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Systematic Review

In Vitro and *In Vivo* Antitumor Activity of Vitamin D3 in Malignant Gliomas: A Systematic Review

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Abstract

Context: The most common type of brain tumors are gliomas, among which glioblastoma multiforme is the most life-threatening. In spite of the existing treatments, malignant gliomas often do not respond to treatment.

Objectives: The aim of this study was to investigate the antitumor activity of vitamin D3 in malignant gliomas through the study of *in vitro* and *in vivo* articles.

Data Sources: The electronic databases, including PubMed, ScienceDirect, Web of Science, Google Scholar, and the Cochrane Central Register of Clinical Trials (last updated April 12th, 2019) were searched, using medical subject headings (MeSH) keywords "vitamin D AND brain malignancy".

Data Extraction: There was no limitation on the date and language of the articles. A review of all the found articles at the level of titles and abstracts was made to find eligible articles. Our work is in accordance with the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines. The quality of the articles was judged in accordance with the GRADE tool for *in vitro* studies and CIRCLES RoB tool for *in vivo* studies.

Results: Totally, 88 articles were identified and after careful examination, 16 eligible articles meeting the inclusion criteria were selected. These studies showed that vitamin D3 and its analogs suppress self-renewal ability, cell viability and/or cell growth, migration rate, and invasive capacity; in addition, they induce autophagy and cell death. They also reduce tumor size in animal models. Evidence was mainly of moderate to high quality.

Conclusions: Based on the *in vitro* and *in vivo* evidence of this systematic review, vitamin D3, compared to the control group, induced cell death and reduced cell growth, invasion, and migratory capacity in gliomas cell culture. As well as, vitamin D analogs were able to show the same effects without the hypercalcemic effect *in vivo*. We suggest performing future high-quality studies with the aim of shedding light on the relevant mechanisms and the effects of vitamin D3 derivatives and its analogs on health status and clinical outcomes in patients with malignant gliomas.

Keywords: Antitumor Activity, Vitamin D3, Vitamin D Analogs, Malignant Gliomas, Glioblastoma, 1,25(OH)2D3

1. Context

The prevalence of gliomas is approximately 50% among all brain tumors and malignant gliomas account for 80% of all brain malignancies (1, 2). These types of brain tumors are categorized into grades I to IV according to World Health Organization (WHO) classification (3). Gliomas grade II or III are very aggressive and the probability of tumor progression is much higher and has a poor prognosis (4, 5). The highest malignancy rate belongs to grade IV gliomas, known as glioblastoma multiforme

(GBM); it has a high proliferation with high adhesion to the surrounding tissue and very hard to remove by en bloc resection, which, in turn, deteriorates the condition (1, 3). Predominantly common risk factors for gliomas are genetic predisposition and previous exposures to radiation, while many of them may occur spontaneously (2). Some evidence also suggests that obesity, vitamin D deficiency, and atopy are causative factors for gliomas risk (2, 6, 7). The median survival rate for patients with GBM is approximately 12 to 15 months and despite the full

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implementation of treatment protocols such as surgery, radiotherapy, and chemotherapy, it remains incurable (1-3). Therefore, adjuvant therapies seem to be necessary. Recently, vitamin D is known as a neurosteroid hormone (8, 9). Hence, it can play varied roles in the brain. Following the exposure to sunlight, 7-dehydrocholesterol in the subcutaneous tissue is turned to cholecalciferol. This is, then, once hydroxylated in the liver becoming 25-hydroxycholecalciferol. Finally, hydroxylation again occurs in the kidneys and 1,25 dihydroxycholecalciferol or calcitriol, which is an active form of vitamin D3, is produced. The other way is to get vitamin via diet or dietary supplements (10, 11). Beyond the calcium balance role of vitamin D and the beneficial effects on bone, due to the existence of vitamin D receptor (VDR) throughout body especially in the brain, vitamin D regulates the various types of function such as cell proliferation and differentiation, apoptosis, angiogenesis, cell invasion, DNA repair, metabolism, and inflammation (12-14). Although antitumor efficacy of vitamin D3 is observed in supraphysiological doses, which in turn may cause hypercalcemia, recent in vivo studies have shown that its analogs have beneficial effects on glioma cells without calcemic effect (15-18). Here, we review studies about the effect of vitamin D3 and its analogs on gliomas with special emphasis on GBM. Because the number of studies on human subjects (19) did not reach the required minimum for a systematic review, we systematically reviewed studies with in vitro and in vivo design.

2. Evidence Acquisition

The electronic databases, including PubMed, ScienceDirect, Web of Science, Google Scholar, and the Cochrane Central Register of Clinical Trials (last updated April 12th, 2019) were searched, using medical subject headings (MeSH) keywords "vitamin D AND brain malignancy". The full strategy of the search is shown in supplementary file Appendix 1. Abstracts from recent international conferences and references of the found articles were also searched for additional related articles. Two investigators independently reviewed the found articles at the title and abstract level. Then, the articles that appeared to be relevant were studied in full-text form. Considering PICOS criteria, articles were included in our study based on the following characteristics: (1) population; in vitro and in vivo samples from humans, rats, and mice with malignant gliomas, (2) intervention; the administration of vitamin D3 and its analogs, (3) comparison intervention; placebo-control, (4) outcomes; self-renewal ability, cell viability and/or cell growth, migration rate and invasive capacity, autophagy, and cell death, and (5) study design; in vitro and in vivo studies. There was no limitation on the date and language of the published articles. Molecular and genetic-based studies that were different from the present study in terms of our main predefined objectives, studies focusing on other living creatures, and studies having different design focusing on other cancers were excluded from the study. Studies that covered all inclusion criteria were selected. In terms of the presence of bias, all eligible studies were evaluated, using the GRADE (20, 21) and CIRCLEs RoB tools (22) for in vitro and in vivo studies, respectively. Given that there are no well-known tools and methods for assessing the quality of in vitro studies, we modified the GRADE tool for in vitro studies like other studies (23, 24). Accordingly, we categorized the articles as "high", "moderate", "low", or "very low" quality based on their methods. For in vivo studies, we rated the articles by "yes", "no", or "unclear". Our work is in accordance with the preferred reporting items for systematic reviews and meta-analyses (PRISMA) guidelines (25).

