soluble tetrazolium salt that reacts with superoxide anions to produce a colored product). In the presence of SOD, superoxide is scavenged, reducing the formation of the colored product; thus, the decrease in absorbance at 450 nm is proportional to SOD activity. The plate was incubated at 20–25 $^{\circ}\text{C}$ for 30 min, and absorbance was measured at 450 nm using a microplate reader (BioTek Instruments Inc., SN 1402047, USA).

The activity of the SOD for each sample was calculated using the equation provided in the kit:

$$\text{SOD activity (units/mL)} = [(A_{\text{NS}} - B) - (A_{\text{S}} - B)] \div (A_{\text{NS}} - B) \times 10, \quad (1)$$

where A_{S} is the absorbance of the sample, A_{NS} is the absorbance of the No SOD control, and B is the absorbance of the blank.

SOD concentrations (units/mL) were determined from the standard curve after blank subtraction. No biological positive control (e.g., medium from cells treated with a known SOD inducer) was included. All measurements were performed in three independent experiments, each with triplicate samples, and data are presented as mean $\pm$ SD.

2.6.3. Measurement of CAT Activity

CAT activity in cell lysates was measured using the CAT Activity Colorimetric Assay Kit (Sigma-Aldrich, Cat. No. MAK381) according to the manufacturer's instructions. H_2O_2 standards were prepared by serial dilution in CAT assay buffer, starting from a 1 mM solution, to generate a standard curve plotted in Microsoft Excel. A blank (B) containing all reagents but no sample was included to correct for background absorbance. Vehicle-treated cell lysates were used as negative (baseline) control. A CAT Positive Control provided in the kit was included to confirm assay performance. Additionally, a Sample High Control (HC) was prepared by pre-incubating the sample with stop solution to completely inhibit CAT activity, serving as a control for maximal H_2O_2 remaining.

For the assay, 78 μL of sample or control was added to each well. To initiate the reaction, 12 μL of freshly prepared 1 mM H_2O_2 was added to all wells. Plates were incubated at 25 $^{\circ}\text{C}$ for 30 min. After incubation, 10 μL of Stop Solution was added to terminate the reaction, followed by 50 μL of Developer Reaction Mix to each well. Plates were incubated at 25 $^{\circ}\text{C}$ for an additional 10 min, and absorbance was measured at 570 nm using a microplate reader (BioTek Instruments Inc., SN, USA).

CAT activity was calculated using

$$\text{CAT activity (mU/mL)} = D_{\text{H}_2\text{O}_2} / (V_{\text{S}} \times T \times C_{\text{PR}}), \quad (2)$$

where $D_{\text{H}_2\text{O}_2}$ is the amount of decomposed H_2O_2 (in nanomoles) determined from the standard curve, v_{S} is the sample volume added to the well (mL), T is reaction time in minutes (30 min), and C_{PR} is the total protein in mg/mL. One unit of CAT is defined as the amount of enzyme that decomposes 1.0 μmol of H_2O_2 per min at pH 4.5 and 25 $^{\circ}\text{C}$. All measurements were performed in three independent experiments, each with triplicate samples, and data are presented as mean $\pm$ SD.

2.7. Data Analysis

Statistical analyses were conducted using Microsoft Excel 2016, version 16.0.4266.1001 (Microsoft Corporation, Redmond, WA, USA) and GraphPad Prism version 10.6.1 (GraphPad Software, San Diego, CA, USA). Graphs and figures were prepared using GraphPad

Prism. Data are presented as mean $\pm$ SD from three independent biological experiments, each performed in three technical replicates. For each experiment, replicate measurements were averaged to generate a single value per condition prior to statistical analysis.

Due to the limited number of independent biological replicates ($n = 3$), formal normality testing was not performed, as such tests lack statistical power at very small sample sizes. Instead, the appropriateness of parametric analysis was evaluated based on the continuous nature of the data, prior evidence of approximate normality in comparable experimental systems, and visual inspection of data distribution.

For comparisons among multiple treatment groups, a one-way analysis of variance (ANOVA) was applied. When a significant overall effect was detected, Dunnett's multiple comparisons test was used to compare each treatment group with the corresponding control. A p -value ≤ 0.05 was considered statistically significant.

3. Results

3.1. The Effect of Vitamin D on ROS Levels (H_2O_2)

The effect of vitamin D on ROS levels was evaluated by measuring H_2O_2 levels in the investigated cancer cell lines following treatment with three concentrations of vitamin D (1 nM, 10 nM, and 100 nM) compared with control cells (Figure 1).

In breast cancer cell lines, MCF-7 cells showed a significant reduction in H_2O_2 levels after treatment with 10 nM and 100 nM vitamin D, reaching 87.68% and 72.70% of control levels, respectively ($p = 0.0164$ and $p = 0.0001$; Figure 1A). In MDA-MB-231 cells, H_2O_2 levels were reduced at all tested concentrations; however, statistical significance was observed at 1 nM and 100 nM, reaching 70.90% and 73.37% of control levels, respectively ($p = 0.0296$ and $p = 0.0439$; Figure 1B).

In CRC cell lines, HT-29 cells showed a significant reduction in H_2O_2 levels only at 100 nM, reaching 74.07% of control levels ($p = 0.0002$; Figure 1C). In contrast, HCT-116 cells exhibited significant reductions at all tested concentrations (1 nM, 10 nM, and 100 nM), with H_2O_2 levels reaching 62.25%, 61.77%, and 48.01% of control levels, respectively ($p = 0.0001$, $p = 0.0001$, and $p < 0.0001$; Figure 1D).

A similar pattern was observed in HNC cell lines. Detroit-562 cells showed significant reductions in H_2O_2 levels at all concentrations, reaching 86.14%, 78.70%, and 77.52% of control levels, respectively ($p = 0.0003$, $p < 0.0001$, and $p < 0.0001$; Figure 1E). Likewise, FaDu cells demonstrated significant reductions at all tested concentrations, reaching 78.09%, 87.22%, and 76.86% of control levels, respectively ($p < 0.0001$, $p = 0.0006$, and $p < 0.0001$; Figure 1F, Table S1).

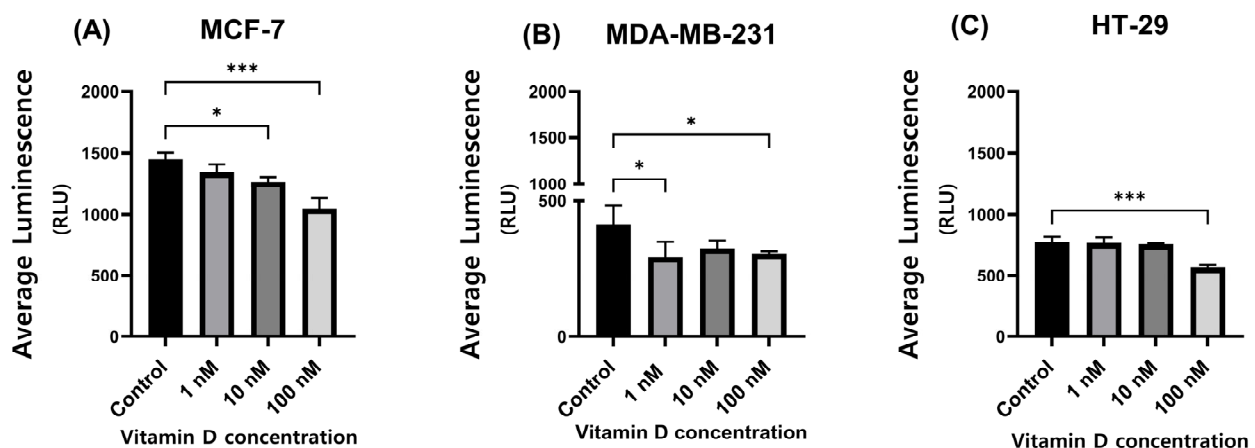


Figure 1. Cont.

