# Expression and Prognostic Significance of the Oncogenic K2P Potassium Channel KCNK9 (TASK-3) in Ovarian Carcinoma

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**Abstract.** *Background/Aims: The TWIK-related acid sensitive $K^+$ channel-3 (TASK-3) is an oncogenic potassium channel. We investigated the expression of TASK-3 in human ovaries, examined its prognostic significance, and determined effects of TASK-3 blockers on cell proliferation and apoptosis. Materials and Methods: Immunofluorescence and western blotting were used to investigate TASK-3 expression in two ovarian cancer cell lines, normal ovarian surface epithelium and cancer. Immunohistochemistry quantified expression in an ovarian cancer tissue microarray. The effect of TASK-3 blocking agents on cell proliferation was investigated with the CellTiter 96® Aqueous Non-Radioactive Cell Proliferation assay and on apoptosis with flow cytometry. Results: TASK-3 expression was confirmed by immunofluorescence in the SKOV-3 and OVCAR-3 cell lines, normal ovaries (n=4) and ovarian tumours (n=4) and by western blotting in normal ovaries (n=6) and ovarian tumours (n=22). Immunohistochemistry demonstrated immunostaining in 99% of tumours (n=230). Increased immunostaining conferred a survival advantage (p=0.002; median survival of >24 months). TASK-3 blockers caused a significant reduction in cell proliferation and an increase in apoptosis in the SKOV-3 and OVCAR-3 cell lines. Conclusion: TASK-3 is expressed in epithelial ovarian cancer, conferring a significant survival advantage on patients with increased expression. TASK-3-modulating agents have a significant effect on cell proliferation and apoptosis. Based on these results, we propose that TASK-3 could prove to be both a novel tumour marker and a new therapeutic target in ovarian cancer, but further investigation is required.*

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*Key Words:* Ovarian cancer, K2P channels, TASK-3, expression, survival, SKOV-3, OVCAR-3 cells.

Ovarian carcinoma (OC) accounts for approximately 4% of all cancers diagnosed in women (1). OC results in more deaths than all other gynaecological cancers combined, and has a 5-year survival rate of only 38% (2). Treatment is based on a combination of platinum chemotherapy with paclitaxel and/or surgery. Platinum-based chemotherapy results in clinical remission in 75% of patients, but the median progression-free survival remains only 16 to 21 months (3). Potassium ($K^+$) channels are encoded for by over 75 genes and form the largest group of ion channels (4). The two-pore $K^+$ (K2P) channels, to which the Tandem of P-domains weakly inward rectifying $K^+$ (TWIK)-related acid sensitive $K^+$ channel -3 (TASK-3) belongs, is the most recently identified group. K2P channels are 'leak' $K^+$ channels that set resting membrane potential and regulate cell excitability (4, 5). TASK-3 is normally found in the adrenal cortex, gastrointestinal tract, neuronal tissue and salivary glands (5, 6), is modulated by alterations in extracellular pH and anaesthetic agents, and plays a role in aldosterone secretion (5, 7, 8, 9). TASK-3 expression has already been defined in several types of cancer. In breast cancer, the potassium channel, subfamily K, member-9 (*KCNK9*) gene encoding TASK-3 is amplified 3- to 10-fold in 10% of tumours and overexpressed by 5- to >100-fold in 44% of tumours (10). TASK-3 expression has also been identified in melanoma, lung and colonic cancers (4, 11, 12). To our knowledge, we have for the first time demonstrated expression of TASK-3 in human OC and identified its potential link with prognosis. We describe the effect of TASK-3 blockers on cell proliferation and apoptosis in OC cell lines.

## Materials and Methods

*Ethical approval*. Samples were collected at the Royal Derby Hospital, UK. Ethical approval was granted by the local research Ethics Committee (08/H0405/71). Patients undergoing surgery for suspected OC (trial group), and oophorectomy for benign reasons (control group) were eligible for recruitment. Patients undergoing debulking surgery following primary chemotherapy were excluded as platinum-based chemotherapy is known to modulate $K^+$ channels (13).

*Culture of cell lines*. The SKOV-3 and OVCAR-3 cell lines (passage 13 and 28, respectively) were supplied by Dimitra Dafou, Translational Research Laboratories, University College Hospital, London, from an authenticated stock provided by the American Type Culture Collection, Teddington, Middlesex, UK. The cell lines were cultured in RPMI-1640 with L-glutamine supplemented with 10% fetal bovine serum (FBS) and 100 U/ml penicillin and 100 μg/ml streptomycin (P&S).

*Primary cell cultures*. OC biopsies (~1 $cm^3$) were finely chopped in filtered collagenase (2 mg/ml) in calcium- and magnesium-free Hanks balanced salt solution (HBSS) and repeatedly brought to 37˚C for 30 s until cells separated when agitated with a Pasteur pipette. The suspension was filtered, washed with HBSS, and centrifuged twice at 90 rcf at 20˚C for 5 min. The pellet was seeded into a T25 flask in 5 ml of RPMI (10% FBS and P&S) and cultured at 37˚C in a humidified atmosphere with 5% $CO_2$. Normal ovarian surface epithelium (OSE) was cultured as previously described with no modifications (14).

*Immunofluorescence*. Cells were grown on glass coverslips in 24-well plates until 80% confluence. Fixation occurred with 4% paraformaldehyde for 20 min at room temperature (RT), and non-specific staining blocked with 3% bovine serum albumin (BSA)/5% glycine followed by 10% goat serum in phosphate-buffered saline (PBS). Cells were incubated overnight at 4˚C with antibody to TASK-3 (1:25) (Abcam plc, Cambridge, UK). After washing with PBS (3× 5 min), FITC-conjugated secondary antibody (1:50) (Sigma Aldrich, Gillingham, UK) was added at RT for 30 min. Cells were also stained for cytokeratin (1:50), vimentin (1:20) and fibroblast (1:50) (all Sigma Aldrich). Cells were observed under a fluorescence microscope (Zeiss Axiovert, Carl Zeiss Ltd., Cambridge, UK) and images were captured using Cell F software (Olympus KeyMed, Southend-on-Sea, UK). The SHSY-5Y neuroblastoma cell line was used as a positive control, and negative control wells contained 10% goat serum only.