3. Results

According to the search strategy described previously, 88 papers were found (Figure 1). Twenty-eight articles with the closest characteristics to the inclusion criteria were selected through reviewing the total articles found at the title and abstract level. Then, these articles were examined more precisely. Two articles were excluded since they focused on tumors rather than gliomas (26, 27). In addition, the reasons for removing the rest of studies excluded from present study are as follows: 1 article studied the insects (28), 1 article did not have control group (29), the objectives and outcomes of 6 studies were different from the predetermined aims of the present study (30-35), 1 article had a randomized clinical trial design (19), and 3 studies were review articles (3, 36, 37). Following the further search, 4 articles were found from the reference list, all of which were eligible in terms of inclusion criteria (1, 38-40). Two out of the 4 articles were already found in our search (38, 39), and 2 other articles were new, which are now included (1, 40). Finally, 11 in vitro studies (Table 1) and 5 in vivo studies (Table 2) were included in this systematic review. In terms of bias quality, given that GRADE scoring was used for quality assessment (Table 3), these studies were mainly of moderate to high quality. To evaluate the quality of animal studies, we used SYRCLE's RoB (Table 4). None of these studies provided a complete description of allocation, randomization, and blinding.

3.1. Results of In Vitro Studies

Emanuelsson et al. (17) evaluated the effects of Tacalcitol and Calcipotriol (as vitamin D analogs) on the via-

No.	Compound	Cells/Animal	Culture	Evaluation	Treatment	Main Results	Effectively	Control	References
1	Tacalcitol, cal- cipotriol, calcitriol	Human glioblastoma cell line T98G	6 days; For cell migration and caspase activity; 24 hours LDH release; 72 - 120 hours. 48 hours for Assay of 3 H-thymidine incorporation	MTT assay, manual cell counting using a Hemocytometer, Wound healing assay, Measurement of LDH release using Cytotoxicity Detection, Caspase-Glo [®] 3/7 assay, RT+CR, Assay of 3 H-thymidine incorporation	1 nmol10 μmol	Calcitriol and both its analogs decreased cell viability and/or growth, doscedependently, mainly inhibited proliferation and reduced the migration rate	Effective	Ethanol	Emanuelsson et al. (17)
2	Calcitriol	Human GBM cell lines U251, U87MG and T98G, and human breast adenocarcinoma cell line T47D	For cell survival assay 96 hours, 17 hours to cell migration and 48 hours for Immunofluores- cence	Tissue microarray construction, Immunohistochemistry, Transfections were carried out, using Lipofectamine 2000, Western blotting (WB), the cells were counted manually using a hemocytometer, wound healing assay, RNA extraction, and reaktime qPCR, Immunofluorescence (IF)	1 μM	Calcitriol increased the expression of VDR in tumor tissue. VDR expression was correlated with an improved outcome in patients with GBM. Treatment with calcitriol decreased the migration rate of T98G cells and retarded their survival.	Effective	Isopropanol	Salomon et al. (41)
3	Calcitriol, tricho- statin A (TSA), 5- azacytidine (5-aza)	GBM cell lines, Tx3868, Tx3095, U87MG, U118 and U373MG	24, 48, and 72 hours	Colorimetric immunoassay, Western blotting, PCR, and flow cytometry	10 ⁻⁶ - 10 ⁻¹⁰ M calcitriol, 5 - 20 ng/mL TSA, 0.1 - 5 μM 5-AZA	Treatment did not result in significant antiproliferative effects. Treatment had not modulatory effects on the expression of key components of the Notch-signaling pathway. Combination therapy with Calcitriol plus TSA or 5-AZA antiproliferative properties.	No effective	Ethanol	Reichrath et al. (42)
4	Calcitriol, Calcidiol	TX3868 and TX3095 were established from glioblastoma and xenografted on mice, COS-1 cells and rat GBM cell line C6	6 days	WST-1 Reagent, nested touchdown reverse transcription-PCR (RT-PCR), real-time RT-PCR, high-performance TLC	10 ⁻¹² to 10 ⁻⁶ mol/L Calcitriol and Calcidiol in a dose of 2.5 × 10 ⁻⁸ mol/L	I6 splice variants of CYP27B1 in GBM with different expressions between tumor and normal tissues were found, Calcitriol had a proliferative effect on some human GBM cell lines, Calcidol had the antiproliferative effect in 6 cell lines and proliferative effect on the remaining three cell lines.	Calcitriol was without the expected effects. Calcidiol was effective	Ethanol	Diesel et al. (43)
5	Cholecalciferol, C2- ceramide	Human glioblastoma cell line Hu197	6 and 12 hours	Light microscopy, DNA extraction & pulsed field gel electrophoresis MTT, assay, and lipids extraction & purification	50 μg of ceramide or 50 μg of cholecalciferol	Cholecalciferol treatment was increased by intracellular ceramide and induced cell death. Ceramide also resulted in induced cell death in the same cells	Effective	Same cells without any inter- vention	Magrassi et al. (44)
6	Calcitriol	The clones C6.9D3Sen and C6.4 D3Res were isolated from the C6 rat glioma cell line	24 hours	Plasmid construction &transfections, MTT assay, DNA fragmentation, southern blot, northern blot analysis, biorad protein assay, radiolabelled ligand assay	10 ⁻¹¹ to 10 ⁻⁷ M	Treatment reduced cell number compared with control cells	Effective	Ethanol	Davoust et al. (38)
7	Calcitriol	Rat glioma C6 cell line	24 hours	cDNA library and high-density filters, hybridization and differential screening analysis, DNA sequencing and computer analysis, RNA isolation for Northern blot analysis and CDNA probe preparation, evaluation of the length of inserts	$5 \times 10^{-8} M$	Calcitriol has reduced the expression of PMP22Jga33, SPARCJON, MAPICJdynein heavy chain, StoO-β, and aldolase C genes, and increased cysteine-rich protein, MGP, β-Tubulin, mortalin	Effective	Ethanol	Baudet et al. (45)
8	Calcitriol	Rat glioma C6.9 and C6.2 cell lines.	24 hours	MTT Assay, DNA Isolation & Analysis, RNA Isolation & Northern Blot, DNA Flow Cytometry, Electron Microscopy	lo ⁻⁷ М	Cell death induced by calcitriol was depended on the synthesis and expression of genes such as c-myc, p53, and gadd45	Effective	Ethanol	Baudet et al. (40)
9	Calcitriol, CB 1093, EB 1089, KH 1060, MC 903, and MC 1288	C6 glioma cell line from rat	24 hours	MTT assay, Northern blot analysis, DNA isolation, and analysis	10 ⁻⁷ -10 ⁻¹¹ M	KH 1060 had the greatest effect in inducing the death of glioma cells. The effect of MC 1288 and CB 1093 was equal to Calcitriol. EB 1089 was partially less effective than Calcitriol and MC 903 had only a subtle activity. Cell death was caused by the c-myc protooncogene induction.	Effective	Ethanol	Baudet et al. (39)
10	Calcitriol, Cholecalcif- erol, All-trans Retinoic Acid (RA)	Human glioblastoma cell lines Hu 70 and Hu 197	1 - 8 hours	MTT and Lactic Dehydrogenase Assays, RNA Analysis and Light Microscopy	0.01-100 µM	Glioblastoma cell growth was reduced even at low concentrations of treatments.	Effective	Same cells without any inter- vention	Magrassi et al. (46)
n	Calcitriol, 24,25(OH)2D3	Rat C6 glioma cell line	6 days	MTT Assay and RNA Analysis; LiCVUrea method, Northern blot analysis	10* ⁸ - 10* ¹² M	Cell death occurred even 24 h after the presence of calcitriol. Calcitriol regulated the expression of its own receptors in C6 glioma cells.	Effective	Ethanol	Naveilhan et al. (47)

