Virchows Archiv (2019) 475:105–114
https://doi.org/10.1007/s00428-019-02584-y

ORIGINAL ARTICLE

# Transient receptor potential vanilloid (TRPV) channel expression in meningiomas: prognostic and predictive significance

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Received: 21 December 2018 / Revised: 24 April 2019 / Accepted: 7 May 2019 / Published online: 22 May 2019


## Abstract

The TRPV1–4 members of TRPV cation channel subfamily are mainly regarded as polymodal receptors that may be activated by diverse changes in cellular microenvironment and endogenous and exogenous agents. Abnormal expression of these channels has been reported in various tumors but not in meningiomas. Meningioma cells are thought to originate from arachnoid cap cells due to cytological and functional similarities between the two types of cells. To investigate the expression profile of TRPV1–4 channels in meningiomas and compare with TRPV1–4 channel expression in leptomeninges, we used immunohistochemistry in formalin-fixed, paraffin-embedded semi-serial tissue sections from 175 meningiomas with different grades and histological subtypes, and normal brain or meningioma specimens that contained leptomeninges. The labeling index (LI), defined as the percentage of positive (labeled) cells out of the total number of tumor cells counted, was determined. Leptomeninges were TRPV1–4 immunonegative. A significant percentage of tumors exhibited TRPV1–4 channel expression which was independent of the proliferation index of the tumors but was significantly associated with histopathological subtypes. The TRPV1 and TRPV3 immunoexpression was decreased whereas TRPV4 immunoexpression was significantly greater in high-grade (WHO, grade II and III) as compared with low-grade (WHO, grade I) meningiomas. Additionally, TRPV4 emerged as an independent predictor for the degree of malignancy using the binary logistic regression model [dependent variable: grade I versus higher grades (II and III)]. Kaplan-Meier analysis for 102 patients showed no significant association of TRPV1–4 expression with overall survival. The above data support that TRPV1–4 channels are implicated in meningioma pathogenesis, and TRPV4 has predictive significance in the disease.

**Keywords** Meningiomas · TRPV1 · TRPV2 · TRPV3 · TRPV4

## Introduction

Meningiomas are considered the neoplastic counterparts of arachnoid cap cells [1]. Apart from its role as a brain surface cover, arachnoid cell layer comprises the structural basis for the arachnoid blood-cerebrospinal fluid (CSF) barrier, which is present from early fetal life [2] whereas arachnoid cells in arachnoid granulations are responsible for CSF drainage into the dural sinuses and veins [3]. Recently, drug transporters have been identified in arachnoid barrier cells [4]. According to World Health Organization (WHO) classification, meningiomas include a wide range of histological subtypes, distributed into three grades [5]. Specific alterations in genome and cell signaling pathways have been proposed to be implicated in meningiomas progression [6]. Thus, evaluation of molecular parameters in addition to histological patterns has been adopted in Classification of Tumors of the Central Nervous System (2007 CNS WHO and updated 2016 CNS WHO) [7, 8], targeting for more accurate diagnosis and prognosis of these tumors.

Transient receptor potential family (TRP) are ligand-gated cation-permeable ion channels (such as $Ca^{2+}$ and $Mg^{2+}$) but also serve as membrane "cellular sensors" that respond to changes in the cellular environment, including temperature,

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stretch/pressure, chemicals, oxidation/reduction, osmolarity, and pH, thus mediating downstream signaling. Importantly, several TRP channels are also activated by natural products, including herbs, spices, venoms, and toxins [9]. Besides their well-documented role in the cell surface, TRP channels are reported to be present in intracellular membranes and interact with proteins which are important for TRP channel trafficking and activity [10]. TRP channels are widely expressed in excitable and non-excitable human cells participating in regulation of tissue-specific processes [9]. Inherited or acquired dysfunction of these channels has been found in diverse pathological states ("channelopathies") [10, 11]. Therefore, TRP channels have been proposed as targets with therapeutic potential for central nervous system, cardiovascular, respiratory, bladder, renal and skin disorders, inflammatory bowel disease, diabetes, and cancer [12, 13].

The vanilloid subfamily of TRP channels was named after its founding member, the vanilloid receptor TRPV1, and consists of six members that are categorized in two groups based on sequence homology, TRPV1–4 and TRPV5–6 [14]. In contrast to TRPV5 and TRPV6 which are highly $Ca^{2+}$-selective channels, the TRPV1–4 subgroup is weakly $Ca^{2+}$-selective but highly sensitive to heat, so they are called "thermo-TRP channels" [15]. TRPV1–4 channels mainly function as polymodal receptors since diverse endogenous or exogenous stimuli like anandamide, pro-inflammatory substances, eicosanoids, capsaicin, and resiniferatoxin for TRPV1 [16]; changes in osmolarity and membrane stretch for TRPV2 [17]; pro-inflammatory agents like bradykinin, histamine, prostaglandin E2 (PGE2), and plant compounds such as oregano, camphor, thyme, and cannabinoids for TRPV3 [18]; and mechanical stretch, hypotonicity, and endogenous substances such as arachidonic acid and epoxyeicosatrienoic acids, endocannabinoids, as well as synthetic alpha-phorbol derivatives for TRPV4 [19] may activate these channels.