*Western blotting*. Western blotting was conducted as previously described by Asher *et al*. (15) with the following modifications. Biopsies were obtained from the ovarian surface aiming to include minimal stromal tissue. Tissue biopsies were freshly snap-frozen in liquid nitrogen. Myometrium was used as a positive control. The membrane was incubated overnight at 4˚C with antibody to TASK-3 (1:200) (Alomone Labs, Jerusalem, Israel). After washing, blots were incubated with goat anti-rabbit secondary antibody linked to alkaline phosphatase (1:30,000) for 90 min at RT, developed using alkaline phosphatase substrate enhancer, viewed using the Chemidoc Imaging System (BioRad Labs Ltd, Hemel Hempstead, UK) and analysed using the QuantityOne software (BioRad Labs Ltd.). The blots were stripped with Mild Reblot (Chemicon Europe Ltd, Chandlers Ford, UK) as per manufacturer's instructions and after 2 h of blocking with 5% Marvel (Premier Foods, Spalding, UK) incubated overnight at 4˚C with an antibody against β-actin (1:8000, Abcam Ltd, UK) and analyzed as previously detailed (15).

*Immunohistochemistry of tissue microarrays (TMA)*. The OC TMA (SDLREC Ref 0205/495) (donated by Dr Spendlove, University of Nottingham, UK) has been previously described (16, 17). It consists of 336 biopsies from patients undergoing surgery for OC between 1st January 1982 and 31st December 1998. Disease-specific survival, defined as the date of diagnosis to the date of death from the cancer, was calculated from the operation date until 31st Dec 2005, when any remaining survivors were censored.

Immunohistochemistry for TASK-3 was conducted according to our previously-described protocol (16). Briefly, antigen retrieval consisted of microwaving tissue sections in 0.1 M sodium citrate buffer (pH 6.0), and antibody to TASK-3 was added at 1:200 (Abcam plc) overnight at 4˚C. The staining was developed using Vectastain ABC kit (Vector Labs Ltd., Peterborough, UK) and diaminobenzidine (DAB) chromogen (DAKO Ltd., Ely, UK), as per the manufacturers' instructions. Single tissue cores were available for analysis. A positive control (rat brain) and a negative control (no primary antibody) were included each time. Staining was initially validated by staining 25 tumours from different parts of the blocks used in constructing the TMA. Staining intensity was graded as: 0, none; 1+, low; 2+, intermediate; and 3+, high, and was scored by three independent observers: a consultant histopathologist (GV) and two researchers (AI and HS).

*Cell proliferation assay*. Proliferation of SKOV-3 and OVCAR-3 cells was measured in the presence and absence of the TASK-3 channel blocking agents zinc (3.3 μM, 10 μM, 33.3 μM, 100 μM and 333 μM) and methanandamide (1 μM, 3.3 μM, 10 μM, 33.3 μM and 100 μM) using the CellTiter 96® Aqueous Non-Radioactive Cell Proliferation assay (Promega, Southampton, UK), according to the manufacturer's instructions. Experiments were otherwise conducted as previously described (15). Each drug was tested in triplicate and experiments were repeated thrice.

*Flow cytometry and apoptosis experiments*. SKOV-3 and OVCAR-3 cells were plated at $5\times10^4$ cells/ml in a 6-well plate and incubated for 24 h after which the media were replaced with RPMI (control wells) or RPMI with TASK-3 blockers including zinc (100 μM) or methanandamide (0 μM, 33 μM and 100 μM), and further incubated for 48 h. The cells were then trypsinised and centrifuged at 90 rcf at 20˚C for 5 min and the pellet washed in 3 ml of PBS at 4˚C. The cell suspension was divided equally into three fluorescence-activated cell sorting (FACS) tubes and centrifuged at 300 rcf for 10 min. TACS®Annexin V Kit (AMS Biotechnology Ltd., Abingdon, UK) was used to determine annexin-V and propidium iodide (PI) binding as per the manufacturer's instructions. Analysis of cellular fluorescence was performed by the Coulter Altra Flow cytometer for 5000 events. Control tubes containing binding buffer only and cells containing annexin-V alone, PI-alone, and annexin-V with PI were used to calibrate the instrument. Data were analyzed with WinMDI 2.9 (available at: (http://facs.scripps.edu/software.html) Copyright© 1993-2000 Joe Trotter).

*Statistical analysis*. Proliferation assays and cell cycle data were analyzed using one-way analysis of variance (ANOVA) with Dunnet's multiple comparison *post hoc* analysis (GraphPad Prism5, GraphPad Software Inc., La Jolla, CA, USA). Results are presented as the mean±standard error of mean (SEM), with $p<0.05$ considered significant. Survival data for the TMA was obtained by Kaplan Meier survival curves and Fisher's exact test was used to assess the interaction of TASK-3 staining with established prognostic variables (SPSS statistics 17.0; IBM, Portsmouth, UK).