Table 1. Summary of *In Vitro* Studies Evaluating the Antitumor Activity of Vitamin D3 Compounds

bility of cells, proliferation, and migratory capacity. T98G cells from human glioblastoma were used in their study. Two analogs and 1 α ,25(OH)2D3 were added to the culture medium in concentrations from 1 nM to 10 μ M. The via-

bility of T98G cells was measured by a colorimetric assay; proliferation was analyzed by manual cell counting, using a hemocytometer and for cell migration "wound healing" assay was taken into account. Both analogs decreased cell

No.	Compound	Animal	Evaluation	Treatment	Main Results	Effectively	Control	References
1	Calcitriol	Mice	Neurosphere formation assay, Tumor xenografts, MRI	Inject 1 µg/kg body weight	Calcitriol reduced the tumor size	Effective	Sesame oil, sterile water	Hu et al. (48)
2	EM1, calcitriol	Mice	WST-1 colorimetric assay (Roche), FACScan flow cytometry, wound healing assay, cell adhesion assay, matrigel-coated; transwell inserts (millipore), gelatin zymography assay, Western blot, immunofluorescence, RNA extraction, and real-time q PCR, plasma calcium levels in mice and liver and kidney histological analysis, molecular docking	0.01 - 100 nmoL in vitro (cells); inject 50 µg/kg body weight in vivo (mice)	EMI and calcitriol treatment reduced cell viability. EM1 had anti-migratory effects and decreased invasive capacity. EM1 bonded to the VDR with greater affinity than calcitriol. EM1 treatment had not induced hypercalcemia or toxicity effects <i>in vivo</i> .	Effective	Isopropanol	Ferronato et al. (18)
3	Calcitriol, TMZ	Rat	Orthotopic xenograft, MRI	Injection of 0.2 $\mu g/kg/day$ of calcitriol dissolved in 200 μ L of saline solution and also received TMZ as described above.	Combined therapy reduced tumor size and prolonged survival duration <i>in vivo</i>	Effective	dimethyl sulfoxide	Bak et al. (1)
4	Calcitriol	Rats	Northern blot & RNase protection, immunostaining methods, DNA transection & chloramphenicol acetyltransferase (CAT) assays	Injection of 20 mg/kg body weight	Treatment resulted in a decrease in the level of P75 ^{NTR} mRNA in the spinal cord.	Effective	Ethanol	Naveilhan et al. (49)
5	ML-344, calcitriol	Mice	WST-1 colorimetric assay (Roche), cell counting, flow cytometry, wound healing assays, matrigel-coated; transwell inserts (millipore), immunofluorescence, plasma calcium levels in mice, molecular docking studies	10^{11} to 10^{-7} moL + 100 nmoL extra <i>in vitro</i> (cells); Inject 5 $\mu g/kg$ body weight <i>in vivo</i> (mice)	Both the ML-344 and calcitriol reduced the cell viability and migration capacity, ML-344 did not hypercalcemic effect <i>in vivo</i> , ML-344 was able to bind to VDR with stronger affinity than calcitriol.	Effective	Isopropanol	Ferronato et al. (16)

viability and/or growth and dose-dependently (P < 0.05and P < 0.001; they also inhibited proliferation (P < 0.001) and decreased cell migration (P < 0.05).

Salomon et al. (41) investigated VDR expression in human glioma and its relevance to cell viability, migration, and patient survival. The cell lines originating from human GBM (U251, U87MG, and T98G) and human breast adenocarcinoma (T47D) were evaluated in this study. Cell lines were treated with calcitriol (1 μ M) or vehicle for 96 hours for cell survival assay. It took 17 hours to check cell migration and 48 hours for immunofluorescence. In comparison with VDR in non-malignant brains, the number of vitamin receptor(s) increased in tumor tissues (P = 0.009). VDR expression was correlated with an improved outcome in patients with GBM (P = 0.046). The results have also shown the modulation of VDR-controlled migration of GBM cells (P = 0.017) and survival (P < 0.001). Treatment with calcitriol decreased the migration rate of T98G cells (P = 0.029).

In an *in vitro* study on several GBM cell lines, Reichrath et al. (42) assessed the effect of 1,25(OH)2D3 on the key components of the Notch-signaling pathway. Tx3868, Tx3095, U87MG, U118, and U373MG were used in this study. Proliferation assay, Western blotting, Polymerase chain reaction (PCR), and Flow cytometry were applied. Various concentrations of 1,25(OH)2D3, trichostatin A (TSA) /5-azacytidine (5-AZA) or their mixture were put on the medium up to 100 μ L volume for 24, 48, and 72 hours. Consequently, some components of Notch-signaling pathways were expressed in some cell lines, but not in some others. Treating GBM cell lines with 1,25(OH)2D3 did not result in significant anti-proliferative effects. The treatment also had no modulatory effect on the gene expression of pivotal components of the Notch-signaling pathway. Combined therapy through 1,25(OH)2D3 plus TSA, or 5-aza did not increase the antiproliferative effects in comparison with TSA or 5-aza alone; it also reduced TSA or 5-aza antiproliferative properties.

Author	Study Limitation	Inconsistency	Indirectness	Imprecision	Publication Bias	Dose Effect	Overall Quality
Hu et al. (48)	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	++++
Ferronato et al. (16)	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	++++
Emanuelsson et al. (17)	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	\times^{b}	+++
Ferronato et al. (18)	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	×c	+++
Bak et al. (1)	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	$ imes^{d}$	+++
Salomon et al. (43)	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	\times^{d}	+++
Reichrath et al. (49)	\checkmark	\checkmark	×e	\times^{f}	\checkmark	\checkmark	++
Diesel et al. (43)	\checkmark	\checkmark	×e	\times^{f}	\checkmark	\checkmark	++
Magrassi et al. (46)	\checkmark	\checkmark	\checkmark	Unclear ^g	\checkmark	\checkmark	+++
Davoust et al. (38)	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	++++
Baudet et al. (45)	\checkmark	\checkmark	$ imes^{h}$	\times^{f}	\checkmark	$ imes^{d}$	+
Baudet et al. (40)	\checkmark	\checkmark	\checkmark	\times^{f}	\checkmark	\times^{d}	++
Naveilhan et al. (49)	\checkmark	\checkmark	$ imes^{h}$	\times^{f}	\checkmark	\checkmark	++
Baudet et al. (39)	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	++++
Magrassi et al. (44)	\checkmark	\checkmark	\checkmark	Unclear ^g	\checkmark	\checkmark	+++
Naveilhan et al. (47)	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	\checkmark	++++

^aGRADE factors: 🗸, no serious limitations; ×, serious limitations; Unclear, unable to rate items based on available information. For overall quality of evidence: +, very low; ++, low; +++, moderate; ++++, high.