Accumulation knowledge implicates alterations of TRPV channel expression in cancer development and progression [20, 21]. Particularly, TRPV1 and TRPV4 are essential for migration of human hepatoblastoma, cervical and bladder cancer cells, and breast tumor-derived endothelial cells, respectively (reviewed in [22]). Additionally, TRPV2 augments growth and invasive properties of prostate and urothelial tumors and may be an early event in bladder carcinogenesis and hepatocarginogenesis [23]. On the other hand, there is evidence for a pro-apoptotic role of TRPV1 and TRPV2 channels in certain normal or tumor-cultured cells (reviewed in [24]). With respect to brain tumors, previous studies have focused on gliomas. Specifically, TRPV1 channel expression was inversely correlated with grading and patients' survival [25, 26] whereas TRPV2 has been reported to promote glioblastoma stem-like cell differentiation and inhibit their proliferation [27]. More recently, overexpression of TRPV4 in gliomas has been associated with poor prognosis of the patients [28]. Vanilloids and cannabinoids are more widely used as therapeutic agents to suppress tumor growth and invasion and promote apoptosis in high-grade astrocytomas, cervical and urothelial cancer cells [29].

The drainage of CSF from arachnoid cells into the dural sinuses [3] indicates that these cells may have transporter mechanisms including channels for water and ions. With this in mind, we aimed to investigate the possible expression of TRPV1, TRPV2, TRPV3, and TRPV4 channels in leptomeninges found in normal brain tissues or in meningioma specimens and the neoplastic counterparts of arachnoid cap cells, meningiomas. The relationship between TRPV1–4 channel expression and patients' clinicopathological characteristics and survival was also studied.

## Materials and methods

### Patients

A total of 175 patients with meningiomas, who underwent surgery at the Neurosurgery Department of Patras University School of Medicine, during a 14-year period, were studied (Table 1). The formalin-fixed, paraffin-embedded archival tissue blocks were retrieved from the archives of the Department of Pathology of the same Hospital, and matching hematoxylin and eosin (H&E)-stained slides were reviewed and screened for representative tumor regions by a pathologist. Tumors were evaluated by routine methods for histopathology, including immunohistochemical staining for Ki-67 index as proliferation marker and graded according to the diagnostic criteria of the WHO classification system [7, 8]. Normal human brain tissue was obtained postmortem (two males, 56 and 28 years). The use of human specimens was in accordance with the University Ethics Commission.

### Immunohistochemistry study

All tissues for immunohistochemistry were fixed in formalin and embedded in paraffin. Consecutive (semi-serial) 4 μm sections of tissue samples were collected on poly-L-lysine-coated slides. One section for each sample was stained with H&E. For immunohistochemical studies, the histological sections were deparaffinized in xylene and rehydrated in graded alcohols up to water. Antigen retrieval was performed by microwaving the slides in 0.01 M citrate buffer (pH 6). Endogenous peroxidase activity was quenched by treatment with 1% hydrogen peroxide for 20 min. Incubation with an appropriate protein-blocking solution was performed. Sections were subsequently incubated with primary antibodies: polyclonal rabbit anti-TRPV1 antibody (cat. no. NBP1-71774; dilution, 1:200) manufactured by Novus Biologicals, Ltd. (Cambridge, UK), polyclonal rabbit anti-TRPV2 (cat. no.

**Table 1** Main demographic and clinical characteristics of patients

| Histology/grading of meningiomas | Age at diagnosis (range; mean) | Gender (no of cases) Male/Female | Race Greek/ other | *N* (%) |
|---|---|---|---|---|
| Grade I | 21–86; 57.97 | 40/97 | 133/4 | 137 (78.2) |
| *Meningothelial* | | | | 92 (52.5) |
| *Transitional* | | | | 10 (5.7) |
| *Microcystic* | | | | 3 (1.7) |
| *Angiomatous* | | | | 1 (0.5) |
| *Secretory* | | | | 3 (1.7) |
| *Fibrous* | | | | 18 (10.2) |
| *Psammomatous* | | | | 10 (5.7) |
| Grade II | 37–81; 65.70 | 15/20 | 35/0 | 35 (20) |
| *Atypical* | | | | 32 (18.2) |
| *Clear cell* | | | | 1 (0.5) |
| *Chordoid* | | | | 2 (1.1) |
| Grade III | | | | |
| *Anaplastic* | 66–78; 74.00 | 0/3 | 3/0 | 3 (1.7) |
| Recurrence | | | | 16 (9.1) |
| Death | | | | 25 (24,5) |
| Follow-up, months (median; range) | | | | 63; 12–288 |

TA317464; dilution 1:200) and monoclonal mouse anti-TRPV3 antibody (cat. no. AM20072PU-N; dilution 1:300) produced by Acris Antibodies GmbH (Herford, Germany), and rabbit polyclonal anti-TRPV4 antibody from Abcam (cat. no. ab39260; Cambridge, UK; dilution 1:200). Detection was carried out using the Envision Plus Detection System kit, according to the manufacturer's instructions (DakoCytomation, USA), with 3,3′-diaminobenzidine (DAB) as a chromogen (which yielded brown reaction products). Sections were counterstained with Mayer's hematoxylin solution, dehydrated and mounted. To ensure antibody specificity, negative controls included the omission of primary antibody and substitution with non-immune serum. Control slides were invariably negative for immunostaining. Positive human inflammatory bowel for TRPV1–4 was used as positive control [30].

