REVIEW ARTICLE

Anti-tumor effects of vitamin D in glioblastoma: mechanism and therapeutic implications

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Glioma is the most prevalent primary brain tumor in adults among which glioblastoma is the most malignant and lethal subtype. Its common resistance to conventional chemotherapeutics calls for the development of alternative or concomitant treatment. Taking advantage of its endocrine function as a neurosteroid, vitamin D has become a target of interest to be used in conjunction with different chemotherapies. In this article, we review the mechanisms through which vitamin D and its analogs induce anti-tumor activity in glioblastoma, and the practical issues relevant to their potential application based on in vitro and in vivo studies. Vitamin D has largely been reported to promote cell cycle arrest and induce cell death to suppress tumor growth in glioblastoma. Glioblastoma cells treated with vitamin D have also shown reduced migratory and invasive phenotypes, and reduced stemness. It is worth noting that vitamin D analogs are able to produce similar inhibitory actions without causing adverse effects such as hypercalcemia in vivo. Upregulation of vitamin D receptors by vitamin D and its analogs may also play a role in enhancing its anti-tumor activity. Based on current findings and taking into consideration its potential cancer-protective effects, the clinical application of vitamin D in glioblastoma treatment and prevention will be discussed. With some study findings subject to controversy, further investigation is warranted to elucidate the mechanism of action of vitamin D and to evaluate relevant issues regarding its treatment efficacy and potential clinical application.

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INTRODUCTION

Gliomas, the most prevalent intracranial neoplasia in adults, account for around 50% of brain tumors[1–3]. Glioblastoma, previously also known as glioblastoma multiforme (GBM), is the most malignant and life-threatening form. Classified as grade IV glioma by the World Health Organization (WHO), glioblastoma is associated with a median patient survival of 14–16 months, and a five-year survival rate of 9.8%[4]. While the new WHO CNS5 classification of gliomas has integrated histological appearances with molecular features and genetic alterations, the most aggressive subtype of glioma being an IDH-wildtype glioblastoma with the presence of at least one of the genetic parameters (TERT promoter mutation, EGFR gene amplification and combined chromosome +chr7/−chr10) in adults[5]. Apart from these genetic markers, other genetic risk factors including the expression of certain vitamin D receptors (VDR) genotypes were reported to be associated with clinical outcome of glioma patients[6]. Glioblastoma is also characterized by high invasiveness and the capability to diffusely invade into the normal cerebral parenchyma. Such an aggressive phenotype poses a hindrance to total en bloc resection and eventually a high risk of tumor relapse[7,8].

Temozolomide (8-carbamoyl-3-methylidazo(5,1-d)-1,2,3,5-terrazin-4(3H)-one; TMZ) is a well-tolerated oral alkylating agent which has the ability to cross the blood-brain barrier[9], making TMZ-based chemoradiotherapy the current gold standard for the treatment of newly diagnosed glioblastoma[10,11]. However, treatment response remains suboptimal and is limited by its relatively short half-life (1.8 h). The expression of the DNA repairing enzyme methylguanine-DNA methyltransferase (MGMT) may compromise the cytotoxic effect of TMZ, leading to a high recurrence rate following TMZ treatment[12]. Strategies to complement response and overcome TMZ resistance are therefore crucial and remain a cornerstone to improving patient prognosis[13,14].

Current studies have suggested different strategies to enhance the chemosensitivity of TMZ. In addition to the use of synthetic compounds, many have studied the potential concomitant use of TMZ with dietary supplements[8]. One of such molecules is vitamin D. Exposure of the skin to sunlight leads to the formation of vitamin D3 (also referred to as cholecalciferol), which then undergoes bioactivation by double hydroxylation in the liver and kidney to form its active metabolite, calcitriol, or 1,25-dihydroxyvitamin D (1,25(OH)2D3)[15,16]. Vitamin D has recently emerged as a neurosteroid hormone in the brain and has been shown to act as a regulator of a variety of brain functions, such as neuroprotection, anti-epileptic and anti-calcification effects, neuro-immunomodulation, and interplay with neurotransmitters and hormones. In particular, calcitriol regulates many cellular physiological processes, including cellular proliferation, differentiation, and apoptosis[16]. Calcitriol has shown anti-tumor effects in cancers of the pancreas, liver, breast, prostate, skin, brain, and myeloid leukemia[17–20]. However, the supraphysiological dosages of calcitriol required to produce the anti-neoplastic activity may cause side effects, including hypercalcemia, and may complicate cancer treatment[17,21]. In light of concerns regarding toxicity and

