Vitamin D Inhibits Ovarian Cancer Cell Line Proliferation in Combination with Celecoxib and Suppresses Cyclooxygenase-2 Expression

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Abstract. *Background: Vitamin D exhibits multiple anti-proliferative and pro-differentiating actions. Prostaglandin-(PG)E2 is a tumor-promoting tissue hormone anabolized by cyclooxygenase-2 (COX-2). Recently, a link between the PG and vitamin D metabolism was reported. Materials and Methods: The influence of calcitriol and celecoxib on the proliferation of ovarian cancer cell lines was measured and the impact of calcitriol on the protein and mRNA expression of COX-2 was quantified by western blot and qRT-PCR, respectively. Results: After COX-2 induction with interleukin (IL)-1β, 10 μM celecoxib did not significantly inhibit the proliferation of OVCAR-3 cells, whereas calcitriol showed such an effect; however, the combination of the two substances had an additive influence. After induction by IL-1β, calcitriol inhibited the COX-2 protein, as well as its mRNA expression significantly in OVCAR-3 and SKOV-3 cells. Conclusion: These data suggest a correlation between PG and vitamin D metabolism in their anti-tumorigenic activity in ovarian carcinomas.*

The estimated annual incidence of ovarian cancer in the USA for 2014 is 21,980 and approximately 14,270 deaths are expected (1). In Germany 7,790 new ovarian cancer cases were diagnozed and the estimated number for 2014 of new ovarian cancer cases in Germany is 7,500. In 2010, 5,599 women died of ovarian cancer (2). Thus, ovarian cancer is the leading cause of death from gynecological malignancies.

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Traditionally, it has been accepted that epithelial ovarian cancer risk is mediated by incessant ovulation and the accompanied inflammatory processes that were caused by repeated trauma to the epithelial surface of the ovary. However, during the last few years there has been a shift in thinking since the carcinogenesis of high-grade serous ovarian carcinoma and candidate precursor lesion was identified in the fallopian tube, called serous tubal intra-epithelial carcinoma (3). For invasive low-grade serous carcinoma, it is known that it arises from serous ovarian borderline tumor.

Nevertheless, inflammatory processes are obligatory for ovarian carcinogenesis and the two isoenzymes cyclooxygenase 1 and 2 (COX-1 and COX-2) are involved in controlling all the inflammatory processes by mediating the prostaglandin (PG) synthesis from arachidonic acid.

Prognostic markers are necessary to estimate the risk of recurrence and mortality. For ovarian cancer, established molecular prognostic parameters, and new bio- and genomic markers that correlate with poor prognosis are missing. Thus, there is an unmet need for new biomarkers and COX-2 could be such a new prognostic marker.

In contradiction to the ubiquitously expressed COX-1 isoenzyme, COX-2 expression is induced in specific tissues by growth factors, cytokines and PGs and is seen as a prognostic factor for malignancy. COX-2 over-expression in ovarian carcinogenesis is associated with increased proliferation, reduced apoptosis and mediation of neoangiogenesis (4-5). Prostaglandin E2 (PGE2), one of the end-products of PG synthesis, regulates several key processes of tumor growth in different carcinomas (6).

The biologically-active form of vitamin D (calcitriol, 1,25-dihydroxycholecaldiferol, $1,25(OH)_2D_3$) has an antiproliferative effect on cancer cells (7). The effect of $1,25(OH)_2D_3$ is mediated by binding to the vitamin D receptor (VDR) that afterwards binds to specific promoter sequences of the genomic

DNA and regulates the transcriptional processing of genes that are relevant for vitamin D metabolism (8).

Obviously, a combination of calcitriol and non-steroidal anti-inflammatory drugs (NSAIDs) has a potential synergistic inhibiting effect on the tumorigenesis of breast cancer. Several studies have revealed that a combination of calcitriol and NSAIDs might be a useful chemopreventive and/or therapeutic strategy in prostate cancer (9-11).

Recently, own studies have suggested a link between the PG and vitamin D metabolisms in breast tumor cells (12) and breast cancer tissue (13); moreover, also in ovarian cancer tissue (14, 15).

Consequently, we hypothesize that a combination of COX-2 inhibition and calcitriol could increase an anti-proliferative effect on ovarian cancer cells and could offer new prevention or treatment approaches in the future. The aim of the present study was to analyze the influence of calcitriol and the selective COX-2 inhibitor celecoxib on the proliferation of ovarian cancer cell lines. We also examined the influence of calcitriol on COX-2 expression in ovarian cancer cell lines.

