Interference of Ca$^{2+}$ with the proliferation of SCCOHT-1 and ovarian adenocarcinoma cells

ANNA OTTE, FINN RAUPRICH, JULIANE VON DER OHE, PETER HILLEMANNS and RALF HASS

Biochemistry and Tumor Biology Laboratory, Department of Obstetrics and Gynecology, Hannover Medical School, Hannover, Germany

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Abstract. A recently established cellular model for the rare small cell carcinoma of the ovary hypercalcemic type (SCCOHT-1) was characterized in comparison to ovarian adenocarcinoma cells (NIH:OVCAR-3 and SK-OV-3). The different cancer populations exhibited a common sensitivity in acidic pH milieu and a continuous proliferation in alkaline medium of pH 8.0-9.0. In the presence of elevated Ca$^{2+}$ concentrations, the ovarian cancer cells demonstrated a progressively reduced proliferation within 72 h in contrast to other tumor types such as breast cancer cells. This significant growth inhibition was calcium-specific since the proliferation was unaffected after culture of the ovarian cancer cells in the presence of similar concentrations of other cations. The Ca$^{2+}$ effects on the ovarian cancer cells were associated with marked differences in the activation of intracellular signaling pathways including enhanced phosphorylation of the p42/44 MAP kinase (Thr202/Tyr204). Further analysis of the signaling pathway revealed a significantly enhanced Ca$^{2+}$-dependent and p42/44 MAP kinase activation-mediated prostaglandin E2 (PGE2) production in SK-OV-3 and SCCOHT-1 and to a lesser extent in NIH-OVCAR-3 cells. Vice versa, exogenous PGE2 did not affect the proliferative capacity of the ovarian cancer cells and inhibition of the Ca$^{2+}$-mediated MAP kinase activation did not abolish the Ca$^{2+}$-mediated cytotoxicity. Collectively, these data suggest that multiple pathways are activated by exogenous Ca$^{2+}$ in the different ovarian cancer cells, including a specific MAP kinase signaling cascade with subsequent PGE2 production and a parallel pathway for the induction of cell death.

Introduction

Ovarian cancer still represents one of the most lethal gynecologic malignancies. Within this type of gynecological cancer the small cell ovarian carcinoma of the hypercalcemic type (SCCOHT) is defined as a rare form of an aggressive ovarian tumor predominantly affecting young women between ages of 13 to 35 which is mostly associated with paraendocrine hypercalcemia (1-3). Following the initial histopathological evaluation of several clinical cases, the SCCOHT has been classified as a separate pathological entity (2). Recent studies revealed a mutation in the 5MARCA4 gene as a potential marker for the SCCOHT (4-6).

The SCCOHT tumor disease is associated with poor prognosis and appears different and clearly distinguishable from other ovarian cancer types such as ovarian epithelial tumors and ovarian germ cell tumors (7). Initial immuno-histochemical analysis of the SCCOHT postulated a germ cell-derived tumor (8). Another study reported SCCOHT as an epithelial-like originating tumor (3). In fact, some cells stained positive for epithelial cell markers, however, the intermediate filament protein vimentin predominantly associated with cells of a mesenchymal phenotype has been identified in the majority of cells in the SCCOHT (9). Further investigations using additional genetic analysis of SCCOHT tumor specimen suggested a heterogeneous tumor entity but did not confirm a germ cell-derived or an epithelial cell-derived tumor origin (9-11). The heterogeneity of these data may be explained in part by the rare and limited tumor material from patients. Considering these controversial reports, the histogenesis of SCCOHT and the mechanism of the development and physiological role of an accompanying hypercalcemia still remain unclear. Likewise, reasonable approaches for a sufficient (chemo)therapeutic management to treat SCCOHT patients are completely unknown. Although a multi-modality platform is suggested including surgery followed by chemotherapy and radiotherapy (12,13), only very few patients survived longer than the following two years (14-17).

Recently, we developed a cellular model for the SCCOHT and the resulting SCCOHT-1 tumor cells were derived from a primary culture of biopsy material from a 31-year-old patient with recurrent SCCOHT. In vivo studies with these primary cells substantiated a SCCOHT phenotype with histopathological similarities between the mouse xenograft-developed...
tumors and the original patient tumor. Moreover, development of SCCOHT-1-induced tumor xenografts displayed an accompanying hypercalcemia in NOD/scid mice with serum calcium levels above 3.5 mmol/l (1).