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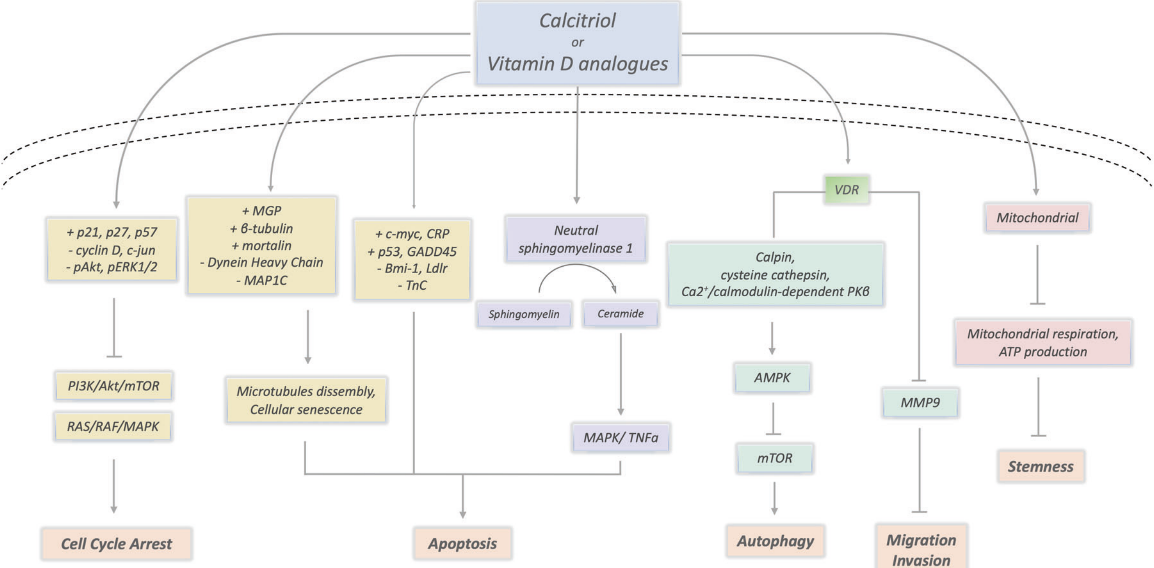

Fig. 1 Vitamin D-induced anti-tumoral actions in cell proliferation, apoptosis, migration, invasion, and stemness. Calcitriol or vitamin D analogs inhibit cell cycle transition, directly through upregulating cyclin-dependent kinase inhibitors, or indirectly through regulating other growth factors. It also induces apoptosis via the activation of intrinsic apoptotic pathways, tumor-suppressor pathways, and the ceramide pathway, and induces VDR-mediated autophagy. In addition, calcitriol acts on VDR to suppress MMP activity to inhibit tumor migration and invasion and interferes with mitochondrial respiration to repress stemness. (+ Upregulation − Downregulation).

calcium homeostasis, the application of vitamin D analogs with milder systemic side effects have also been proposed as an alternative. In addition to empirical evidence substantiating its anti-tumor effects, empirical and epidemiological studies have proposed that vitamin D may play a chemopreventive role in prostate, breast, colorectal cancers and gliomas, and put forth a possibility of application in cancer prevention[18,22]. In this review article, we discuss how vitamin D exerts its function in glioblastoma cells, with a focus on the molecular mechanisms underlying its anti-tumor effects, and most importantly, the therapeutic implications.

GENOMIC AND NON-GENOMIC ACTIONS OF VITAMIN D

Classically, vitamin D and its analogs act through the genomic pathway which involves vitamin D receptors (VDRs). VDRs are present in more than 30 different tissues throughout the body, including the intestine, bone, brain, stomach, heart, pancreas, skin, activated T and B lymphocytes, and gonads[23]. Upon binding of the hormonally active vitamin D ($1{,}25(OH)_2D3$) to VDR, which is a ligand-activated transcription factor in the inner nuclear membrane microdomains, retinoid-X receptor (RXR) or in some cases the retinoid-A receptor (RAR) facilitates its binding to the vitamin D response element (VDRE) in target gene promoters[24–26]. The assembly of VDR/RXR heterodimers can activate transcription in different genes containing functional VDREs, such as 25(OH)D3 24-hydroxylase[27], and those involved in cell proliferation, including p21[28], GADD45[29], and TNFα[30].