Materials and Methods

Cell culture and treatment. The ovarian cancer cell lines SKOV-3 and OVCAR-3 were purchased from LGC-Standards (Wesel, Germany). The cells were cultured in RPMI-1640 (Life Technologies, Karlsruhe, Germany) with 10% fetal calf serum (FCS) and 1% penicillin/streptomycin (both from PAA-Laboratories, Cölbe, Germany) at 37˚C in a humidified atmosphere containing 5% CO_2. For treatment, the cells were plated in full media overnight in 96-well or 6-well dishes to perform growth-assays or protein and mRNA analyses, respectively. The next day media were changed to 3% FCS containing the test-substances. For every concentration used, the substances were dissolved and diluted in pure DMSO at 1,000 × stocks for 1α,25-dihydroxyvitamin D3 (calcitriol) and 10,0000 × stocks for celecoxib (both from Sigma, Taufkirchen, Germany). Therefore, 0.1% DMSO was used as a general solvent control.

Proliferation assays. To measure the influence of calcitriol on the proliferative activity in ovarian cancer cell lines 20,000 and 3,000 cells per well for BrdU and MTT-assays, respectively, were plated on 96-well plates in media with 10% FCS. After 24 h, the media were exchanged with treatment media containing 3% FCS. Cells treated with calcitriol and/or celecoxib concentrations ranging from 0.1 to 10 μM were compared to solvent controlled cells with 0.1% DMSO added. After 24 h of treatment, the Cell Proliferation ELISA, BrdU (Roche, Mannheim, Germany) was performed following the manufacturer's recommendations. For MTT-assays, the media were exchanged after 72 h of treatment to 100 μl colorless media containing 10% MTT solution (5 mg/ml thiazolyl blue in PBS; Sigma) and cultivated for 4 h. Then, the reaction was stopped and dissolution of formazan crystals was performed in the dark overnight by adding 100 μl stop-solution (10% (w/v) SDS, 50% N,N-dimethylformamide, pH 4.7). A microplate-reader was used to measure the absorption of the wells at 560 nm (Dynatech Laboratories-MRX, DPC Biermann, Bad Nauheim, Germany).

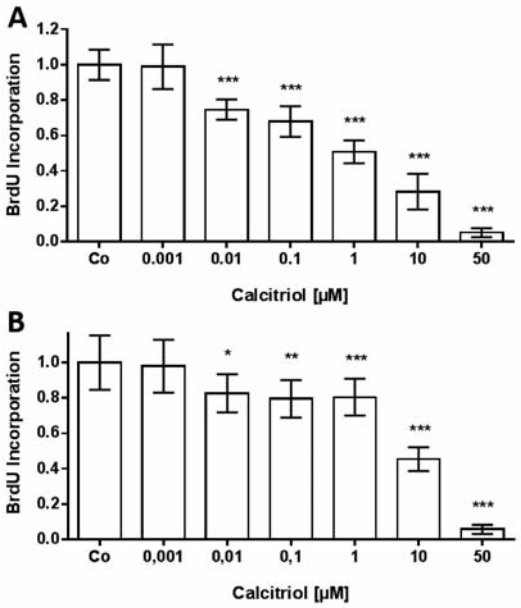

Figure 1. *Calcitriol inhibits the proliferation of ovarian cancer cell lines. The BrdU-ELISA in (A) OVCAR-3 and (B) in SKOV-3 cell line decreases proliferative activity after 24 h of treatment. Statistical significance is shown at p-values of * <0.05; ** <0.01 and *** <0.001.*

Immunochemical detection. The content of COX-2 protein in cells after treatment was compared to solvent controlled cells on western blots using a monoclonal COX-2 antibody (clone 33; BD-Transduction Laboratories, Heidelberg, Germany) and a β-actin antibody (clone AC-15; Sigma). Two hundred thousand cells were cultured in full media for 24 h and then exchanged with treatment media containing 3% FCS. Total protein was isolated 24 h later with MPER-buffer (GE healthcare, München, Germany). Equal amounts of proteins were resolved in 10% SDS-polyacrylamid gelelectrophoresis and transferred to nitrocellulose membranes. Blocking in TBST with 5% nonfat dry milk was followed by antibody incubation overnight at 4˚C. Using horseradish-peroxidase labeled sheep anti-mouse antibodies (GE Healthcare), the immunoreactive bands were detected with the Immobilon-Western-Chemiluminescent-HRP-Substrate (Merck Millipore, Schwalbach, Germany) on Hyperfilm-ECL-Performance-Chemiluminescent-Film (GE Healthcare). The films were scanned and afterwards densitometrically analyzed with the Easy-Win software (Herolab, Wiesloch, Germany).

