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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 ($25(OH_2)D_3$) and prostaglandin E_2 (PGE_2) serum level in ovarian cancer patients☆

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ABSTRACT

Ovarian carcinomas are associated with increased inflammation which is based upon an up-regulation of inducible cyclooxygenase-2 (COX-2). Moreover, based on our previous published data, the extra-renal vitamin D metabolism seems to be dysregulated in comparison to healthy tissue. In order to gain further insight into the prostaglandin (PG)- and vitamin D-metabolism in ovarian carcinomas, the study aimed to evaluate the expression of the PG metabolising enzymes COX-2 and 15-hydroxyprostaglandin dehydrogenase (15-PGDH) compared to the vitamin D receptor (VDR) in benign and malignant ovarian tissues. Additionally, we determined the 25-hydroxycholecalciferol ($25(OH_2)D_3$) serum levels. Expression of VDR, COX-2 and 15-PGDH was determined by Western blot analysis. Serum levels of $25(OH_2)D_3$ and PGE_2 were measured by chemiluminescence-based and colorimetric immunoassay. We detected significantly higher expressions of the PG metabolising enzymes 15-PGDH and COX-2 in malignant tissue and PGE_2 serum levels were 2-fold higher in tumour patients. Furthermore, we found an inverse correlation to the VDR-expression which was 62.1% lower in malignant tissues compared to that in benign tissues. Surprisingly, we could not detect any differences between the $25(OH_2)D_3$ serum levels in either group ($n = 20$). These data suggest a correlation between PG- and vitamin D-metabolism in ovarian carcinomas.

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1. Introduction

Several hypotheses have been proposed to explain ovarian carcinogenesis. The most widely accepted is that epithelial ovarian cancer risk is mediated by incessant ovulation and the accompanied inflammatory processes caused by repeated trauma to the epithelial surface of the ovary. The two isoenzymes COX-1 and COX-2 are involved in controlling all the inflammatory processes by mediating between the PG synthesis and the arachidonic acid. COX-1 seems to be linked to homeostatic processes and is ubiquitary expressed while COX-2 expression is stimulated by different growth factors, cytokines and prostaglandins and is seen as a prognostic factor for malignancy. COX-2 overexpression in ovarian carcinogenesis is associated with increased proliferation, reduced apoptosis and mediation of neoangiogenesis [1–3]. PGE_2, one of the end products of PG synthesis, regulates several key processes of tumour growth in different carcinomas [4]. 15-PGDH is the key enzyme for biological inactivation of PGs.

Based on the antiproliferative effects in tumour cells, the biologically active form of vitamin D (calcitriol, 1,25-dihydroxycholecaldiferol, $1,25(OH)_2D_3$), is a relevant factor in tumour prevention and therapy. Its synthesis is catalysed by the 1-α-hydroxylase which is predominantly expressed in the kidneys, but an extra-renal expression has also been detected in prostate, lung, pancreas, parathyroid, monocytes and breast and influences cell cycle arrest, induction of apoptosis and cell differentiation [5]. The signalling pathway of $1,25(OH)_2D_3$ is mediated by binding to the VDR that afterwards binds to specific DNA sequences and regulates the transcriptional processing of its target genes [6].

Recently, some studies have suggested a link between the PG- and vitamin D-metabolism in prostate cancer [7] and in breast cancer tumour cells as well [8]. Consequently, there seems to be a strong evidence for an interaction between VDR, associated target genes and PG in cancer. Arising from the need for further

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investigation concerning carcinogenesis of other tumours, the aim of this study was to analyse the expression of PG metabolising enzymes and VDR in both benign and malignant ovarian tissues, and $25(OH_2)D_3$ and PGE_2 serum level in healthy and tumour patients.

2. Materials and methods

2.1. Tissue samples

We analysed a non-consecutive series of primary ovarian carcinomas ($n = 13$) ranging in age from 38 to 80 years (median age, 63.6 years) as well as normal-appearing ovarian epithelia ($n = 13$) that were obtained from our institutional tumour bank. Tissue samples were frozen in liquid nitrogen.

