

Expression and Function of the Vitamin D Receptor in Malignant Germ Cell Tumour of the Testis

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Abstract. Testicular germ cell tumours (TGCTs) are the most common malignancy in young men aged 18-35 years. They are clinically and histologically subdivided into seminomas and non-seminomas. 1,25-Dihydroxyvitamin D ($1,25(\text{OH})_2\text{D}_3$) is the active form of vitamin D and exerts its actions via a specific intracellular vitamin D receptor (VDR). Several investigations in the recent years have revealed, in addition to a physiological occurrence of the VDR in various tissues, VDR expression in different human malignancies. Furthermore, $1,25(\text{OH})_2\text{D}_3$ plays an important role in the regulation of cell proliferation and differentiation. In different normal and malignant cell types, antiproliferative and pro-differentiating effects of $1,25(\text{OH})_2\text{D}_3$ are described. We investigated whether TGCT express the VDR, whether differences exist between the histological subtypes and if vitamin D has a function on the proliferation of tumour cells. Furthermore, we investigated the potential function of the vitamin D-regulated genes nuclear receptor co-repressor 1 (NCOR1), nuclear receptor co-repressor 2 (NCOR2), thyroid receptor interacting protein 15 (TRIP15), Growth Arrest and DNA Damage (GADD45), MAP kinase-activated protein kinase 2 (MAPKAPK2), Cytochrome P450, family 24, subfamily A, polypeptide 1 (CYP24A1) and Cytochrome P450, family 27, subfamily B, polypeptide 1 (CYP27B1) in the pathogenesis of TGCT. We demonstrate, for the first time, that primary TGCT as well as TGCT cell lines, express VDR mRNA and protein. Vitamin D and VDR may play a role in the pathogenesis of TGCTs. Furthermore, vitamin D inhibits proliferation of TGCT cell-lines, potentially via an increase in expression of GADD45. Our data suggest that vitamin D could play a role in antitumour therapy.

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Key Words: TGCT, Vitamin D, VDR, GADD45, immunohistochemistry, PCR, Western blot.

Testicular germ cell tumours (TGCTs) represent the most common malignant tumours in men aged 18 to 35 years and comprise 98% of all testicular malignancies. The incidence is constantly increasing in Europe as well as in the U.S.A., having doubled over the last 40 years (1). Histologically, TGCTs are divided into two main groups, seminomas and nonseminomas, the nonseminomas being subdivided into embryonal carcinomas, yolk sac tumours, chorioniocarcinomas and teratomas (2). All invasive testicular germ cell tumours arise from intratubular germ cell neoplasia, unclassified type, (IGCNU) which represent the precursor lesions of invasive TGCT (3).

1,25-Dihydroxyvitamin D ($1,25(\text{OH})_2\text{D}_3$) is the active form of vitamin D and exerts its action via a specific intracellular vitamin D receptor (VDR). The VDR belongs to the steroid, thyroid and retinoid receptor gene family and regulates the transcriptional activity of $1,25(\text{OH})_2\text{D}_3$ -influenced genes by complexing with vitamin D-responsive elements (VDREs). VDREs are located in the promoter region of DNA of target genes and are able to induce the transcription of the target genes (4-6). Several investigations have shown VDR expression in different tumour types, including leukemia cells, colon and breast cancer cells, prostate carcinoma cells and thyroid carcinomas (7-11). Besides the action of $1,25(\text{OH})_2\text{D}_3$ on calcium and phosphate homeostasis, it has been demonstrated that $1,25(\text{OH})_2\text{D}_3$ is a significant modulator of cellular differentiation and proliferation in a number of different normal and malignant cells (12).

In regulating the cell cycle, signal transduction and interaction with thyroid hormone receptor in addition to the VDR, different genes are involved. Cytochrome P450, family 27, subfamily B, polypeptide 1 (CYP27B1) and Cytochrome P450, family 24, subfamily A, polypeptide 1 (CYP24A1) are two key enzymes of $1,25(\text{OH})_2\text{D}_3$ synthesis and act at different points in regulating the metabolism of vitamin D_3 (6). Nuclear receptor co-repressor 1 (NCOR1) and nuclear receptor co-repressor 2 (NCOR2) are nuclear co-repressors which support nuclear receptors in the down-regulation of target gene expression (13, 14). The protein thyroid receptor

interacting protein 15 (TRIP15), also called CSN2, is involved as part of COP9/signalosome complex in the regulation of different cell-signalling pathways and the cell cycle. It influences ligand binding to thyroid hormone and retinoic acid X receptors (RXR) (15, 16). MAP kinase-activated protein kinase 2 (MAPKAPK2) is involved in p38 MAPK signalling pathway and regulates gene expression (18, 19). Growth Arrest and DNA Damage (GADD45) is involved in the replication of DNA, the G1 cell cycle arrest and repression of proto-oncogenes in exerting antiproliferative effects in tumour cells. It directly affects proapoptotic actions and functions of DNA repair (20, 21). We investigated these genes for their potential role in the pathogenesis of TGCT. Furthermore, we analysed the expression and function of VDR and its co-regulators in primary TGCT and two TGCT cell lines.