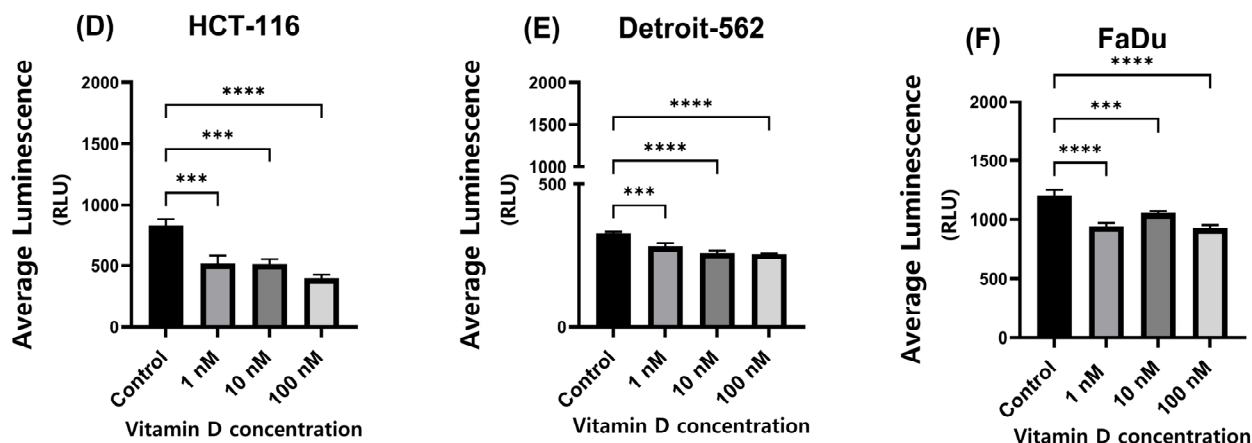


Figure 1. Effect of vitamin D treatment on reactive oxygen species (ROS) generation, measured as H_2O_2 levels, in a panel of cancer cell lines. Cells were treated with 1 nM, 10 nM, or 100 nM $1,25(\text{OH})_2\text{D}_3$ for 48 h. H_2O_2 levels were quantified using a luminescence-based assay (ROS-Glo™ H_2O_2 assay) and are expressed as relative luminescence units (RLU). (A) MCF-7, (B) MDA-MB-231, (C) HT-29, (D) HCT-116, (E) Detroit-562, and (F) FaDu. Data represent the mean $\pm$ SD of three independent experiments. Statistical significance was determined relative to control cells. * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$.

3.2. The Effect of Vitamin D on Oxidative Stress Biomarkers

3.2.1. The Effect of Vitamin D on PC Levels

In breast cancer cells, a significant reduction in PC levels was observed in MDA-MB-231 cells at all tested concentrations (1 nM, 10 nM, and 100 nM), reaching 18.24%, 17.94%, and 5.70% of control levels, respectively ($p < 0.0001$; Figure 2B). In contrast, no significant changes were detected in MCF-7 cells (Figure 2A).

In CRC cell lines, no significant differences in PC levels were observed in either HT-29 or HCT-116 cells (Figure 2C,D). Among HNC cells, Detroit-562 exhibited significant reductions in PC levels at 1 nM, 10 nM, and 100 nM, reaching 54.24%, 30.94%, and 27.67% of control levels, respectively ($p = 0.0451$, $p = 0.0037$, and $p = 0.0026$; Figure 2E), whereas FaDu cells showed no significant changes (Figure 2F, Table S2).

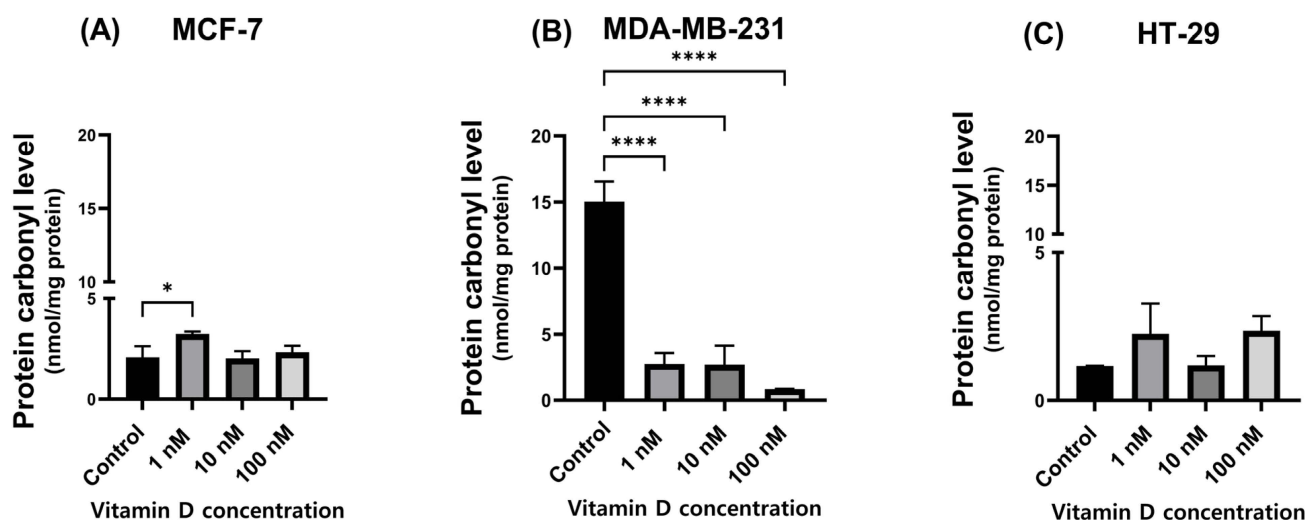


Figure 2. Cont.

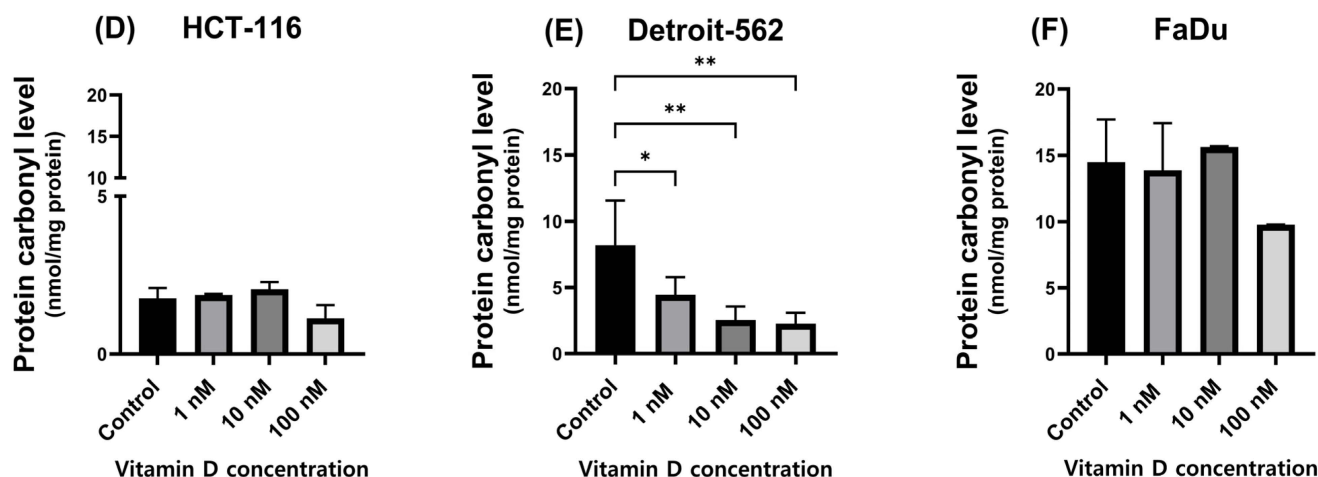


Figure 2. Effect of vitamin D treatment on protein carbonyl (PC) levels in a panel of cancer cell lines. Cells were treated with 1 nM, 10 nM, or 100 nM $1,25(\text{OH})_2\text{D}_3$ for 48 h. PC levels are expressed as nmol/mg protein. (A) MCF-7, (B) MDA-MB-231, (C) HT-29, (D) HCT-116, (E) Detroit-562, and (F) FaDu. Data represent the mean $\pm$ SD of three independent experiments. Statistical significance was determined relative to control cells. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$.

