Glucocorticoid Regulation of the Vitamin D Receptor

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Abstract

Many studies indicate calcitriol has potent anti-tumor activity in different types of cancers. However, high levels of vitamin D can produce hypercalcemia in some patients. Glucocorticoids are used to ameliorate hypercalcemia and to enhance calcitriol anti-tumor activity. Calcitriol in combination with the glucocorticoid dexamethasone (Dex) increased vitamin D receptor (VDR) protein levels and ligand binding in squamous cell carcinoma VII (SCC). In this study we found that both calcitriol and Dex induce VDR- and glucocorticoid receptor (GR)-mediated transcription respectively, indicating both hormone receptors are active in SCC. Pre-treatment with Dex increases VDR-mediated transcription at the human \textit{CYP24A1} promoter. Whereas, pre-treatment with other steroid hormones, including dihydrotestosterone and R1881, has no effect on VDR-mediated transcription. Real-time PCR indicates treatment with Dex increases \textit{Vdr} transcripts in a time-dependent manner, suggesting Dex may directly regulate expression of \textit{Vdr}. Numerous putative glucocorticoid response elements (GREs) were found in the \textit{Vdr} gene. Chromatin immunoprecipitation (ChIP) assay demonstrated GR binding at several putative GREs located within the mouse \textit{Vdr} gene. However, none of the putative GREs studied increase GR-mediated transcription in luciferase reporter assays. In an attempt to identify the response element responsible for \textit{Vdr} transcript regulation, future studies will continue to analyze newly identified GREs more distal from the \textit{Vdr} gene promoter.

1. Introduction

Calcitriol (1\alpha,25(OH)\textsubscript{2}D\textsubscript{3}), the active form of vitamin D has an essential role in calcium homeostasis [1]. Calcitriol promotes cell differentiation and cell cycle arrest, while inhibiting cell growth in a number of cancer cell types. Numerous studies demonstrate calcitriol has significant anti-tumor activity both \textit{in vivo} and \textit{in vitro} in a number of tumor models including squamous cell carcinoma, breast, colon and prostate [2]. In addition to a direct anti-proliferative effect on tumor cells, our laboratory recently demonstrated that calcitriol inhibits proliferation of tumor derived endothelial cells and tumor angiogenesis [3–5]. The action of calcitriol is exerted through binding to the vitamin D receptor (VDR), a member of the nuclear receptor superfamily. In the presence of ligand, VDR binds to vitamin D response elements (VDREs) to either increase or repress transcription of target
genes [2,6,7]. VDR levels are known to be regulated at the level of transcription by a number of transcription factors including VDR itself. In addition, treatment with calcitriol stabilizes VDR and increases its own activity by phosphorylation at several residues [8–10]. Recent findings also indicate micro-RNAs may target VDR mRNAs in human cells. For example micro-RNA-125b decreases the amount of VDR protein [11].

Hypercalcemia, the dose-limiting toxicity of vitamin D, could limit use as an anti-cancer agent. Glucocorticoids, such as dexamethasone (Dex), have been shown to inhibit calcium absorption by intestine and increase calcium excretion in kidney. Thus, glucocorticoids are utilized to overcome hypercalcemic effects of calcitriol [12,13]. Previously, our laboratory examined effects of glucocorticoids, such as Dex, and demonstrated Dex increases VDR protein levels and anti-proliferative effects of calcitriol in vitro and in vivo by using the SCC model [14,15]. We present here data exploring regulation of VDR by glucocorticoids.

2. Expression and activity of VDR

Effects of vitamin D are mediated by the active metabolite calcitriol, a secosteroid hormone, which binds to intracellular VDR. VDR, a ligand dependent transcription factor, forms a heterodimer with retinoid X receptor (RXR). VDR-RXR heterodimers in turn bind to VDREs on the promoter region to either increase or repress transcription of target genes [16]. Calcitriol and VDR are critical for calcium homeostasis and normal mineralization of bone [1,2]. Effects of calcitriol and VDR activity have been explored in vivo using VDR knockout (VDR KO) mice models. VDR KO mice are hypocalcemic and exhibit a Type II rickets phenotype. In VDR KO mice, impaired calcium absorption has been proposed as the primary defect responsible for the phenotype [1,17]. Primary target organs of calcitriol include the small intestine to regulate calcium absorption, bone where it regulates calcium deposition and kidney where calcitriol regulates its metabolism [1,2,18]. In addition to calcium homeostasis, a number of observations indicate calcitriol plays a role in regulation of cellular differentiation, cell cycle arrest, proliferation and apoptosis in different tissues and cell types where VDR is expressed.