Materials and Methods

Tissue samples of primary TGCT. Tumour tissues were acquired from 80 patients from the Federal Armed Forces Hospital, Hamburg, Germany. The mean age of patients was 33.86 years. An overview of patient ages and the different tumour types is presented in Table I. Ethical approval for using the human material in the present study was obtained from the Ethics Committee of the Federal Armed Forces Hospital, Hamburg. Tumour tissues from each testis were immediately fixed in HOPE (Hepes–glutamic acid buffer-mediated organic solvent protection effect) solution (DCS Diagnostic Systems, Hamburg, Germany) as described previously (22, 23).

Immunohistochemistry. Paraffin sections were used to perform the immunohistochemical reactions for VDR. The sections used were dewaxed, rehydrated and incubated with a polyclonal antibody against human VDR for one hour, at room temperature, in 1:200 dilution (Santa Cruz, CA, USA). Afterwards, the sections were incubated with the REAL Link/Label detection system (Dako, Hamburg, Germany) as described by the manufacturer. The signals were visualised with 3,3-diaminobenzidine (DAB; Dako). All sections were analysed by light microscopy after counterstaining with Meyer's hematoxylin. The immunohistochemical staining was evaluated according to the Remmele score in the diagnosis of breast cancer using immunohistochemical multiplying scores. This is the product of the percentage of positive tumour cells and the staining intensity (Table II) (24).

RNA isolation and quantitative real time polymerase chain reaction (qRT-PCR). RNA from HOPE-fixed paraffin-embedded tissues and TGCT cells were isolated with the RNeasy Mini Kit (Qiagen, Hilden, Germany). RNA integrity and quantity were measured with an Agilent Bioanalyzer 2100 using an RNA 6000 Nano LabChip-Kit (Agilent Technologies, Waldbronn, Germany). For reverse transcription, 500 ng of total cellular RNA were transcribed with random hexamer primers using an Omniscript RT Kit (Qiagen). Analysis of gene expression was performed with an iCycler iQ real-time detection system (Bio-Rad, Munich, Germany) and the QuantiTectSYBR Green Kit (Qiagen). The 20 µl reaction mixture from the kit was supplemented with 2 µl cDNA and 0.6 µM gene-specific primers. Primers (MWG, Ebersberg, Germany) were designed using the Primer 3 on-line primer design

Table I. *The different histological types and number of investigated tumours, and mean patient ages.*

Histology	n	Mean age (years)
Seminoma	57	35.43±9.417
Embryonic carcinoma	13	28.57±8.446
Teratoma	8	29.63±7.308
Chrorioncarcinoma	1	27±0
Yolk sac tumour	1	34±0
Total	80	33.86±9.276

programme (<http://frodo.wi.mit.edu/primer3/>) and the Operon oligo tool kit (<http://www.operon.com>). The primer sequences used are shown in Table III.

Culture and drug treatment of TGCT cell lines. The human TGCT cell lines used in the present study were NTERA-2 (CRL 1973) and NCCIT (CRL 2073) (American Type Culture Collection, Manassas, VA, USA). The explored cell lines were maintained in HEPES-buffered RPMI-1640 (Biochrom, Berlin, Germany) supplemented with foetal calf serum (FCS, 10%; CC Pro, Neustadt, Germany), penicillin (100 IU/ml; Sigma, Munich, Germany), streptomycin (100 µg/ml; Sigma) and L-glutamine (2 mM; Biochrom, Berlin, Germany). The incubation temperature was 37°C in a humid atmosphere with 5% carbon dioxide in air. For stimulation with 1,25(OH)₂D₃, the cells were plated sub-confluently in 75 ml flasks and cultured in serum-free medium for one day prior to treatment, the latter step to synchronize the cells and exclude influence of serum components on proliferation of tumour cells.

Proliferation assay. The proliferation of the TGCT cell lines was measured after stimulation with 50, 100 or 200 nM 1,25(OH)₂D₃ for 24 and 48 h with a BrdU-proliferation assay (Roche, Mannheim, Germany) as described by the manufacturer. The absorbance was determined in an ELISA reader (BioRad, Munich, Germany) at a wavelength of 450nm and a reference length from 655nm.

Western blotting. Tumour cells were harvested at 10⁷ cells in 500 µl lysis buffer (0.4% sodium deoxycholate, 1% NP-40, 50 mM EGTA, pH 7.4, 10 mM Tris, pH 7.4, 1 mM phenylsulphonyl fluoride (PMSF), 10 µg/ml leupeptin, 10 µg/ml aprotinin). Thereafter, cell proteins were isolated as described previously (25).