## Scoring of immunohistochemical staining

To determine labeling index (LI) (% labeled cells) for each antibody, two observers independently assessed ten non-overlapping, random fields (× 400 total magnification) for each case and manually counted 100 tumor cells in each field with the aid of an ocular grid. Immunopositive endothelial and stromal cells were excluded from the cell counts. Immunoexpression of proteins was examined in adjacent (semi-serial) sections of each sample. The mean values of LIs (32.85, 35.93, 32.93, and 16.12 for TRPV4, TRPV1, TRPV3, and TRPV2, respectively) were used as cut-off points to classify tumors as exhibiting low and high protein expression. Microphotographs were obtained using a Nikon DXM 1200C digital camera mounted on a Nikon Eclipse 80i microscope and ACT-1C software (Nikon Instruments Inc., Melville, NY, USA).

## Statistical analysis

Non-parametric methods were used for statistical analysis of the results. Median comparisons were performed with Wilcoxon's rank-sum test (equivalent to the Mann-Whitney *U* test) and the Kruskal-Wallis test between cohorts where $N \geq 10$. Spearman correlation was used to assess the significance of associations between LIs. Binary logistic regression model was used to investigate the predictive value of TRPV1, TRPV2, TRPV3, and TRPV4 expression levels regarding the grade [dependent variable: grade I versus higher grades (II and III)]. Follow-up for 102 patients was evaluated as the number of months from the date of the diagnostic surgical procedure to that of death or date of last follow-up (April 2019). The median follow-up period was 63 months (range 12–288 months). Overall survival (OS) was analyzed using the Kaplan-Meier method, and differences between subgroups (LI low vs. LI high stained cohorts) were compared with the long-rank-test. Cox proportional hazard univariate analysis was performed to identify predictors of survival and the relative risk was calculated with 95% confidence interval (CI). *P* values < 0.05 were considered significant. Statistical analysis was performed using SPSS package (version 23.0; SPSS, Inc., Chicago, IL, USA).

## Results

### Immunolocalization of TRPV1–4 in meningiomas and leptomeninges

TRPV1–4 channel expression was not detected in leptomeninges found in normal human brain tissues or meningioma specimens. However, neurons and a few glial cells in normal brain showed TRPV1 and TRPV4 cytoplasmic immunoreactivity. In meningiomas, TRPV1–4 channel cytoplasmic expression was observed over a wide range in the tumor body but its expression in tumoral tissues nearby to or invading the meninges increased significantly. TRPV1–4 immunoreactivity was detected in vessels of meningiomas. Furthermore, in some samples—irrespective of the TRPV immunoreactivity of tumor cells—the endothelium of tumor capillaries displayed TRPV4 immunoreactivity. TRPV4 nuclear immunostaining was also noticed in certain tumor cells (Figs. 1, 2, and 3).

### Quantitative analyses of the immunohistochemical findings

Immunohistochemical findings are illustrated in Table 2. TRPV1 and TRPV3 expression levels were decreased in grade III meningiomas compared with grade I and grade II meningiomas although the differences were not statistically significant ($p \geq 0.05$). There was a strong statistically significant correlation between TRPV1 and TRPV3 expression (Spearman of TRPV1 with TRPV3 = 0.782, $p = 0.00$). TRPV4 LIs in high-grade (WHO, grade II and grade III) meningiomas were significantly higher compared with grade I meningiomas ($p < 0.05$). Interestingly, the anaplastic meningioma (WHO, grade III) that displayed the highest TRPV4 expression (LI = 80) was a recurrent meningioma. Comparison of median LIs revealed that TRPV1 and TRPV3 expression was significantly increased than TRPV4 and TRPV2 channel expression in grade I meningiomas (Fig. 4). Quantitative analysis of TRPV1–4 channel expression between histological subtypes showed that besides atypical (WHO, grade II), clear cell (WHO, grade II), and anaplastic meningiomas (WHO, grade III), certain histological subtypes, in the group of grade I meningiomas such as transitional, microcystic, and angiomatous meningiomas, displayed higher expression for TRPV4 compared with meningothelial, fibrous, psammomatous, and secretory meningiomas (Fig. 5). Furthermore, high expression for TRPV1 and TRPV3 in transitional meningiomas and high expression for TRPV2 in