3. Glucocorticoids regulate VDR expression

Anti-proliferative effects of calcitriol in combination with glucocorticoids have been studied in vivo and in vitro. The glucocorticoid Dex sensitizes SCC cells to anti-tumor effects of calcitriol, increases VDR-ligand binding and increases VDR protein levels. Further studies indicate treatment with calcitriol or Dex alone induces cell cycle arrest in SCC, which is further enhanced upon combination treatment. Activation of apoptosis was also studied in SCC cells where Dex dramatically increases calcitriol-induced apoptosis [14,15]. To further investigate how glucocorticoids increase VDR and calcitriol effects, SCC cells were treated with Dex. Dex increased de novo Vdr transcription as seen by real-time PCR in the SCC model. Induction with 500 nM Dex increased Vdr transcript after 6, 12 or 24h of treatment with an induction of 4–6 times compared to ethanol control (Figure 1). These results are consistent with a recent finding showing that calcitriol and glucocorticoids regulate mouse VDR at the level of mRNA in adipocytes [19]. Mice harboring subcutaneously implanted SCC tumors were used to study effects of Dex on calcitriol anti-tumor activity. In addition to increased anti-proliferative effects of calcitriol in mice treated with a combination of calcitriol-Dex, there was an increase in VDR measured by hormone binding in SCC cells from implanted tumors. Interestingly, hormone binding was increased in the kidney but reduced in intestinal mucosa. Indicating differential regulation of VDR by Dex is tissue specific. Tissue specific VDR regulation may result in both an anti-proliferative effect and reduction of calcitriol induced hypercalcemia within mice as Dex reduces calcium absorption in the intestine [12,15,20–22]. This preclinical data and the need to develop new
anti-cancer therapies led to evaluate the combination treatment in clinical trials and to extend the study to different models [5,13]. Increased anti-proliferative effects of calcitriol and VDR by Dex, has also been observed in tumor associated endothelial cells [5]. Contrarily, effects of Dex on calcitriol-mediated actions in prostate cells indicate Dex does not increase calcitriol anti-proliferative effects (Figure 2) or VDR-mediated transcription (data not shown). This observation is further supported by data showing decreased GR expression in prostate as prostate cancer progresses [23]. Calcitriol plus Dex treatment of androgen-independent prostate cancer patients was demonstrated to be safe, feasible and has anti-tumor activity. Although we believe a better prognosis can be predicted in light of GR status in each individual. In the SCC model it has been shown that RU486, an anti-glucocorticoid that binds GR, inhibits the ability of Dex to enhance anti-proliferative effects, cell cycle arrest, and apoptosis produced by calcitriol [14]. Results from studies using RU486 indicate a direct effect of glucocorticoids/GR in modulating actions of calcitriol. We examined the mouse Vdr gene for presence of glucocorticoids response elements (GREs) using the online suite Nubiscan [24]. Twenty putative GREs with a score of 0.6 or higher were identified between the upstream sequence (5 kb) and the first 4 introns of the Vdr gene. Chromatin immuno-precipitation (ChIP) assays demonstrate GR occupancy at several putative GREs after 2 or 3h of treatment with 100 nM Dex (data not shown). However, none of the putative GREs were able to significantly increase GR-mediated transcription in the presence of 100 nM Dex by using luciferase reporter assays (Figure 3). Previous studies indicate the sequence immediately upstream of exon 1c of the human VDR gene may function as a hormone-responsive TATA-containing promoter that responds to Dex [6]. However, this promoter region is not present in the mouse Vdr gene [25]. Our studies and the studies of others consistently suggest a direct regulation of VDR by glucocorticoids via GR in mouse and human cells. However, the mechanism involved remains unknown. It is possible that one or more GREs located farther from the mouse Vdr transcription start site may act as enhancers. Other possible mechanisms include changes in Vdr mRNA/protein stability and changes in expression/activity of transcription cofactors that may modulate Vdr expression.