Results

TGCTs express VDR. For expression analysis of the VDR protein, we investigated HOPE-fixed and paraffin-embedded TGCT tissue samples by immunohistochemistry. The tumour-free normal testicular tissue (n=80) showed no expression of VDR in the germ cells or cells of sex cord stroma (Leydig cells, Sertoli cells). IGCNU (n=37) as a non-invasive TGCT precursor lesion, exhibited slight cytoplasmatic expression of VDR in the atypical germ cells. In all examined seminomas (n=57), only slight cytoplasmatic expression of VDR protein was detected in the tumour cells (Figure 1A). All 23

Table II. Evaluation score for the immunohistochemical studies.

Percentage of positive	Points	Intensity	Points	Score
None	0	None	0	Group I
<10% positive cells	1	Low	1	Group II
10-50%	2	Moderate intensity	2	Group III
50-80%	3	Strong	3	
>80%	4			

Table III. Sequences of investigated genes with forward and reverse primers used.

Gene	Forward	Reverse
VDR	5'-GCC CAC CAT AAG ACC TAC GA-3';	5'-AGA TTG GAG AAG CTG GAC GA-3'.
CYP 24 A1	5'-GAG ACT GGT GAC ATC TAC GGC GTA CA-3'	5'-CCA TAA AAT CGG CCA AGA CCT CAT TG-3
CYP27B1	5'-CAG AGG CAG CCA TGA GGA AC-3'	5'-GGG TCC CTT GAA GTG GCA TAG-3'
GADD45	5'-GCC TGT GAG TGA GTG CAG AA-3'	5'-CCC CAC CTT ATC CAT CCT TT-3'
MAPKAPK2	5'-TCA TGA AGA GCA TCG GTG AG-3'	5'-TCA AAG AGT TGT GGC TGG TG-3
NCOR1	5'-AAA GTG TGG AGA CCC AGG TG-3'	5'-ACC CTC ACT TCA ACG TCC AC-3'
NCOR2	5'-AAG TCC ATC CTC ACG TCC AC-3'	5'-TGA AGC ACA CTG GGT CTC TG-3'
TRIP15	5'-AGG GGT GGT GCA CGA TAT AC-3'	5'CAG GAC ACA TCA ACC GAC AG-3'
ARP	5'-CGA CCT GGA AGT CCA ACT AC-3'	5'-ATC TGC TGC ATC TGC TTG-3'

examined nonseminomas, embryonic carcinoma (n=13), teratomas (n=8), chorionic carcinoma (n=1) and yolk sac tumours (n=1) exhibited strong cytoplasmatic and nuclear VDR expression (Figure 1B). Table IV gives an overview of the tissues divided into the different groups, investigated by immunohistochemistry. TGCTs were also analysed by qRT-PCR. An expression of VDR mRNA was demonstrated in TGCT but not in tumour-free testis. Seminomas exhibited a moderate expression of VDR, whereas nonseminomas exhibit a significantly higher expression of the VDR in the investigated tissues (data not shown).

VDR expression in TGCT cell lines. In addition to the studies in 80 primary TGCTs, the expression of VDR was investigated in the TGCT cell lines NCCIT and NTERA-2 by qRT-PCR. The qRT-PCR showed that the tumour cell lines express VDR mRNA (Figure 2A). To verify the results on translational level, VDR protein expression was examined in the TGCT cell lines NCCIT and NTERA-2 by western blot. Both TGCT cell lines express VDR protein (Figure 2B).

Proliferative behaviour of NCCIT and NTERA-2 cells after vitamin D stimulation. The proliferative behaviour of the two TGCT cell lines NCCIT and NTERA-2 was investigated after stimulation with 50, 100 or 200 nM of 1,25(OH)₂D₃ for 24 and 48 h. After 24 h, the tumour cell line NCCIT exhibited a significant decrease in proliferation by up to 44% ($p<0.05$) at a concentration of 200 nM 1,25(OH)₂D₃ and a reduction of proliferation by 13% after 48 h stimulation with 200 nM

Table IV. The investigated patient samples divided into different the groups after immunohistochemical analysis.

Tumour entity	IHC scoring		
	Group I	Group II	Group III
Tumour free	80	0	0
IGCNU	0	37	0
Seminoma	0	57	0
Embryonic carcinoma	0	1	12
Teratoma	0	0	8
Chorioncarcinoma	0	0	1
Yolk sac tumour	0	0	1

(Figure 3A). The proliferative behaviour after 24 h of the TGCT cell line NTERA-2 exhibited a statistically significant decrease up to 39% ($p<0.05$) at a concentration of 200 nM vitamin D and after 48 h stimulation with 200 nM 1,25(OH)₂D₃ proliferation was reduced up to 9% (Figure 3B).