Using this unique cellular model, we examined in the present study the effects of exogenous calcium representing a hypercalcemia on SCCOHT-1 in comparison to established human ovarian adenocarcinoma cell lines including NIH:OVCAR-3 and SK-OV-3 cells. Moreover, different calcium-mediated signaling pathways were analysed in these ovarian cancer cells, which may be supportive in search of an appropriate therapeutic approach, particularly in SCCOHT.

Materials and methods

Cell culture
Primary human SCCOHT-1 cells. SCCOHT-1 cells were derived as a spontaneous, permanently growing primary culture from a tumor biopsy after surgery of a 31-year-old patient with recurrent SCCOHT (1). Informed written consent was obtained from the patient for the use of this material and the study was approved by the Ethics Committee of Hannover Medical School, Project #3916, June 15, 2005. The SCCOHT-1 cells were cultured in RPMI-1640 supplemented with 1 or 10% (v/v) fetal calf serum, 100 U/ml L-glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin. The tissue culture was performed at 37°C in a humidified atmosphere of 5% (v/v) CO2 and the medium was changed at intervals of 3 to 4 days. For subculture, the cells were centrifuged (320 g/6 min) and resuspended in growth medium and the proliferative capacity at various conditions and the population doublings in parallel to the cell viability during culture was determined in a hemocytometer using the trypan blue exclusion test. In an alternative fluorescence-based proliferation assay the SCCOHT-1 cells have been transduced with a 3rd generation lentiviral SIN vector containing the eGFP gene (SCCOHT-1^{eGFP}) as previously described for these cells (1).

Human ovarian adenocarcinoma cell lines. Human NIH:OVCAR-3 ovarian cancer cells (ATCC® #HTB-161™) were commercially obtained in passage 76 (P76) from the Institute for Applied Cell Culture (IAZ), Munich, Germany. The SK-OV-3 ovarian cancer cells (ATCC® #HTB-77™) were commercially obtained in P25 from the ATCC, Manassas, VA, USA. These ovarian adenocarcinoma cell lines were originally established from the malignant ascites of a patient with progressive adenocarcinoma of the ovary, respectively. The cells were cultured at about 1,750 cells/cm² in RPMI-1640 supplemented with 1 or 10% (v/v) fetal calf serum, 100 U/ml L-glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin. Subculture was performed by trypsin/EDTA (Biochrom GmbH, Berlin, Germany) treatment for 5 min at 37°C. For the experiments NIH:OVCAR-3 cells were used in P86 to P118 and SK-OV-3 cells were used in P37 to P39. For fluorescence measurement in an appropriate proliferation assay the NIH:OVCAR-3 as well as the SK-OV-3 cells were also transduced with a eGFP gene vector (NIH:OVCAR-3^{eGFP} and SK-OV-3^{eGFP}) similar to SCCOHT-1^{eGFP} cells.

Human breast cancer cell line. Human MDA-MB-231 breast cancer cells (MDA) were obtained from the ATCC (#HTB-26). This cell line was analyzed in a short tandem repeat (STR)-based authentication by the Institute for Legal Medicine at the University Hospital Schleswig-Holstein as recently documented (18). MDA cells were cultivated at about 1,500 cells/cm² in Leibovitz's L-15-medium (Invitrogen) with 10% (v/v) FCS, 2 mM L-glutamin and 1 mM penicillin/streptomycin. For fluorescence measurement MDA-MB-231^{eGFP} cells were also generated after transduction with the eGFP gene vector.

Cell line authentication. Authentication of SCCOHT-1, NIH:OVCAR-3, SK-OV-3, and MDA-MB-231 cells was performed by short tandem repeat (STR) fragment analysis using the GenomeLab human STR primer set (Beckman Coulter Inc., Fullerton, CA, USA). Following DNA isolation of the cell lines and amplification by polymerase chain reaction (PCR) with the STR primer set, the appropriate PCR products were sequenced in the CEQ8000 Genetic Analysis System (Beckman Coulter) using the GenomeLab DNA size standard kit-600 (Beckman Coulter). Comparison of the sequencing results from SCCOHT-1 were similar to the original SCCOHT patient cells cultured in our lab. Moreover, the NIH:OVCAR-3, SK-OV-3 and MDA-MB-231 cell lines demonstrated a similar STR pattern according to the STR database provided by the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany) for these cell lines.