2.2. Blood samples

Blood samples were taken from both healthy patients ($n = 20$) and tumour patients ($n = 20$) in order to evaluate $25(OH)_2D_3$ plasma levels and PGE_2 plasma levels. Blood samples were centrifuged at 4 °C, 4000 rpm for 10 min and frozen at −80 °C until measurement. We excluded blood samples from patients with chronic diseases.

2.3. Immunoblotting

COX-2 and 15-PGDH protein expression was determined by cytosolic and membrane proteins of deep frozen tissue samples being isolated with Qproteome cell compartment kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. The detection of VDR was performed with whole cell lysates of deep frozen tissue samples lysed in sample buffer (125 mM Tris, 30% glycerine, 8% SDS, PH 6.8). The subjected proteins were blotted onto nitrocellulose and PVDF membranes (Schleicher Schuell, Dassel, Germany), respectively. After blocking in 5% non fat dry milk in PBST (1× PBS, 0.2% Tween), membranes were incubated with the primary antibodies for human COX-2 and 15-PGDH (Cayman Chemicals, Ann Arbor, MI, USA) in a dilution of 1:1000 and VDR antibody (rabbit-anti human IgG, Cat. no. DLN-13560, Dianova, Hamburg, Germany, prepared at 1 mg/ml in 10 mM PBS, pH 7.4 with 0.2% BSA & 0.09% sodium) was used in a dilution of 1:10,000 in blocking reagent over night at 4 °C. Subsequently, membranes were incubated with secondary antibodies conjugated to horseradish peroxidase. The obtained signals were visualised with the enhanced chemiluminescence (ECL) detection system (Millipore, Schwalbach, Germany) and were densitometrically analysed and normalised to β-actin as a loading control. Finally, the data of the benign were compared to malignant tissue samples.

2.4. Quantification of prostaglandin E_2 and $25(OH)_2D_3$

PGE_2 levels of serum samples were determined by PGE_2 monoclonal enzyme immunoassay (Biozol, Munich, Germany) according to the protocol of the manufacturer. Serum concentrations of $25(OH)_2D_3$ were assessed with Elecsys Vitamin D_3 chemiluminescent immunoassay (Roche Diagnostics, Mannheim, Germany) by a photomultiplier (Elecsys 2010, Hitachi, Tokyo, Japan).

2.5. Statistical significance

Statistical analysis of real-time PCR, Western blots results and $25(OH)_2D_3$ and PGE_2 measurements were performed using Student's *t*-test. All experiments were performed in duplicates and repeated twice. Data represented as mean relative to healthy subjects as control group.