Vitamin D₃-regulated genes in TGCT cell lines. After stimulation with 1,25(OH)₂D₃, the tumour cell lines NCCIT and NTERA-2 were investigated by qRT-PCR for an expression of the vitamin D₃-regulated genes VDR, NCOR1, NCOR2, TRIP15, GADD45, MAPKAPK2, CYP24A1 and CYP27B1. The stimulation was performed with 50, 100 or 200 nM 1,25(OH)₂D₃ for 24 h. After stimulation with 50 and 100 nM 1,25(OH)₂D₃, the tumour cell line NCCIT

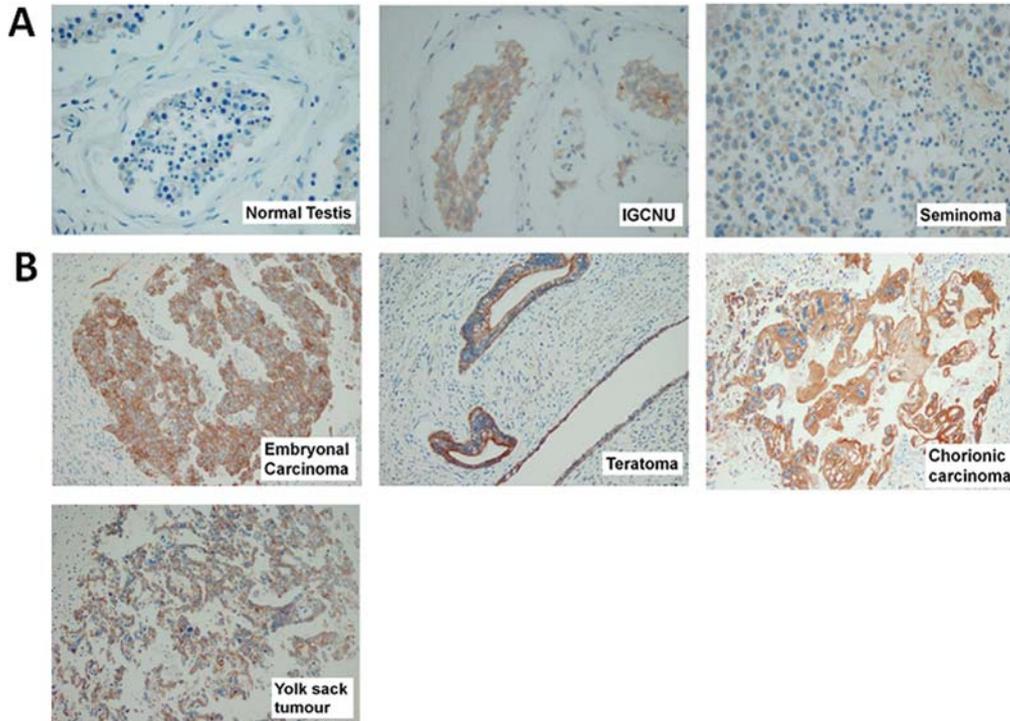


Figure 1. Vitamin D Receptor (VDR) expression in Testicular Germ Cell tumours (TGCT). The VDR expression in TGCT was investigated by immunohistochemistry. No expression of VDR is seen in the normal testis (n=80), while representative tissue of intratubular germ cell neoplasia unclassified type (IGCNU) (n=37) and seminoma (n=57) demonstrate a slight cytoplasmic expression of VDR protein (A). In contrast, nonseminomas (n=23), chorionic carcinoma (n=1), embryonal carcinoma (n=13), teratoma (n=8) and yolk sack tumours (n=1) exhibit strong cytoplasmic and nuclear VDR protein expression (B).

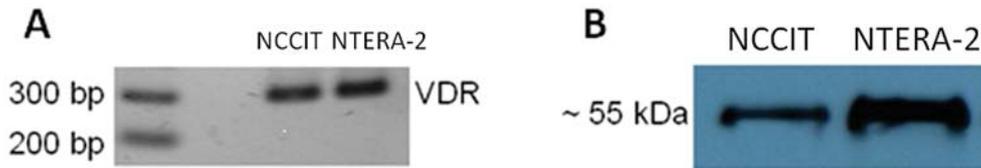


Figure 2. VDR mRNA and protein expression in TGCT cell lines. VDR expression was studied by qRT-PCR in TGCT cell lines NCCIT and NTERA-2. Both TGCT cell lines express VDR mRNA with no difference in the expression level between NCCIT and NTERA-2 (A). In addition, expression of VDR was examined in both TGCT cell lines by western blot, which showed that both TGCT cell lines also express VDR protein (B).

showed no statistical change in the expression of the examined genes. After stimulation with 200 nM $1,25(\text{OH})_2\text{D}_3$ VDR, NCOR1 and MAPKAPK2 gene expression significantly increased, whereas that of NCOR2 statistically decreased (Figure 4A). After stimulation with 50 and 100 nM $1,25(\text{OH})_2\text{D}_3$, the tumour cell line NTERA-2 sustained no statistical change in the expression of the examined genes. After stimulation with 200 nM $1,25(\text{OH})_2\text{D}_3$ of the NTERA-2 cell line, there was a further significant rise in expression of VDR and GADD45, while CYP27B1 gene expression decreased (Figure 4B).