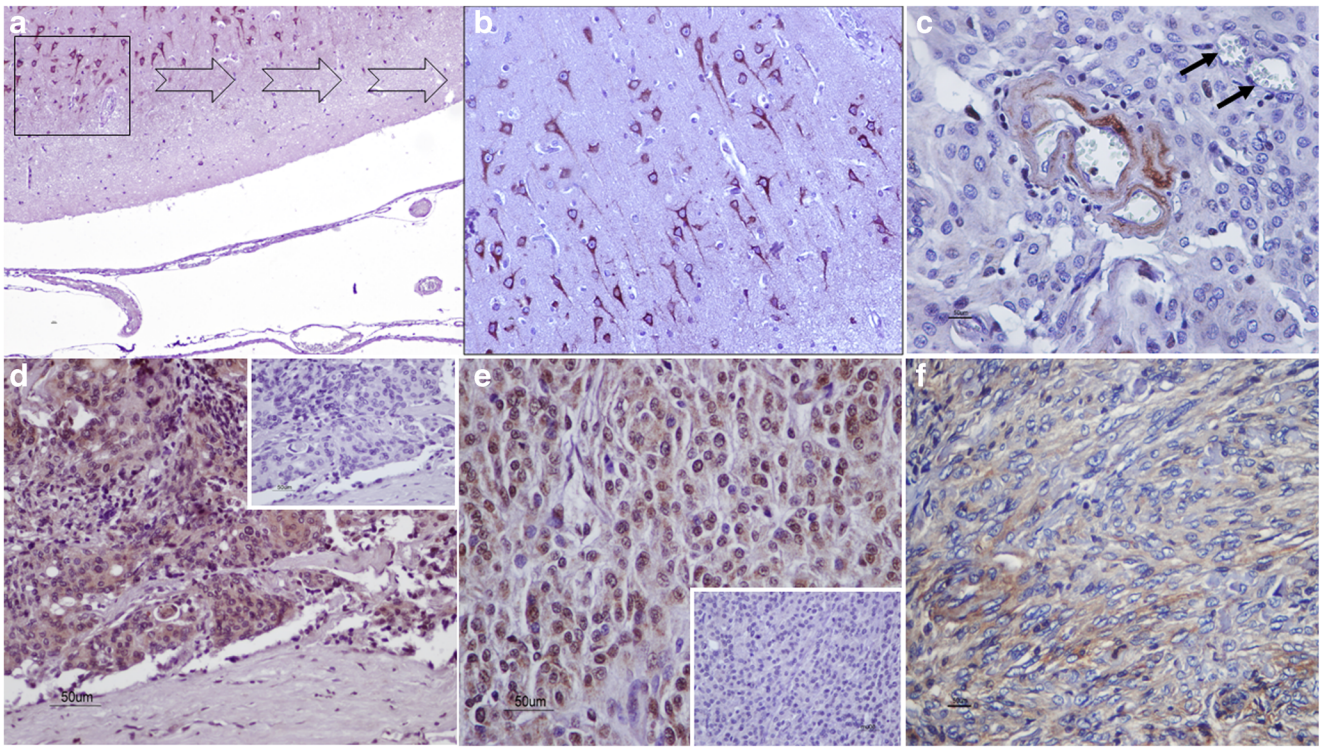


**Fig. 1** TRPV4 channel expression in normal brain tissue: Leptomeninges are TRPV4-immunonegative, × 100, (**a**). Neurons in cerebrum demonstrate strong cytoplasmic TRPV4 immunoreactivity (**b**), × 200. Certain vessels display TRPV4 immunoreactivity in a meningothelial meningioma (WHO, grade I) whereas the tumor cells and some vessels (*arrows*) are TRPV4-immunonegative (**c**) × 400. Strongly TRPV4-immunopositive tumor cells nearby the dura meninge in an atypical meningioma (WHO, grade II) (**d**), × 200. Immunostaining is absent in negative control sections (**d**, insert). Abundant cytoplasmic and nuclear TRPV4 immunoreactivity (LI = 80) in an anaplastic meningioma (WHO, grade III) (**e**), × 400. Immunostaining is absent in negative control sections (**e**, insert). Weak to moderate immunostaining for TRPV2 (LI = 20) in a fibrous meningioma (WHO, grade I) (**f**), × 400. Counter stain, hematoxylin; scale bar 50 μm; TRPV, Transient receptor potential cation channel subfamily V