^bDose-dependency about LDH-release and migration was not implied.

^cDose-dependency only about cell viability was implied.

^dNo dose-dependency was observed.

^eOur pre-defined outcomes comprehensively were not examined.

^fNo statistical test was used.

^gStatistical analysis for considered results is not shown.

^hOutcomes differ from those of primary interest.

Table 4.	Risk of Bias	Assessment	for In Vivo	Studies A	According to	SYRCLE's ROB	Criteria

Item	Bak et al. (1)	Ferronato et al. (16)	Ferronato et al. (18)	Naveilhan et al. (47)	Hu et al. (48)
1. Was the allocation sequence adequately generated and applied?	U	U	U	U	U
2. Were the groups similar at baseline or were they adjusted for confounders in the analysis?	Y	Y	Y	Y	Y
3. Was the allocation to the different groups adequately concealed?	U	U	U	U	U
4. Were the animals randomly housed during the experiment?	U	Y	Y	U	U
5. Were the caregivers and/or investigators blinded from knowledge which intervention in each animal received during the experiment?	U	U	U	U	U
6. Were animals selected at random for the outcome assessment?	U	U	U	U	U
7. Was the outcome assessor blinded?	U	U	U	U	U
8. Were incomplete outcome data adequately addressed?	U	U	U	U	U
9. Are reports of the study free of selective outcome reporting?	U	U	U	U	U
10. Was the study apparently free of other problems that could result in high risk of bias?	U	U	U	U	U

Abbreviations: Y, yes; N, no; U, unclear.

Diesel et al. (43) examined the role of vitamin D3 metabolism in glioblastoma multiforme. TX3868 and TX3095 were xenografted on mice. The rest of the GBM cell lines did not undergo xenografting; COS-1 cells and rat GBM cell line C6 were also used in that study. Proliferation assays, nested touchdown reverse transcription-PCR (RT-PCR), real-time RT-PCR, and high-performance TLC were applied. Calcitriol and calcidiol were used in concentrations of 10⁻¹² to 10⁻⁶ mol/L and 2.5 \times 10⁻⁸ mol/L, respectively. The medium culture was restored daily with fresh vitamin D3



metabolites content within 6 days. This study reported the 16 splice variants of CYP27B1 in GBM, which were differentially expressed in cancerous and normal tissues. They expressed the enzymatic activity of endogenous CYP27B1 in GBM cell lines. The proliferative effect of calcitriol on some cell lines from human GBM was reported, but it had no significant effect on the rest of them. In addition, calcidiol had an anti-proliferative effect on 6 out of 9 cell lines and a proliferative effect on the remaining 3 cell lines.

Magrassi et al. (44) examined the effect of cholecalciferol on glioblastoma cells death. Hu197, which is a stable human glioblastoma cell line, was used in this study. In this study, light microscopy, DNA extraction and pulsedfield gel electrophoresis, MTT assay and lipids extraction, and purification were applied; 50 μ g of ceramide and 50 μ g of cholecalciferol were used separately compared with the control within 6 and 12 hours. After cholecalciferol treatment, intracellular ceramide was increased and induced cell death was observed compared with the control (P < 0.05). Ceramide also resulted in induced cell death in the same cells compared with the control (P < 0.005). These results have shown that the sphingomyelin pathway could be involved in the antitumor activity of vitamin D metabolites.

Davoust et al. (38) reported that the transfection of VDR into a colony of rat glioma cells resistant to 1,25-D3 increased the sensitivity to the cytotoxic effect of this vitamin. C6 cell line from rat glioma was supplied with the C6.9D3Sen and C6.4 D3Res clones. Cell cultures were treated with 10^{-11} to 10^{-7} M ethanol 0.1% (vehicle) or 1,25-D3. The duration of treatment was 24 hours. The assays applied in this study included Plasmid construction and transfections, MTT cell viability assay, DNA fragmentation, southern blot, northern blot analysis, and the measurement of 1,25(OH)2D3 specific binding. The treatment of C6.9D3Sen cells for 24 hours with 1,25-D3 at defined concentrations significantly reduced cell numbers compared with control cells (P < 0.01).

Baudet et al. (45), showed antineoplastic effects of 1,25-D3 by examining the genes, which in adherence to the induced cell death by 1,25-D3 were differentially expressed. Rat glioma C6 cell line in the culture medium was treated with 1,25-D3 at 5 \times 10⁻⁸ M for 24 hours or with vehicle alone (ethanol). Rat glioma C6.9 involved in the cell death program was examined in terms of gene expression through the differential analysis of a rat brain cDNA library, using probes containing control and 1,25-D3treated cells. Their methods included the following assays: cDNA library and high-density filters, hybridization and differential screening analysis, DNA sequencing and computer analysis, Northern blot analysis and cDNA probing, and the evaluation of the length of inserts. Consequently, 61 differentially expressed cDNAs were reported. Among them, 1,25-D3 was able to down-regulate the expression of peripheral myelin protein 22 (PMP22/gas3), secreted protein acidic and rich in cysteine/osteonectin (SPARC/ON) MAP1C/dynein heavy chain, S100 β and aldolase C genes, and was able to up-regulate cysteine-rich protein, matrix gamma carboxyglutamic acid protein (MGP), β -Tubulin, and mortalin. These changes caused the programmed cell death induced by 1,25-D3.

Baudet et al. (40) investigated the mechanisms leading to glioma cell death induced by 1,25(OH)D3 in the rat. C6.9 and C6.2 clones were used. Cells were exposed to 10⁻⁷ M 1,25(OH)2D3, or vehicle for 24 hours. MTT assay, DNA isolation and analysis, RNA isolation and northern blot, DNA flow cytometry, and electron microscopy were applied. The findings of this study showed that cell death induced by 1,25(OH),D3 depends on the synthesis of certain proteins along with some genes expression, including c-myc, p53, and gadd45. After the addition of 1,25(OH)2D3, the expression of two genes encoding the interleukin-6 and vasoendothelial growth factor were also increased.