Discussion

Expression of VDR in TGCT. In addition to the physiological occurrence of the VDR in various tissues, several research groups have demonstrated the presence of VDR in various malignancies in recent years. VDR expression has been studied in early and well-differentiated colon carcinoma (8), breast cancer (9), in prostate cancer (10), in papillary thyroid carcinoma (11), basal cell carcinoma (17), and in tumours of the lung (26). The results presented here show the expression of VDR in TGCT for the first time.

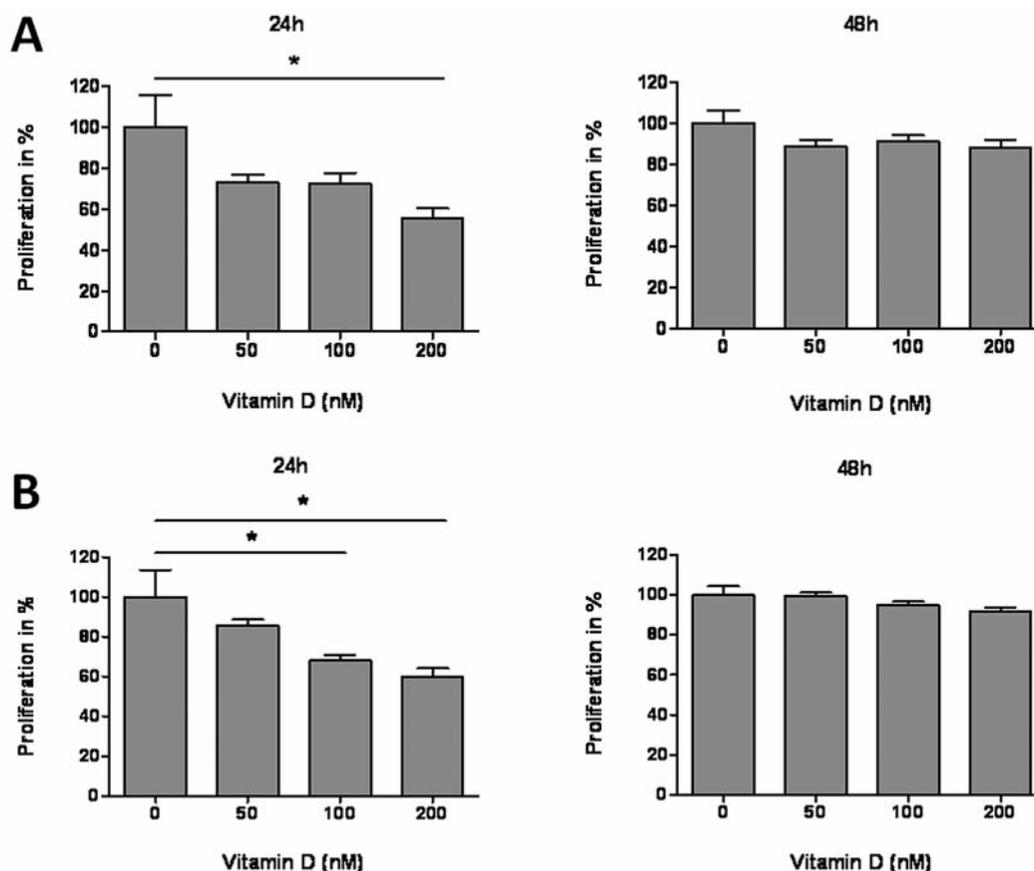


Figure 3. Stimulation with vitamin D in TGCT cell lines results in reduced proliferation. NCCIT and NTERA-2 were stimulated with $1,25(\text{OH})_2\text{D}_3$ (50, 100 or 200 nM). The proliferation of both TGCT cell lines was examined by BrdU ELISA after 24 h and 48 h. After stimulation with 200 nM $1,25(\text{OH})_2\text{D}_3$, proliferation of the tumour cell line NCCIT significantly decreased by up to 42% after 24 h and up to 19% after 48 h (A). After stimulation with 200 nM, proliferation of the tumour cell line NTERA-2 significantly decreased up to 29% after 24 h and up to 16% after 48 h (B).

Early and well-differentiated colon carcinomas express VDR. Interestingly, this VDR expression was largely lost in late stages and poorly differentiated colon carcinomas (27, 28). This supports the hypothesis of Khadzokou *et al.* (11), that $1,25(\text{OH})_2\text{D}_3$ in connection with the VDR may have a promoting effect on the differentiation and proliferation of tumour cells. The variety of different tumour types in which VDR is expressed speaks for its importance in differentiation and proliferation in the pathogenesis of tumours.

Proliferative behaviour of TGCT cell lines in response to vitamin D stimulation and therapeutic aspects of vitamin D.