Proliferation and cell cycle analysis
For fluorescence measurement the different eGFP-transduced ovarian cancer populations were cultured in flat bottom 96-well plates (Nunc/ThermoFischer, Roskilde, Denmark) and incubated with 1.6, 3.2 and 6.4 mM Ca^{2+}, respectively, for 24 to 72 h. At different time points, the medium was removed and the cells were lysed with 5% (w/v) sodium dodecylsulfate (SDS). Therefore, the fluorescence intensities of GFP in the cell homogenate which corresponded to the appropriate cell number of ovarian cancer cells, were measured at excitation 485 nm/emission 520 nm using the Fluoroscan Ascent Fl (Thermo Fisher Scientific) fluorescence plate reader.

To substantiate these results in an alternative assay, wild-type ovarian cancer populations were incubated similarly with 1.6, 3.2 and 6.4 mM Ca^{2+}, respectively, for 24 to 72 h and the cells were counted at the appropriate time points in a hemocytometer following trypan blue staining.

The cell cycle analysis was performed as described previously (19) using untreated compared to 1.6 mM Ca^{2+} and 6.4 mM Ca^{2+}-stimulated SCCOHT-1^{eGFP}, NIH:OVCAR-3^{eGFP} and SK-OV-3^{eGFP} ovarian cancer cells after 48 h. Briefly, 5x10⁶ cells were fixed in 70% (v/v) ice-cold ethanol at 4°C for 24 h. Thereafter, the fixed cells were stained with CyStain DNA 2 step kit (Partec GmbH, Münster, Germany) and filtered through a 50 µm filter. The samples were then analyzed in a Galaxy flow cytometer (Partec) using the MultiCycle cell cycle software (Phoenix Flow Systems Inc., San Diego, CA, USA).

Immunoblot analysis
Following culture of SCCOHT-1^{eGFP} cells in culture medium with 1% FCS, untreated control cells and Ca^{2+}-stimulated cells were washed three times in ice-cold PBS and lysed in a reswelling buffer containing 8 M urea (Carl Roth GmbH Co KG, Karlsruhe, Germany), 1% CHAPS (3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate) (Carl Roth GmbH
Co KG), 0.5% (v/v) pharmalyte 3-10 (GE Healthcare Europe GmbH, Freiburg, Germany), 0.002% (w/v) bromophenol blue (Serva Electrophoresis GmbH, Heidelberg, Germany) and freshly prepared 0.4% (w/v) dithiothreitol (DTT) (Carl Roth GmbH Co KG). Protein concentration was adjusted using the colorimetric BCA-assay (Perbio Science Deutschland, Bonn, Germany), subjected to SDS-polyacrylamide gel electrophoresis and transferred to a Hybond C Extra Nitrocellulose membrane (GE Healthcare Life Science). The membranes were blocked with PBS containing 5% FCS and 0.05% Tween-20 (PBS/Tween). After washing four times with PBS/Tween, the membranes were incubated with the primary antibodies (polyclonal anti-phospho-p44/42 MAPK Thr202/Tyr204 (Cell Signaling Technology Inc.); polyclonal anti-Stim-1 (clone D88E10; Cell Signaling Technology Inc.); polyclonal anti-IP3 receptor (clone D53A5; Cell Signaling Technology Inc.); monoclonal anti-GAPDH (clone 6C5, Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 4˚C overnight. Thereafter, the membranes were washed four times with PBS/Tween and incubated with the appropriate horseradish peroxidase-conjugated secondary antibody (all from Santa Cruz Biotechnology) for 1 h/25˚C. The membranes were washed with PBS/Tween and visualized by autoradiography using the ECL-detection kit (GE Healthcare Europe GmbH).

Prostaglandin E2 (PGE2) ELISA
SCCOHT-1WT, SK-OV-3WT and NIH:OVCAR-3WT cells were seeded in 24-well plates at 10^6 cells/well (Nunc/ThermoFischer, Roskilde, Denmark) with 500 µl culture medium per well. In comparison to untreated control cells, the populations were stimulated with 1.6, 3.2 and 6.4 mM Ca^{2+}, respectively, in the absence or presence of a 1-h pre-incubation with 50 µM of the MAP kinase inhibitor PD98059 (Cell Signaling Technology Inc.). The conditioned medium was collected after 12 and 24 h, respectively, and centrifuged at 1,000 rpm/10 min. Thereafter, 50 µl aliquots of the supernatant were applied to the appropriate PGE2 measurements which were performed in an ELISA system according to the manufacturer's recommendation (R&D Systems Ltd., Abingdon, UK).