Baudet et al. (39), in an in vitro study, evaluated the effects of different vitamin D3 analogs with the lowest calcemic effect in vivo on the growth of glioma cells. C6 glioma cell line from rat was used. Various concentrations of 1,25(OH)2D3, its analogs, or ethanol (as a vehicle) were put on the medium culture for 24 hours. The analogs used in this study included CB 1093, EB 1089, KH 1060, MC 903, and MC 1288. The most effective doses were approximately 10⁻⁹ M and 10⁻¹⁰ M for 1,25(OH)2D3 and KH 1060, respectively (P < 0.001). MTT assay, northern blot analysis, DNA isolation, and analysis were applied in the study. The compound KH 1060 had the greatest effect in inducing the death of glioma cells (P < 0.001). The effect of MC 1288 and CB 1093 was equal to 1,25(OH)2D3. The effectiveness of EB 1089 was partially less than 1,25(OH)2D3. MC 903 had only a subtle activity on C6.9 cells. DNA fragmentation and induction of the c-myc protooncogene were also accompanied by this cytotoxicity effect.

Magrassi et al. (46), in an *in vitro* study, showed the effects of vitamin D and its metabolites along with retinoic acid on human glioblastoma cells. Hu 70 and Hu 197 were used in the study. 1,25(OH)2D3, Cholecalciferol, and all-trans retinoic acid (RA) were purchased. MTT and Lactic Dehydrogenase assays, RNA analysis, and light microscopy were applied in their study. Both substances reduced glioblastoma cell growth at a concentration of more than 5 μ M. This was significantly inhibited after the dilution of 25-dihydroxy vitamin D3 and combination with 1 μ M of all trans-retinoic acid even after adding vitamin D at the concentration range of nanomolar.

Naveilhan et al. (47) conducted a study aimed at *in vitro* investigating the effects of 1,25(OH)2D3 on cell lines from glioma. Cell culture was provided through the rat C6 glioma cell line. The 1,25(OH)2D3 and 24,25(OH)2D3 were used in the study. MTT assay and RNA analysis were applied. 1,25(OH)2D3 remained in the medium culture for 3 days, the cytotoxic effects were mediated by 1,25(OH)2D3, which were detected in approximately 10⁻⁸ M of this vitamin (P < 0.001 compared to control). However, the continuous stay of 1,25(OH)2D3 in the medium was not necessary because cell death happened when 1,25(OH)2D3 presented only for 24 hours and, then, the cells were cultured without the 1,25(OH)2D3. Furthermore, 1,25(OH)2D3 could regulate its own receptors in C6 cells.

3.2. Results of In Vivo Studies

Hu et al. (48) conducted a study on human glioma cell lines (U87MG and T98G), U251 cells, and normal cells originated from human astrocytes. They used 10 and 100 nmoL 1,25(OH)2D3 (calcitriol) to treat stem cell-like glioma cells (SLCs) for 4, 8, 12, 24, and 48 hours in the circumstances with pH 7.4 and 6.8. The expression of stemness markers, self-renewal ability of SLCs, and pre-treated mitochondrial oxygen consumption rates of SLCs were measured. Calcitriol could suppress the stemness of SLCs to some extent. Furthermore, self-renewal ability was repressed in pH 7.4 compared with pH 6.8 (P < 0.01) and in two groups of calcitriol compared with the control (P < 0.001). The basal and maximal respirations of these cells were retarded with calcitriol treatment (P < 0.01). In addition, according to the photos of magnetic resonance imaging, the in vivo treatment of 1 μ g/kg calcitriol on mice reduced the tumor size. These results suggested that calcitriol could be a novel and safe treatment for malignant glioma.

Ferronato et al. (18) evaluated the effects of an analog of calcitriol called EM1 on GBM cells in the *in vitro*, in silico, and *in vivo* manner. Human U251 and T98G GBM, in addition to human primary astrocytes, were used in their study. With the range of concentrations from 0.01 to 100 nM of EM1, calcitriol or vehicle was used to cell treat for 120 hours. EM1 and calcitriol treatment reduced cell viability compared with the vehicle (P < 0.001). EM1 also had anti-migratory effects on GBM cells (P < 0.01) and decreased their invasive capacity (P < 0.001). In silico EM1 had a higher affinity for the VDR than calcitriol. In the end, mice were injected with 50 μ g/kg from EM1 within 21 days without any raise in the serum level of calcium or toxicity.

Bak et al. (1) conducted a study with the aim of examining the antitumor efficacy of combination therapy with vitamin D and temozolomide in glioblastoma. The glioblastoma cell (C6) origin from rat was used. The treatment was taken for 24 hours with dimethyl sulfoxide (DMSO) alone (as a control group), 1 mM TMZ, 100 nM Vitamin D, or a mixture from 1 mM TMZ plus 100 nM Vitamin D. Moreover, 24 male 2-month-old Sprague-Dawley rats with 250 g to 300 g body weight were examined in this study. The assays demonstrated that the treatment of C6 cell line with TMZ and vitamin D resulted in significant increased antitumor effects compared with each of them alone (P < 0.001). This is also true for autophagy (P < 0.001). Furthermore, combined therapy with TMZ and vitamin D significantly restrained tumor advancement and induced a longer lifespan compared with TMZ alone in the rat (P < 0.001).

In an *in vitro* and *in vivo* study, Naveilhan et al. (49) reported the vitamin D3 and its metabolite's effects on the regulation of low-affinity neurotrophin receptor. They also revealed whether 1,25-(OH)2D3 could regulate their expression in primary astrocytes and in C6 cells that undergo the program of cell death caused by 1,25-(OH)2D3. C6 cell lines and primary cultures of astrocytes from rats were used. The concentration of 10⁻⁸ M 1,25-(OH)2D3 or vehicle was used for 24 hours. To run in vivo section of the study, female 15-day-old rats (Sprague-Dawley) were injected with 20 mg/kg1,25-(OH)2D3 or vehicle alone for 24 hours. Northern blot and RNase protection, immunostaining methods and DNA transection, and chloramphenicol acetyltransferase (CAT) assays were applied. Even low amounts of 1,25-(OH)2D3 and its metabolites could increase the gene expression and protein levels of low-affinity neurotrophin receptor (P75NTR) in C6 cell lines. They also induced the expression of its own receptor in a similar manner to the P75NTR mRNA. Even the high concentrations of 1,25-(OH)2D3 in the primary cultures of astrocytes did not result in the regulation of P75NTR. In vivo 1,25-(OH)2D3 has decreased the expression of the P75NTR gene in the spinal cord in healthy samples, but it did not affect its expression in dorsal root ganglion or sciatic nerve. In this study, it was revealed that based on the 1,25-(OH)2D3 activity on C6 cell lines, it could be a promising approach for describing the act of P75NTR in cell death.