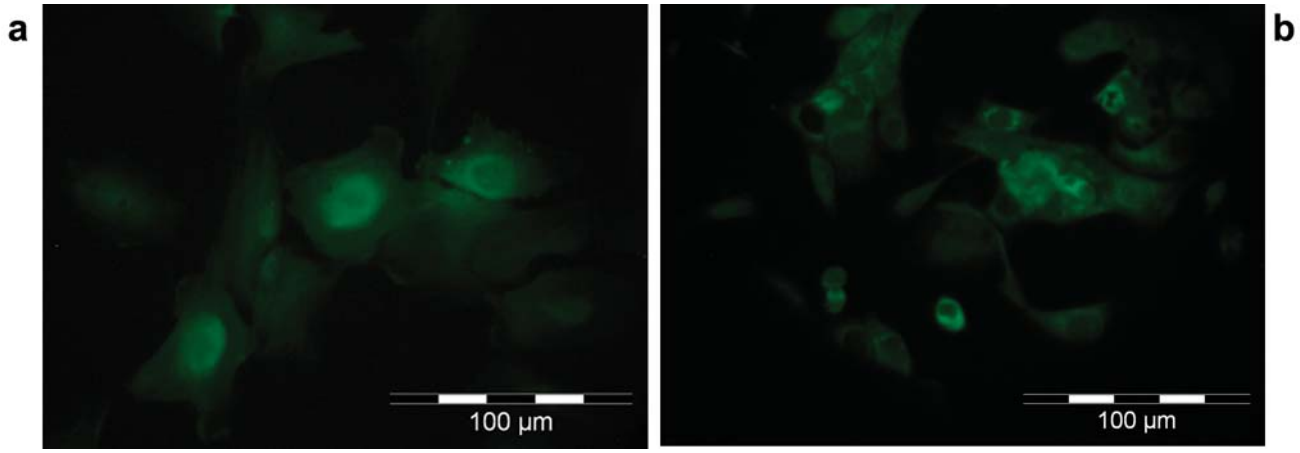


Figure 1. *a Immunofluorescence staining for TASK-3 in normal OSEs. This demonstrates TASK-3 IF staining in normal OSEs obtained from a patient with histologically-normal ovaries. Positive staining is present throughout the cells with intense perinuclear staining. b Immunofluorescence staining for TASK-3 in ovarian cancer. This demonstrates TASK-3 IF staining in a moderately differentiated serous adenocarcinoma. There appears to be positive staining throughout the cells with sparing of the nucleus.*

## Results

*TASK-3 is expressed in normal ovarian surface epithelium and OC*. Immunofluorescence demonstrated positive staining for TASK-3 for the SKOV-3 cell line (n=3), OVCAR-3 cell line (n=3), cultured normal OSE cells (n=4; Figure 1a) and cultured OC samples (not age-matched to controls) (n=4; Figure 1b). Western blotting identified TASK-3 expression in normal ovaries (n=6) and cancer (n=22) (Figure 2).

*Immunohistochemistry demonstrated a significant survival advantage of increased TASK-3 expression*. Out of the 336 tumour biopsies, 230 cores were suitable for analysis (Table I). Overall, 99.1% of tumours exhibited positive immunostaining for TASK-3: 0: 0.87% (n=2); 1+: 31.7%, (n=73); 2+: 25.2%, (n=58); 3+: 42.2%, (n=97). An example of immunostaining is demonstrated in Figure 3. Kaplan Meier analysis demonstrated significantly improved survival in patients with 3+ staining ($p$=0.002; Figure 4). Median survival according to staining was as follows: 1+: 18.6 (95% confidence interval CI=10.3-26.8) months; 2+: 15.6 (95% CI=4.7-26.5) months; 3+: 43.3 (95% CI=22.4-64.2) months. Fisher's exact test did not demonstrate a significant correlation of TASK-3 staining with tumour stage ($p$=0.204), grade ($p$=0.593), histology ($p$=0.45) or residual disease ($p$=0.166).

*TASK-3 channel blockers significantly inhibit cell proliferation*. SKOV-3 cells demonstrated a significant reduction in proliferation at 72 h with 100 µM ($p$<0.05) and 333 µM ($p$<0.001) of zinc, representing a reduction in proliferation of >25% and >50% compared to the control. Similarly, OVCAR-3 cells demonstrated a significant reduction in proliferation at 72 h

Table I. *Demographics of patients from whom samples were analyzed by immunohistochemistry on tissue microarrays.*

| Patients' demographics | n=230 |
|---|---|
| Median age at diagnosis, years (IQR) | 62 (24-89) |
| Median overall survival, months (IQR) | 24.0 (8-74) |
| | n (%) |
| FIGO stage | |
| 1 | 58 (25.2) |
| 2 | 27 (11.7) |
| 3 | 117 (50.8) |
| 4 | 24 (10.4) |
| Missing data | 4 (1.7) |
| Histological subtype | |
| Serous | 119 (51.7) |
| Mucinous | 26 (11.3) |
| Endometroid | 28 (12.2) |
| Clear-cell | 18 (7.8) |
| Undifferentiated | 36 (15.7) |
| Missing data | 3 (1.3) |
| Tumour grade | |
| G1 | 30 (13.0) |
| G2 | 48 (20.8) |
| G3 | 144 (62.6) |
| Missing data | 8 (3.5) |
| Residual disease | |
| No macroscopic disease | 93 (40.4) |
| Macroscopic disease remaining | 130 (56.5) |
| Missing data | 7 (3.0) |
| Chemotherapy | |
| Yes | 171 (74.3) |
| No | 57 (24.8) |
| Missing data | 2 (0.9) |