Apart from the classical VD-VDR response, vitamin D can also activate receptor-independent non-genomic pathways to regulate cytoplasmic signaling and mediate cellular functions. These signaling pathways include protein kinase C[31], Ras and mitogen-activated protein kinase (MAPK)[31,32], and the ceramide pathway[33,34]. Such non-genomic actions lead to rapid intracellular changes in calcium, as well as the activation or deactivation of proteins like Bcl-2 and c-jun, thereby regulating cellular growth, differentiation, and apoptosis, independently or in cooperation with the classical genomic VDR pathway[21].

MECHANISMS OF VITAMIN D ANTI-TUMOR ACTIVITY

A number of vitamin D studies performed on in vitro and in vivo cancer models have proposed several mechanisms in explanation of the tumor-suppressive activity of vitamin D and its analogs in glioma. Figure 1 illustrates the anti-tumoral mechanisms of vitamin D in glioblastoma, and Table 1 summarizes the anti-tumor mechanisms of calcitriol and its analogs.

CELL CYCLE ARREST

Cell cycle arrest is one of the most well-studied mechanisms accounting for the anti-tumor activity of vitamin D in gliomas. Several studies have demonstrated that calcitriol-induced arrest in the G0/G1 phase in various glioblastoma cell lines[35–37]. Treatments with its analogs have shown similar cytostatic effects. For example, EM1 and tacalcitol inhibited glioblastoma cell proliferation in T98G cell line[35,38], and ML-344 induced cell cycle arrest in the murine GL261 and human U251 cell lines[36]. Such inhibitory effects on cell cycle progression were suggested to be induced by the simultaneous activation of cyclin-dependent kinase (CDK) inhibitors p21, p27 and p57[6,21,38–40], as well as the reduction in cyclin D1 and c-jun expressions, and the reduction in phosphorylation of pAkt (S473) and pERK1/2 (Thr202/Tyr204)[6,34,38], thereby affecting downstream signal transduction to suppress glioblastoma cell proliferation[38]. Known to play a crucial role in glioma tumorigenesis, the overexpression and/or mutation of epidermal growth factor receptor (EGFR) can activate downstream targets via multiple signaling pathways, including the phosphatidylinositol 3-kinase (PI3K)/Akt/rapamycin-sensitive mTOR-complex (mTOR) pathway, the RAS/RAF/MAPK pathway, together with their key phosphorylated kinases[38,41,42]. By delaying the phosphorylation of Akt at Ser473 (p-Akt-S473) and ERK1/2 at Thr202/Tyr204 (pERK1/2), and simultaneously decreasing the expression of nuclear transcription factor c-jun, a downstream substrate of MAPKs, calcitriol and its analogs may interfere with signal transduction and ultimately inhibit proliferation of glioma cells.

Table 1. Anti-tumor mechanisms of calcitriol and its analogs - in vitro and in vivo studies.