Ferronato et al. (16) examined the effect of a synthetic

novel derivative of vitamin D3 called ML-344 on in vitro antitumor activity and in vivo calcemic effects. They also had the computational studies to examine its affinity for VDR through in silico assays. They focused on various types of cancer cell lines, including glioblastoma cells (U251, GL26, and T98G). To measure cell viability, cells were cultured in medium with 10⁻¹¹ to 10⁻⁷ M of calcitriol, ML-344, and vehicle for 120 hours. In addition, to evaluate the migration of cells through "wound healing" assays, the extra 100 nM of ML-344 or calcitriol was added to the medium. To check the calcemic effect of ML-344, they continued their investigation by injecting a 5 μ g/kg body weight of the ML-344 in mice with ML-344, calcitriol or vehicle. Cell viability and migration capacity were retarded by both ML-344 (P< 0.001) and calcitriol treatment (P < 0.01) compared with the control. In addition, despite calcitriol, ML-344 did not cause hypercalcemic effects in CF1 mice (P < 0.05 and P < 0.001). ML-344 was able to bind to VDR with a stronger affinity than calcitriol.

4. Conclusions

This systematic review has provided in vitro and in vivo evidence to investigate the effects of vitamin D3 and its analogs on malignant gliomas. The majority of our findings suggest that vitamin D3 and its analogs, compared with the control, induced cell death and apoptosis and reduced cell growth, invasion, and migratory capacity in gliomas cell culture. Some in vivo studies indicate that calcitriol administration was accompanied by a reduction in the tumor size, and vitamin D3 analogs were able to show the same effects without the hypercalcemic effect in vivo. This systematic review can be a platform for researchers to sum up the antitumor effects of vitamin D3 and consider vitamin D3 and its analogs as a safe curable supplement along with routine therapies for malignant gliomas. However, there is a necessity for high-quality studies in the future to better understand the mechanisms and to investigate the effects of this vitamin on health status and clinical outcomes in patients with malignant gliomas.

Supplementary Material

Supplementary material(s) is available here [To read supplementary materials, please refer to the journal website and open PDF/HTML].

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Melika

Footnotes

Authors' Contribution:

Hajimohammadebrahim-Ketabforoush and Zahra Vahdat Shariatpanahi created the concept and design of this study. Full search of databases was done by Melika Hajimohammadebrahim-Ketabforoush and Mohammadreza Shahmohammadi. Technical methods and critical appraisal of papers, abstracting data, and writing all parts of the manuscript were carried out by Melika Hajimohammadebrahim-Ketabforoush. Zahra Vahdat made the final edit. Support, coordination, and supervising this study were done by Mohammadreza Shahmohammadi. All authors read and approved the final manuscript.

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References

- Bak DH, Kang SH, Choi DR, Gil MN, Yu KS, Jeong JH, et al. Autophagy enhancement contributes to the synergistic effect of vitamin D in temozolomide-based glioblastoma chemotherapy. *Exp Ther Med.* 2016;11(6):2153-62. doi: 10.3892/etm.2016.3196. [PubMed: 27313664]. [PubMed Central: PMC4888049].
- Howell AE, Zheng J, Haycock PC, McAleenan A, Relton C, Martin RM, et al. Use of mendelian randomization for identifying risk factors for brain tumors. *Front Genet*. 2018;9:525. doi: 10.3389/fgene.2018.00525. [PubMed: 30483309]. [PubMed Central: PMC6240585].
- Elmaci I, Ozpinar A, Ozpinar A, Perez JL, Altinoz MA. From epidemiology and neurometabolism to treatment: Vitamin D in pathogenesis of glioblastoma multiforme (GBM) and a proposal for Vitamin D + all-trans retinoic acid + Temozolomide combination in treatment of GBM. *Metab Brain Dis.* 2019;**34**(3):687-704. doi: 10.1007/s11011-019-00412-5. [PubMed: 30937698].
- Elmaci I, Altinoz MA. A metabolic inhibitory cocktail for grave cancers: Metformin, pioglitazone and lithium combination in treatment of pancreatic cancer and glioblastoma multiforme. *Biochem Genet.* 2016;54(5):573–618. doi: 10.1007/s10528-016-9754-9. [PubMed: 27377891].
- Fu Y, Zheng Y, Chan KG, Liang A, Hu F. Lithium chloride decreases proliferation and migration of C6 glioma cells harboring isocitrate dehydrogenase 2 mutant via GSK-3beta. *Mol Biol Rep.* 2014;**41**(6):3907–13. doi: 10.1007/s11033-014-3258-7. [PubMed: 24549719].
- Sergentanis TN, Tsivgoulis G, Perlepe C, Ntanasis-Stathopoulos I, Tzanninis IG, Sergentanis IN, et al. Obesity and risk for brain/CNS tumors, gliomas and meningiomas: A meta-analysis. *PLoS One*. 2015;**10**(9). e0136974. doi: 10.1371/journal.pone.0136974. [PubMed: 26332834]. [PubMed Central: PMC4558052].
- 7. Takahashi H, Cornish AJ, Sud A, Law PJ, Kinnersley B, Ostrom QT, et al. Mendelian randomisation study of the relationship between vitamin

D and risk of glioma. *Sci Rep.* 2018;**8**(1):2339. doi: 10.1038/s41598-018-20844-w. [PubMed: 29402980]. [PubMed Central: PMC5799201].