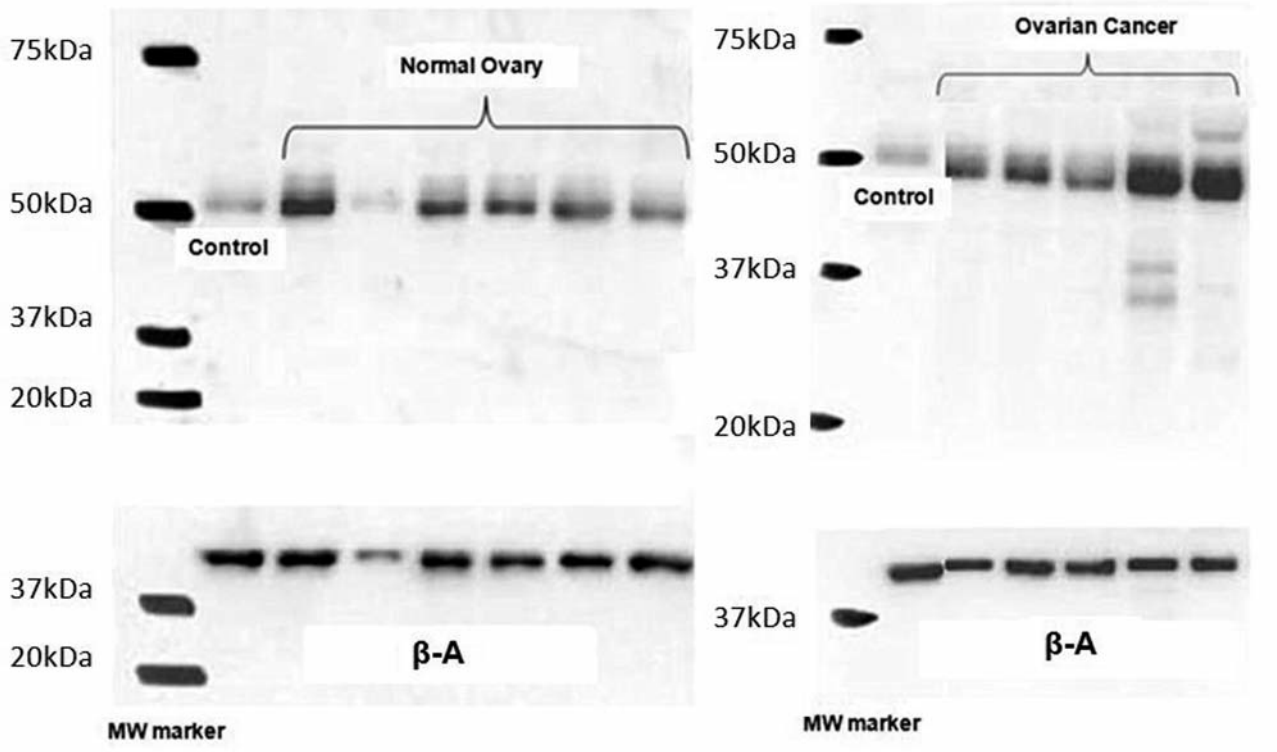


Figure 2. *Western blotting for TASK-3 expression in ovarian cancer and normal ovaries. This demonstrates a band visible at approximately 50 kDa, which is at the expected molecular weight of TASK-3. The β-actin (β-A) loading control is demonstrated in the blot below.*

with 33.3 μM ($p<0.01$), 100 μM ($p<0.01$) and 333 μM ($p<0.001$), representing a reduction of >25% for concentrations above 33 μM and >75% for 100 μM (Figure 5). This effect was also seen at 96 h with a significant inhibition of proliferation at concentrations of above 33 μM ($p<0.01$) in both SKOV-3 and OVCAR-3 cells. At 72 h methanandamide had a significant inhibitory effect on proliferation of SKOV-3 cells when applied at 10 μM ($p<0.001$), 33 μM ($p<0.001$) and 100 μM ($p<0.01$), causing a reduction in proliferation of >50% for 10 μM and 33 μM and >25% for 100 μM. This effect persisted at 96 h with concentrations above 3.3 μM ($p<0.001$). At 100 μM, there was an initial increase in proliferation at 48 h which was maintained when compared with 10 μM and 33 μM, although proliferation was still significantly reduced at 96 h ($p<0.001$, >50%) when compared with the control. Similarly, OVCAR-3 cells demonstrated a reduction in proliferation with concentrations of 33 μM ($p<0.001$) and 100 μM ($p<0.001$) at both 72 and 96 h (percentage reduction of >75% for all concentrations) (Figure 5).

*TASK-3 blockers have a significant effect on early and late apoptosis of SKOV-3 and OVCAR-3 cells*. The effect of 10 μM, 33 μM and 100 μM of methanandamide on apoptosis was investigated on the SKOV-3 and OVCAR-3 cell lines. A concentration of 10 μM significantly increased early apoptosis of SKOV-3 cells ($p<0.05$, mean increase of >25% compared to control). Concentrations of 33 μM and 100 μM however, had no effect (Figure 6a). There were no significant effects observed on early apoptosis in the OVCAR-3 cells at any of the concentrations tested (Figure 6b). All three concentrations caused a significant increase in late apoptosis of the SKOV-3 cells (10 μM, $p<0.01$; 33 μM, $p<0.001$; and 100 μM, $p<0.01$), representing a mean increase over the control of >25% (Figure 6c). An increase in late apoptosis was only apparent at a concentration of 33 μM ($p<0.05$, mean increase over control >50%) in the OVCAR-3 cells (Figure 6d). Applying 100 μM of zinc, the lowest concentration that had a significant effect on proliferation with both SKOV-3 and OVCAR-3 cells, had no significant effect on early apoptosis in either cell line, but late apoptosis was significantly increased in SKOV-3 ($p<0.001$, mean increase over control >50%) and OVCAR-3 ($p<0.05$, mean increase over control >50%).

## Discussion

As far as we know, here we demonstrated for the first time expression of the oncogenic TASK-3 channel in OC and normal OSEs, described the effects of TASK-3 blockers on proliferation and apoptosis of OC cells, and confirmed a link of TASK-3

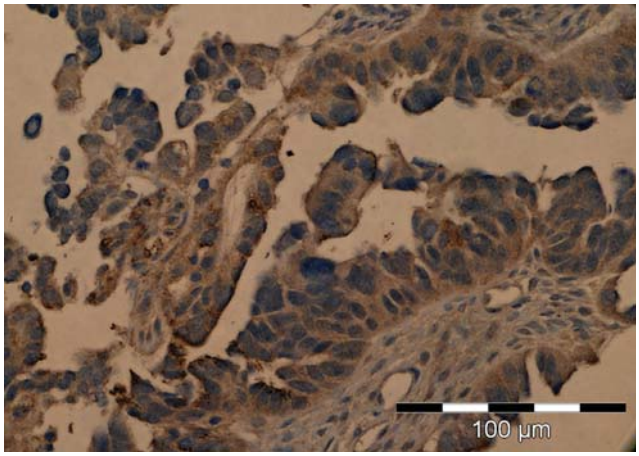


Figure 3. *Immunohistochemistry staining for TASK-3 in ovarian cancer. This demonstrates 2+ staining for TASK-3 in a serous adenocarcinoma. The staining is uniform and well-demarcated throughout the epithelium.*