In a variety of normal and malignant cell types, antiproliferative and pro-differentiating effects of $1,25(\text{OH})_2\text{D}_3$ have been shown (29). In studies of prostate and breast cancer, significant antitumour effects of $1,25(\text{OH})_2\text{D}_3$ and vitamin D analogues are reported (30, 31). $1,25(\text{OH})_2\text{D}_3$ and its derivatives have an effect on the regulation of proliferation, apoptosis and angiogenesis (32, 33). Here, we demonstrate a significant antiproliferative effect

while stimulating the TGCT cell lines NCCIT and NTERA-2 with $1,25(\text{OH})_2\text{D}_3$. The described antiproliferative effects open up possible opportunities for a potential pharmacological use of $1,25(\text{OH})_2\text{D}_3$ for patients with TGCT.

The role of GADD45 in the decrease of proliferation after stimulation with $1,25(\text{OH})_2\text{D}_3$.

The observations regarding the expression of *GADD45* are also noteworthy. $1,25(\text{OH})_2\text{D}_3$ induces up-regulation of *GADD45* in ovarian tumours, colon cancer and leukaemia cells. *GADD45* is involved in the activation of various genes. These genes are involved, among other functions, in the replication of DNA and G_1 cell cycle arrest, and by repression of proto-oncogenes, *GADD45* has antiproliferative effects in tumor cells. It acts directly proapoptotically and affects DNA repair (34). Both investigated TGCT cell lines, NCCIT and NTERA-2, express *GADD45*. Interestingly, there is an up-regulation of *GADD45* in both TGCT cell lines after stimulation with $1,25(\text{OH})_2\text{D}_3$. These findings may indicate that $1,25(\text{OH})_2\text{D}_3$ has the ability to increase the expression of *GADD45* and its effect on

