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Atypical nuclear localization of VIP receptors in glioma cell lines and patients

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ABSTRACT

An increasing number of G protein-coupled receptors, like receptors for vasoactive intestinal peptide (VIP), are found in cell nucleus. As VIP receptors are involved in the regulation of glioma cell proliferation and migration, we investigated the expression and the nuclear localization of the VIP receptors VPAC1 and VPAC2 in this cancer. First, by applying Western blot and immunofluorescence detection in three human glioblastoma (GBM) cell lines, we observed a strong nuclear staining for the VPAC1 receptor and a weak nuclear VPAC2 receptor staining. Second, immunohistochemical staining of VPAC1 and VPAC2 on tissue microarrays (TMA) showed that the two receptors were expressed in normal brain and glioma tissues. Expression in the non-nuclear compartment of the two receptors significantly increased with the grade of the tumors. Analysis of nuclear staining revealed a significant increase of VPAC1 staining with glioma grade, with up to 50% of GBM displaying strong VPAC1 nuclear staining, whereas nuclear VPAC2 staining remained marginal. The increase in VPAC receptor expression with glioma grades and the enhanced nuclear localization of the VPAC1 receptors in GBM might be of importance for glioma progression.

1. Introduction

The G protein-coupled receptors (GPCR) superfamily is classified into six groups (classes A–F) on the basis of sequence homology and functional criteria [1]. A growing set of evidences demonstrates a nuclear localization for several members of the three first classes of GPCRs, including receptors for neurotransmitters, hormones, lipid ligands and a number of bioactive polypeptides. These nuclear GPCRs have been observed in normal cells and in various cancer cell lines deriving from different tissues [2]. The perinuclear or nuclear localization of these receptors was demonstrated by means of immuno-cyto and -histo chemistry, autoradiography using radioligands, or ligand binding on functional assays [3]. A minority of nuclear GPCRs possess at least one nuclear localization sequence (NLS) and thus can be imported in the nucleus by the canonical Ran/GTP importin pathway but it cannot be excluded that atypical NLS could be found in other GPCRs [4,5]. Small hydrophobic mediators such as prostaglandins or lysophosphatidic acid can freely cross the membrane and trigger their nuclear GPCRs [6], leading to transactivation of target genes [7]. For non-hydrophobic ligands, different mechanisms could account for their intracrine action: endocytosis of receptor-bound ligand, intracellular synthesis and retention of ligand, as demonstrated for endothelin-1, and transmembrane transport (as for example for adrenaline) (reviewed in [2]). Some GPCRs have also been demonstrated to enter the nucleus in the absence of their ligand [8]. Effectors associated with plasma membrane GPCRs are also found in the nucleus: G proteins, adenylate-cyclase, phospholipases, ion channels. Intranuclear production of second messengers, including cAMP, IP3 and DAG is also well documented (reviewed in [2]). The nuclear GPCRs are thought to regulate cell proliferation and survival, DNA replication, transcription, inflammation and tumorigenesis [2,3].

Vasoactive intestinal peptide (VIP), and pituitary adenylate cyclase-activating polypeptide (PACAP) belong to a family of structurally related peptides including also secretin and glucagon. Both neuropeptides exert a wide spectrum of biological functions through their interaction with three members of the class B of heptahelical GPCRs: VPAC1 and VPAC2 that share similar affinity for VIP and PACAP, and PAC1 displaying higher affinity for PACAP than for VIP. After binding to these plasma membrane receptors, these

Abbreviations: GBM, glioblastoma; GPCR, G protein-coupled receptor; TMA, tissue microarray; VIP, vasoactive intestinal peptide.

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peptides trigger different signaling pathways, including the cAMP/PKA, the Ca++/PKC and the PI3K/Akt cascades [9,10]. The overexpression of these receptors and their involvement in the progression of a number of cancer types and in tumor angiogenesis, have been extensively demonstrated. Consequently, strategies aiming to target these receptors for tumors imaging or therapy are under consideration [11]. An atypical nuclear localization of VIP receptors has been reported in a human colonic adenocarcinoma cell line [12] and VPAC1 nuclear receptors have been observed in human breast cancer [13] and renal carcinoma [14] cells. It has been hypothesized that nuclear membrane VPAC1/2 receptors could be involved in the proliferation, survival and differentiation of Th2 lymphocytes [15]. So far, little is known about the possible involvement of nuclear GPCRs in tumor progression. Our group and others have demonstrated that glioblastoma (GBM) cells express VIP or PACAP and their receptors, allowing an autocrine/paracrine regulation of proliferation and migration [16–22]. The aim of the present study was to analyze the expression and the subcellular localization of VPAC1/2 receptors in glioma cell lines and tissue microarrays (TMA) from gliomas of different grades of malignancy.