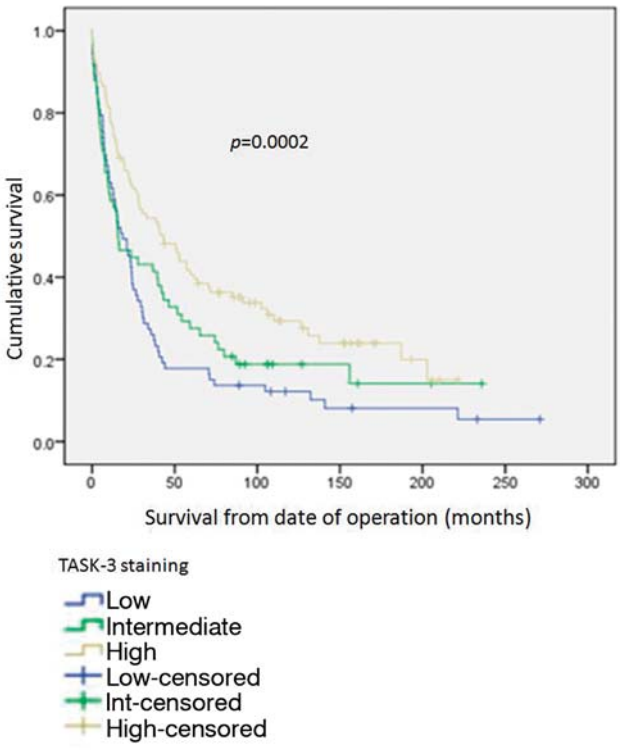


Figure 4. *Kaplan Meier survival analysis for TASK-3 immunohistochemistry staining. This demonstrates significantly improved survival in patients with 3+ staining (p=0.002).*

staining intensity with prognosis in OC. TASK-3 has been shown to have oncogenic potential as illustrated by work undertaken by Pei *et al*. (18). A point-mutation (*G95E*) within the consensus filter of *KCNK9* abolishes TASK-3 activity and oncogenic functions, including proliferation in low serum (1% fetal bovine serum), resistance to apoptosis and tumour cell growth (18). Embryonic mouse fibroblast cells (C8) expressing the mutation proliferated at a much slower rate than cells expressing wild-type *KCNK9* and overexpression of wild-type resulted in a 50% reduction of tumour necrosis factor (TNF)-induced apoptosis (18). Injecting athymic nude mice with C8 cells-expressing wild-type *KCNK9* caused tumour development, whereas injecting with C8 cells expressing vector alone or mutant *KCNK9* caused smaller, slower growing tumours (18). Finally, introducing *KCNK9*-G95E to the human lung carcinoma cell line Ben, which overexpresses TASK-3, reduced cell proliferation within 48 h (18). In keeping with this, as we identified TASK-3 expression in OC, we could suggest that TASK-3 is associated with malignant transformation in OC. However, TASK-3 expression was also found in normal OSEs. Similarly, TASK-3 is expressed in benign melanocytes and melanoma, with no difference in expression between the two (6, 11, 12). It is interesting to note that TASK-3 immunohistochemistry displayed strong nuclear and peri-nuclear staining in benign naevi, melanomas and colorectal tumours (6, 12). Our findings are in keeping with this. As a membrane protein, TASK-3 would be expected to be expressed on the cell surface. However, evidence is emerging of intracellular expression of TASK-3 due to mitochondrial expression of the channel (19). Functional properties of mitochondrial TASK-3 remain to be investigated (20). Our most pertinent findings are that 99% of tumours expressed TASK-3 and that increased TASK-3 immunopositivity was associated with clinically-significant increased survival, suggesting there is a role for TASK-3 to be considered as a new prognostic marker. As TASK-3 is oncogenic, increased expression might be expected to correlate with reduced survival. There is a paradox when considering $K^+$ channels, as although increased expression can result in increased cell proliferation, it can also result in increased apoptosis (21, 22). Therefore $K^+$ channels can have a dual role, and for example, there is evidence from the oncogenic voltage-gated $K^+$ channel, human ether-à-go-go related gene (HERG), that it plays an active role in apoptosis, increasing the rate of cell death, but conversely during cell proliferation, it acts as a membrane-anchoring protein, recruiting growth receptors to the membrane (21). Asher *et al*. demonstrated that blocking HERG in the SKOV-3 cell line resulted in a significant reduction in cell proliferation and accumulation of cells in the S and $G_2$/M phases, but no significant effect on apoptosis was observed, demonstrating that results of $K^+$ channel modulation can be unpredictable (15). One of the most significant studies relevant to our findings was published in 2012 (23). The study investigated gene and protein expression of *KCNK9* in two breast cancer cell lines, MCF-7 (non-invasive) and MDA-MB-231 (invasive) (23). The relationship between TASK-3 expression and cell migration was analysed using gene overexpression and knock-down techniques