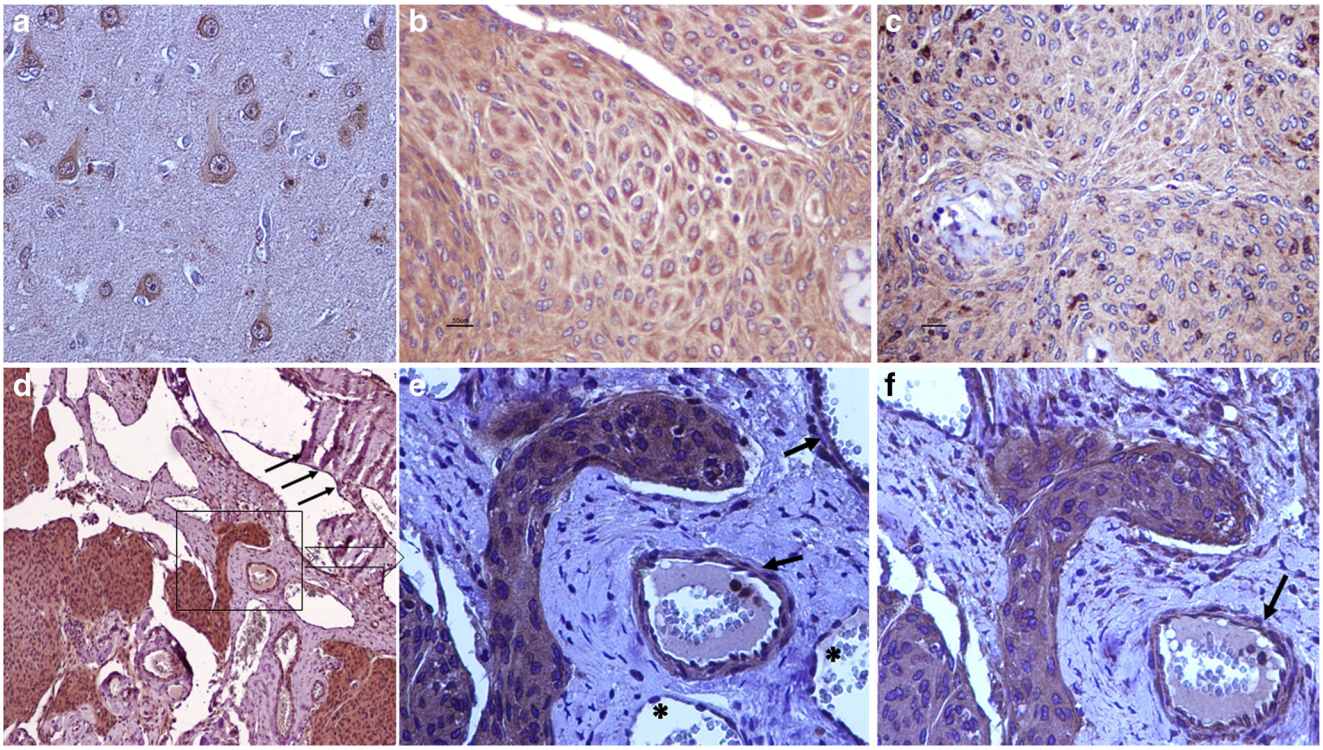


**Fig. 2** **a** TRPV1 cytoplasmic immunolocalization in neurons and a few glial cells of normal brain tissue. **b**, **c** Strong cytoplasmic immunoreactivity for TRPV1 (LI = 80) and TRPV3 (LI = 95) in homologs fields of immediately adjacent (semi-serial) sections of a meningothelial meningioma (WHO, grade I) (the same sample of Fig. 1c). Note that some meningioma cells demonstrate conspicuous TRPV3 immunopositivity (**c**). **d**–**f** Photomicrographs from homolog fields of immediately adjacent (semi-serial) sections of a meningothelial meningioma (WHO, grade I); TRPV3 (**d**, **e**) and TRPV1 (**f**) channel cytoplasmic expression in a wide range of the tumor body and in tumoral tissues invading the meninges (**d**, *arrows*). Note that certain vessels are intensely immunostained for TRPV3 (**e**, *arrows*), and TRPV1 (**f**, *arrow*) whereas some vessels are TRPV-immunonegative (**e**, *asterisks*). Counter stain, hematoxylin; original magnification × 400; scale bar 50 μm; TRPV, Transient receptor potential cation channel subfamily V

psammomatous meningiomas was detected in the group of WHO, grade I meningiomas (data not shown). No association emerged between TRPV1–4 channel expression and Ki67 expression ($p \geq 0.05$) or clinical features of the patients (e.g., age, gender, recurrence).

### TRPV1–4 expression as a predictive and prognostic factor

We used the binary logistic regression model to investigate the predictive value of TRPV1, TRPV2, TRPV3, and TRPV4 expression levels in the diagnosis of grade of meningiomas. Among all TRPV channels examined, only TRPV4 is independent predictor for grade of meningiomas. Specifically, high TRPV4 expression was associated with an 81.6% probability of accurately distinguishing grade I from high-grade (WHO, grade II and III) meningiomas ($p = 0.03$). Additionally, to test the relationship between TRPV1–4 expression and overall survival, survival curves according to the Kaplan-Meier method were plotted. Stratification according to the two molecular subgroups (low and high TRPV channel expression) revealed no statistically significant association with OS (long-rank $p \geq 0.05$).

## Discussion

TRPV1–4 subgroup of TRPV channels is mainly known for the function of its members as polymodal receptors sensing changes in cellular microenvironment whereas pharmacological modulation of these channels in cancer cells has been reported [29]. Very little is known about the expression of TRPV1–4 channels in meningiomas. In the present study, a high percentage of meningiomas demonstrated expression of TRPV1–4 channels whereas leptomeninges were TRPV1–4 immunonegative. The hypothesis that meningiomas originate from arachnoid cap cells is based on cytological and functional similarities of meningioma cells with arachnoid cells [8]. Therefore, this discrepancy regarding TRPV1–4 channel expression may reflect the existence of possible mechanisms that activate the expression of TRPV1–4 channels, even in benign meningiomas (WHO, grade I), due to the cancerous context of meningioma cells. Besides that, it is well documented that TRP channel family appears to have a role in tumor growth [20, 21].

It is worth noting that TRPV1, TRPV3, and TRPV4 channel expression was differentiated in high-grade (WHO, grade II and III) compared with benign meningiomas (WHO, grade