	Compound	Cell line (in vitro/in vivo)	VD treatment	Evaluation	Key findings	Ref.
1	Calcitriol	Wild-type p53 GL15, mutant p53 U251 and LN18 GBM cell line (in vitro)	10^{-7}–4×10^{-7}M	MTT assay, RT-qPCR, western blot, enzyme activity assay, immunofluorescence, xenotransplantation in chorioallantoic membrane, immunohistochemistry	Calcitriol slows proliferation and stimulates differentiation of GBM cells with mutated p53, via neutral sphingomyelinase 1.	34
2	Calcitriol	Human U251, T98G, murine GL26 GBM cell line; LM3, 4T1 breast cancer cell line; HN12 head and neck squamous cell carcinoma cell line (in vitro & in vivo)	10^{-11}–10^{-7}M in vitro (cells); Inject 50 µg/kg body weight in vivo (mice)	WST-1 colorimetric assay (Roche), manual counting with haemocytometer, FACS flow cytometry, wound healing assay, Matrigel-coated transwell inserts (millipore), immunofluorescence	Both calcitriol and ML-344 reduced cell viability of U251 and GL26 cell lines, but showed no effect on T98G. ML-344 caused cell arrest in G0/G1 phase.	36
					Both calcitriol and ML-344 retarded cell migration of U251 and T98G cells. ML-344 reduced invasive capacity of LM3 and 4T1 cell lines, by inducing rearrangement of actin cytoskeleton in LM3 cells.	
3	Calcitriol, NAPOE-VD3 NPs	C6 glioma cell line (in vitro)	10^{-6}M	Fluorescence spectroscopy, MTT assay, flow cytometry, nuclear staining with acridine orange, colony formation assay, real-time qPCR	Targeted delivery of calcitriol produced G1 arrest and apoptosis in C6 glioma cells, by downregulating Bmi-1, p53, Ldlr, and Cdh-1.	37
4	Calcitriol	Human T98G GBM cell line (in vitro)	10^{-9}–10^{-5}M	MTT assay, manual counting with haemocytometer, wound healing assay, LDH cytotoxicity assay, Caspase-Glo 3/7 assay, RT-PCR, ^{3}H-thymidine incorporation assay	Calcitriol and both of its analogs reduced cell viability/growth in a dose-dependent manner. Tacalcitol-treated cells showed significant suppression of ^{3}H-thymidine incorporation, suggesting growth suppression. They produced no caspase activity.	35
	Calcipotriol				All three compounds reduced migration rate of T98G cells.	
5	Calcitriol	Human U251, T98G; murine GL26 GBM cell line (in vitro & in vivo)	10^{-11}–10^{-7}M in vitro (cells); Inject 50 µg/kg body weight in vivo (mice)	WST-1 colorimetric assay (Roche), manual counting with haemocytometer, FACS flow cytometry, wound healing assay, cell adhesion assay, Matrigel-coated transwell inserts (Millipore), gelatin zymography assay, western blot, immunofluorescence, RNA extraction and real-time qPCR, plasma calcium evaluation in mice, liver and kidney histological analysis, molecular docking	Calcitriol and EM1 reduced cell viability. EM1 caused G0/G1 arrest in T98G cells, by increasing p21, p27 and p57, and decreasing cyclin D1. EM1 delayed pAkt-S473 and pERK1/2 in T98G cells, and decreased c-jun expression.	38
					EM1 produced anti-migratory effects and reduced invasive capacity, by reducing MMP-9 proteolytic activity.	
					EM1 bonded to VDR with greater affinity than calcitriol, and did not induce hypercalcaemia or toxicity effects in vivo.	
6	Calcitriol	Human U251, U87MG, T98G GBM cell lines; T47D breast adenocarcinoma cell line (in vitro)	10^{-6}M	Tissue microarray construction, immunohistochemistry, transfection using Lipofectamine 2000, western blot, manual counting with haemocytometer, wound healing assay, RNA extraction and real-time qPCR, immunofluorescence	Calcitriol increased VDR expression in tumor tissue. VDR expression was correlated with an improved outcome in GBM patients.	6
					Calcitriol retarded the survival and reduced the migration rate of T98G cells.	
7	Calcitriol	Rat C6 glioma cell line (in vitro)	5×10^{-8}M	cDNA library and high-density filters, hybridization and differential screening analysis, DNA sequencing and computer	Calcitriol reduced the expression of PMP22/gas3, SPARC/ON, MAP1C/ dynein heavy chain, S100B, and	51