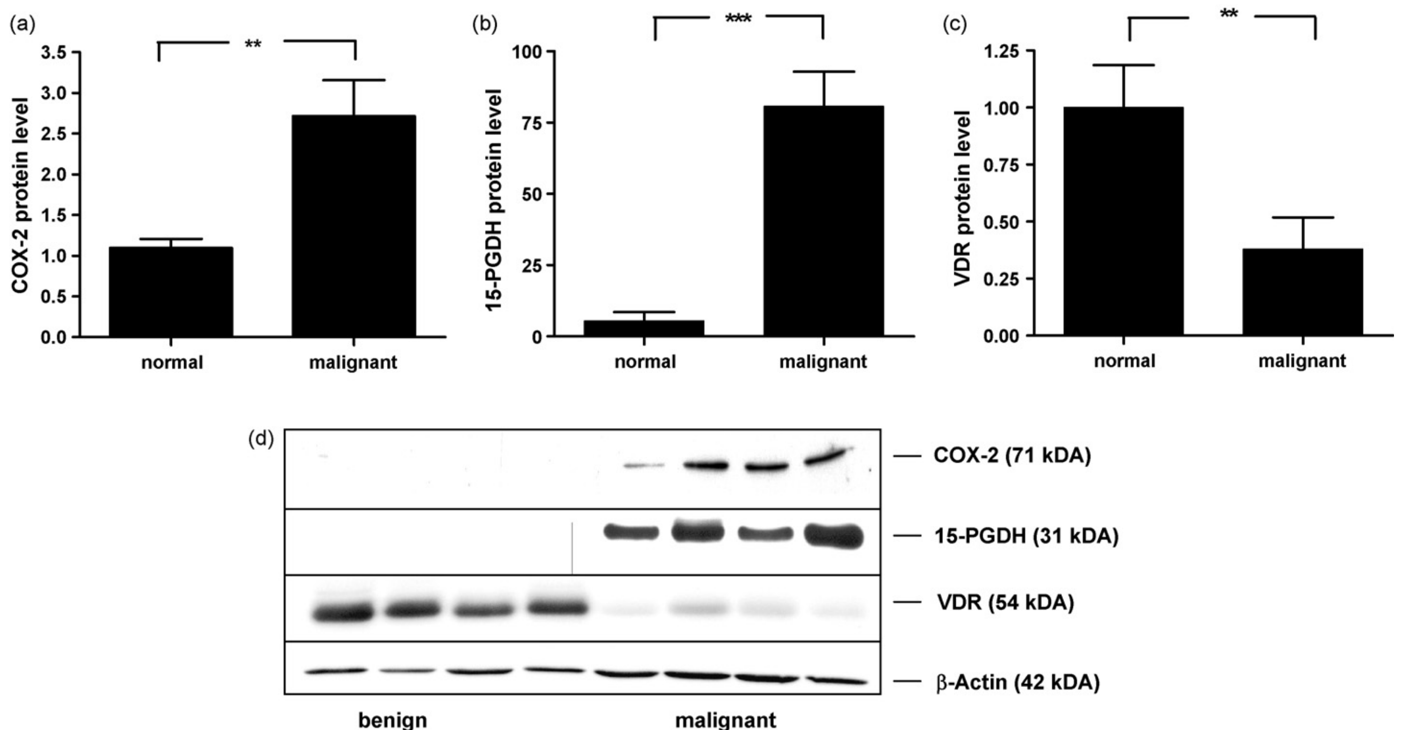

Fig. 1. (a–d) Increased COX-2 expression in malignant ovarian tissues compared to normal tissue. Protein expression of COX-2 from normal ($n = 7$) and malignant ($n = 10$), 15-PGDH of normal ($n = 8$) and malignant ($n = 8$) and VDR from normal ($n = 11$) and malignant ($n = 9$) tissue samples was densitometrically analysed and normalised to β-actin as loading control. $^{**}p < 0.01$ and $^{***}p < 0.001$.

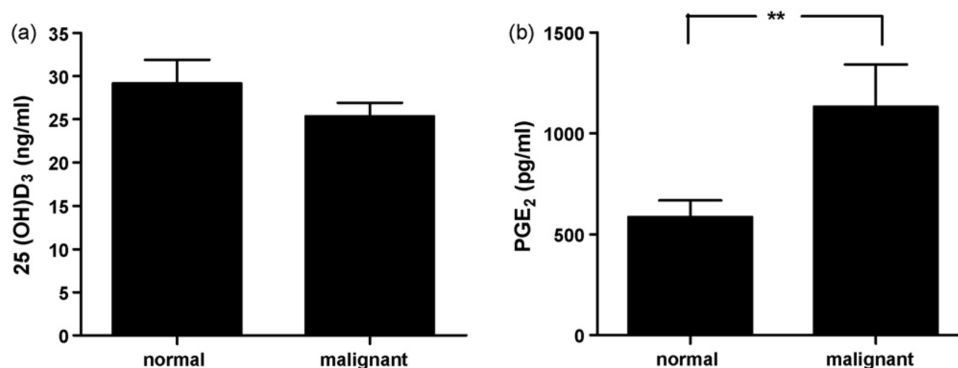

Fig. 2. (a and b) 25(OH)2D3 (a) and PGE2 (b) serum level from healthy women and from women with ovarian cancer (older than 45 years) diagnosed during wintertime. Blood samples were taken and analysed at the same time. **$p < 0.01$.