Quantitative reverse transcription polymerase chain reaction (qRT-PCR). The mRNA expression of the COX-2 gene was evaluated by means of the quantitative reverse transcription polymerase chain reaction. Two hundred thousand cells were plated per well on a 6-well plate in growth media and replaced after 24 h by media containing 3% FCS and the substances. After an incubation period of 4 h, total RNA was isolated by using QIAzol (Qiagen, Hilden, Germany). One μg RNA was reverse transcribed with Superscript-II (Invitrogen, Karslruhe, Germany) using random-primers. QPCR was performed by using the Platinum-SYBR-Green-qPCR-Super-Mix-UDG (Invitrogen) on the realtime-PCR-system Opticon 2 (BioRad, Munich, Germany). To quantify the COX-2 gene, the Hs_PTGS2_1_SG-QuantiTect-

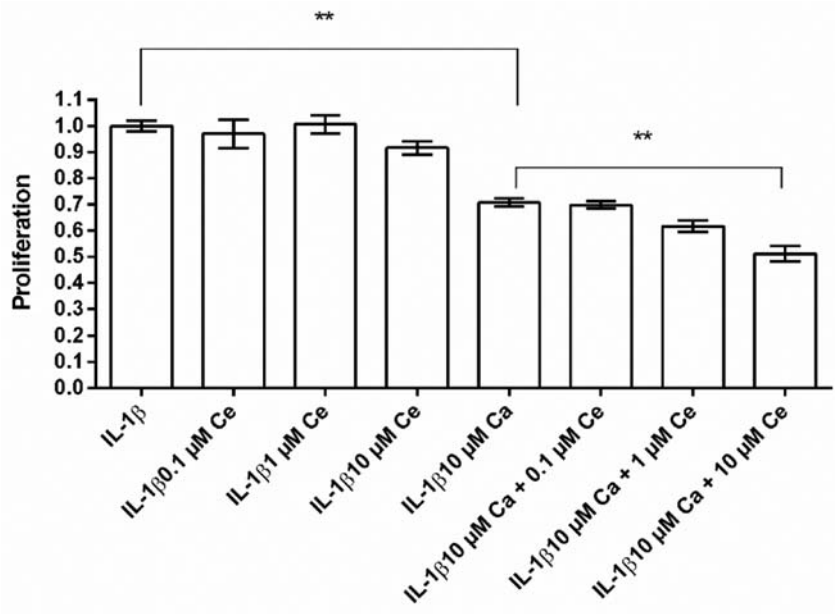

Figure 2. *A combination of celecoxib and calcitriol additively inhibits the proliferation of the OVCAR-3 cell line. The influence of a combination of calcitriol and celecoxib in the presence of IL-1β was measured after 72 h treatment by the MTT-assay. Statistical significance is shown at a p-value of ** <0.01. Ce, celecoxib; Ca, calcitriol.*

Primer-Assay (Qiagen) was used and for normalization a sequence of the hypoxanthine-guanine phosphoribosyltranferase 1 (*HPRT1*) was amplified with the primers forward: 5'-TCA GGC AGT ATA ATC CAA AGA TGG T-3' and reverse: 5'-AGT CTG GCT TAT ATC CAA CAC TTC G-3' (synthesized at Metabion, Martinsried, Germany). The PCR protocol consisted of 42 cycles for 15 s at 95˚C and 30 s at 60˚C. Each sample was tested in triplicates. The reaction efficiency of each primer pair had been determined using dilution series with a representative pool of cDNAs as proposed by Pfaffl (16). Data were further processed with the excel-based program REST-MCS©-version 2 (www.gene-quantification.info).

Results

Influence of calcitriol on proliferation. Calcitriol significantly inhibits the proliferation of both tested ovarian cancer cell lines at concentrations of 10 nM to 50 μM measured in BrdU-ELISA (Figure 1). At 10 μM calcitriol OVCAR-3 and SKOV-3 were inhibited to 28% and 45%, respectively, compared to the solvent control.

Growth influence of combined treatment with calcitriol and celecoxib. The combination of calcitriol and celecoxib in OVCAR-3 cells was measured by the MTT-assay after 72-h treatment. Results are shown in Figure 2. For the activation of COX-2 expression and to imitate an inflammatory environment, the cells were treated in parallel with 1 ng/ml IL-1β. Celecoxib at 0.1, 1 and 10 μM could not significantly influence the growth of OVCAR-3 cells. Ten μM calcitriol was inhibiting the proliferation of OVCAR-3 cells to 70% compared to the solvent control IL-1β-treated cells. Combining celecoxib with 10 μM calcitriol, a significant additive effect could be measured at 10 μM celecoxib when compared to calcitriol treatment alone.