3.2.2. The Effect of Vitamin D on TBARS Levels

In breast cancer cell lines, MCF-7 cells showed a significant reduction in TBARS levels, following treatment with 1 nM, 10 nM, and 100 nM vitamin D, reaching 55.04%, 54.63%, and 39.75% of control levels, respectively ($p = 0.0331$, $p = 0.0012$, and $p < 0.0001$; Figure 3A). In MDA-MB-231 cells, TBARS levels decreased to 92.96%, 63.82%, and 66.14% of control levels at the same concentration, respectively ($p = 0.0226$, $p < 0.0001$, and $p < 0.0001$; Figure 3B).

In CRC cell lines, HT-29 cells exhibited significant reductions in TBARS levels, reaching 76.37%, 71.87%, and 43.93% of control levels following treatment with 1 nM, 10 nM, and 100 nM, respectively ($p = 0.0376$, $p = 0.0127$, and $p = 0.0005$; Figure 3C). HCT-116 cells showed significant decreases at 10 nM and 100 nM, reaching 91.40% and 87.92% of control levels, respectively ($p = 0.0079$ and $p = 0.009$; Figure 3D).

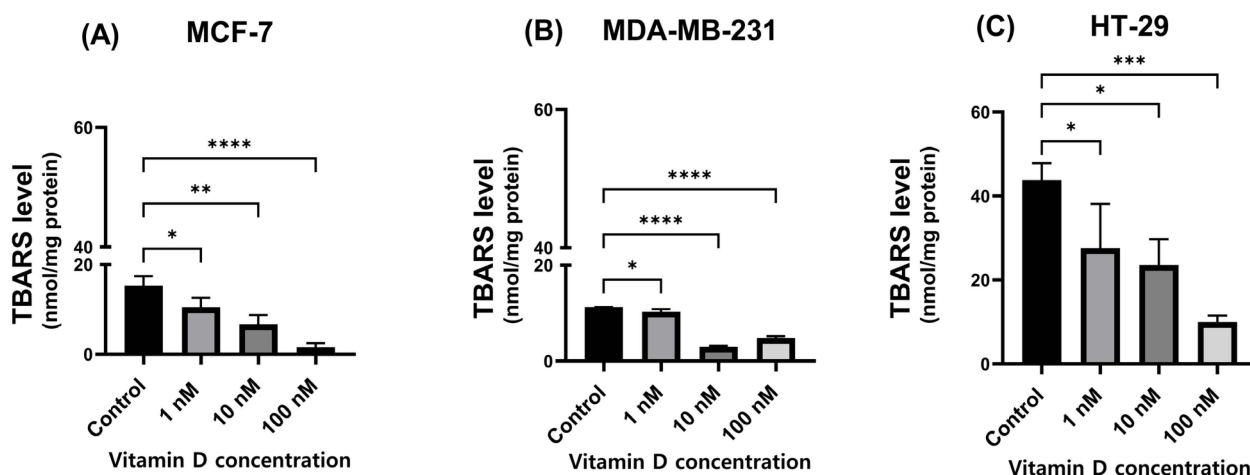


Figure 3. Cont.

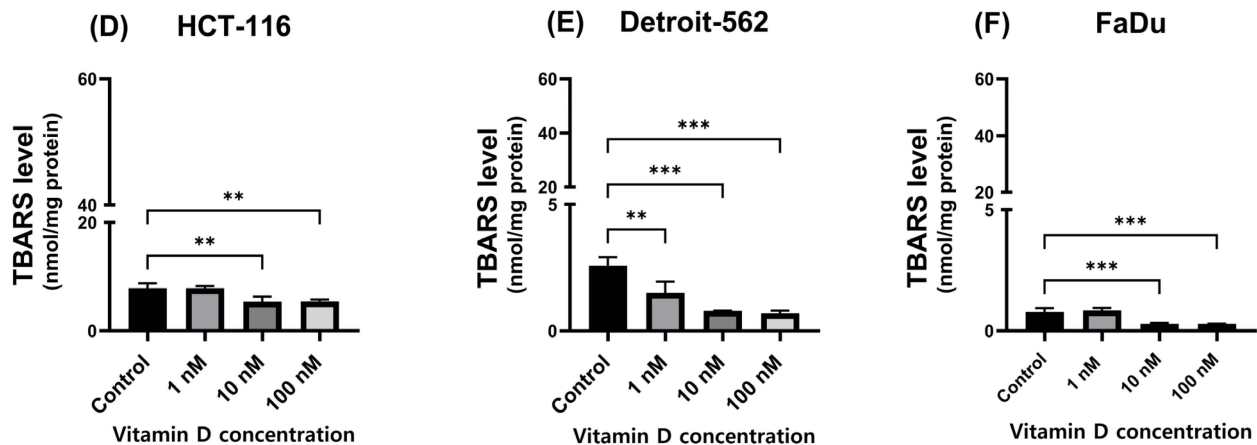


Figure 3. Effect of vitamin D treatment on thiobarbituric acid reactive substances (TBARS) levels in a panel of cancer cell lines. Cells were treated with 1 nM, 10 nM, or 100 nM $1,25(\text{OH})_2\text{D}_3$ for 48 h. TBARS levels are expressed as nmol/mg protein. (A) MCF-7, (B) MDA-MB-231, (C) HT-29, (D) HCT-116, (E) Detroit-562, and (F) FaDu. Data represent the mean $\pm$ SD of three independent experiments. Statistical significance was determined relative to control cells. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

In HNC cell lines, Detroit-562 cells demonstrated significant reductions in TBARS levels at all tested concentrations, reaching 69.27%, 58.02%, and 30.01% of control levels, respectively ($p = 0.0041$, $p = 0.0001$, and $p = 0.0001$; Figure 3E). In FaDu cells, treatment with 10 nM and 100 nM of vitamin D significantly reduced TBARS levels to 53.43% and 45.57% of control levels, respectively ($p = 0.0007$ and $p = 0.0008$; Figure 3F, Table S3).

3.2.3. The Effect of Vitamin D on 8-OHdG Levels

In breast cancer cell lines, MCF-7 cells exhibited significant reductions in 8-OHdG levels following treatment with 1 nM, 10 nM, and 100 nM vitamin D, reaching 88.63%, 88.86%, and 79.90% of control levels, respectively ($p < 0.0001$; Figure 4A). Similarly, MDA-MB-231 cells showed pronounced reductions in 8-OHdG levels to 58.32%, 66.12%, and 36.42% of control levels, respectively ($p < 0.0001$; Figure 4B).