2. Materials and methods

2.1. Cell culture

M059K and M059J human GBM cell lines were maintained in high glucose (4.5 g/l) DMEM: Ham F12 (1:1) with GlutaMAX™ I (Invitrogen). Human U87MG cell line was maintained in low glucose (1 g/l) DMEM (Invitrogen). Both media were supplemented with 10% fetal bovine serum (Lonza), 100 U/ml penicillin and 100 μg/ml streptomycin (Lonza). Cells were incubated in a humidified 95% air, 5% CO_2 controlled atmosphere at 37 °C. Medium was changed every 3 or 4 days.

2.2. Protein preparation and Western blotting

Nuclear and non nuclear extracts were prepared as described before [23]. Protein concentration of each fraction was determined by using the BCA protein assay kit (Pierce), and samples were normalized accordingly. Proteins of the non nuclear fraction (40 μg) and of the nuclear fraction (20 μg) were resolved on a 10% SDS–PAGE gel and transferred to a polyvinylidene difluoride (PVDF) membrane. The membrane was blocked for 1 h at room temperature, by using 5% non fat milk in phosphate-buffered saline (PBS) and incubated overnight with polyclonal rabbit antibodies raised against VPAC1 (1:1000) or VPAC2 (1:1000) (PA3–113 and PA3–114, respectively, Pierce). Antibodies raised against Lamin A/C (1:1000) and GAPDH (1:20,000) (ab8984 and ab8245 respectively, Abcam) were both used to assess the quality of nuclear protein extraction and for loading controls. After 1 h incubation at room temperature with goat anti-rabbit or goat anti-mouse secondary antibodies (1:50,000) (A0545 and A2554 respectively, Sigma–Aldrich), proteins were detected using enhanced chemiluminescence (ECL, Luminata Crescendo, Millipore).

2.3. Immunofluorescence and confocal imaging

Cells were cultured on a coverslip for 48 h, then rinsed in PBS, fixed with 4% paraformaldehyde for 30 min, and permeabilized with 0,3% Triton X-100 in PBS for 30 min. After blockade of unspecific sites with 3% bovine serum albumin in PBS, rabbit polyclonal antibodies against VPAC1 or VPAC2 (1:1000) were incubated overnight at 4 °C. Goat anti-rabbit secondary antibodies conjugated with Alexa Fluor 488 (1:500, Invitrogen) and DRAQ5 for nuclear labeling (1:500) (Cell Signaling) were incubated 1 h at room temperature. Cells were examined with a spectral confocal FV-1000 station installed on an inverted microscope IX-81 (Olympus) with an UplanSapo x60 oil, 1.2 NA, objective lens. Multiple fluorescence signals were acquired sequentially to avoid cross-talk between image channels. Fluorophores were excited with the 488 nm line of an argon laser (Alexa fluor 488) and 633 line of an HeNe laser (DRAQ5). The emitted fluorescences were detected through spectral detection channels between 500 and 530 nm for green fluorescence and through a 650 nm long pass filter for far red fluorescence.

2.4. Tissue microarrays and immunohistochemistry

TMAs were obtained from US Biomax Inc. Each TMA (slides used: BS17016a, GL807a, GL241a, T175) comprised 0.6-mm cores taken from paraffin-embedded specimens that represent a total of 73 glioma tissues (grade I-IV) and 13 samples of adjacent normal tissues and normal tissues. Cases details are presented in Table 1. Slides were deparaffinized and heated in citrate buffer pH 6 for antigenic retrieval. Immunohistochemistry was performed using the streptavidin–biotin-peroxidase method with diaminobenzidine as the chromogen (Kit LSAB, Dakocytomotion). The primary antibodies VPAC1 (1:1000) and VPAC2 (1:1000) (PA3–113 and PA3–114, respectively, Pierce) were incubated overnight at 4 °C.