- Kalueff AV, Tuohimaa P. Neurosteroid hormone vitamin D and its utility in clinical nutrition. *Curr Opin Clin Nutr Metab Care*. 2007;**10**(1):12–9. doi: 10.1097/MCO.0b013e328010ca18. [PubMed: 17143049].
- Annweiler C. Vitamin D in dementia prevention. Ann N Y Acad Sci. 2016;1367(1):57–63. doi: 10.1111/nyas.13058. [PubMed: 27116242].
- Holick MF. Vitamin D deficiency. N Engl J Med. 2007;357(3):266–81. doi: 10.1056/NEJMra070553. [PubMed: 17634462].
- Henry HL. Regulation of vitamin D metabolism. *Best Pract Res Clin Endocrinol Metab.* 2011;25(4):531–41. doi: 10.1016/j.beem.2011.05.003. [PubMed: 21872796].
- Campbell FC, Xu H, El-Tanani M, Crowe P, Bingham V. The yin and yang of vitamin D receptor (VDR) signaling in neoplastic progression: Operational networks and tissue-specific growth control. *Biochem Pharmacol.* 2010;**79**(1):1–9. doi: 10.1016/j.bcp.2009.09.005. [PubMed: 19737544]. [PubMed Central: PMC2824849].
- Bandera Merchan B, Morcillo S, Martin-Nunez G, Tinahones FJ, Macias-Gonzalez M. The role of vitamin D and VDR in carcinogenesis: Through epidemiology and basic sciences. J Steroid Biochem Mol Biol. 2017;167:203–18. doi: 10.1016/j.jsbmb.2016.11.020. [PubMed: 27913313].
- Diaz L, Diaz-Munoz M, Garcia-Gaytan AC, Mendez I. Mechanistic effects of calcitriol in cancer biology. *Nutrients*. 2015;7(6):5020–50. doi: 10.3390/nu7065020. [PubMed: 26102214]. [PubMed Central: PMC4488829].
- Salomon DG, Mascaro E, Grioli SM, Ferronato MJ, Vitale CA, Radivoy GE, et al. Phosphonate analogues of 1alpha, 25 dihydroxyvitamin D3 are promising candidates for antitumoural therapies. *Curr Top Med Chem.* 2014;14(21):2408–23. doi: 10.2174/1568026615666141208101418. [PubMed: 25486937].
- Ferronato MJ, Obiol DJ, Alonso EN, Guevara JA, Grioli SM, Mascaro M, et al. Synthesis of a novel analog of calcitriol and its biological evaluation as antitumor agent. *J Steroid Biochem Mol Biol*. 2019;**185**:118–36. doi: 10.1016/j.jsbmb.2018.08.006. [PubMed: 30125657].
- Emanuelsson I, Wikvall K, Friman T, Norlin M. Vitamin D analogues tacalcitol and calcipotriol inhibit proliferation and migration of T98G human glioblastoma cells. *Basic Clin Pharmacol Toxicol*. 2018;**123**(2):130–6. doi: 10.1111/bcpt.13007. [PubMed: 29575677].
- Ferronato MJ, Alonso EN, Salomon DG, Fermento ME, Gandini NA, Quevedo MA, et al. Antitumoral effects of the alkynylphosphonate analogue of calcitriol EM1 on glioblastoma multiforme cells. *J Steroid Biochem Mol Biol.* 2018;**178**:22–35. doi: 10.1016/j.jsbmb.2017.10.019. [PubMed: 29102624].
- Trouillas P, Honnorat J, Bret P, Jouvet A, Gerard JP. Redifferentiation therapy in brain tumors: Long-lasting complete regression of glioblastomas and an anaplastic astrocytoma under long term 1-alpha-hydroxycholecalciferol. J Neurooncol. 2001;51(1):57-66. doi: 10.1023/a:1006437003352. [PubMed: 11349882].
- Guyatt G, Oxman AD, Akl EA, Kunz R, Vist G, Brozek J, et al. GRADE guidelines: 1. Introduction-GRADE evidence profiles and summary of findings tables. *J Clin Epidemiol*. 2011;64(4):383–94. doi: 10.1016/j.jclinepi.2010.04.026. [PubMed: 21195583].
- Huguet A, Hayden JA, Stinson J, McGrath PJ, Chambers CT, Tougas ME, et al. Judging the quality of evidence in reviews of prognostic factor research: Adapting the GRADE framework. *Syst Rev.* 2013;2:71. doi: 10.1186/2046-4053-2-71. [PubMed: 24007720]. [PubMed Central: PMC3930077].
- Hooijmans CR, Rovers MM, de Vries RB, Leenaars M, Ritskes-Hoitinga M, Langendam MW. SYRCLE's risk of bias tool for animal studies. *BMC Med Res Methodol*. 2014;14:43. doi: 10.1186/1471-2288-14-43. [PubMed: 24667063]. [PubMed Central: PMC4230647].
- Borges GA, Rego DF, Assad DX, Coletta RD, De Luca Canto G, Guerra EN. In vivo and in vitro effects of curcumin on head and neck carcinoma: A systematic review. J Oral Pathol Med. 2017;46(1):3–20. doi: 10.1111/jop.12455. [PubMed: 27219631].

- Pavan LM, Rego DF, Elias ST, De Luca Canto G, Guerra EN. In vitro anti-tumor effects of statins on head and neck squamous cell carcinoma: A systematic review. *PLoS One*. 2015;10(6). e0130476. doi: 10.1371/journal.pone.0130476. [PubMed: 26098683]. [PubMed Central: PMC4476585].
- Liberati A, Altman DG, Tetzlaff J, Mulrow C, Gotzsche PC, Ioannidis JP, et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate healthcare interventions: Explanation and elaboration. *BMJ*. 2009;**339**:b2700. doi: 10.1136/bmj.b2700. [PubMed: 19622552]. [PubMed Central: PMC2714672].
- Rcom-H'cheo-Gauthier AN, Meedeniya AC, Pountney DL. Calcipotriol inhibits alpha-synuclein aggregation in SH-SY5Y neuroblastoma cells by a Calbindin-D28k-dependent mechanism. J Neurochem. 2017;141(2):263–74. doi: 10.1111/jnc.13971. [PubMed: 28164279].
- van Ginkel PR, Yang W, Marcet MM, Chow CC, Kulkarni AD, Darjatmoko S, et al. 1 alpha-Hydroxyvitamin D2 inhibits growth of human neuroblastoma. *J Neurooncol*. 2007;85(3):255–62. doi: 10.1007/s11060-007-9418-z. [PubMed: 17603751].
- Mandalaywala NV, Chang S, Snyder RG, Levendusky MC, Voigt JM, Dearborn RE Jr. The tumor suppressor, vitamin D3 upregulated protein 1 (VDUP1), functions downstream of REPO during Drosophila gliogenesis. *Dev Biol.* 2008;315(2):489–504. doi:10.1016/j.ydbio.2008.01.010. [PubMed: 18262515].
- Zou J, Landy H, Feun L, Xu R, Lampidis T, Wu CJ, et al. Correlation of a unique 220-kDa protein with vitamin D sensitivity in glioma cells. *Biochem Pharmacol.* 2000;60(9):1361–5. doi: 10.1016/s0006-2952(00)00438-x. [PubMed: 11008130].
- Yague JG, Garcia-Segura LM, Azcoitia I. Selective transcriptional regulation of aromatase gene by vitamin D, dexamethasone, and mifepristone in human glioma cells. *Endocrine*. 2009;35(2):252–61. doi:10.1007/s12020-008-9134-2. [PubMed: 19116788].
- Parry PV, Engh JA. Calcitriol enhances 5-aminolevulinic acidinduced fluorescence and the effect of photodynamic therapy in human glioma. *Neurosurgery*. 2014;74(4):N8–9. doi: 10.1227/NEU.000000000000310. [PubMed: 24642995].
- Yagishita T, Kushida A, Tamura H. Vitamin D(3) enhances ATRAmediated neurosteroid biosynthesis in human glioma GI-1 cells. *J Biochem.* 2012;**152**(3):285–92. doi: 10.1093/jb/mvs074. [PubMed: 22761456].
- Chen X, Wang C, Teng L, Liu Y, Chen X, Yang G, et al. Calcitriol enhances 5-aminolevulinic acid-induced fluorescence and the effect of photodynamic therapy in human glioma. *Acta Oncol*. 2014;53(3):405–13. doi: 10.3109/0284186X.2013.819993. [PubMed: 24032442].
- Maas RM, Reus K, Diesel B, Steudel WI, Feiden W, Fischer U, et al. Amplification and expression of splice variants of the gene encoding the P450 cytochrome 25-hydroxyvitamin D(3) 1,alpha-hydroxylase (CYP 27B1) in human malignant glioma. *Clin Cancer Res.* 2001;7(4):868–75. [PubMed: 11309335].
- 35. Diesel B, Seifert M, Radermacher J, Fischer U, Tilgen W, Reichrath J, et al. Towards a complete picture of splice variants of the gene for 25-hydroxyvitamin D3talpha-hydroxylase in brain and skin cancer. J Steroid Biochem Mol Biol. 2004;89-90(1-5):527-32. doi: 10.1016/j.jsbmb.2004.03.062. [PubMed: 15225832].
- Elias J, Marian B, Edling C, Lachmann B, Noe CR, Rolf SH, et al. Induction of apoptosis by vitamin D metabolites and analogs in a glioma cell line. *Recent Results Cancer Res.* 2003;**164**:319–32. doi: 10.1007/978-3-642-55580-0_22. [PubMed: 12899531].