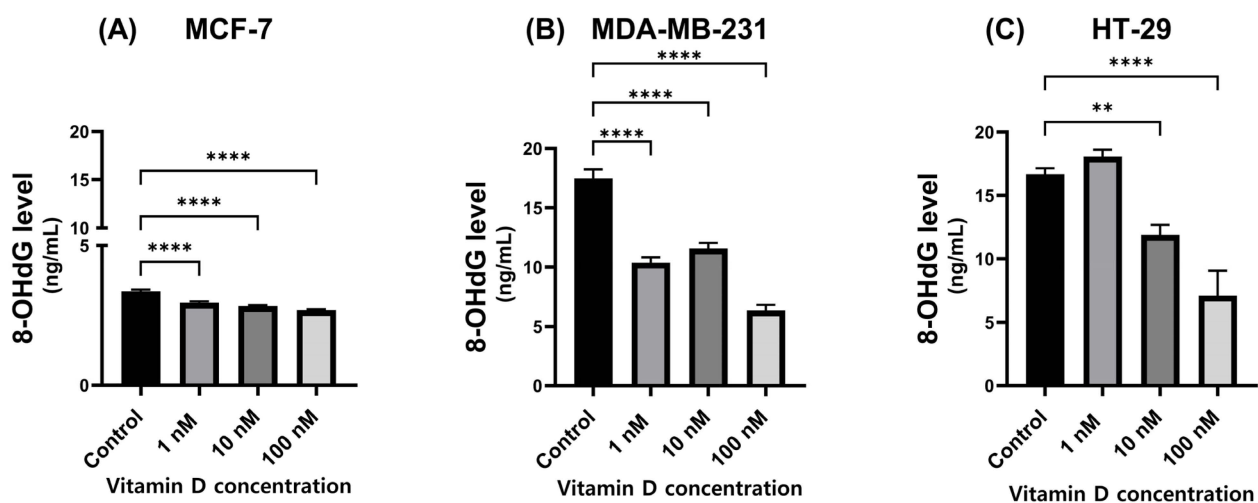


Figure 4. Cont.

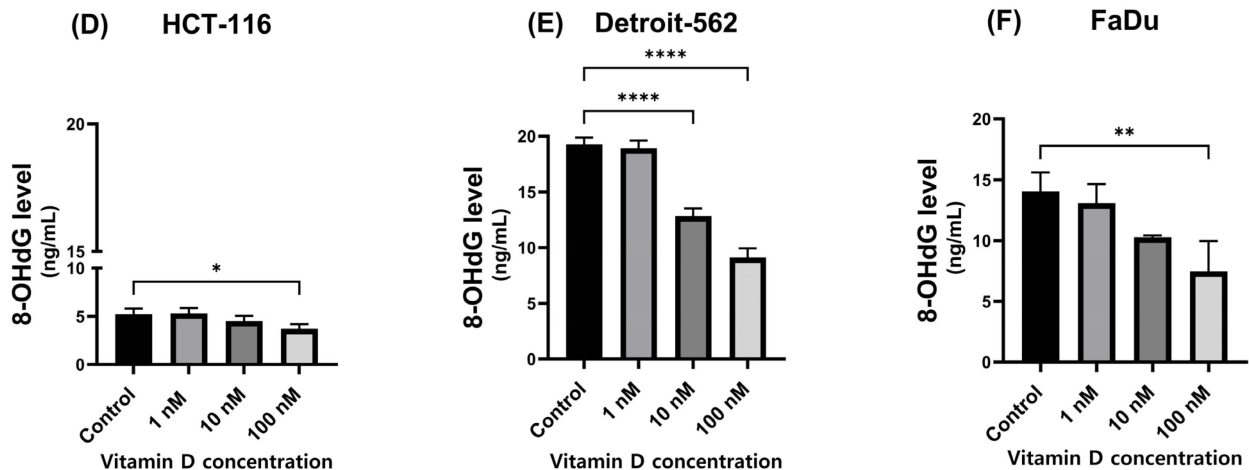


Figure 4. Effect of vitamin D treatment on oxidative DNA damage, assessed by 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels, in a panel of cancer cell lines. Cells were treated with 1 nM, 10 nM, or 100 nM $1,25(\text{OH})_2\text{D}_3$ for 48 h. 8-OHdG levels are expressed as ng/mL. (A) MCF-7, (B) MDA-MB-231, (C) HT-29, (D) HCT-116, (E) Detroit-562, and (F) FaDu. Data represent the mean $\pm$ SD of three independent experiments. Statistical significance was determined relative to control cells. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$.

In CRC cell lines, HT-29 cells demonstrated significant reductions in 8-OHdG levels at 10 nM and 100 nM, reaching 71.31% and 42.61% of control levels, respectively ($p = 0.0019$ and $p < 0.0001$; Figure 4C). In contrast, HCT-116 cells showed a significant reduction only at 100 nM, with levels reduced to 70.99% of control levels ($p = 0.0233$; Figure 4D).

In HNC cell lines, Detroit-562 cells exhibited significant reductions in 8-OHdG levels at 10 nM and 100 nM, reaching 66.55% and 47.40% of control levels, respectively ($p < 0.0001$; Figure 4E). In FaDu cells, a significant reduction was observed only at 100 nM, where 8-OHdG levels decreased to 53.18% of control levels ($p = 0.0035$; Figure 4F, Table S4).

3.3. The Effect of Vitamin D on Oxidative Stress–Related Enzymes

3.3.1. Effect of Vitamin D on XOD Levels

XOD levels in conditioned cell culture medium were significantly reduced to 76.23% of control levels in MCF-7 cells treated with 10 nM vitamin D compared with control cells ($p = 0.0151$; Figure 5A), whereas no significant changes were observed in MDA-MB-231 cells (Figure 5B).

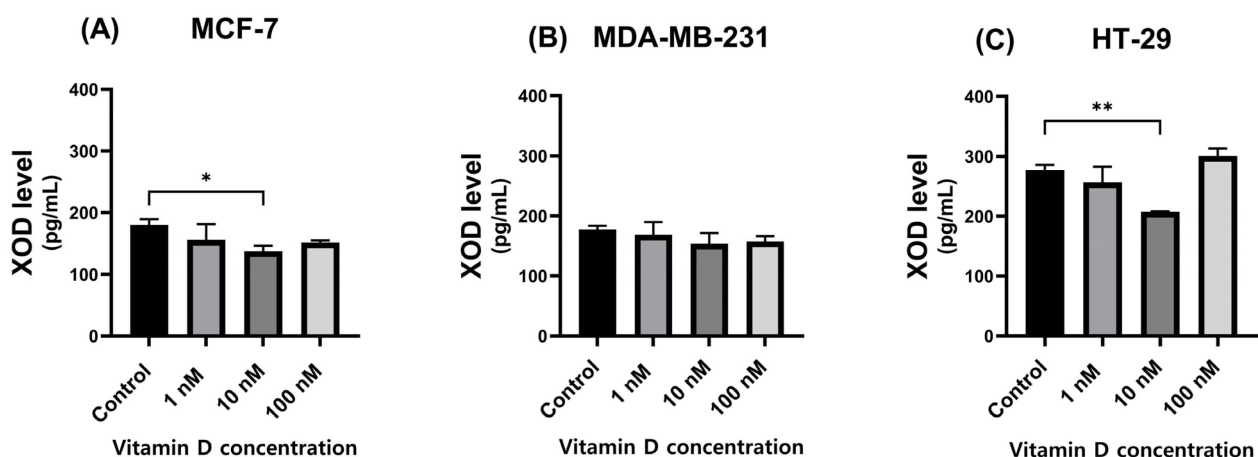


Figure 5. Cont.