2.5. Scoring of antibody staining and statistical analysis

Immunoreactivity in TMA samples was evaluated independently by two biologists in a blinded fashion. The cases with discrepant scores were discussed in order to obtain a consensus. Staining intensity in the non-nuclear compartments and nuclei was graded using a scale from 0 to 3 (0, no immunostaining; 1, light brown color; 2, medium brown color, and 3, dark brown color). The percentage of positively stained nuclei was scored as follows: 0, no staining; 1, ⩽50% of the tumor cells; 2, 50%–90% of the tumor cells; 3, >90% of the tumor cells. For the nuclei, the intensity score and proportion score were multiplied in order to generate an immunoreactive score (IS). Negative expression was defined as an intensity score lower than 2 for the non-nuclear compartments and as an IS lower than 4 for the nuclei. The differences between groups were compared with the χ^2 test using Prism (GraphPad Software, La Jolla, USA).

3. Results

3.1. Detection of a nuclear localization signal sequence in the VPAC1 receptor sequence

Several members of the G Protein Coupled Receptor (GPCR) family have been recently observed in the nucleus of cells, including in cancer cells [2]. We investigated the nuclear localization of the two VIP receptors VPAC1 and VPAC2 in glioma cell lines and patients, as these receptors are known to be involved in GBM progression [17–20]. First, we searched for the presence of a nuclear localization sequence (NLS) signal in the amino-acid sequence of the two receptors. Using the prediction software NucPred [24], a weak NLS signal was found in the intracytoplasmic C-terminal domain of the VPAC1 receptor. This signal is composed of the last three residues of the intracytoplasmic eighth alpha helix ($R^{400}R^{401}K^{402}$, Fig. 1A). Close to these three residues, two other basic residues R^{404} and R^{405} were not underlined by the NucPred software but could be of importance. Indeed, the alignment of the VPAC1 sequence with other members of the B GPCRs family, using the GPCR database tool [25], revealed a conservation of these residues for some members (Fig. 1A, *gray box*). Moreover, those

Table 1
Case details of TMAs used.