COX-2 expression under the influence of calcitriol. The expression of the COX-2 protein in the ovarian cancer cells after treatment with IL-1β (Figure 3; part A; lane 3) was activated as shown in the western blot to a strong band at 72 kDA in the OVCAR-3, as well as in SKOV-3 cell line, while DMSO (lane 2) had no such effect. Ten μM calcitriol added to IL-1β inhibited the COX-2 protein expression compared to IL-1β alone to an obviously diminished band (lane 5) in both cell lines. Calcitriol alone (lane 4) had no effect on COX-2 expression. The expression of the *COX-2* gene is strongly activated after treatment with IL-1β as shown in Figure 3, part B for OVCAR-3 and in part C for SKOV-3 cells compared to the solvent containing control cultures. The addition of 10 μM calcitriol to IL-1β inhibited the activation of *COX-2* mRNA expression from 10 times to 4 times and from 4 times to 2 times in OVCAR-3 and SKOV-3 cells, respectively. Calcitriol alone had no effect on the *COX-2* expression.

Discussion

In the present study, we report for the first time, an additive inhibition of ovarian cancer cell proliferation by the combination of calcitriol and the selective COX-2 inhibitor celecoxib.

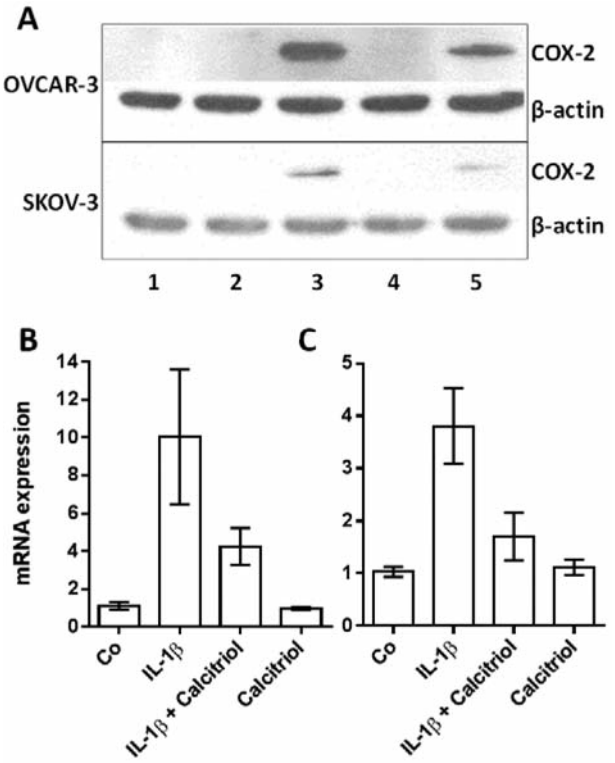

Figure 3. *Expression of COX-2 protein and COX-2 mRNA after treatment with calcitriol and celecoxib in the presence of the COX-2 activator IL-1β. (A) Western blot: lane 1, control; lane 2, DMSO control; lane 3, IL-1β; lane 4, 10 μM calcitriol; and lane 5, IL-1β and 10 μM calcitriol. β-actin antibody reaction is shown as a loading control of the Western blots. (B) and (C): relative mRNA expression of COX-2, whereas (B) is OVCAR-3 and (C) is SKOV-3. Co, control.*

As we treated ovarian cancer cells with calcitriol, we received a strong inhibition of proliferation measured in the very sensitive BrdU incorporation assay. This proves the effect, which has been published by other groups before in various cancer cell lines derived from *e.g.* breast cancer (17-19), as well as in ovarian cancer cell lines (20). To reach the next step, we focused on the effects of a combination of celecoxib and calcitriol in ovarian cancer cells and if this combination interferes with the PG pathway. Whereas calcitriol again exhibited a significant reduction of cell growth, this response was much smaller than that in the BrdU-ELISA used in the previous experiment. Celecoxib was not significantly decreasing the ovarian cancer cell growth, although there was an inhibiting tendency of 10%. Herein, we used the less sensitive MTT-assay after 72 h of treatment, which might have been unfavorable. Kim *et al.* (21) found a 20% growth inhibition and the induction of apoptosis by 10 μM celecoxib. Therefore, a growth inhibiting effect can be assumed. The most meaningful result from this combination experiment was the significant inhibition of the combined application with celecoxib and calcitriol over calcitriol alone. Furthermore, we were asking if there were an interaction between the pathways of vitamin D and PG and we measured the COX-2 expression under the influence of calcitriol. At a first attempt, we could not detect any visual band in western blots using a COX-2 antibody neither in SKOV-3 nor in OVCAR-3 cells. The COX-2 expression had to be induced by simulating an inflammatory environment, which could be successfully created by the application of IL-1β. The combined application of calcitriol with IL-1β strongly interfered with the induction of the COX-2 expression. This could be shown on protein level, as well at the level of mRNA expression (*COX-2*). Therefore, we presume a genomic regulation of the *COX-2* gene by calcitriol. Recent publications reported of this regulative interaction found in MCF-7 breast cancer cells (22-23). Krishnan *et al.* performed a microarray based expression analysis of calcitriol application in various cancer cells and they found a reduction of the COX-2 mRNA in prostate cancer cells (24).