3. Results

3.1. COX-2, 15-PGDH and VDR protein expression in normal and malignant ovarian tissue

In the Western blot analysis, COX-2 protein levels were significant being more than two times higher ($p < 0.01$) in the malignant tissue (2.71 ± 0.44) compared to that of the normal tissue samples (1.09 ± 0.11) (Fig. 1a). In the malignant ovarian tissue samples (80.73 ± 12.11), the protein level of 15-PGDH was even more significantly ($p < 0.001$) higher compared to the normal tissue (5.49 ± 3.05) (Fig. 1b). A highly significant lower VDR-expression ($p < 0.01$) was found in the malignant tissue samples (0.37 ± 0.13) compared to the normal tissue (1.00 ± 0.18) and therefore an inverse correlation to the COX-2 and 15-PGDH protein expression (Fig. 1c) was found.

3.2. $25(OH)_2D_3$ and PGE_2 serum levels in healthy women and ovarian cancer patients

We observed no significant differences in 25-$(OH)_2D_3$ serum levels in women either older than or younger than 45 years, and with or without ovarian cancers that were diagnosed and sampled during the wintertime months (October–February). We found $25(OH)_2D_3$ serum levels as follows: healthy women: 29.15 ± 2.74 ng/ml and ovarian cancer patients: 25.32 ± 1.57 (Fig. 2a). The PGE_2 levels were significantly higher ($p < 0.01$) only in ovarian cancer patients older than 45 years diagnosed and sampled in the wintertime (1132.0 ± 210.1 pg/ml) compared to the healthy women with the same age (587.0 ± 82.28 pg/ml) (Fig. 2b).

4. Discussion

In the present study we report for the first time an inverse correlation between the VDR with both the COX-2 and 15-PGDH expression. The data regarding COX-2 expression are inconsistent but several studies may suggest that an overexpression of COX-2 is associated with the carcinogenesis and chemoresistance of ovarian carcinomas [1–3]. Thus, our results are in line with the literature [4,9] as we detected a significantly higher COX-2 expression in the malignant tissue. To our knowledge there is nothing known about 15-PGDH expression in ovarian carcinomas, however a 14.7-fold higher protein level of 15-PGDH was found in the present study. These results are consistent with our unpublished data in breast cancer. An explanation for these increased levels might be a compensation of higher COX-2 and consecutive, PG levels by 15-PGDH.

VDR, detected by immunohistochemistry (IHC), was found to be down-regulated in colon and breast tumours but was found to be up-regulated in ovarian tumours [10]. In contrast, in our Western blot analyses we observed a decreased VDR-expression in the tumour tissue compared with the normal tissue samples and therefore an inverse correlation to the COX-2 expression. These findings are coherent with the results by Fischer et al. [11,12] who observed decreased target genes (*cyp27B1*, *cyp24*) in malignant ovarian samples.

The circulating concentration of $25(OH)_2D_3$ is more useful in investigating a potential relationship between ovarian cancer and the availability of vitamin D from diet and supplements and from synthesis in the skin. To date, there have been several epidemiologic studies about the association between vitamin D and ovarian cancer risk, however, their results have not been consistent [13]. In our investigation we found no association between ovarian cancer patients and healthy women in either summer or winter, however, we observed a trend to reduced $25(OH)_2D_3$ serum levels in tumour patients older than 45 years that were sampled in the winter months. In the same subgroup of patients we found 2-fold higher PGE_2 serum levels.

The obtained data suggest a possible link between PG- and vitamin D-metabolism in ovarian cancer. Recent studies have investigated the potential of COX-2 inhibitors (NSAIDs) [14] and vitamin D analogues in the treatment of ovarian cancer partly with encouraging results [15]. Further research is needed if the synergistic effect of these approaches is to be a useful therapeutic strategy in the treatment of ovarian cancer.

Conflict of interest statement

The authors declare no conflict of interest relevant to this article.

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