Results

Proliferation. All three ovarian cancer cell types exhibited sensitivity for an acidic culture milieu and continued maximal proliferation in alkaline medium of approximately pH 9.0 (Fig. 2). The proliferative capacity of SCCOHT-1 and NIH:OVCAR-3 cells was progressively inhibited by about 80% at pH 6.0 within 72 h (n=5) whereas SK-OV-3 cells demonstrated more stability with a growth reduction of about 30% (n=6). At pH 10.0 the proliferation progressively declined by 59±4% (n=5) in SCCOHT-1 cells after 72 h. A higher sensitivity with 80±2% (n=5) was observed in NIH:OVCAR-3 cells at pH 10.0 after 72 h and SK-OV-3 cells revealed 41±6% (n=5) growth inhibition at similar conditions (Fig. 1).

According to the hypercalcemia associated with SCCOHT, exogenous stimulation with calcium was tested and revealed a significant growth inhibition in all three ovarian carcinoma cell types in a concentration- and time-dependent manner. Whereas the culture medium constitutively contained about 0.8 mM Ca^{2+} and Mg^{2+} during steady-state culture conditions, the proliferative capacity of SK-OV-3 cells after exogenous addition of 1.6 mM Ca^{2+} up to 6.4 mM Ca^{2+} was progressively reduced to 17.8±6.2% (n=10) within 72 h. These growth-inhibitory effects of 6.4 mM Ca^{2+} were even more pronounced in SCCOHT-1 with growth reduction down to 11.4±5.0% (n=9) and were maximal in NIH:OVCAR-3 cells reaching only 3.8±0.5% (n=10) of proliferative capacity after 72 h as compared to control cells cultured at pH 7.4 for 24 to 72 h. Every 24 h, the medium was exchanged with newly established pH following appropriate adjustments by HCl or NaOH, respectively. Data represent the mean ± SD (n=5).
presence of 6.4 mM Ca\textsuperscript{2+} was associated with a growth rate of 68.1±13.2% (n=6) compared to a control culture after 72 h (Fig. 2A). Similar results of a marked Ca\textsuperscript{2+}-mediated concentration- and time-dependent growth inhibition were also obtained with the appropriate wild-type ovarian cancer cell populations by cell counting in a trypan blue exclusion assay (Fig. 2B). The results from the proliferation assays were also accompanied by appropriate morphological changes. Whereas the different ovarian cancer cell types demonstrated their typical morphology in phase contrast microscopy of the control cultures together with a GFP expression of the lentiviral eGFP-transduced cultures, a significant cell death with rounded and granulated cell bodies was observed in NIH:OVCAR-3\textsuperscript{GFP} cells following exposure to 6.4 mM Ca\textsuperscript{2+} for 72 h (Fig. 3, upper panel). Moreover, little if any fluorescence was detectable anymore in NIH:OVCAR-3\textsuperscript{GFP} cells. Only few GFP-expressing viable cells remained in the SCCOHT-1\textsuperscript{GFP} culture after incubation with 6.4 mM Ca\textsuperscript{2+} for 72 h (Fig. 3, middle panel). SK-OV-3\textsuperscript{GFP} cells also exhibited a significant granulation after incubation with exogenous Ca\textsuperscript{2+} with some more GFP-positive viable cells which substantiated the results of the proliferation assay (Fig. 3, lower panel).