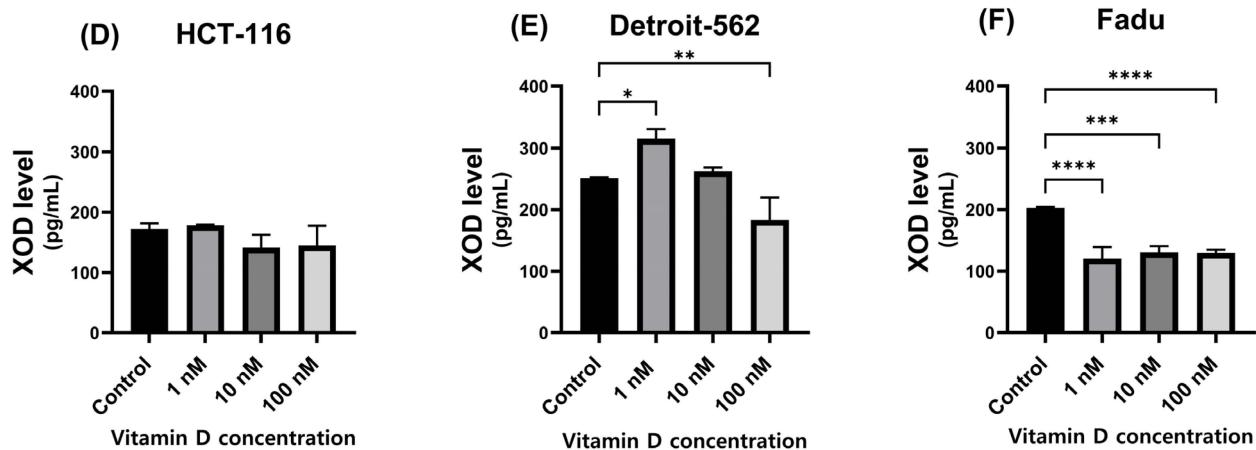


Figure 5. Effect of vitamin D treatment on xanthine oxidase (XOD) levels in a panel of cancer cell lines. Cells were treated with 1 nM, 10 nM, or 100 nM $1,25(\text{OH})_2\text{D}_3$ for 48 h. XOD levels are expressed as pg/mL. (A) MCF-7, (B) MDA-MB-231, (C) HT-29, (D) HCT-116, (E) Detroit-562, and (F) FaDu. Data represent the mean $\pm$ SD of three independent experiments. Statistical significance was determined relative to control cells. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

In CRC cell lines, a significant reduction in XOD levels was detected only in HT-29 cells treated with 10 nM, reaching 74.99% of control levels ($p = 0.0013$; Figure 5C), with no significant effects observed in HCT-116 cells (Figure 5D).

In HNC cell lines, Detroit-562 cells exhibited a significant reduction in XOD levels to 73.29% of control following treatment with 100 nM vitamin D ($p = 0.008$; Figure 5E). In contrast, FaDu cells showed significant reductions at all tested concentrations (1 nM, 10 nM, and 100 nM), reaching approximately 60% of control levels ($p < 0.0001$, $p = 0.0001$, and $p < 0.0001$, respectively; Figure 5F, Table S5).

3.3.2. The Effect of Vitamin D on SOD Level and Activity

Among breast cancer cell lines, MCF-7 cells showed a significant increase in both SOD protein levels and enzymatic activity following treatment with 10 nM and 100 nM vitamin D. SOD levels and activity increased by 226.75% and 187.37%, respectively, compared to control cells ($p = 0.0049$ and $p = 0.0143$; Figures 6A and 7A). No significant changes were observed in MDA-MB-231 cells (Figures 6B and 7B).

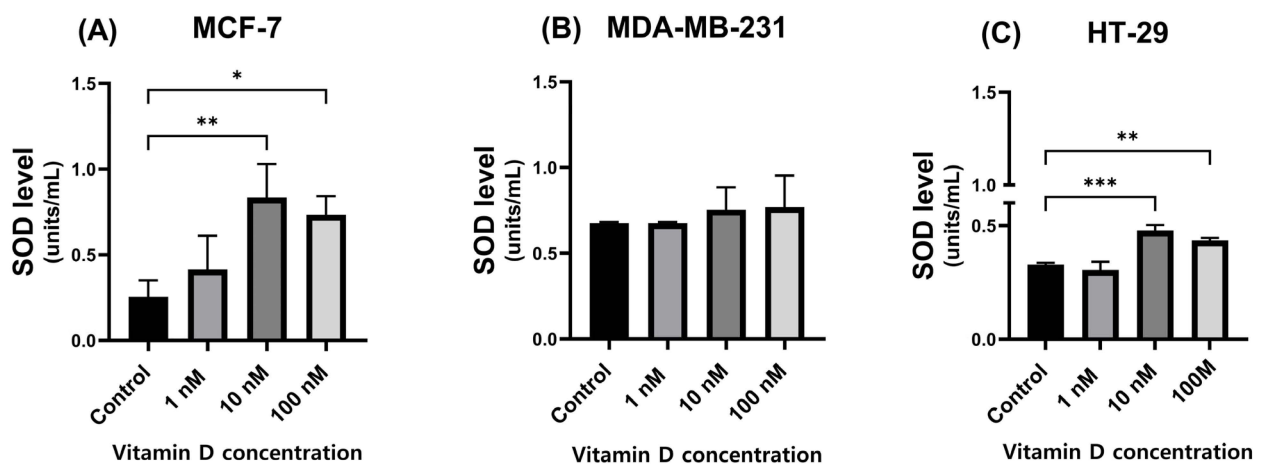


Figure 6. Cont.

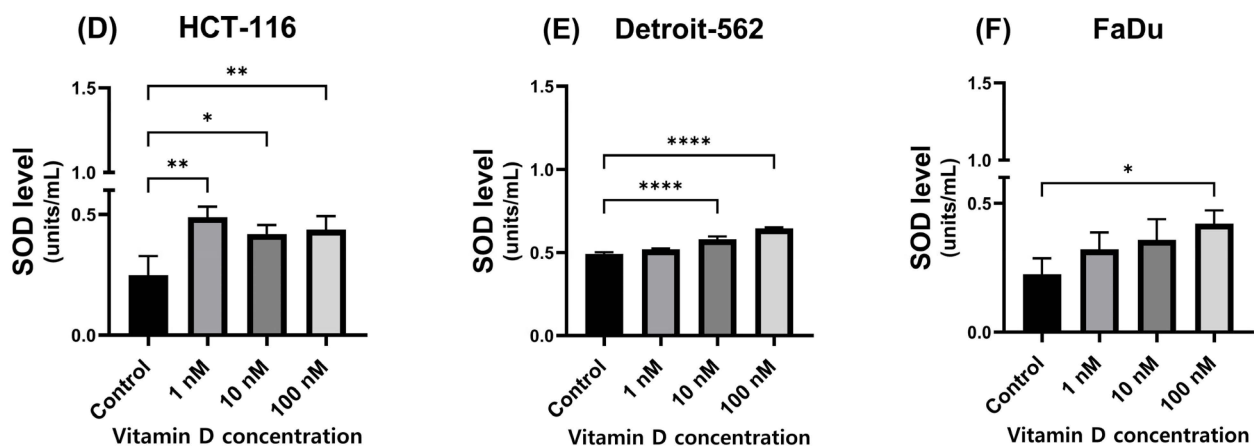


Figure 6. Effect of vitamin D treatment on superoxide dismutase (SOD) protein levels in a panel of cancer cell lines. Cells were treated with 1 nM, 10 nM, or 100 nM $1,25(\text{OH})_2\text{D}_3$ for 48 h. SOD levels are expressed as units/mL. (A) MCF-7, (B) MDA-MB-231, (C) HT-29, (D) HCT-116, (E) Detroit-562, and (F) FaDu. Data represent the mean $\pm$ SD of three independent experiments. Statistical significance was determined relative to control cells. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