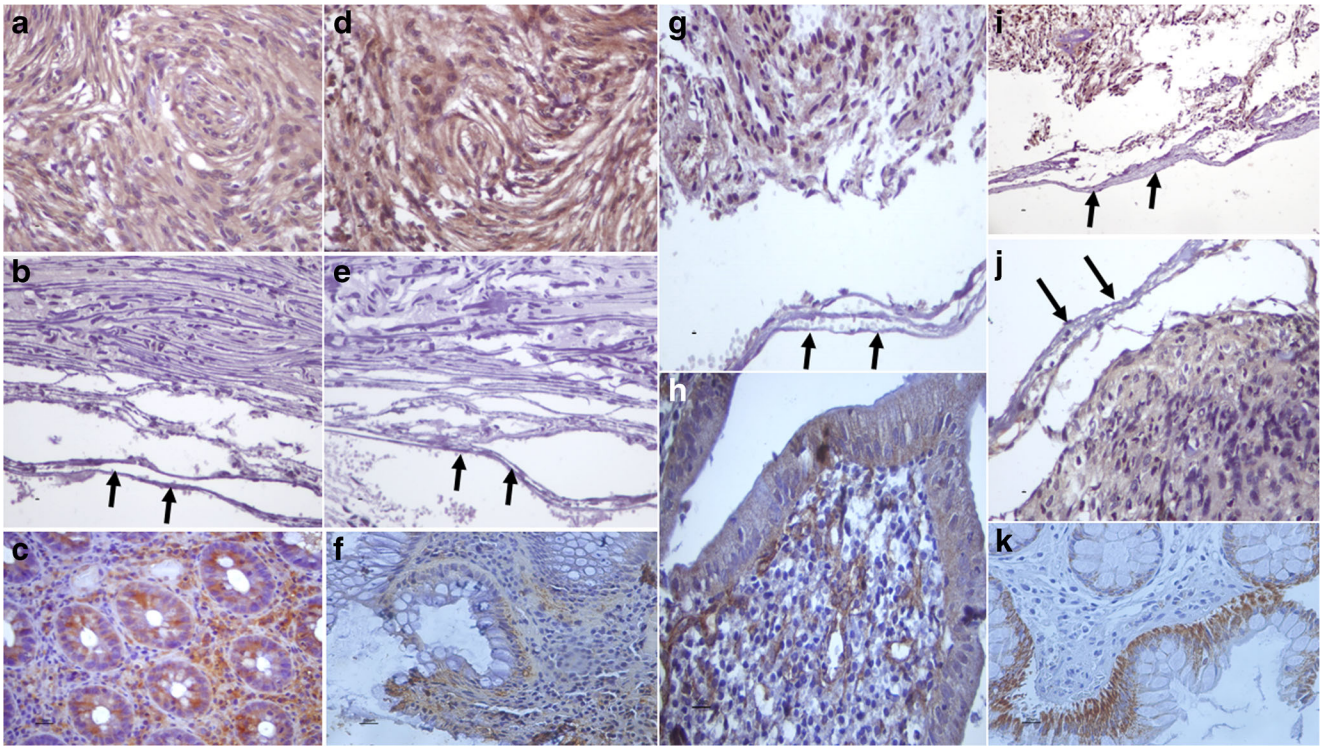


**Fig. 3** . TRPV1 (**a**, **b**) and TRPV3 (**d**, **e**) channel expression in homolog fields of immediately adjacent (semi-serial) sections of a meningioma WHO, grade I. Note that leptomeninges (**b**, **e**, *arrows*) are TRPV1 and TRPV3-immunonegative whereas the cells in the tumor body (**a**, **d**) display strong TRPV1 and TRPV3 immunoreactivity. Human inflammatory bowel specimens were used as positive controls for TRPV1 (**c**) and TRPV3 (**f**) expression. Leptomeninges demonstrate TRPV2 immunostaining in a meningioma sample WHO, grade I (**g**, *arrows*) in contrast with tumor cells which are TRPV2-immunopositive. Human inflammatory bowel was used as positive control for TRPV2 expression (**h**). (**i**, **j**) TRPV4 channel expression is observed in meningioma specimens WHO, grade I whereas leptomeninges are TRPV4-immunonegative (**i**, **j**, *arrows*). Human inflammatory bowel was used as positive control for TRPV4 expression (**k**). Counter stain, hematoxylin; original magnification × 400; scale bar 50 $\mu$m; TRPV, Transient receptor potential cation channel subfamily V

I). Particularly, strongly decreased TRPV1 expression in grade III meningiomas compared with grade I or grade II meningiomas was observed (although no statistical analysis was performed). Previous studies have also shown that

**Table 2** TRPV channels labeling index (LI) and immunopositive tumors in different grades of meningiomas. The (non-parametric) Wilcoxon's Rank-Sum test was performed and the level of significant was defined as $p < 0.05$

| Meningiomas | TRPV4 Mean ± SD range immunopositive tumors (%) | TRPV1 Mean ± SD range immunopositive tumors (%) | TRPV3 Mean ± SD range immunopositive tumors (%) | TRPV2 Mean ± SD range immunopositive tumors (%) |
|---|---|---|---|---|
| Grade I | 30.34 ± 31.22[a, b] | 37.84 ± 27.84 | 34.77 ± 27.78 | 15.24 ± 17.59 |
| | (0–95) | (0–95) | (0–100) | (0–80) |
| | 75.93 | 90.27 | 83.33 | 66.21 |
| Grade II | 47.84 ± 33.33 | 35.38 ± 26.33 | 30.85 ± 16.23 | 25.71 ± 30.05 |
| | (0–90) | (5–80) | (10–60) | (0–75) |
| | 87.17 | 92.30 | 100 | 71.42 |
| Grade III | 55.00 ± 35.00 | 4.00 ± 5.29 | 1.00 ± 1.14 | 15.00 ± 21.21 |
| | (15–80) | (2–10) | (0–2) | (0–30) |
| | 100 | 33.33 | 0 | 50 |

Meningiomas were regarded as immunopositive when more than or equal to 5% of tumor cells were immunoreactive (LIs ≥ 5)

*LI* the percentage of positively (labeled) cells out of the total number of tumor cells counted, *Mean* mean labeling index, *SD* standard deviation

[a] $p = 0.004$ vs. TRPV4 expression in grade II meningiomas

[b] $p = 0.002$ vs. TRPV4 expression in the group of grade II and grade III meningiomas)