Cell cycle analysis revealed a significant arrest of SCCOHT-1\textsuperscript{GFP} cells in the G\textsubscript{2} phase after 48 h in the presence of 1.6 mM Ca\textsuperscript{2+}. An elevation to 6.4 mM Ca\textsuperscript{2+} was associated with increased cell death by an accumulation of SCCOHT-1\textsuperscript{GFP}...
Similar findings were observed in 6.4 mM Ca\textsuperscript{2+}-exposed NIH:OVCAR-3\textsuperscript{GFP} cells with significantly elevated levels of cells in the subG\textsubscript{1} phase after 48 h (Fig. 5B and C). Ca\textsuperscript{2+}-sensitizing proteins were also investigated, including stromal interaction molecule-1 (Stim-1) which determines differences in [Ca\textsuperscript{2+}] in the endoplasmic reticulum and can oscillate for stimulatory interactions with the ORAI1 calcium ion channels to the plasma membrane (20). The Stim-1 expression was enhanced between 1 and 2 h of 1.6 mM Ca\textsuperscript{2+} treatment of SCCOHT-1 cells (Fig. 5A), whereas little, if any, different Stim-1 protein levels were observed in NIH:OVCAR-3 and SK-OV-3 cells until a decrease was observed after 24 h (Fig. 5B and C). With respect to the IP3 receptor which releases Ca\textsuperscript{2+} from endoplasmic storage compartments upon phospholipase C-mediated PI-breakdown and inositol trisphosphate generation, there was only marginal if any change in the IP3 receptor protein levels following Ca\textsuperscript{2+} stimulation of the ovarian cancer cells. The unaltered GAPDH expression served as loading control (Fig. 5A).

Ca\textsuperscript{2+}-mediated phosphorylation of p42/44 MAP kinase (Thr202/Tyr204) was associated with enhanced PGE2 production in the ovarian cancer cells. Thus, stimulation of SK-OV-3 cells with increasing [Ca\textsuperscript{2+}] between 1.6 and 6.4 mM exhibited progressively increasing PGE2 release after 12 h, which was significantly further elevated after 24 h following Ca\textsuperscript{2+} stimulation (Fig. 6A). Moreover, pre-treatment with the p42/44 MAP kinase inhibitor PD98059 completely abolished even the highest levels of Ca\textsuperscript{2+}-mediated PGE2 production (Fig. 6A). Similar results were obtained in SCCOHT-1 cells with 40.4±24.1 pg/ml PGE2 after 1.6 mM Ca\textsuperscript{2+} and 232.5±37.9 pg/ml PGE2 after 6.4 mM Ca\textsuperscript{2+} stimulation for 12 h. Likewise, NIH:OVCAR-3 cells produced 41.2±0.1 pg/ml PGE2 after 1.6 mM Ca\textsuperscript{2+} and 48.4±0.1 pg/ml PGE2 after 6.4 mM Ca\textsuperscript{2+} incubation within 24 h whereas non-stimulated control cells displayed 12.1±0.1 pg/ml PGE2 and the PGE2 concentrations in the presence of the MAP kinase inhibitor PD98059 were below detection limit. To test any potential growth-inhibitory effects of PGE2 on the different ovarian cancer populations, SCCOHT-1, NIH:OVCAR-3 and SK-OV-3 cells were incubated with various PGE2 concentrations between 1 pg/ml to 10 ng/ml and revealed little if any effect on the proliferative capacity of the tumor cells (Fig. 6B). Together, these findings suggested that the Ca\textsuperscript{2+}-mediated phosphorlp42/44 MAP kinase phosphorylation and subsequent stimulation of PGE2 production was independent of the Ca\textsuperscript{2+}-mediated growth inhibition. Indeed, this suggestion was substantiated by the MAP kinase inhibitor PD98059 which completely abolished the Ca\textsuperscript{2+}-mediated p42/44 MAP kinase phosphorylation in SCCOHT-1 cells (Fig. 7A). Moreover, MAP kinase inhibition did not demonstrate any effect on the increased G\textsubscript{2} arrest by 1.6 mM Ca\textsuperscript{2+} nor on the pronounced cell death of SCCOHT-1 cells in subG\textsubscript{1} phase by 6.4 mM Ca\textsuperscript{2+} after 48 h similar to the results in Fig. 4 (Fig. 7B).
Discussion

Ovarian cancer represents the predominant cause of gynecological cancer-related deaths affecting approximately 65,000 females in economically-developed countries in 2011 (21). As a rare form and special kind of ovarian cancer, the SCCOHT represents an aggressive tumor with poor prognosis and characteristics as compared to other ovarian carcinoma types remain unclear. The in vitro results in this study revealed common pH sensitivity in acidic milieu and continuous proliferation in neutral/low alkaline environment. Whereas young patients diagnosed with SCCOHT often present with a concomitant serum hypercalcemia, it was of interest to focus on calcium effects in ovarian cancer cells.