4. VDR targets and calcitriol effects are modulated by dexamethasone

Glucocorticoids sensitize SCC cells to anti-tumor effects of calcitriol along with increasing VDR protein levels. Consequent increases in VDR by Dex also increase VDR-mediated transcription. Pre-treatment with Dex significantly increased VDR-mediated transcription at the human CYP24A1 promoter. Pre-treatment with other steroid hormones including dihydrotestosterone and R1881 has no effect on VDR-mediated transcription, indicating effects of Dex on VDR-mediated transcription are specific to glucocorticoids. Also in vivo results show glucocorticoids increase VDR and its target CYP24A1. Mice receiving 2 mg/kg per day for 5 days increased both Vdr mRNA and markedly increase Cyp24a1 at the level of mRNA and enzymatic activity [26]. Previous studies indicate treatment with calcitriol or Dex produces a decrease of p-Erk and p-Akt survival signals in SCC which is further potentiated by combination treatment. Activation of apoptosis was also examined by measuring cleavage of the effector caspase-3. Dex increased calcitriol-induced cleavage of caspase-3 and combination of calcitriol with Dex led to cleavage of full length PARP.

5. Conclusions

Our recent results indicate Dex potentiates calcitriol effects by increasing VDR. Increases in VDR are proposed to occur at the level of transcription. Treatment of SCC cells with Dex produces an important increase of Vdr transcripts. Similar effects have been observed in mouse adipocytes and human breast cancer cell lines. The Vdr gene contains a number of putative GREs. Rapid increase in Vdr transcript levels may indicate glucocorticoids directly
induce \textit{Vdr de novo} transcription. Several putative GREs shows GR occupancy by ChIP assay, however none of the putative GREs show enhanced transcription by using luciferase reporter assays. Future directions include studying the direct role of GR in dexamethasone effect by using siRNAs targeting GR, potential distal enhancers, and whether glucocorticoids modulate VDR stability at either protein or mRNA levels.

6. Addendum in proof

The regulation of \textit{Vdr} expression in mouse by glucocorticoids/GR signaling axis was recently addressed by the group of Dr. Pike (Lee A. Zella, Mark B. Meyer, Robert D. Nerenz, Seong Min Lee, Melissa L. Martowicz and J. Wesley Pike, Molecular Endocrinology 24 (1): 128–147, 2010). In this work ChIP on Chip was used to identify GR binding sites that may regulate \textit{Vdr} expression in mouse cells. A fragment of 2.2 kb, that binds GR, located 5.1 kb upstream of \textit{Vdr}’s transcription start site was found to be transcriptionally active in a luciferase reporter assay [27].

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References


Figure 1. Dexamethasone induces VDR transcripts
SCC cells were treated for 6, 12 and 24h with 500 nM Dex and 10 nM calcitriol. mRNA was measured using TaqMan® real-time PCR. Both 500 nM Dex and 10 nM calcitriol increased Vdr mRNA expression. Vdr mRNA transcripts levels were normalized to Gapdh expression. The results are expressed as relative quantitation compared to the ethanol control. Results of one representative experiment.
Figure 2. Dexamethasone does not increase the anti-proliferative effect of calcitriol in C4-2 cells
2×10^5 cells were seeded in 12-well plates using RPMI-10%FBS. Cells were allowed to
attach for 24h and were treated with 10, 100, or 1000 nM Dex plus increasing concentrations
of calcitriol or ethanol (control). After 6 days of treatments cells were trypsinized and cell
viability was assessed by trypan blue dye exclusion assay. Results of one representative
experiment.
Figure 3. Analysis of putative glucocorticoids response elements present in the mouse Vdr gene
GREs found between the first 5kb upstream of mouse Vdr transcription start site and in
between the first 4 introns were cloned into the minimal promoter vector pGL4.23
(Promega). The constructs were named by the intron where the putative GREs were found,
follow by the region cloned between the same intron (e.g. pGL4.23-1.1 contains the GRE
present in the intron 1 located at most 5’ position and pGL4.23-0.1 contains the 2 GREs
found in the upstream region). SCC cells were transfected and recovered overnight in
RPMI-10% charcol stripped FBS medium. After 24h of treatment with 100 nM Dex, cells
were lysed and assess for luciferase activity to test the ability of GREs to enhance
transcription. The results of one representative experiment are shown as units of luciferase normalized by the renilla reporter control.