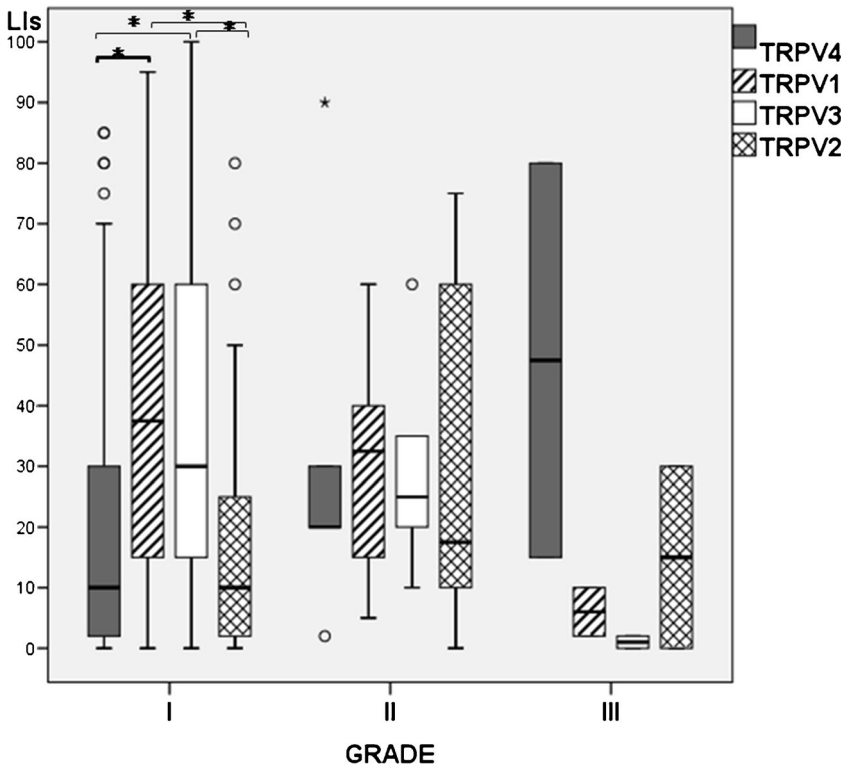


**Fig. 4** TRPV1, TRPV2, TRPV3, and TRPV4 channel expression in correlation to the grade for meningiomas. The (non-parametric) Wilcoxon's rank-sum test was used and the level of significant was defined as $p < 0.05$. *Statistically significant differences between TRPV channel expression in the group of grade I meningiomas were detected ($p < 0.01$)

TRPV1 expression was inversely correlated with cancer progression in gliomas [25, 26] as well as in renal cell [31] and bladder transitional cell carcinomas [32]. Based on the above-mentioned data, the reduction of TRPV1 channel expression in anaplastic meningiomas (WHO, grade III) may indicate an association of these channels with meningioma cell differentiation although the number of this cohort of meningiomas was very low ($n = 3$), in this study, and further experiments with larger series of anaplastic meningiomas are necessary. In addition to its role in generation and transduction of pain through nociceptive sensory neurons [12], accumulating experimental data implicate TRPV1 in cancer pain [20]. Although TRPV1 was not detected in leptomeninges, previous studies have shown TRPV1 channel-immunopositive nerve fibers and blood vessels in dura matter which may contribute to the pathophysiology of migraine [33, 34].

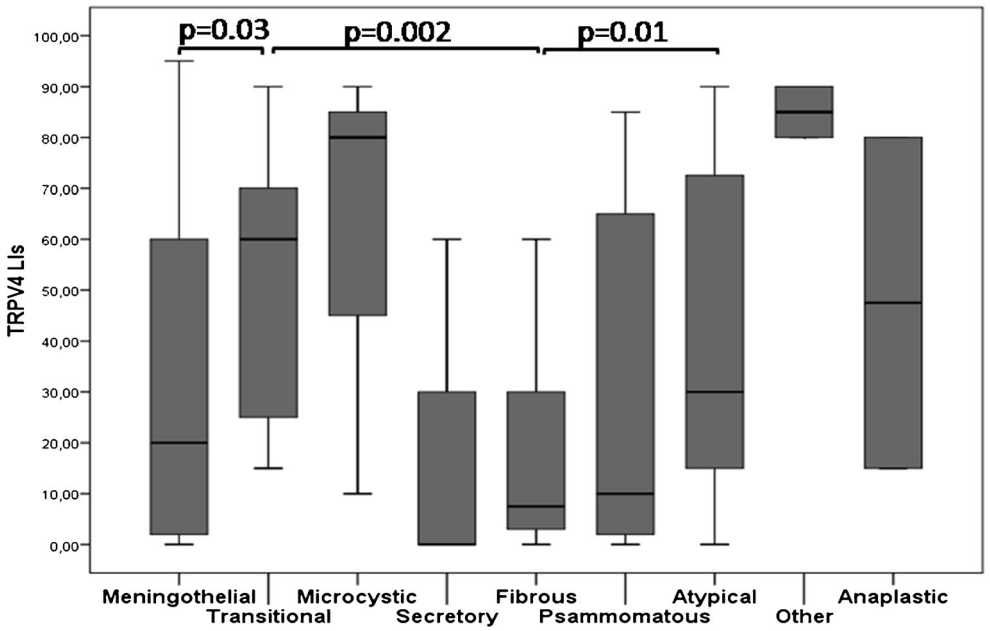


**Fig. 5** Comparison of TRPV4 channel expression between histological subtypes of meningiomas. The (non-parametric) Wilcoxon's rank-sum test was used in groups of histological subtypes where $N \geq 10$. The level of significant was defined as $p < 0.05$. Other: one angiomatous meningioma (WHO, grade I), one clear cell meningioma (WHO, grade II), and two chordoid meningiomas (WHO, grade II) were included in this cohort

Furthermore, anandamide has been demonstrated to cause vasodilation of dural blood vessels in rats by activating TRPV1 channels [34]. Interestingly, TRPV1 channel expression was detected in blood vessels of meningiomas. Functional studies could provide the therapeutic utility of TRPV1 inhibitors in management of pain, e.g., headache, in patients with meningiomas.