Table 1 continued

	Compound	Cell line (in vitro/in vivo)	VD treatment	Evaluation	Key findings	Ref.
				analysis, RNA isolation for northern blotting and CDNA probe preparation, evaluation of the length of inserts	aldolase C genes, while increasing MGP, Β-tubulin, mortalin, and CRP.	
8	Calcitriol	Rat C6.9 and C6.2 glioma cell line (in vitro)	10^{-7}M	MTT assay, DNA isolation and analysis, RNA isolation and northern blot, DNA flow cytometry, electron microscopy	Calcitriol-induced cell death was dependent on protein synthesis and expression of genes including c-myc, p53, and gadd45.	52
9	Calcitriol, CB1093, EB1089, KH1060, MC903, MC1288	Rat C6 glioma cell line (in vitro)	10^{-11}–10^{-7}M	MTT assay, northern blot, DNA isolation and analysis	KH1060 showed the greatest efficacy in inducing cell death, while MC1288 and CB1093 produced similar effects as calcitriol. EB1089 and MC903 had lesser effects. Cell death was accompanied by c-myc induction.	53
10	Calcitriol, retinoic acid	Rat C6 glioma cell line (in vitro)	10^{-10}–10^{-6}M	RNA isolation and northern blot, immunoprecipitation	Calcitriol and retinoic acid produced dose-dependent inhibition of Tenascin-C expression, prior to a delayed cytotoxic effect.	55
11	Calcitriol TMZ	Rat C6 glioma cell line (in vitro & in vivo)	10^{-7}M in vitro (cells); Inject 0.2 μg/kg/day of calcitriol in 200 μl saline solution, and/or 10 mg/kg/day TMZ in 200 μl DMSO	MTT assay, colony formation assay, wound healing assay, Hoechst 33258 staining immunofluorescence with LC3, flow cytometry, electron microscopy, western blot, orthotopic xenograft, MRI	Combined therapy of calcitriol and TMZ enhanced cytotoxicity in vitro, by inducing autophagy. Co-treatment also significantly inhibited tumor growth and prolonged survival in vivo.	43
12	Chole-calciferol, C2-ceramide	Human Hu197 GBM cell line (in vitro)	50 μg of chole-calciferol, or 50 μg of ceramide	Light microscopy, DNA extraction and pulsed-field gel electrophoresis, MTT assay, lipids extraction, and purification	Cholecalciferol increased cellular ceramide and decreased sphingomyelin, and induced cell death. Ceramide also induced cell death.	48
13	Calcitriol, 24,25(OH)2D3, 25(OH)D3	Rat C6 glioma cell line (in vitro & in vivo)	10^{-11}–10^{-7}M in vitro; Inject 2 or 20 mg/kg body weight in vivo (rat)	Northern blot and RNase protection assays, immunoprecipitation, immunohistochemistry, DNA transfection, and chloramphenicol acetyltransferase (CAT) assays	Calcitriol, even at low concentrations, increased $p75^{NTR}$ mRNA and protein levels in C6 glioma cells, but the opposite was observed in the spinal cord in vivo, suggesting a restricted regulatory effect of calcitriol on $p75^{NTR}$ expression.	56
14	Calcitriol, Chole-calciferol, All-trans retinoic acid (ATRA)	Human Hu70 and Hu197 GBM cell line (in vitro)	10^{-8}–10^{-4}M	MTT assay, LDH cytotoxicity assay, RNA analysis, light microscopy	Calcitriol and cholecalciferol both reduced GBM cell growth. When combined with ATRA, growth inhibition was significant even at low concentrations of calcitriol or cholecalciferol.	76
15	Calcitriol, 24,25(OH)2D3	Rat C6 glioma cell line; human GHD glioma cell line (in vitro)	10^{-12}–10^{-8}M	MTT assay, RNA isolation by LiCl/Urea method and northern blot	Calcitriol produced cell death in C6 glioma cells within 24h, and upregulated the expression of its own receptors.	63
16	Calcitriol	Human U87MG, T98G, U251 GBM cell line (in vitro & in vivo)	10^{-8}–10^{-7}M in vitro (cells); Inject 1 μg/kg body weight in vivo (mice)	Neurosphere formation assay, western blot, immunostaining, mitochondrial respiration analysis, real-time RT-qPCR, LncRNA and mRNA arrays, 1α,25(OH)2D3 quantification, subcutaneous and intracranial xenografts, MRI	Calcitriol suppressed stemness markers expression, reduced acidosis-induced increase of self-renewal ability and restrained mitochondrial respiration in vitro. Calcitriol reduced the size of tumors in vivo.	62