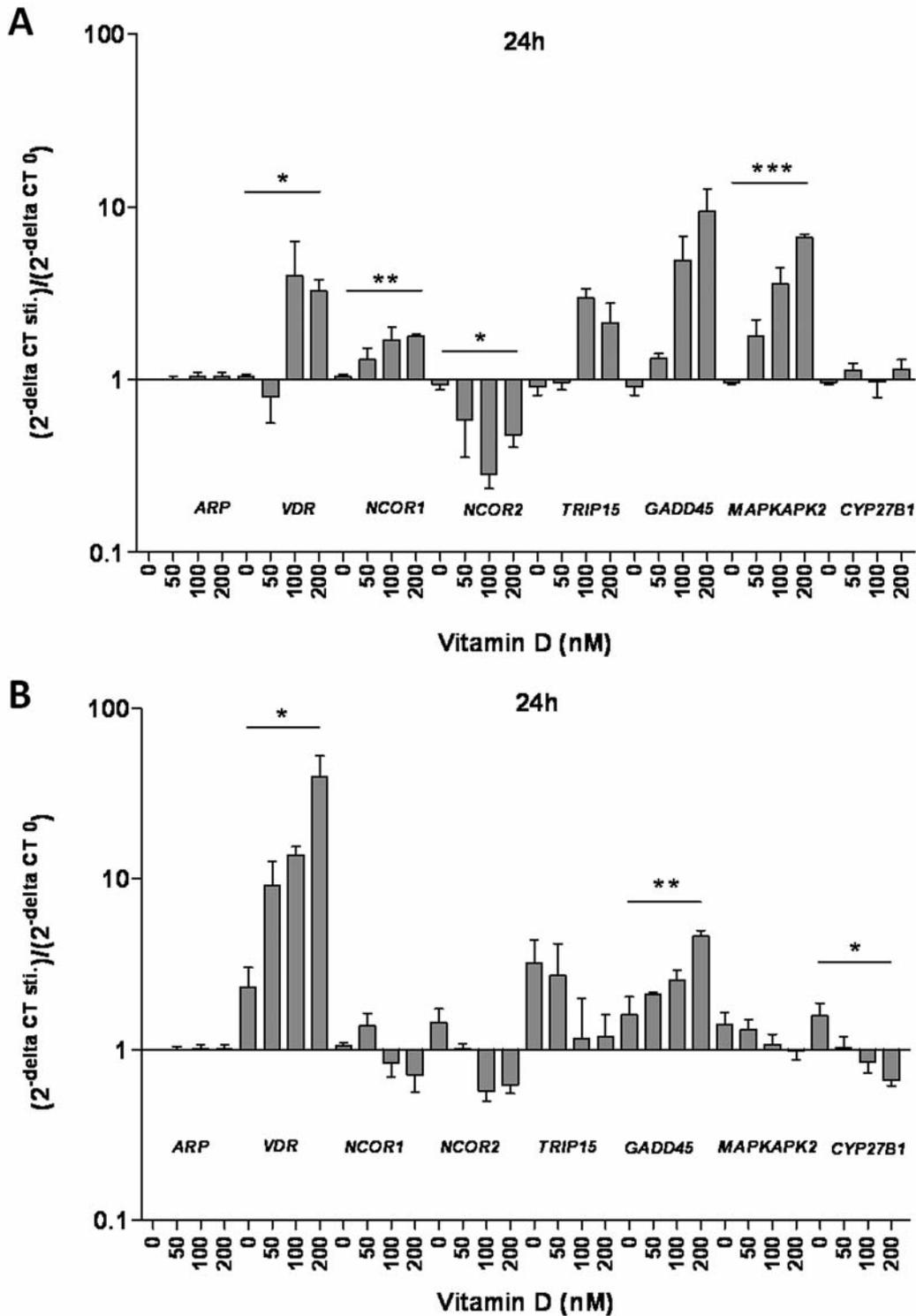


Figure 4. mRNA expression of $1,25(\text{OH})_2\text{D}_3$ regulated genes in TGCT cell lines after $1,25(\text{OH})_2\text{D}_3$ stimulation. The expression of VDR and co-regulators was investigated by qRT-PCR in the TGCT cell line NCCIT. The tumour cell line expresses VDR and co-regulators. After stimulation with 50, 100 and 200 nM $1,25(\text{OH})_2\text{D}_3$, VDR, nuclear receptor co-repressor 1 (NCOR1) and MAP kinase-activated protein kinase 2 (MAPKAPK2) gene expression significantly increased, while expression of nuclear receptor co-repressor 2 (NCOR2) significantly decreased. The other co-regulators showed no significant change in expression after $1,25(\text{OH})_2\text{D}_3$ stimulation (A). The tumour cell line NTERA-2 also expresses VDR and co-regulators. After stimulation with 50, 100 and 200 nM $1,25(\text{OH})_2\text{D}_3$, there was a small increase of VDR expression. GADD45 significantly increased in expression after stimulation with $1,25(\text{OH})_2\text{D}_3$, whereas a slight decrease of CYP27B1 expression was observed. The other co-regulators showed no significant change in gene expression (B).

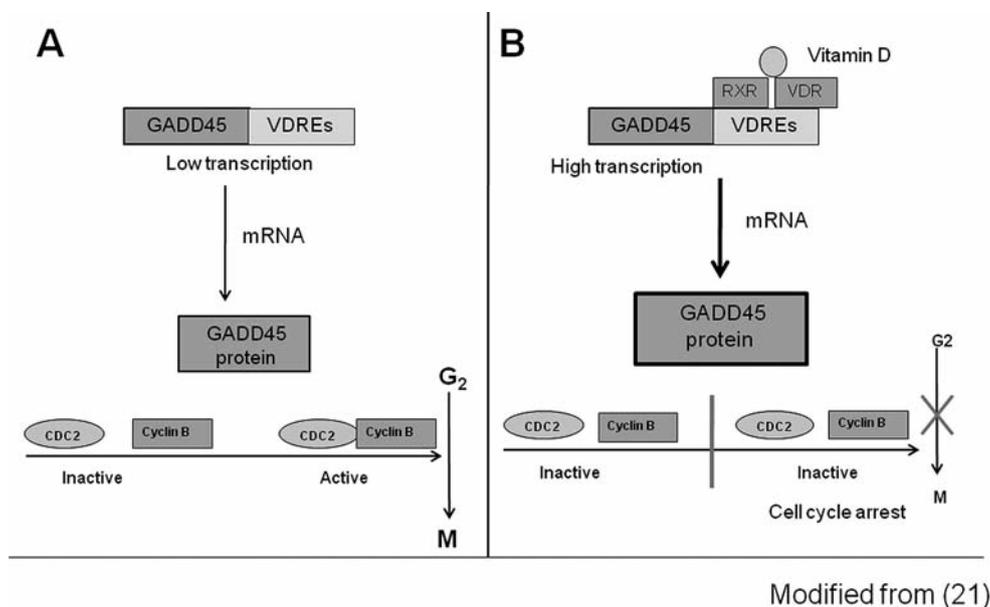


Figure 5. Stimulation with $1,25(\text{OH})_2\text{D}_3$ results in a GADD45-mediated cell cycle arrest. Cyclin B is a regulatory subunit of Cyclin-dependent kinase 1 (encoded by gene *CDC2*). The active CDC2/cyclin B complex mediates the transition from the G₂ to the M phase of the cell cycle (A). Stimulation with vitamin D induces the transcription of GADD45, while binding at VDREs. GADD45 prevents the connection between CDC2 and cyclin B and mediates cell cycle arrest (B).

differentiation and growth of TGCT. Interestingly, vitamin D directly induces an increase in transcription of *GADD45*. The increase in *GADD45* protein expression leads to a decrease of cyclin B, the regulatory subunit of Cyclin-dependent kinase 1 (encoded by gene *CDC2*). This induces a cell cycle arrest in the transition from the G₂ into the M phase (21, 35). This mechanism may also explain the decrease in proliferation after vitamin D stimulation in both investigated TGCT cell lines (Figure 5).