According to normal serum calcium levels of 2 to 2.5 mmol/l, hypercalcemia is considered as a mild type at concentrations between 2.5 to 3.0 mmol/l serum calcium and as a moderate type at concentrations between 3.0 to 3.5 mmol/l serum calcium. Patients with serum calcium levels above 3.5 mmol/l are diagnosed with a hypercalcemic crisis. Of interest, a recent study in a variety of ovarian cancer patients reports elevated blood calcium levels whereby \([\text{Ca}^{2+}]\) was proposed a potential predictive marker for ovarian cancer (22).

To test different levels of hypercalcemia in vitro, calcium concentrations of 1.6, 3.2 and 6.4 mmol/l were applied to the various ovarian cancer cells and revealed already significant growth-inhibitory effects between mild and moderate hypercalcemia. These growth-inhibitory effects were calcium-specific, since none of these results were obtained with similar concentrations of \(\text{Mg}^{2+}\) or further cations. Moreover, other tumor types such as breast cancer cells demonstrated much less responsiveness to high \(\text{Ca}^{2+}\) concentrations as compared to the different ovarian cancer cells. This calcium sensitivity of ovarian cancer cells suggested that elevated \([\text{Ca}^{2+}]\) is supportive for a therapeutic approach particularly in SCCOHT. Additional examination of these growth-inhibitory effects of high \([\text{Ca}^{2+}]\) in vitro demonstrated a morphological disintegration of the ovarian cancer cells. This was associated with increased cell death as revealed by cell cycle analysis. Interference with the calcium homeostasis can induce cell damage and eventually initiate cell death (23), whereby
recent studies proposed a process of programmed necrosis (necroptosis) upon cytosolic calcium accumulation in mouse xenografts of human neuroblastoma (24). These findings further substantiate our hypothesis that suitable chemotherapeutic compounds in combination with increased calcium levels contribute to an enhanced promotion of tumor cell death particularly in SCCOHT-1 cells. In this context, the hypercalcemia associated with SCCOHT may reflect a physiological anti-tumor response. However, due to a certain protection of the tumor cells within the tumor microenvironment and potential interactions with other cell types including immune cells and mesenchymal stem/stroma cells as documented for other tumor types such as breast cancer (18,25,26), the hypercalcemic effects achieve only a limited threshold and therefore, remain unresponsive and inefficient without further support to directly target the SCCOHT cancer cells.

At the molecular level, high [Ca^{2+}] was associated with increased activation/phosphorylation of the p42/p44 MAPK in the ovarian cancer cells. Activated p42/p44 MAPK can further relay phosphorylation signals to eventually stimulate phospholipase A2 (27). Upon cleavage of polyunsaturated fatty acids including arachidonic acid from the C2-position of membrane phospholipids by activated phospholipase A2, the elevated levels of arachidonic acid can be further metabolized via cyclooxygenase isoforms (COX-1, COX-2) into prostaglandins and predominantly PGE2 (28,29). Indeed, the data obtained in the present study substantiated such a signaling pathway, whereby stimulation of the ovarian cancer cells with increasing calcium concentration resulted in appropriately increasing PGE2 production both, in a concentration- and time-dependent manner. Enhanced PGE2-synthesis accompanied by an increased expression of COX-1 and COX-2 has been documented in certain epithelial ovarian cancer (30) indicating potential metabolic alterations with the malignant transformation and progression. PGE2 can mediate suppressive effects via ligation to the E prostanoid receptors EP2 and EP4 followed by decreased cAMP levels. Therefore, PGE2-mediated immune-modulation by tumors can alter immune surveillance by re-educating the infiltrating inflammatory and immune cells to support tumorigenesis (31).Whereby no direct proliferative effects of PGE2 on the ovarian cancer cells were detectable, our work also demonstrated...
that the calcium-mediated PGE2 production was p42/p44 MAPK-dependent since MAPK inhibition abolished the PGE2 production in the different ovarian cancer cells. Together, increased calcium concentrations can specifically stimulate PGE2 production via p42/p44 MAPK activation and in parallel, contribute to the induction of cell death in ovarian cancer cells, whereby these calcium-mediated effects are relayed via different signaling pathways. Although the appearance of a serum hypercalcemia in SCCOHT patients and a variety of other ovarian cancer patient exhibit only a limited and insufficient threshold, the present findings indicate that elevated Ca$^{2+}$ levels can enhance a physiological antitumor strategy for SCCOHT in support of a combined therapeutic approach against this rare but severe type of ovarian cancer.

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References