AUTOPHAGY AND APOPTOSIS

Cytotoxic autophagy has been suggested to be an important apoptotic mechanism of calcitriol in glioblastoma, where it demonstrates synergistic effects with TMZ[43]. Bak et al. observed in calcitriol-treated C6 cells the ultrastructure of autophagosomes and found an increase in size and number, along with an increase in conversion of microtubule-associated protein 1 light chain 3 puncta LC3-I to LC3-II, indicating the activation of autophagy[43]. Consistently, in breast cancer cells treated with calcitriol and its analog EB1089, Høyer-Hansen et al. found damaged mitochondria containing markers indicative of autolysosomes, including cathepsin D, heat-shock protein 60, and cytochrome *c*[44]. It has been suggested that VDR mediated the elevation of cytoplasmic Ca^{2+} concentration by altering calcium-regulating protein expression. Such an increase induces and activates calpain, cysteine cathepsins, and Ca^{2+}/calmodulin-dependent protein kinase-β which subsequently activates AMP-activated kinase (AMPK)[44,45]. AMPK acts as a potent inducer of autophagy via the inhibition of mammalian target of rapamycin (mTOR) complex 1, followed by the activation of several autophagy-associated proteins, leading to massive cell death[45,46]. This autophagy-induced anti-tumor effect has been proposed to be beclin-dependent, with the alteration of Bcl-1/Bcl-2 balance restoring autophagic activity to normal levels in tumor cells. Bcl-1 is a tumor suppressor and is negatively regulated by Bcl-2, an anti-apoptotic protein overexpressed in many tumors[44]. Høyer-Hansen et al. demonstrated an increase in Bcl-1 activity which enhanced DNA fragmentation and chromatin condensation in breast cancer cells, and Guzey et al. demonstrated the downregulation of originally overexpressed Bcl-2 in several prostate cancer cell lines, in response to calcitriol treatment[44,47]. This cell death process may be accelerated by an accompanying increase in autophagic stress, further raising autophagic activity above normal levels[43]. While the precise pathway by which enhanced autophagic activity promotes glioma cell death remains to be determined, these findings nonetheless support its key involvement in inducing apoptosis in glioma.

Another apoptotic pathway activated by calcitriol is the ceramide pathway. Cataldi et al. demonstrated in glioblastoma cell lines U251 and LN18 that upon calcitriol treatment, neutral sphingomyelinase 1 degraded sphingomyelin which increased intracellular ceramide level[34]. Cholecalciferol, a vitamin D metabolite, activated the sphingomyelin pathway and led to a prominent increase in ceramide levels in a similar manner in Hu197 cells, followed by massive cell death[48]. This was first shown by Okazaki et al. in leukemia cell line HL-60[33,49], and it was proposed that the dysregulation of the balance between ceramide and other sphingolipids exerting opposing effects on MAPKs may activate an intrinsic cell death pathway[48]. Pirianov et al. further demonstrated in breast cancer cells that vitamin D analog CB1093 was able to potentiate TNFα-induced cytotoxicity, possibly by promoting C_2-ceramide-induced DNA fragmentation and cytosolic phospholipase A2 ($cPLA_2$) activation[50]. Despite the limited understanding of the interaction between VD, ceramide and its downstream signals, it is surmised that vitamin D activates sphingomyelinase to induce intracellular ceramide, that in turn promotes apoptosis through TNFα/MAPK pathways in glioblastoma cells.

VD exerts its effect via multiple modes of action, suggesting the involvement of various apoptotic players in addition to the autophagic and ceramide pathways. Calcitriol has been reported to alter the expression of several house-keeping genes involved in maintaining cell structure, motility and defense. Baudet et al. demonstrated in C6 rat glioma that calcitriol upregulated matrix Gla protein (MGP), β-tubulin and mortalin, causing microtubule disassembly, cellular senescence and cell death[51]. It simultaneously downregulated Dynein Heavy Chain/MAP1C, responsible for the microtubules-associated intracellular movements. Hence, calcitriol introduced perturbation in basic cellular functions that led to glioblastoma cell death. This is consistent with the observed reduction in osteonectin/SPARC expression, which primarily functions to enhance cell viability under stressful conditions.

On the other hand, calcitriol has been shown to regulate the expression of genes that participate in the course of apoptosis or programmed cell death. Calcitriol increased the expression of the proto-oncogene c-myc and its primary response gene cysteine-rich protein (CRP), tumor-suppressor gene p53, and growth arrest and DNA damage-inducible gene GADD45, hence inducing apoptosis or programmed cell death following irreversible DNA damage[52,53], while simultaneously downregulating proto-oncogene Bmi-1 and Ldlr responsible for the energy nourishment of glioma cells[37,54]. In addition, Alvarez-Dolado et al. demonstrated that calcitriol suppressed the expression of Tenascin-C (Tn-C), an extracellular matrix glycoprotein normally involved in glial cell development but substantially reactivated in glioma cells, triggering tumor growth, invasion, and angiogenesis. Its downregulation subsequently produced delayed cytotoxicity in C6 rat glioma cells[55].