Regulation of vitamin D₃ polymorphism in TGCT. As described above, *NCOR1* and *NCOR2* are nuclear co-repressors which support nuclear receptors in the down-regulation of target gene expression. Tzelepi *et al.* (36) correlated *NCOR1* expression with morbidity-free interval. It seems that the impaired regulation of vitamin D₃-induced genes such as *NCOR1* contributes to the initiation and progression of colorectal cancer (36). The expression of *NCOR1* in TGCT demonstrated here may play a role in a better prognosis for patients with TGCT, especially in nonseminoma. The decrease of *NCOR2* gene expression in both TGCT cell lines after stimulation with $1,25(\text{OH})_2\text{D}_3$ could have a regulatory effect on the interaction between VDR/ retinoic acid-X receptors (RXR).

TRIP15 is a protein involved in the regulation of various cell signalling pathways and the cell cycle. It also has an influence on thyroid hormone and RXR (37). Both investigated TGCT cell lines express TRIP15, but there was

only a slight increase of mRNA expression in the NCCIT cell line after stimulation with $1,25(\text{OH})_2\text{D}_3$. Whether TRIP15, as a subunit of the CSN2 COP9/signalosome (38), influences the transcription of the VDR has not been studied so far. These findings, however, could be used as an approach for further investigations.

Kumar *et al.* (39) demonstrated the role of MAPKAPK2 in bladder cancer and confirmed the results presented by Xu and colleagues on the role of MAPKAPK2 in the invasion of prostate cancer (40). Kotlyarov and colleagues (41) also underlined the importance of MAPKAPK2 in the metastasis of tumour cells. Interestingly with regard to the TGCT cell lines expressing MAPKAPK2, there was a significant increase in *MAPKAPK2* mRNA concentration after stimulation with $1,25(\text{OH})_2\text{D}_3$ in the NCCIT cell line. MAPKAPK2 expression in TGCT cell lines may play a role in the pathogenesis of TGCT and may have an effect in metastasis.

CYP27B1 and *CYP24A1* are two key genes in the regulation of vitamin D₃ metabolism. In our examination of NTERA-2, we recorded expression of *CYP24A1* after stimulation with vitamin D₃. However vitamin D₃ had no effect on the protein concentration of *CYP24A1* in the TGCT cell lines in cell culture experiments. *CYP24A1* is not expressed in the tumour cell line NCCIT. Besides its occurrence in the kidneys, 1α -hydroxylase is also found in extrarenal tissues. It is possible that local conversion of $25(\text{OH})\text{D}_3$ to $1,25(\text{OH})_2\text{D}_3$ takes place in various tissues such as colon, brain, placenta, pancreas, lymph nodes and

skin. In the early stages of well-differentiated colon cancer, there is an increased expression of *CYP27B1*. In poorly differentiated stages of colon cancer, a decrease of *CYP27B1* expression was observed (28, 42). Increased expression of *CYP27B1* in cancer tissue speaks for a local conversion of 25(OH)D₃ to 1,25(OH)₂D₃ and, in theory, 25(OH)D₃ and 1,25(OH)₂D₃ have a chemo preventative role in these tumours (43). An increased *CYP24A1* mRNA expression in tumours results in reduced 1,25(OH)₂D₃ concentration. A high *CYP24A1* mRNA expression may counteract the antiproliferative effect of 1,25(OH)₂D₃.

Cross *et al.* (44) showed that the increased expression of *CYP24A1* would support a simultaneous reduction of the gene product of *CYP27B1* in high-grade colon cancer. While only a low expression of *CYP24A1* mRNA was recorded in normal ovary, lung, colon and breast tissues, increased expression of *CYP24A1* mRNA occurred in tumour tissues from lung, ovary and colon. In tumour tissue of the breast, however, there was a decrease in expression. This suggests that 1,25(OH)₂D₃ levels might be reduced in these cases (44-48).

In conclusion, our data suggest that TGCT express VDR. While IGCNU and seminomas exhibited only a slight cytoplasmic expression of VDR, all investigated nonseminomas had a strong cytoplasmic and nuclear expression. The data also suggest that vitamin D could have an inhibitory effect on proliferation *via* an increase expression of *GADD45* and therefore might have a role in antitumour therapy. We demonstrated that primary TGCT and TGCT cell lines express VDR and that the stimulation of TGCT cells with 1,25(OH)₂D₃ leads to up-regulation of VDR and inhibition of proliferation in tumour cells.

Conflict of Interest Statement

In regard to the present publication, there is no conflict of interest with other institutions or companies.

Acknowledgements

The Authors thank Anke Klages for technical assistance.

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Received September 29, 2011

Revised November 2, 2011

Accepted November 3, 2011