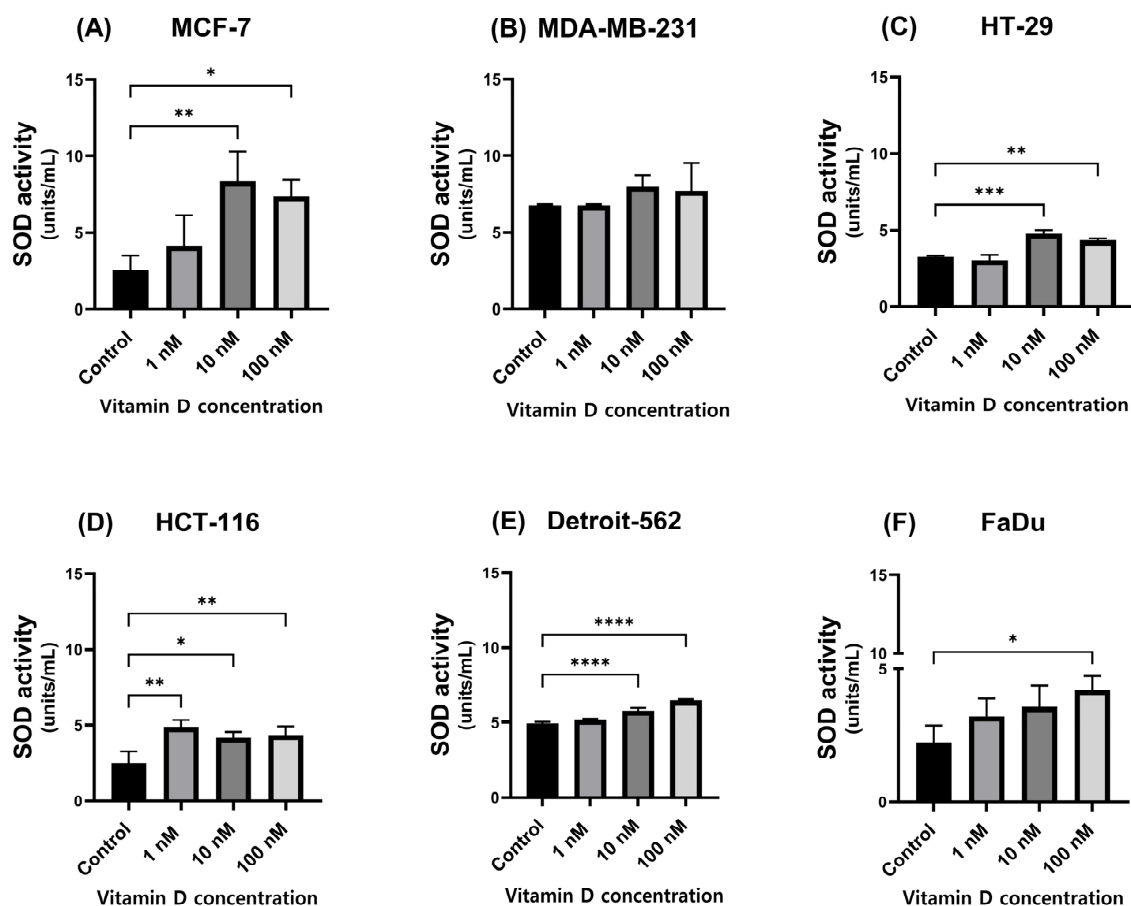


Figure 7. Effect of vitamin D treatment on superoxide dismutase (SOD) enzymatic activity in a panel of cancer cell lines. Cells were treated with 1 nM, 10 nM, or 100 nM $1,25(\text{OH})_2\text{D}_3$ for 48 h. SOD activity is expressed as units/mL. (A) MCF-7, (B) MDA-MB-231, (C) HT-29, (D) HCT-116, (E) Detroit-562, and (F) FaDu. Data represent the mean $\pm$ SD of three independent experiments. Statistical significance was determined relative to control cells. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

In CRC cell lines, HT-29 cells exhibited significant increases in SOD levels and activity by 34.41% and 32.46%, respectively, following treatment with 10 nM and 100 nM vitamin D ($p = 0.0001$ and $p = 0.0012$; Figures 6C and 7C). HCT-116 cells showed significant increases at all tested concentrations, with SOD levels and activity increasing by 96.84%, 68.74%, and 76.36% at 1 nM, 10 nM, and 100 nM, respectively ($p = 0.0020$, $p = 0.0149$, and $p = 0.0086$; Figures 6D and 7D).

In HNC cell lines, Detroit-562 cells demonstrated significant increases in SOD expression and activity of 13.97% at 10 nM and 31.12% at 100 nM ($p < 0.0001$ for both; Figures 6E and 7E). FaDu cells showed a significant increase only at 100 nM, with an 87.81% rise in both SOD expression and activity ($p = 0.0163$; Figures 6F and 7F, Tables S6 and S7).

3.3.3. The Effect of Vitamin D on CAT Activity

In breast cancer cell lines, MDA-MB-231 cells exhibited a significant increase in CAT activity following treatment with 10 nM and 100 nM vitamin D, reaching 108.04% and 535.72% of control levels, respectively ($p = 0.0006$ and $p < 0.0001$; Figure 8B). No significant changes were detected in MCF-7 cells (Figure 8A).

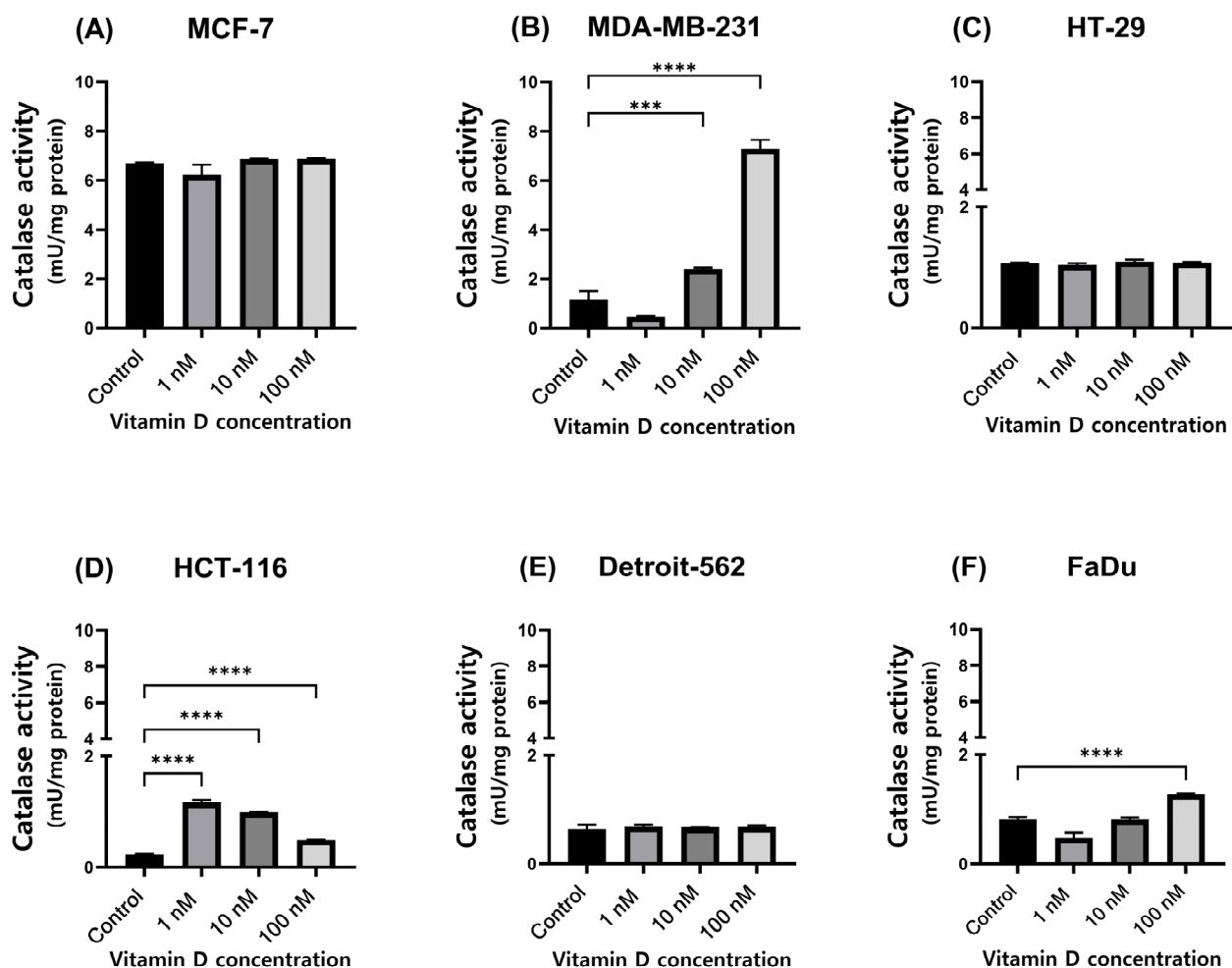


Figure 8. Effect of vitamin D treatment on catalase (CAT) activity in a panel of cancer cell lines. Cells were treated with 1 nM, 10 nM, or 100 nM $1,25(\text{OH})_2\text{D}_3$ for 48 h. CAT activity is expressed as mU/mg protein. (A) MCF-7, (B) MDA-MB-231, (C) HT-29, (D) HCT-116, (E) Detroit-562, and (F) FaDu. Data represent the mean $\pm$ SD of three independent experiments. Statistical significance was determined relative to control cells. *** $p < 0.001$, **** $p < 0.0001$.