- So JY, Suh N. Targeting cancer stem cells in solid tumors by vitamin D. J Steroid Biochem Mol Biol. 2015;148:79–85. doi: 10.1016/j.jsbmb.2014.10.007. [PubMed: 25460302]. [PubMed Central: PMC4361233].
- Davoust N, Wion D, Chevalier G, Garabedian M, Brachet P, Couez D. Vitamin D receptor stable transfection restores the susceptibility to 1,25-dihydroxyvitamin D3 cytotoxicity in a rat glioma resistant clone. J Neurosci Res. 1998;52(2):210–9. doi: 10.1002/(SICI)1097-4547(19980415)52:2<210::AID-JNR9>3.0.CO;2-D. [PubMed: 9579411].
- Baudet C, Chevalier G, Naveilhan P, Binderup L, Brachet P, Wion D. Cytotoxic effects of 1 alpha,25-dihydroxyvitamin D3 and synthetic vitamin D3 analogues on a glioma cell line. *Cancer Lett.* 1996;**100**(1-2):3-10. doi: 10.1016/0304-3835(95)04054-4. [PubMed: 8620449].
- Baudet C, Chevalier G, Chassevent A, Canova C, Filmon R, Larra F, et al. 1,25-Dihydroxyvitamin D3 induces programmed cell death in a rat glioma cell line. *J Neurosci Res.* 1996;46(5):540–50. doi: 10.1002/(SICI)1097-4547(19961201)46:5<540::AID-JNR3>3.0.CO;2-J. [PubMed: 8951666].
- Salomon DG, Fermento ME, Gandini NA, Ferronato MJ, Arevalo J, Blasco J, et al. Vitamin D receptor expression is associated with improved overall survival in human glioblastoma multiforme. *J Neurooncol.* 2014;**118**(1):49–60. doi: 10.1007/s11060-014-1416-3. [PubMed: 24584679].
- Reichrath S, Muller CS, Gleissner B, Pfreundschuh M, Vogt T, Reichrath J. Notch- and vitamin D signaling in 1,25(OH)2D3-resistant glioblastoma multiforme (GBM) cell lines. J Steroid Biochem Mol Biol. 2010;121(1-2):420–4. doi: 10.1016/j.jsbmb.2010.02.028. [PubMed: 20206691].
- Diesel B, Radermacher J, Bureik M, Bernhardt R, Seifert M, Reichrath J, et al. Vitamin D(3) metabolism in human glioblastoma multiforme: Functionality of CYP27B1 splice variants, metabolism of calcidiol, and effect of calcitriol. *Clin Cancer Res.* 2005;11(15):5370–80. doi: 10.1158/1078-0432.CCR-04-1968. [PubMed: 16061850].
- 44. Magrassi I, Adorni L, Montorfano G, Rapelli S, Butti G, Berra B, et al. Vitamin D metabolites activate the sphingomyelin pathway and induce death of glioblastoma cells. *Acta Neurochir (Wien)*. 1998;**140**(7):707-13. discussion 713-4. doi: 10.1007/s007010050166. [PubMed: 9781285].
- Baudet C, Perret E, Delpech B, Kaghad M, Brachet P, Wion D, et al. Differentially expressed genes in C6.9 glioma cells during vitamin D-induced cell death program. *Cell Death Differ*. 1998;5(1):116–25. doi: 10.1038/sj.cdd.4400327. [PubMed: 10200452].
- Magrassi L, Butti G, Pezzotta S, Infuso L, Milanesi G. Effects of vitamin D and retinoic acid on human glioblastoma cell lines. *Acta Neurochir (Wien)*. 1995;133(3-4):184–90. doi: 10.1007/bf01420072. [PubMed: 8748764].
- Naveilhan P, Berger F, Haddad K, Barbot N, Benabid AL, Brachet P, et al. Induction of glioma cell death by 1,25(OH)2 vitamin D3: Towards an endocrine therapy of brain tumors? *J Neurosci Res.* 1994;**37**(2):271– 7. doi: 10.1002/jnr.490370212. [PubMed: 8151734].
- Hu P, Li S, Tian N, Wu F, Hu Y, Li D, et al. Acidosis enhances the self-renewal and mitochondrial respiration of stem cell-like glioma cells through CYP24AI-mediated reduction of vitamin D. *Cell Death Dis.* 2019;**10**(1):25. doi: 10.1038/s41419-018-1242-1. [PubMed: 30631035]. [PubMed Central: PMC6328565].
- Naveilhan P, Neveu I, Baudet C, Funakoshi H, Wion D, Brachet P, et al. 1,25-Dihydroxyvitamin D3 regulates the expression of the low-affinity neurotrophin receptor. *Brain Res Mol Brain Res.* 1996;41(1-2):259–68. doi: 10.1016/0169-328x(96)00103-9. [PubMed: 8883959].