TRPV3 expression levels, similarly to TRPV1, strongly decreased in grade III meningiomas compared with grade I or grade II meningiomas. Furthermore, a strong significant relationship between TRPV3 and TRPV1 channel expression was detected which is justified due to the proximity of chromosomal location of these genes and the heterotetramerization of TRPV1 and TRPV3 [10]. Regarding the TRPV3 channel, not much is known about its role in cancer. A recent study reports that overexpression of TRPV3 is correlated with tumor progression in non-small cell lung cancer [35]. Considering that TRPV3 is mainly expressed in keratinocytes where it plays several roles in skin functions such as cutaneous sensations, hair development, and barrier function [18], it is challenging to research possible analogue roles in tumors.

In contrast with TRPV1 and TRPV3 channels, a statistically increased expression of TRPV4 in high-grade meningiomas (WHO, grade II and grade III) compared with grade I meningiomas was detected. Moreover, only TRPV4 emerged as an independent predictor factor for grade in meningiomas. Similarly, recent research has reported that increased TRPV4 expression levels were associated with the histological grade of gliomas and worse prognosis of the patients [28]. Although TRPV4 has been well documented as a physiological sensor for temperature, osmotic pressure, and mechanical deformation, there are little data about its implication in cancer. Particularly, TRPV4 promotes the migration and invasion of human glioma [28] and breast cancer cells [36]. According to the above findings, TRPV4 may be involved in the progression of meningiomas. Indeed, apart from other malignant characteristics, e.g., high mitotic index, hypercellularity, and cytological atypia, high-grade meningiomas may also display brain invasiveness [7, 8]. Additionally, it is well established that multiple as well as recurrent meningiomas are of clonal origin and represent metastatic meningeal seeding through dural spread [6]. In this study, strongly TRPV4-immunopositive cells localized nearby or invading the meninges were found. Interestingly, these cells also displayed co-expression of TRPV1 and TRPV3 channels implying that TRPV1, TRPV3, and TRPV4 may be implicated in meningioma cell migration. The nuclear expression of TRPV4 found in certain cells has been reported previously [37] and may reflect a mechanism which implicates TRPV4 in nuclear functions [38].

TRPV4 has been proposed as a promising molecular target for antiangiogenic treatments since it acts as a mechanosensor in the vascular endothelium during cell swelling and shear stress [19] leading to smooth muscle cell hyperpolarization and vasodilation [39]. An increasing number of reports also highlight the role of TRPV4 in endothelial cell proliferation [40] and migration [41]. Although the molecular mechanisms for TRPV4 functions in endothelial cells are under investigation, it has been demonstrated that an ultra rapid TRPV4-mediated Ca2+ influx occurs upon exposure to shear flow [42]. Importantly, TRPV4 immunoexpression in vessels was independent from TRPV4 immunopositivity in tumor cells of meningiomas. Apart from TRPV4, certain tumor vessels also displayed TRPV1, TRPV2, and TRPV3 immunoreactivity, providing a tool for considering novel combinatorial drugs targeted at these channels in vessels of meningiomas.

Finally, no difference was detected for TRPV2 expression levels between grades I, II, and III in meningiomas. As for TRPV1, there are contradictory data about the role of TRPV2 in cancer. In gliomas, TRPV2 has been described as a regulator of glioblastoma stem-like cell differentiation [27], whereas in prostate and bladder cancers, TRPV2 has been associated with stimulation of cell migration and invasion [43]. Moreover, TRPV2 has been shown to promote apoptosis in human bladder cancer cells [44] and glioma cells [45]. Although low levels of TRPV2 channel expression were detected in meningiomas, its functional role in these tumors needs to be investigated.

In this study, TRPV1–4 channel expression was not associated with patient survival suggesting that TRPV1–4 channels do not serve as prognostic indicators. Nevertheless, this finding needs confirmation with further research in a larger number of patients with high-grade meningiomas since only three anaplastic meningiomas (WHO, grade III) and 35 WHO, grade II, in the total of 175 meningiomas, were included in the present investigation.

## Conclusions

The current study presented a wide TRPV1–4 expression in meningiomas with different expression profiles of these channels in high-grade counterparts. However, the mechanisms underlying these alterations at the molecular level remain to be elucidated. Although meningiomas display mainly benign characteristics, their biological behavior diverse and clinical outcome, in a minority of meningiomas, is difficult to predict [1, 5–8]. Therefore, any additional data with prognostic and/or predictive value contributes to accurate personalized management of these patients. Since TRP channels are considered as possible therapeutic targets for tumors, the above findings provide evidence for including meningiomas in this therapeutic approach.

**Authors' contributions** SG, CT, AP, and AM carried out the immunostaining and performed the analysis of immunohistochemical findings

and statistical analyses. VZ carried out the pathological evaluation of the specimens. GG provided clinical information of the patients. MA conceived and designed the experiments, performed the analysis of immunohistochemical and statistical findings, and wrote the manuscript.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

**Research involving human participants and/or animals** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the Helsinki declaration (as revised in Edinburgh 2000).

**Retrospective study** For this type of study, formal consent is not required. This study has been reviewed and approved by the Ethics Committee of the University Hospital of Patras.

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