In CRC cell lines, HCT-116 cells showed significant increases in CAT activity at all tested concentrations, with increases of 425.01%, 347.36%, and 122.93% following treatment with 1 nM, 10 nM, and 100 nM vitamin D, respectively ($p < 0.0001$; Figure 8D). In contrast, no significant changes were observed in HT-29 cells (Figure 8C).

Among HNC cell lines, FaDu cells showed a significant increase in CAT activity only at 100 nM, reaching 57.41% above control levels ($p < 0.0001$; Figure 8F), whereas no significant changes were detected in Detroit-562 cells (Figure 8E, Table S8).

4. Discussion

Vitamin D has been widely documented to regulate cell growth and differentiation across many cell types, including cancer cells, generating considerable interest in its potential role in cancer prevention and therapy [21]. Accumulating evidence also indicates that vitamin D plays an important role in maintaining the balance between antioxidant and oxidant systems [26,28]. Oxidative stress, resulting from excessive ROS production or impaired antioxidant defenses, is a key contributor to cancer initiation and progression [2]. In this *in vitro* study, we evaluated the effect of $1,25(\text{OH})_2\text{D}_3$ on oxidative stress in breast cancer (MCF-7, MDA-MB-231), CRC (HCT-116, HT-29), and HNC (Detroit-562, FaDu) cell lines, for which evidence regarding vitamin D-mediated modulation of oxidative stress remains limited. Accordingly, a panel of complementary assays was employed to characterize oxidative stress, including measurements of intracellular redox status, oxidative stress biomarkers, and oxidant and antioxidant enzymes.

For all experiments, $1,25(\text{OH})_2\text{D}_3$ was applied at concentrations of 1 nM, 10 nM, and 100 nM for 48 h. The concentrations were selected to cover a biologically relevant and commonly used range in *in vitro* studies while allowing assessment of potential dose-dependent effects [32]. The lower concentrations (1 nM and 10 nM) reflect biologically relevant levels sufficient to activate vitamin D receptor (VDR)-mediated antioxidant signaling, whereas the higher concentration (100 nM) was included to assess maximal cellular responsiveness [32]. The 48 h treatment duration was chosen to allow sufficient time for VDR-mediated genomic effects to occur. Time-course transcriptomic studies have demonstrated that $1,25(\text{OH})_2\text{D}_3$ induces progressively broader gene expression changes over time, with substantially more VDR-responsive genes detected at 48 h compared with earlier time points [33], supporting this duration as appropriate for assessing downstream antioxidant and DNA-protective effects.

The effect of vitamin D on ROS generation was first assessed using the ROS-Glo™ H_2O_2 assay. In this study, 20 μL of the ROS-Glo™ H_2O_2 substrate solution was used, as recommended by the manufacturer. This volume provides an appropriate concentration to reliably detect ROS without inducing cytotoxicity, consistent with established *in vitro* oxidative stress models [34]. Control cells, which are expected to exhibit higher H_2O_2 levels than vitamin D-treated cells, showed absorbance values consistent with the assay standards, confirming the reliability of ROS detection. Vitamin D significantly reduced ROS (H_2O_2) in all cell lines in a dose-dependent manner, with the greatest effect at 100 nM. Although supra-physiological doses of vitamin D have been suggested to induce cytotoxic or pro-oxidant effects in certain experimental settings [35], the consistent reduction in ROS observed here indicates preserved antioxidant activity. Mechanistically, vitamin D may reduce ROS through multiple pathways, including upregulation of antioxidant enzymes such as SOD and CAT [36], downregulation of pro-oxidant enzymes such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidases [37], and activation of the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway [38]. The more pronounced effect observed at 100 nM likely reflects enhanced VDR-mediated transcriptional activity at higher ligand concentrations [39]. Our findings are consistent with previous work showing

that $1,25(\text{OH})_2\text{D}_3$ at 1 nM had minimal effects on H_2O_2 -induced ROS in HL60-G leukemia cells, whereas 100 nM significantly reduced ROS levels [40].

Based on the observed reduction in ROS, we further examined the effect of vitamin D on established oxidative stress biomarkers, including protein damage (PC) [6], lipid peroxidation (TBARS) [4], and DNA damage (8-OHdG) [5].

Vitamin D significantly reduced PC levels in MDA-MB-231 and Detroit-562 cells but not in the other cell lines, suggesting a cell-type-specific effect. Mechanistically, this reduction may result from decreased intracellular ROS levels [29,41,42] and/or VDR-mediated inhibition of redox-sensitive transcription factors, including nuclear factor (NF- κ B), which downregulates pro-oxidant gene expression and indirectly limits oxidative protein damage [43]. Furthermore, limiting lipid peroxidation may reduce the formation of secondary carbonyls derived from lipid oxidation products, thereby preserving protein integrity and reducing overall PC levels [44]. These observations are supported by *in vitro* and clinical studies demonstrating decreased PC levels following vitamin D or analog supplementation in skeletal muscle, hemodialysis patients, and individuals with low back pain or osteoarthritis [45–48]. Collectively, these findings suggest that vitamin D mitigates oxidative protein damage by reducing intracellular ROS and strengthening antioxidant defenses, although the magnitude of this effect appears to vary among cell types, potentially reflecting differences in VDR expression or redox-sensitive signaling pathways. To our knowledge, this is the first *in vitro* study to evaluate PC modulation by vitamin D in these cancer cell lines.

TBARS levels were significantly reduced in all cell lines, with greatest effects observed at 10 nM and 100 nM, reflecting dose-dependent protection. Although the TBARS assay is widely used, it is relatively non-specific for lipid peroxidation, as thiobarbituric acid can react with aldehydes other than MDA [4]. Nevertheless, the consistent reduction in TBARS levels observed across all cell lines supports a vitamin D-mediated attenuation of lipid peroxidation. Reduced lipid peroxidation may also limit the formation of reactive aldehydes that contribute to secondary protein carbonylation, thereby preserving protein integrity [44]. Future studies could validate these findings using more specific lipid peroxidation markers such as 4-hydroxynonenal (4-HNE) and F_2 -isoprostanes, which are considered more specific and mechanistically informative biomarkers of oxidative damage than TBARS [49]. Mechanistically, the reduction in TBARS may reflect decreased ROS generation and/or modulation of pro-oxidant and antioxidant enzymes. Increased XOD activity has been associated with elevated lipid peroxidation in cancer tissues [50], whereas enhanced antioxidant enzyme activities, including SOD and CAT, are linked to reduced lipid peroxidation [51]. Clinical studies support these findings, reporting decreased TBARS levels following vitamin D supplementation in patients with non-melanoma skin cancer and in vitamin D-deficient individuals [52,53]. Together with the observed reduction in PC, these data suggest a coordinated antioxidant effect of vitamin D across multiple molecular targets.

We further assessed the impact of vitamin D on DNA integrity by measuring 8-OHdG levels [30]. Vitamin D treatment significantly reduced 8-OHdG levels in a dose-dependent manner across all cell lines, indicating protection against ROS-induced DNA damage. Similar findings have been reported clinically, where vitamin D and calcium supplementation reduced 8-OHdG in colorectal adenoma patients [30]. Mechanistically, this protection might involve VDR-dependent genomic signaling. VDR-knockout colon cancer mouse models exhibited elevated 8-OHdG [54], and $1\alpha(\text{OH})\text{ase}^-/-$ mammary tumor models showed increased ROS and 8-OHdG, which were restored by $1,25(\text{OH})_2\text{D}_3$ supplementation alongside induction of SOD-2 [55]. The dose-dependent decrease in 8-OHdG observed here likely reflects progressive activation of VDR signaling at higher

ligand concentrations [56], resulting in enhanced antioxidant defenses and reduced ROS-mediated DNA damage. Additionally, vitamin D signaling has been implicated in the regulation of DNA repair processes, including base excision repair, which is responsible for removing oxidized DNA bases such as 8-OHdG [57]. These findings complement the observed effects on PC and TBARS, reinforcing a broad modulatory role of vitamin D on oxidative stress regulation.