The upregulation of neurotrophin expression has also been linked to calcitriol-induced cell death. The overexpression of low-affinity neurotrophin receptor $p75^{NTR}$ following calcitriol treatment, in the absence of its ligand, can induce apoptosis directly, or indirectly via the activation of the ceramide pathway given its structural homology with the TNF receptor (TNFR)[56–58]. IL-6, an inflammatory cytokine with reported neurotrophic properties, was upregulated by calcitriol and may act to cause cell death by modifying the host-glioma immune interaction[52]. These are consistent with the observed increase, in response to calcitriol, in vascular endothelial growth factor (VEGF) expression, a hypoxia-inducible neurotrophic factor upregulated in stressful conditions such as programmed cell death. The role of certain calcitriol-induced neurotrophins in glioma is, however, reported to be contradictory to its potential cell-protective effects and will be further discussed in this article.

ANTI-MIGRATORY AND ANTI-INVASIVE EFFECTS

Glioblastoma is known for its highly invasive nature, and is characterized by its extensive infiltration into the surrounding normal brain tissue[59]. Its invasion is often described as a multistep process, that involves first the adhesion of tumor cells to the extracellular matrix (ECM), the secretion of matrix metalloproteinases (MMPs) to degrade the local ECM, and subsequently the migration into the proteolytically modified ECM[60]. Calcitriol and its analogs have demonstrated anti-migratory effects and decreased invasive capacity in this regard. Salomón et al. demonstrated that calcitriol lowered T98G cell migration rate, and that the reduction was VDR-dependent[6]. Such suppression of migration by calcitriol through VDR may be attributed to the translocation of tumor-suppressor p27 to the nucleus[6,61]. Ferronato et al., on the other hand, demonstrated that calcitriol analog EM1 reduced MMP9 proteolytic activity in T98G cells, hence suppressing glioblastoma infiltration through the surrounding ECM into normal tissue[38]. Similarly, ML-344 has been shown to retard migration and reduce invasiveness in U251 and T98G glioma cell lines, as well as breast and head and neck cancer cell lines[36].

REPRESSION OF STEMNESS

The acidic microenvironment in glioblastoma is associated with the maintenance of cancer stemness and facilitates tumor progression. CYP24A1 encoding for 25-hydroxyvitamin D3-24-hydroxylase, which catalyzes the fast degradation of calcitriol, was overexpressed in stem cell-like glioma cells (SLCs) under acidosis and was accounted for their stemness and metabolism changes. Hu et al. revealed that calcitriol could repress stemness and the self-renewal ability of SLCs, by inhibiting mitochondrial respiration

and ATP production[62]. It is suggested that calcitriol acts to inhibit stemness markers expression and attenuate the acidosis-induced increase of self-renewal ability and mitochondrial respiration of glioma stem cells (GSCs) in malignant glioma, in a similar manner.

THE ROLE OF VDR IN GLIOMA

VD has been shown to upregulate VDR expression in tumor tissues, which further enhances its anti-neoplastic effects. Salomón et al. showed that silencing of VDR significantly increased cellular survival of T98G. However, subsequent exposure to calcitriol augmented VDR mRNA and protein levels and suppressed glioma cell survival in U251, U87MG and T98G cell lines, suggesting anti-tumor signaling mediated by VDR[6]. Such an increase in VDR expression stimulated by calcitriol has also been demonstrated in C6 glioma cells where it was accompanied by apoptotic cell death, characterized by DNA fragmentation and elevated proto-oncogenes p53 and c-myc expression[52,63]. This is in agreement with that observed in other cervical, mammary and ovarian cancers[64–66]. In addition, Davoust et al. demonstrated that stable transfection of VDR into a VD-resistant rat glioma clone was able to restore its sensitivity to calcitriol-induced cytotoxicity[67], not only corroborating the role of VDR in producing VD-mediated anti-tumor activity in glioma, but also proposing a solution to overcoming resistance to vitamin D by inducing VDR expression in glioma.

CONTRADICTORY PROTECTIVE EFFECTS

On the other hand, calcitriol has been reported to exert contradictory protective effects on glioma cells, mainly through the effect of neurotrophins. Calcitriol stimulates the production of numerous trophic factors including nerve growth factors (NGFs)[68], neurotrophin-3 (NT-3)[69], and glial cell line-derived neurotrophic factor (GDNF)[70], all of which enhance survival of glioma cells. NGFs have previously been observed to increase the proliferation of human glioma cell lines U87, U251 and U373[71], possibly via α9B1 integrin[72]. NT-3 has been shown to be protective of cells with the potential to initiate glial tumor growth[73], and of glioma cells in a hypoxic environment[74]. Blockage of GDNF- and GDNF receptor-α1 expression has been reported to inhibit C6 rat glioma cell proliferation[75], suggesting the potential promoter role of GDNF in glioma progression. Conversely, calcitriol has also been shown to downregulate NT-4 and to have no effect on the expression of BDNF[69]. Accumulating evidence suggested the potential role of neurotrophins in glioma progression, but such oncogenic functions remain largely unknown. Further investigation is needed to evaluate the net consequences on cell survival contributed by these neurotrophins.