In several studies, COX-2 over-expression is associated with an increased proliferation and neoagiogenesis and, therefore, with a poor outcome of ovarian cancer patients (4-5, 25) and the antiproliferative effects of COX-2 inhibitors on cancer cells *in vitro* and *in vivo* are well known (21, 26-27). These facts lead to the idea of a COX-2 inhibition to gain a survival benefit for ovarian cancer patients. The selective COX-2 inhibitor celecoxib is able to induce apoptosis in different types of cancer (21, 28). Promising clinical data were presented by Legge *et al.* who included heavily pre-treated patients in a phase II study. The authors observed a good tolerability and activity of celecoxib added to carboplation chemotherapy (29). However, a recently published phase II study by Reyners *et al.* did not show significant effects of 400 mg celecoxib on disease-free and overall survival when added to first-line chemotherapy in ovarian cancer patients (FIGO IC-FIGO IV) after surgery (30).

Several epidemiological studies have been published regarding the association between vitamin D and ovarian cancer risk; however all studies showed either no, or not a clear or only marginal associations of serum vitamin D levels with the risk of ovarian cancer (14-15, 31-32). Additionally, a meta-analysis by Yin *et al.* could not find any statistical significant inverse association between 25(OH)D serum level and ovarian cancer risk (33). In an actually published meta-analysis about *VDR* polymorphisms (vitamin D receptor) and ovarian cancer risk, only the *FokI* polymorphisms (in the vitamin D receptor gene (*VDR*)) were associated with an increased ovarian cancer risk (34). However, experimental data support a role of the vitamin D pathway in ovarian carcinogenesis. A number of studies have observed that 1,25-dihydroxyvitamin D inhibits ovarian cancer cell growth (35-

37) and increases apoptosis (38). The Vitamin D analog EB1089 inhibits the proliferation of multiple ovarian cancer cell lines (including OVCAR-3) and reduces the growth of OVCAR-3 tumor xenografts in nude mice (39). In SCOV-3 cells, the first non-hypercalcaemic vitamin-D derivative MT19c had a higher cytotoxicity than EB1089 (40). Furthermore, Lange *et al*. utilized the calcidiol derivative B3CD in SKOV-3 and OVCAR-3 cells and reported a concentration-dependent cytotoxic and growth inhibitory effect in both cell lines (20). Moreover, high 1,25-dihhydroxyvitamin D levels are able to increase VDR expression in ovarian cancer cell lines (38). As ovarian tissue expresses the VDR (15, 35, 37), existing data suggest that ovarian cancer has a functional vitamin D pathway that could potentially be a target for treatment or prevention (32). However, in our previous work we observed a significant lower VDR expression in ovarian cancer compared to normal tissue (15).

Based on several studies, summarized in a recent published review by Ma and co-workers, that have described the anti-proliferative and pro-apoptotic effect of calcitriol, calcitriol depicts a promising approach for the treatment of cancer (41). Calcitriol acts in multiple ways to influence cancer cell proliferation. It controls genes in PG metabolism, supports anti-inflammatory effects by an up-regulation of the mitogen-activated protein kinase phosphatase-5 expression (42), it influences the activation of nuclear factor-kappaB (NF-κB) (43). NF-κB is a transcription factor that regulates several genes involved in inflammatory and immune processes and likely plays a key role in the development from inflammation to carcinogenesis (44).

Calcitriol has been utilized in a number of pre-clinical and clinical studies as an antitumor agent in different carcinomas but not in ovarian cancer (45). Pre-clinical data suggest that calcitriol has to be given in very high amounts to induce antitumor effects and clinical data do indicate that very high doses of calcitriol (>100 μg weekly, intravenously and 0.15 μg/kg weekly orally) can be given safely (45). However, the maximum tolerated dose (MTD) of calcitriol is unclear. Because of the potential hypercalcaemic impact and the lack of a suitable available oral preparation, application is restricted and vitamin D analogues should be preferred (46-47). Some clinical trials regarding the use of calcitriol in combination with chemotherapy for cancer treatment exist, but the results are not satisfying (45). In the ASCENT I trial (AIPC Study of Calcitriol Enhancing Taxotere), pre-treated and advanced prostate cancer patients were treated with docetaxel + calcitriol but the study arm treatment was associated with shorter survival than the control arm (48).

Experiments with prostate cancer cells have shown that calcitriol inhibits cancer cell proliferation by influencing the PG metabolism: suppression of COX-2 expression, up-regulation of 15-hydroxyprostaglandindehydrogenase (15-PGDH) and reduction of PG receptors (9-11, 49).