Given the reductions in ROS and oxidative stress biomarkers, we investigated whether vitamin D may exert these effects through modulation of oxidant and antioxidant enzymes [27,28]. Specifically, we evaluated the protein expression of the pro-oxidant enzyme XOD, the activity of CAT, and both the protein expression and activity of SOD. To our knowledge, this is the first *in vitro* study to assess the effect of vitamin D on XOD expression in a cancer context. Vitamin D significantly reduced XOD protein levels at 10 nM and 100 nM, with the most pronounced effects observed in HNC cell lines compared with CRC and breast cancer cells. Mechanistically, suppression of XOD activity may occur through VDR-mediated transcriptional responses that attenuate pro-oxidant and inflammatory signaling pathways. XOD expression may also be indirectly regulated via inhibition of redox-sensitive transcription factors such as active-protein-1 (AP-1), which has been implicated in XOD upregulation under pro-inflammatory conditions [58]. However, a direct transcriptional regulation of XOD by vitamin D/VDR signaling has not yet been established. By reducing XOD levels, vitamin D likely limits intracellular ROS production, thereby decreasing oxidative damage to proteins, lipids, and DNA. These findings are consistent with previous studies reporting reduced XOD activity following vitamin D treatment in mesencephalic cultures and in patients with type 2 diabetes [29,59].

Vitamin D significantly increased SOD activity and protein expression in all cell lines and increased CAT activity in all cell lines except Detroit-562, with the strongest effects observed at 10 nM and 100 nM. These findings align with *in vivo* studies demonstrating elevated SOD activity in rat models of hepatocarcinogenesis treated with $1,25(\text{OH})_2\text{D}_3$ [60], as well as *in vitro* studies showing that vitamin D analogs increase CAT expression and activity in canine bladder carcinoma cells [61]. These effects are likely mediated through VDR-dependent transcriptional activation [61]. Together with suppression of XOD, the coordinated upregulation of SOD and CAT promotes efficient detoxification of superoxide and H_2O_2 , shifting cellular redox homeostasis toward a less oxidative and more cytoprotective state.

Notably, cell-type-specific responses were observed across XOD, CAT, and SOD. These differences may reflect variations in VDR expression [62] or differential activation of redox-sensitive pathways among cancer types [11]. Clinically, such variability may influence the magnitude of antioxidant protection conferred by vitamin D [63], underscoring the importance of cellular context when evaluating its therapeutic potential and translational relevance.

Overall, the antioxidant effects of vitamin D appear to involve dual mechanisms: downregulation of the pro-oxidant XOD to limit ROS generation and upregulation of antioxidant enzymes to enhance ROS detoxification. These mechanisms explain the observed reductions in ROS, PC, TBARS, and 8-OHdG in a dose- and cell-type-dependent manner. These redox-modulating effects may represent early upstream events through which vitamin D influences additional cancer-related pathways. The antioxidant effects observed in this study appear to be mediated, at least in part, through genomic actions following VDR activation, as supported by the coordinated modulation of oxidant and antioxidant enzymes after 48 h of treatment. Previous studies have demonstrated transcriptional regulation of redox homeostasis by vitamin D. For example, microarray analysis in human prostatic epithelial cells revealed a ~3-fold upregulation of thioredoxin reductase-1 (TR1) following vitamin D treatment [64]. In MG-63 osteosarcoma cells, 10 nM $1,25(\text{OH})_2\text{D}_3$ increased SOD2 expression while decreasing SOD1 expression over 48 h [28]. Vitamin D

has also been shown to protect pancreatic cells from H₂O₂-induced cytotoxicity by reducing ROS and DNA damage while upregulating antioxidant genes including *CAT*, *SOD1–3*, and *GPx-3* [36]. Collectively, these observations reinforce the role of vitamin D as a modulator of redox-sensitive signaling and genomic stability in cancer cells.

5. Conclusions

The findings of this study support a potential protective role of vitamin D against oxidative stress in a panel of cancer cell lines derived from breast, colorectal, and head and neck cancers. These effects were reflected by reduced ROS generation and decreased levels of oxidative stress biomarkers, including PC, TBARS and 8-OHdG. The observed antioxidant effects may be partially explained by modulation of redox-related enzymes characterized by reduced expression of the pro-oxidant enzyme XOD and increased activity and/or expression of the antioxidant enzymes CAT and SOD. However, the magnitude of these effects varied across cancer models, indicating cell-specific responsiveness to vitamin D.

Overall, these findings support a potential role for vitamin D in modulating oxidative stress in cancer cells. Further *in vitro* and *in vivo* studies are warranted to clarify the underlying mechanisms, evaluate functional consequences for cancer progression, and determine the clinical relevance of these antioxidant effects.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/biomedicines14020469/s1>, Table S1: Effect of vitamin D treatment on reactive oxygen species (ROS) generation, measured as H₂O₂ levels in a panel of cancer cell lines (mean of relative luminescence units (RLU) ± standard deviation).; Table S2: Effect of vitamin D treatment on protein carbonyl (PC) levels in a panel of cancer cell lines (mean (nmol/mg protein) ± standard deviation).; Table S3: Effect of vitamin D treatment on thiobarbituric acid reactive substances (TBARS) levels in a panel of cancer cell lines (mean (nmol/mg protein) ± standard deviation).; Table S4: Effect of vitamin D treatment on oxidative DNA damage, assessed by 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels, in a panel of cancer cell lines (mean (ng/mL) ± standard deviation).; Table S5: Effect of vitamin D treatment on xanthine oxidase (XOD) levels in a panel of cancer cell lines (mean (pg/mL) ± standard deviation).; Table S6: Effect of vitamin D treatment on superoxide dismutase (SOD) protein levels in a panel of cancer cell lines (mean (units/mL) ± standard deviation).; Table S7: Effect of vitamin D treatment on superoxide dismutase (SOD) enzymatic activity in a panel of cancer cell lines (mean (units/mL) ± standard deviation).; Table S8: Effect of vitamin D treatment on catalase (CAT) activity in a panel of cancer cell lines (mean (mU/mg protein) ± standard deviation).

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Abbreviations

μL	Microliter
1,25(OH) ₂ D ₃	1,25-dihydroxyvitamin D
8-OHdG	8-Hydroxy-2'-deoxyguanosine
AP-1	Active protein-1
CAT	Catalase
CRC	Colorectal cancer
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
FBS	Fetal bovine serum
GPx	Glutathione peroxidase
h	Hour
H ₂ O ₂	Hydrogen peroxide
HNC	Head and neck cancer
MDA	Malondialdehyde
MEM	Minimum essential medium
mg	Milligram
mg prot	Milligram protein
min	Minute
mL	Milliliter
NADPH	Nicotinamide adenine dinucleotide phosphate
ng	Nanogram
nM	Nanomolar
nm	Nanometer
nmol	Nanomol
Nrf2	Nuclear factor erythroid 2-related factor 2
OD	Optical density
PBS	Phosphate-buffered saline
PC	Protein carbonyl
pg	Picogram
RIPA	Radioimmunoprecipitation Assay
RLU	Relative luminescence units
ROS	Reactive oxygen species
RPM	Revolutions per minute
SD	Standard deviation
SOD	Superoxide dismutase
TBARS	Thiobarbituric acid reactive substances
VDR	Vitamin D receptor
XOD	Xanthine oxidase

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