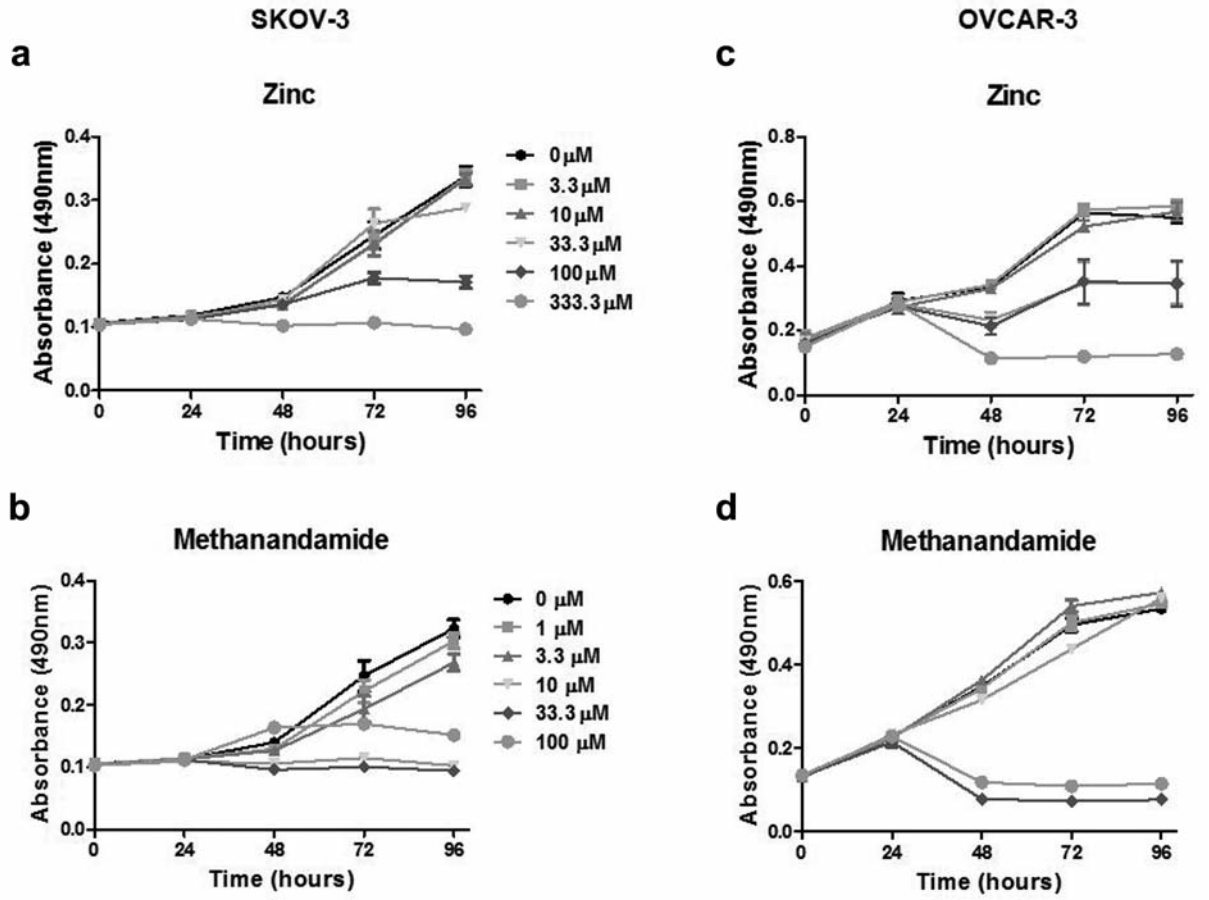


Figure 5. *Effect of zinc and methanandamide on SKOV-3 and OVCAR-3 cell proliferation. This demonstrates the effect of the TASK-3 blockers zinc and methanandamide on cell proliferation as measured by the CellTiter 96® Aqueous Non-Radioactive Cell Proliferation assay.*

and the functional expression of TASK-3 in both cell lines was recorded using patch-clamping (23). The study found that MCF-7 cells express TASK-3 more highly than MDA-MB-231 cells and that overexpression reduced cell migration and invasion; conversely, silencing *KCNK9* increased migration and invasion (23). These results are opposite to the effect one would expect, making TASK-3 an extremely interesting channel in the context of cancer. Inhibition of tumour cell proliferation by blocking $K^+$ channels has been demonstrated in melanoma (24, 25), small cell lung cancer (26), breast cancer (27) and prostate cancer (28). We confirmed a significant reduction in cell proliferation in OC cell lines with TASK-3 blockers, but unfortunately, there are no known specific K2P channel blockers and these drugs exhibit other actions in addition to TASK-3 blockade. Methanandamide is a cannabinoid that potently blocks TASK-3 and also acts at cannabinoid receptor type-1 and -2 (CB1 and CB2) receptors (29). CB1 and CB2 receptors have been identified in the human ovarian cortex and medulla (30), and evidence from rat oocytes confirms the presence of CB1 receptors in the surface epithelium (31). While methanandamide is a cannabinoid receptor agonist, previous studies have shown a variable effect of methanadamide *via* CB1/CB2-mediated pathways on tumour cells (32, 33). Zinc plays an important role in transcription factor function, antioxidant defence and DNA repair in cells (34). Zinc also results in the activation of the mitogen-activated protein kinase pathway (35). Therefore, although our observations demonstrate that TASK-3 blockers have a variable effect on early and late apoptosis in the SKOV-3 and OVCAR-3 cell lines, it is unclear how this relates to cancer progression, given that channel blockade inhibited cell proliferation. To complicate the matter further, there is evidence that the tumour microenvironment plays a significant role in tumourigenesis and metastatic spread (36). Intracellular alkalinity of cancer cells underlies neoplastic progression and this disturbance in the acid-base balance of tumour cells appears to be universal for all solid tumours, including OC (36). TASK-3 is modulated by changes in extracellular pH, closing with extracellular acidification (36). It may, therefore, be that in addition to regulating tumour cell apoptosis and