While inhibition of COX-2 in ovarian cancer is already under clinical evaluation, data to an inhibition with calcitriol, or even with the combination of calcitriol and COX-2 inhibition, are lacking. Our group published the only data showing a link between the PG and vitamin D metabolism in ovarian cancer cells and tissue. We demonstrated an inverse correlation between the vitamin D receptor (VDR) and the COX-2 expression and a lower PGE2 EP2 and EP4 receptor expression in ovarian cancer tissue (50). Moreover, we published data of ovarian cancer patients who had a higher PGE_2 level compared to healthy women (14).

Our data suggest a correlation between vitamin D activities and the PG metabolism in ovarian carcinomas. Further research is required if the synergistic effect of this approach is a useful therapeutic strategy in treatment of ovarian cancer.

Acknowledgements

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References

1 Cancer Facts & Figures 2014. American Cancer Society, Atlanta, 2014.
2 Krebs in Deutschland 2009/2010. Vol. 9 (Robert Koch-Institut (Hrsg) und die Gesellschaft der epidemiologischen Krebsregister in Deutschland e.V. (Hrsg), Berlin, 2013).
3 Vang R, Shih Ie M and Kurman RJ: Fallopian tube precursors of ovarian low- and high-grade serous neoplasms. Histopathology *62*: 44-58, 2013.
4 Fujimoto J, Toyoki H, Sato E, Sakaguchi H, Jahan I, Alam SM and Tamaya T: Expression of cyclooxygenase-2 related to angiogenesis in uterine cervical cancers. J Biomed Sci *13*: 825-832, 2006.
5 Yoshida A, Sarian LO, Andrade LA, Pignataro F, Pinto GA and Derchain SF: Cell proliferation activity unrelated to COX-2 expression in ovarian tumors. Int J Gynecol Cancer *17*: 607-614, 2007.
6 Rask K, Zhu Y, Wang W, Hedin L and Sundfeldt K: Ovarian epithelial cancer: a role for PGE2-synthesis and signalling in malignant transformation and progression. Mol Cancer *5*: 62, 2006.
7 Dusso AS: Update on the biologic role of the vitamin d endocrine system. Curr Vasc Pharmacol *12*: 272-277, 2014.
8 Mamede AC, Tavares SD, Abrantes AM, Trindade J, Maia JM and Botelho MF: The role of vitamins in cancer: a review. Nutr Cancer *63*: 479-494, 2011.
9 Krishnan AV and Feldman D: Molecular pathways mediating the anti-inflammatory effects of calcitriol: implications for prostate cancer chemoprevention and treatment. Endocr Relat Cancer *17*: R19-38, 2010.
10 Krishnan AV, Moreno J, Nonn L, Swami S, Peehl DM and Feldman D: Calcitriol as a chemopreventive and therapeutic agent in prostate cancer: role of anti-inflammatory activity. J Bone Miner Res *22(Suppl 2)*: V74-80, 2007.

11 Moreno J, Krishnan AV, Swami S, Nonn L, Peehl DM and Feldman D: Regulation of prostaglandin metabolism by calcitriol attenuates growth stimulation in prostate cancer cells. Cancer Res *65*: 7917-7925, 2005.

12 Thill M, Fischer D, Becker S, Cordes T, Dittmer C, Diedrich K, Salehin D and Friedrich M: Prostaglandin metabolizing enzymes in correlation with vitamin D receptor in benign and malignant breast cell lines. Anticancer Res *29*: 3619-3625, 2009.

13 Cordes T, Fischer D, Thill M, Becker S, Friedrich M and Salehin D: Vitamin D-1alpha-hydroxylase and vitamin D-24-hydroxylase in benign and malign breast tissue. Eur J Gynaecol Oncol *31*: 151-155, 2010.

14 Cordes T, Hoellen F, Dittmer C, Salehin D, Kummel S, Friedrich M, Koster F, Becker S, Diedrich K and Thill M: Correlation of prostaglandin metabolizing enzymes and serum PGE2 levels with vitamin D receptor and serum 25(OH)2D3 levels in breast and ovarian cancer. Anticancer Res *32*: 351-357, 2012.

15 Thill M, Fischer D, Kelling K, Hoellen F, Dittmer C, Hornemann A, Salehin D, Diedrich K, Friedrich M and Becker S: Expression of vitamin D receptor (VDR), cyclooxygenase-2 (COX-2) and 15-hydroxyprostaglandin dehydrogenase (15-PGDH) in benign and malignant ovarian tissue and 25-hydroxycholecalciferol (25(OH2)D3) and prostaglandin E2 (PGE2) serum level in ovarian cancer patients. J Steroid Biochem Mol Biol *121*: 387-390, 2010.