THERAPEUTIC IMPLICATIONS

Empirical evidence shows that calcitriol and cholecalciferol exert therapeutic effects on its own at concentrations of 10^{-8} to 10^{-4} M, which carry a potential risk of causing hypercalcemia[21]. Therefore, efforts have been made to identify safer alternatives. Several vitamin D synthetic analogs have been examined, including tacalcitol, calcipotriol, ML-344, EM1, CB1093, EB1089, KH1060, MC903 and MC1288, all of which have proved to be able to produce similar therapeutic effects without giving rise to severe hypercalcemic side effects.

When combined with other known chemotherapeutic agents, calcitriol and cholecalciferol have demonstrated synergy in terms of therapeutic efficacy. Examples include the combination of calcitriol with temozolomide, and calcitriol or cholecalciferol with retinoic acid[43,55,76]. Concomitant treatment with vitamin D or vitamin D on its own may serve as a new therapeutic paradigm to overcoming chemoresistance in glioblastoma. Several phase I/II clinical trials on the combination therapy of calcitriol and other chemotherapeutic agents on glioma and other solid tumors have been taking place. A phase I/II trial is in progress to assess the efficacy and toxicity of long-term high-dose vitamin D3 with concurrent chemoradiotherapy/adjuvant chemotherapy containing TMZ in patients with newly diagnosed glioblastoma, evaluating doses as high as 4000IU[77]. Another phase I trial also underway aims to determine the effectiveness and maximum tolerated doses of subcutaneous and/or oral calcitriol combined with intravenous carboplatin in treating advanced solid tumors, including brain tumors[78]. While these ongoing clinical trials are still awaiting results, they shall provide valuable information regarding the efficacy, toxicity, and other practical issues to evaluate the potential clinical application of VD.

Based on the role of VDR as a regulator of autophagy, recent studies have also suggested a potential cancer-preventive effect of VD. Tavera-Mendoza et al. observed that in addition to inducing autophagy in breast cancer cells, dietary vitamin D supplementation in mice was able to increase basal autophagy levels even in the normal mammary gland, highlighting its protective effect against cancer[79]. Indeed, epidemiological studies have reported an inverse relationship between pre-diagnostic 25(OH)D levels and glioblastoma risk[22]. However, empirical glioma studies are required to elucidate this preventive effect in glioblastoma. VDR gene polymorphisms Fok-I (rs2228570) and Taq-I (rs731236) have also been reported as a genetic risk factor in various cancer types. Toptas et al. demonstrated a positive correlation between a VDR Fok-I ff genotype and the risk of meningioma[80]. Despite there being no significant difference observed in glioma patients, a larger scale genome-wide association study between VDR polymorphism and glioma susceptibility is needed. The functional and prognostic significance of VDR is becoming more prominent as VDR expression was also associated with KRAS mutation in several cancers[81]. Together, these findings point to the potential dual role of vitamin D as both a therapeutic and prophylactic agent against glioblastoma and support the clinical application of vitamin D or VDR as a novel biomarker in this regard.

To date, no unifying theories regarding the anti-tumor activity of vitamin D is available. Nevertheless, the mechanism by which vitamin D produces anti-tumor activity in glioma is likely to entail both genomic and non-genomic pathways and involve a vast number of downstream mediators and effectors. Robust evidence supports that vitamin D and its analogs may act to produce cell cycle arrest, apoptosis, anti-migratory and anti-invasive effects and repression of stemness, while upregulating VDR to further enhance anti-tumor response. On the other hand, some, though limited, evidence suggests a protective role of vitamin D on cancer patient survival. More investigation into the potential dual role and subsequent net effect of vitamin D on glioma progression is warranted. Regardless, current evidence presents a favorable and promising outlook on the therapeutic application of vitamin D and its analogs as a supplement to standard glioblastoma therapy, along with a potential application in glioblastoma prevention, offering insight into new means of overcoming chemoresistance and improving glioma patient survival.

DATA AVAILABILITY

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

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