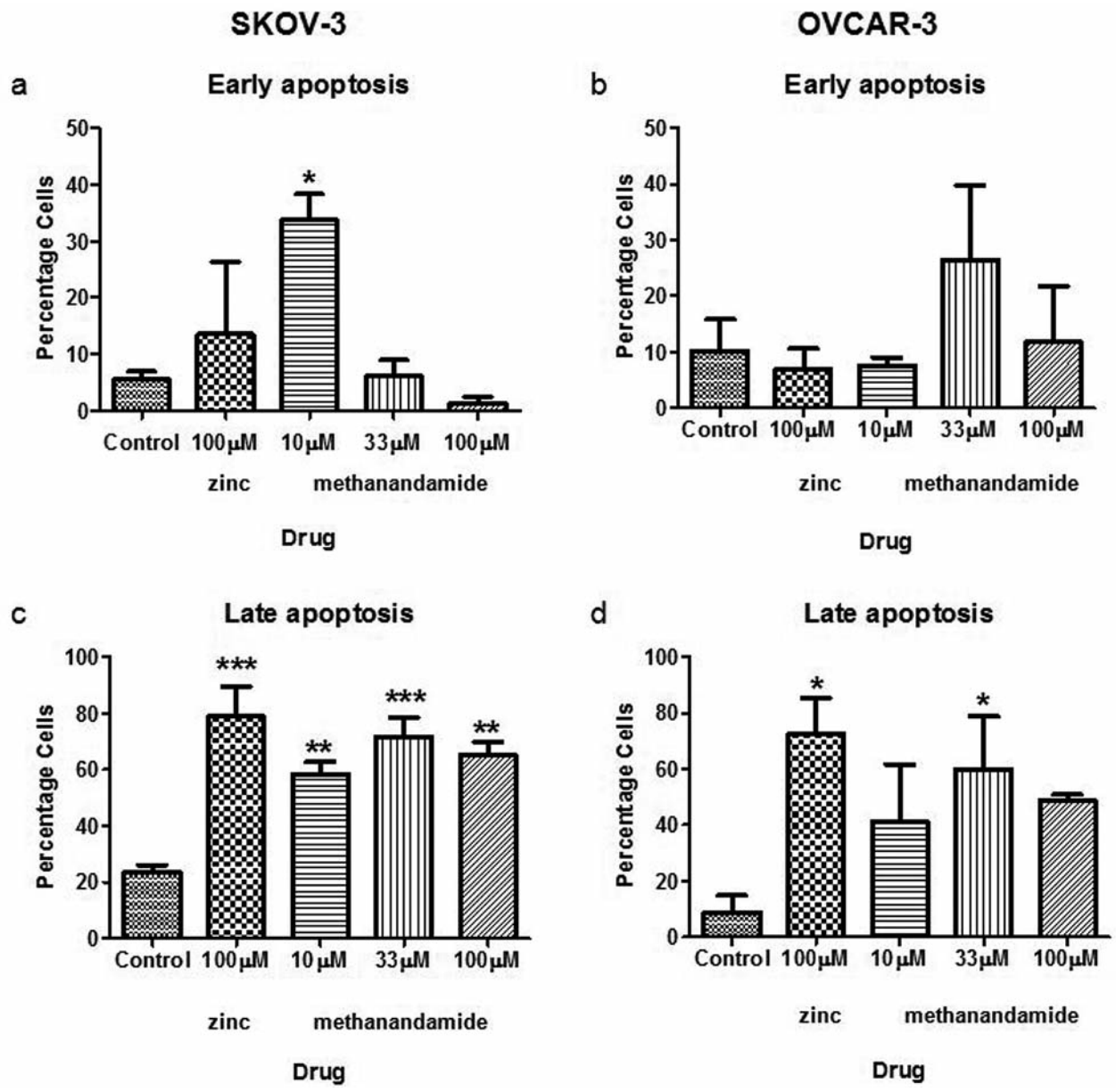


Figure 6. *Effect of zinc and methanandamide on early and late apoptosis in the SKOV-3 and OVCAR-3 cell lines. The graphs demonstrate the effect of the drugs on early and late apoptosis. Significance was defined as: *p<0.05; **p<0.01; ***p<0.001.*

proliferation, TASK-3 channels are involved in regulating cancer progression due to their pH-sensitive properties. It is this complex interplay of the acid-base balance with these pH-sensing channels that may well provide new insight into the role of TASK-3 in tumour progression and biology, and will form the basis of our future research.

## Conflicts of Interest

We have no conflicts of interest to declare.

## References

1 Cancer Research UK: Ovarian Cancer - UK Incidence Statistics: Cancer Research UK. http://www.cancerresearchuk.org/cancer-info/cancerstats/types/ovary/incidence/uk-ovarian-cancer-incidence-statistics. Last accessed 26th January, 2012.

2 Cancer Research UK: Ovarian Cancer Survival Statistics: Cancer Research UK. http://www.cancerresearchuk.org/cancer-info/cancerstats/types/ovary/survival/ovarian-cancer-survival-statistics. Last accessed 26th January, 2012.

3 Ozols RF: Challenges for chemotherapy in ovarian cancer. Ann Oncol *17(Suppl 5)*: v181-187, 2006.

4 Felipe A, Vicente R, Villalonga N, Roura-Ferrer M, Martinez-Marmol R, Sole L, Ferreres JC and Condom E: Potassium channels: new targets in cancer therapy. Cancer Detect Prev *30*: 375-385, 2006.

5 Bayliss DA and Barrett PQ: Emerging roles for two-pore-domain potassium channels and their potential therapeutic impact. Trends Pharmacol Sci *29*: 566-575, 2008.

6 Kovacs I, Pocsai K, Czifra G, Sarkadi L, Szucs G, Nemes Z and Rusznak Z: TASK-3 immunoreactivity shows differential distribution in the human gastrointestinal tract. Virchows Arch *446*: 402-410, 2005.

7 Bayliss DA, Sirois JE and Talley EM: The TASK family: two-pore domain background $K^+$ channels. Mol Interv *3*: 205-219, 2003.

8 Ekhterae D, Platoshyn O, Zhang S, Remillard CV and Yuan JX: Apoptosis repressor with caspase domain inhibits cardiomyocyte apoptosis by reducing $K^+$ currents. Am J Physiol Cell Physiol *284*: C1405-1410, 2003.

9 Patel AJ and Lazdunski M: The 2P-domain $K^+$ channels: role in apoptosis and tumorigenesis. Pflugers Arch *448*: 261-273, 2004.

10 Mu D, Chen L, Zhang X, See LH, Koch CM, Yen C, Tong JJ, Spiegel L, Nguyen KC, Servoss A, Peng Y, Pei L, Marks JR, Lowe S, Hoey T, Jan LY, McCombie WR, Wigler MH and Powers S: Genomic amplification and oncogenic properties of the KCNK9 potassium channel gene. Cancer Cell *3*: 297-302, 2003.

11 Kim CJ, Cho YG, Jeong SW, Kim YS, Kim SY, Nam SW, Lee SH, Yoo NJ, Lee JY and Park WS: Altered expression of *KCNK9* in colorectal cancers. APMIS *112*: 588-594, 2004.