16 Pfaffl MW: A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res *29*: e45, 2001.

17 Moreno J, Krishnan AV, Peehl DM and Feldman D: Mechanisms of vitamin D-mediated growth inhibition in prostate cancer cells: inhibition of the prostaglandin pathway. Anticancer Res *26*: 2525-2530, 2006.

18 Swami S, Raghavachari N, Muller UR, Bao YP and Feldman D: Vitamin D growth inhibition of breast cancer cells: gene expression patterns assessed by cDNA microarray. Breast Cancer Res Treat *80*: 49-62, 2003.

19 Thill M, Cordes T, Hoellen F, Becker S, Dittmer C, Kummel S, Salehin D, Friedrich M, Diedrich K and Koster F: Influence of calcitriol on prostaglandin- and vitamin D-metabolising enzymes in benign and malignant breast cell lines. Anticancer Res *32*: 359-365, 2012.

20 Lange TS, Stuckey AR, Robison K, Kim KK, Singh RK, Raker CA and Brard L: Effect of a vitamin D(3) derivative (B3CD) with postulated anti-cancer activity in an ovarian cancer animal model. Invest New Drugs *28*: 543-553, 2010.

21 Kim HJ, Yim GW, Nam EJ and Kim YT: Synergistic Effect of COX-2 Inhibitor on Paclitaxel-Induced Apoptosis in the Human Ovarian Cancer Cell Line OVCAR-3. Cancer Res Treat *46*: 81-92, 2014.

22 Wang Q, He Y, Shen Y, Zhang Q, Chen D, Zuo C, Qin J, Wang H, Wang J and Yu Y: Vitamin D inhibits COX-2 expression and inflammatory response by targeting thioesterase superfamily member 4. J Biol Chem *289*: 11681-11694, 2014.

23 Yuan L, Jiang R, Yang Y, Ding S and Deng H: 1,25-Dihydroxyvitamin D3 inhibits growth of the breast cancer cell line MCF-7 and downregulates cytochrome P4501B1 through the COX-2/PGE2 pathway. Oncol Rep *28*: 2131-2137, 2012.

24 Krishnan AV, Shinghal R, Raghavachari N, Brooks JD, Peehl DM and Feldman D: Analysis of vitamin D-regulated gene expression in LNCaP human prostate cancer cells using cDNA microarrays. Prostate *59*: 243-251, 2004.

25 Lee JY, Myung SK and Song YS: Prognostic role of cyclooxygenase-2 in epithelial ovarian cancer: a meta-analysis of observational studies. Gynecol Oncol *129*: 613-619, 2013.

26 Narayanan BA, Narayanan NK, Pittman B and Reddy BS: Regression of mouse prostatic intraepithelial neoplasia by nonsteroidal anti-inflammatory drugs in the transgenic adenocarcinoma mouse prostate model. Clin Cancer Res *10*: 7727-7737, 2004.

27 Srinath P, Rao PN, Knaus EE and Suresh MR: Effect of cyclooxygenase-2 (COX-2) inhibitors on prostate cancer cell proliferation. Anticancer Res *23*: 3923-3928, 2003.

28 Jendrossek V: Targeting apoptosis pathways by Celecoxib in cancer. Cancer Lett *332*: 313-324, 2013.

29 Legge F, Paglia A, D'Asta M, Fuoco G, Scambia G and Ferrandina G: Phase II study of the combination carboplatin plus celecoxib in heavily pre-treated recurrent ovarian cancer patients. BMC Cancer *11*: 214, 2011.

30 Reyners AK, de Munck L, Erdkamp FL, Smit WM, Hoekman K, Lalisang RI, de Graaf H, Wymenga AN, Polee M, Hollema H, van Vugt MA, Schaapveld M and Willemse PH: A randomized phase II study investigating the addition of the specific COX-2 inhibitor celecoxib to docetaxel plus carboplatin as first-line chemotherapy for stage IC to IV epithelial ovarian cancer, Fallopian tube or primary peritoneal carcinomas: the DoCaCel study. Ann Oncol *23*: 2896-2902, 2012.

31 Toriola AT, Surcel HM, Agborsangaya C, Grankvist K, Tuohimaa P, Toniolo P, Lukanova A, Pukkala E and Lehtinen M: Serum 25-hydroxyvitamin D and the risk of ovarian cancer. Eur J Cancer *46*: 364-369, 2010.

32 Tworoger SS, Gates MA, Lee IM, Buring JE, Titus-Ernstoff L, Cramer D and Hankinson SE: Polymorphisms in the vitamin D receptor and risk of ovarian cancer in four studies. Cancer Res *69*: 1885-1891, 2009.