12 Pocsai K, Kosztka L, Bakondi G, Gonczi M, Fodor J, Dienes B, Szentesi P, Kovacs I, Feniger-Barish R, Kopf E, Zharhary D, Szucs G, Csernoch L and Rusznak Z: Melanoma cells exhibit strong intracellular TASK-3-specific immunopositivity in both tissue sections and cell culture. Cell Mol Life Sci *63*: 2364-2376, 2006.

13 Marklund L, Henriksson R and Grankvist K: Cisplatin-induced apoptosis of mesothelioma cells is affected by potassium ion flux modulator amphotericin B and bumetanide. Int J Cancer *93*: 577-583, 2001.

14 Shepherd TG TB, Campbell EL and Nachtigal MW: Primary culture of ovarian surface epithelial cells and ascites-derived ovarian cancer cells from patients. Nature Protocols *1*: 2643-2649, 2007.

15 Asher V, Warren A, Shaw R, Sowter H, Bali A and Khan R: The role of Eag and HERG channels in cell proliferation and apoptotic cell death in SKOV-3 ovarian cancer cell line. Cancer Cell Int *11*: 6, 2011.

16 Asher V, Khan R, Warren A, Shaw R, Schalkwyk GV, Bali A and Sowter HM: The Eag potassium channel as a new prognostic marker in ovarian cancer. Diagn Pathol *5*: 78, 2010.

17 Kononen J, Bubendorf L, Kallioniemi A, Barlund M, Schraml P, Leighton S, Torhorst J, Mihatsch MJ, Sauter G and Kallioniemi OP: Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med *4*: 844-847, 1998.

18 Pei L, Wiser O, Slavin A, Mu D, Powers S, Jan LY and Hoey T: Oncogenic potential of TASK3 (*KCNK9*) depends on $K^+$ channel function. Proc Natl Acad Sci USA *100*: 7803-7807, 2003.

19 Rusznak Z, Bakondi G, Kosztka L, Pocsai K, Dienes B, Fodor J, Telek A, Gonczi M, Szucs G and Csernoch L: Mitochondrial expression of the two-pore domain TASK-3 channels in malignantly transformed and non-malignant human cells. Virchows Arch *452*: 415-426, 2008.

20 Szewczyk A, Jarmuszkiewicz W and Kunz WS: Mitochondrial potassium channels. IUBMB Life *61*: 134-143, 2009.

21 Wang H, Zhang Y, Cao L, Han H, Wang J, Yang B, Nattel S and Wang Z: HERG $K^+$ channel, a regulator of tumor cell apoptosis and proliferation. Cancer Res *62*: 4843-4848, 2002.

22 Wang Z: Roles of $K^+$ channels in regulating tumour cell proliferation and apoptosis. Pflugers Arch *448*: 274-286, 2004.

23 Lee GW, Park HS, Kim EJ, Cho YW, Kim GT, Mun YJ, Choi EJ, Lee JS, Han J and Kang D: Reduction of breast cancer cell migration *via* up-regulation of TASK-3 two-pore domain $K^+$ channel. Acta Physiol (Oxf) *204*: 513-524, 2012.

24 Lepple-Wienhues A, Berweck S, Bohmig M, Leo CP, Meyling B, Garbe C and Wiederholt M: $K^+$ channels and the intracellular calcium signal in human melanoma cell proliferation. J Membr Biol *151*: 149-157, 1996.

25 Nilius B and Wohlrab W: Potassium channels and regulation of proliferation of human melanoma cells. J Physiol *445*: 537-548, 1992.

26 Pancrazio JJ, Tabbara IA and Kim YI: Voltage-activated $K^+$ conductance and cell proliferation in small-cell lung cancer. Anticancer Res *13*: 1231-1234, 1993.

27 Woodfork KA, Wonderlin WF, Peterson VA and Strobl JS: Inhibition of ATP-sensitive potassium channels causes reversible cell-cycle arrest of human breast cancer cells in tissue culture. J Cell Physiol *162*: 163-171, 1995.

28 Skryma RN, Prevarskaya NB, Dufy-Barbe L, Odessa MF, Audin J and Dufy B: Potassium conductance in the androgen-sensitive prostate cancer cell line, LNCaP: involvement in cell proliferation. Prostate *33*: 112-122, 1997.

29 Veale EL, Buswell R, Clarke CE and Mathie A: Identification of a region in the TASK-3 two-pore domain potassium channel that is critical for its blockade by methanandamide. Br J Pharmacol *152*: 778-786, 2007.

30 El-Talatini MR, Taylor AH, Elson JC, Brown L, Davidson AC and Konje JC: Localisation and function of the endocannabinoid system in the human ovary. PLoS One *4*: e4579, 2009.

31 Bagavandoss P and Grimshaw S: Temporal and spatial distribution of the cannabinoid receptors (CB1, CB2) and fatty acid amide hydroxylase in the rat ovary. Anat Rec *293*: 1425-1432, 2010.

32 Gustafsson K, Christensson B, Sander B and Flygare J: Cannabinoid receptor-mediated apoptosis induced by R(+)-methanandamide and Win55,212-2 is associated with ceramide accumulation and p38 activation in mantle cell lymphoma. Mol Pharmacol *70*: 1612-1620, 2006.

33 Olea-Herrero N, Vara D, Malagarie-Cazenave S and Diaz-Laviada I: The cannabinoid $R^+$ methanandamide induces IL-6 secretion by prostate cancer PC3 cells. J Immunotoxicol *6*: 249-256, 2009.

34 Ho E: Zinc deficiency, DNA damage and cancer risk. J Nutr Biochem *15*: 572-578, 2004.

35 Daniels WM, Hendricks J, Salie R and van Rensburg SJ: A mechanism for zinc toxicity in neuroblastoma cells. Metab Brain Dis *19*: 79-88, 2004.

36 Harguindey S, Orive G, Luis Pedraz J, Paradiso A and Reshkin SJ: The role of pH dynamics and the $Na^+/H^+$ antiporter in the etiopathogenesis and treatment of cancer. Two faces of the same coin-one single nature. Biochim Biophys Acta *1756*: 1-24, 2005.


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