33 Yin L, Grandi N, Raum E, Haug U, Arndt V and Brenner H: Meta-analysis: Circulating vitamin D and ovarian cancer risk. Gynecol Oncol *121*: 369-375, 2011.

34 Xu Y, He B, Pan Y, Deng Q, Sun H, Li R, Gao T, Song G and Wang S: Systematic review and meta-analysis on vitamin D receptor polymorphisms and cancer risk. Tumour Biol *35*: 4153-4169, 2014.

35 Ahonen MH, Zhuang YH, Aine R, Ylikomi T and Tuohimaa P: Androgen receptor and vitamin D receptor in human ovarian cancer: growth stimulation and inhibition by ligands. Int J Cancer *86*: 40-46, 2000.

36 Jiang F, Li P, Fornace AJ Jr., Nicosia SV and Bai W: G2/M arrest by 1,25-dihydroxyvitamin D3 in ovarian cancer cells mediated through the induction of GADD45 via an exonic enhancer. J Biol Chem *278*: 48030-48040, 2003.

37 Saunders DE, Christensen C, Lawrence WD, Malviya VK, Malone JM, Williams JR and Deppe G: Receptors for 1,25-dihydroxyvitamin D3 in gynecologic neoplasms. Gynecol Oncol *44*: 131-136, 1992.

38 Jiang F, Bao J, Li P, Nicosia SV and Bai W: Induction of ovarian cancer cell apoptosis by 1,25-dihydroxyvitamin D3 through the down-regulation of telomerase. J Biol Chem *279*: 53213-53221, 2004.

39 Zhang X, Jiang F, Li P, Li C, Ma Q, Nicosia SV and Bai W: Growth suppression of ovarian cancer xenografts in nude mice by vitamin D analogue EB1089. Clin Cancer Res *11*: 323-328, 2005.

40 Brard L, Lange TS, Robison K, Kim KK, Ara T, McCallum MM, Arnold LA, Moore RG and Singh RK: Evaluation of the first Ergocalciferol-derived, non hypercalcemic anti-cancer agent MT19c in ovarian cancer SKOV-3 cell lines. Gynecol Oncol *123*: 370-378, 2011.
41 Ma Y, Trump DL and Johnson CS: Vitamin D in combination cancer treatment. J Cancer *1*: 101-107, 2010.
42 Nonn L, Peng L, Feldman D and Peehl DM: Inhibition of p38 by vitamin D reduces interleukin-6 production in normal prostate cells via mitogen-activated protein kinase phosphatase 5: implications for prostate cancer prevention by vitamin D. Cancer Res *66*: 4516-4524, 2006.
43 McCarty MF: Targeting multiple signaling pathways as a strategy for managing prostate cancer: multifocal signal modulation therapy. Integr Cancer Ther *3*: 349-380, 2004.
44 Maeda S and Omata M: Inflammation and cancer: role of nuclear factor-kappaB activation. Cancer Sci *99*: 836-842, 2008.
45 Trump DL, Deeb KK and Johnson CS: Vitamin D: considerations in the continued development as an agent for cancer prevention and therapy. Cancer J *16*: 1-9, 2010.
46 Aparna R, Subhashini J, Roy KR, Reddy GS, Robinson M, Uskokovic MR, Venkateswara Reddy G and Reddanna P: Selective inhibition of cyclooxygenase-2 (COX-2) by 1alpha,25-dihydroxy-16-ene-23-yne-vitamin D3, a less calcemic vitamin D analog. J Cell Biochem *104*: 1832-1842, 2008.
47 Masuda S and Jones G: Promise of vitamin D analogues in the treatment of hyperproliferative conditions. Mol Cancer Ther *5*: 797-808, 2006.
48 Scher HI, Jia X, Chi K, de Wit R, Berry WR, Albers P, Henick B, Waterhouse D, Ruether DJ, Rosen PJ, Meluch AA, Nordquist LT, Venner PM, Heidenreich A, Chu L and Heller G: Randomized, open-label phase III trial of docetaxel plus high-dose calcitriol versus docetaxel plus prednisone for patients with castration-resistant prostate cancer. J Clin Oncol *29*: 2191-2198, 2011.
49 Peehl DM, Krishnan AV and Feldman D: Pathways mediating the growth-inhibitory actions of vitamin D in prostate cancer. J Nutr *133*: 2461S-2469S, 2003.
50 Thill M, Fischer D, Hoellen F, Kelling K, Dittmer C, Landt S, Salehin D, Diedrich K, Friedrich M and Becker S: Prostaglandin metabolising enzymes and PGE2 are inversely correlated with vitamin D receptor and 25(OH)2D3 in breast cancer. Anticancer Res *30*: 1673-1679, 2010.

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