Case number	Sex	Age	Organ	Pathology	Grade	Type
1	M	22	Cerebrum	Astrocytoma	1	Malignant
2	F	35	Cerebrum	Astrocytoma	2	Malignant
3	M	52	Cerebrum	Astrocytoma	1	Malignant
4	F	45	Cerebrum	Astrocytoma	1	Malignant
5	F	34	Cerebrum	Astrocytoma	1	Malignant
6	F	63	Cerebrum	Astrocytoma	2	Malignant
7	M	32	Cerebrum	Astrocytoma	2	Malignant
8	F	30	Cerebrum	Astrocytoma	1	Malignant
9	M	27	Cerebrum	Astrocytoma	2	Malignant
10	M	44	Cerebrum	Astrocytoma	2	Malignant
11	M	66	Cerebrum	Astrocytoma	3	Malignant
12	F	26	Cerebrum	Astrocytoma	2	Malignant
13	M	24	Cerebrum	Astrocytoma	2	Malignant
14	M	43	Cerebrum	Astrocytoma	2	Malignant
15	M	42	Cerebrum	Astrocytoma	3	Malignant
16	F	49	Cerebrum	Astrocytoma	1	Malignant
17	M	51	Cerebrum	Astrocytoma	2	Malignant
18	F	18	Cerebrum	Astrocytoma	2	Malignant
19	M	39	Cerebrum	Astrocytoma	2	Malignant
20	F	49	Cerebrum	Glioblastoma multiforme	4	Malignant
21	F	39	Cerebrum	Astrocytoma	3	Malignant
22	M	36	Cerebrum	Astrocytoma	4	Malignant
23	F	58	Cerebrum	Glioblastoma	4	Malignant
24	F	43	Cerebrum	Glioblastoma multiforme	4	Malignant
25	F	63	Cerebrum	Glioblastoma	4	Malignant
26	M	65	Cerebrum	Glioblastoma	4	Malignant
27	F	61	Cerebrum	Glioblastoma with necrosis (sparse)	4	Malignant
28	M	42	Cerebrum	Glioblastoma	4	Malignant
29	M	9	Cerebrum	Glioblastoma	4	Malignant
30	F	44	Cerebrum	Glioblastoma	4	Malignant
31	M	59	Cerebrum	Glioblastoma	4	Malignant
32	M	25	Cerebrum	Glioblastoma	4	Malignant
33	F	8	Cerebrum	Cancer adjacent normal brain tissue	–	NAT
34	M	77	Cerebrum	Cancer adjacent normal brain tissue	–	NAT
35	M	63	Cerebrum	Cancer adjacent normal brain tissue	–	NAT
36	F	49	Cerebrum	Cancer adjacent normal brain tissue	–	NAT
37	M	38	Cerebrum	Astrocytoma	2	Malignant
38	M	57	Cerebrum	Astrocytoma	2	Malignant
39	F	39	Cerebrum	Astrocytoma	1	Malignant
40	M	38	Cerebrum	Astrocytoma	1	Malignant
41	F	44	Cerebrum	Astrocytoma	1	Malignant
42	F	47	Cerebrum	Astrocytoma	1	Malignant
43	M	56	Cerebrum	Astrocytoma	1	Malignant
44	M	52	Cerebrum	Astrocytoma	2	Malignant
45	F	30	Cerebrum	Astrocytoma	2	Malignant
46	M	44	Cerebrum	Astrocytoma	3	Malignant
47	M	47	Cerebrum	Astrocytoma	2	Malignant
48	M	36	Cerebrum	Astrocytoma	2	Malignant
49	F	36	Cerebrum	Astrocytoma	2	Malignant
50	M	33	Cerebrum	Astrocytoma	1	Malignant
51	F	50	Cerebrum	Astrocytoma	3	Malignant
52	F	40	Cerebrum	Astrocytoma	3	Malignant
53	M	50	Cerebrum	Astrocytoma	2	Malignant
54	F	38	Cerebrum	Astrocytoma	3	Malignant
55	M	55	Cerebrum	Glioblastoma	4	Malignant
56	M	51	Cerebrum	Astrocytoma	3	Malignant
57	M	54	Cerebrum	Astrocytoma	3	Malignant
58	M	44	Cerebrum	Glioblastoma	4	Malignant
59	F	41	Cerebrum	Glioblastoma	4	Malignant
60	F	61	Cerebrum	Glioblastoma	4	Malignant
61	M	53	Cerebrum	Glioblastoma	4	Malignant
62	M	20	Cerebrum	Glioblastoma	4	Malignant
63	M	34	Cerebrum	Glioblastoma	4	Malignant
64	M	26	Cerebrum	Glioblastoma	4	Malignant
65	F	31	Cerebrum	Glioblastoma	4	Malignant
66	F	45	Cerebrum	Giant cell glioblastoma	4	Malignant
67	M	15	Cerebrum	Giant cell glioblastoma	4	Malignant
68	M	30	Cerebrum	Normal brain tissue	–	Normal
69	M	52	Cerebrum	Normal brain tissue	–	Normal
70	M	39	Cerebrum	Normal brain tissue	–	Normal
71	F	24	Cerebrum	Normal brain tissue	–	Normal
72	M	49	Cerebrum	Normal brain tissue	–	Normal
73	M	33	Cerebrum	Astrocytoma	1	Malignant
74	F	44	Cerebrum	Astrocytoma	1	Malignant

Table 1 (*continued*)

Case number	Sex	Age	Organ	Pathology	Grade	Type
75	M	53	Cerebrum	Astrocytoma	2	Malignant
76	M	34	Cerebrum	Astrocytoma	2	Malignant
77	F	28	Cerebrum	Astrocytoma	1	Malignant
78	M	35	Cerebrum	Astrocytoma	2	Malignant
79	F	48	Cerebrum	Astrocytoma	3	Malignant
80	M	36	Cerebrum	Astrocytoma	3	Malignant
81	F	44	Cerebrum	Cancer adjacent normal brain tissue	–	NAT
82	F	28	Cerebrum	Cancer adjacent normal brain tissue	–	NAT
83	F	43	Brain	Astrocytoma	2	Malignant
84	M	35	Brain	Glioblastoma	4	Malignant
85	F	50	Brain	Normal brain tissue	–	Normal
86	M	46	Brain	Normal brain tissue	–	Normal

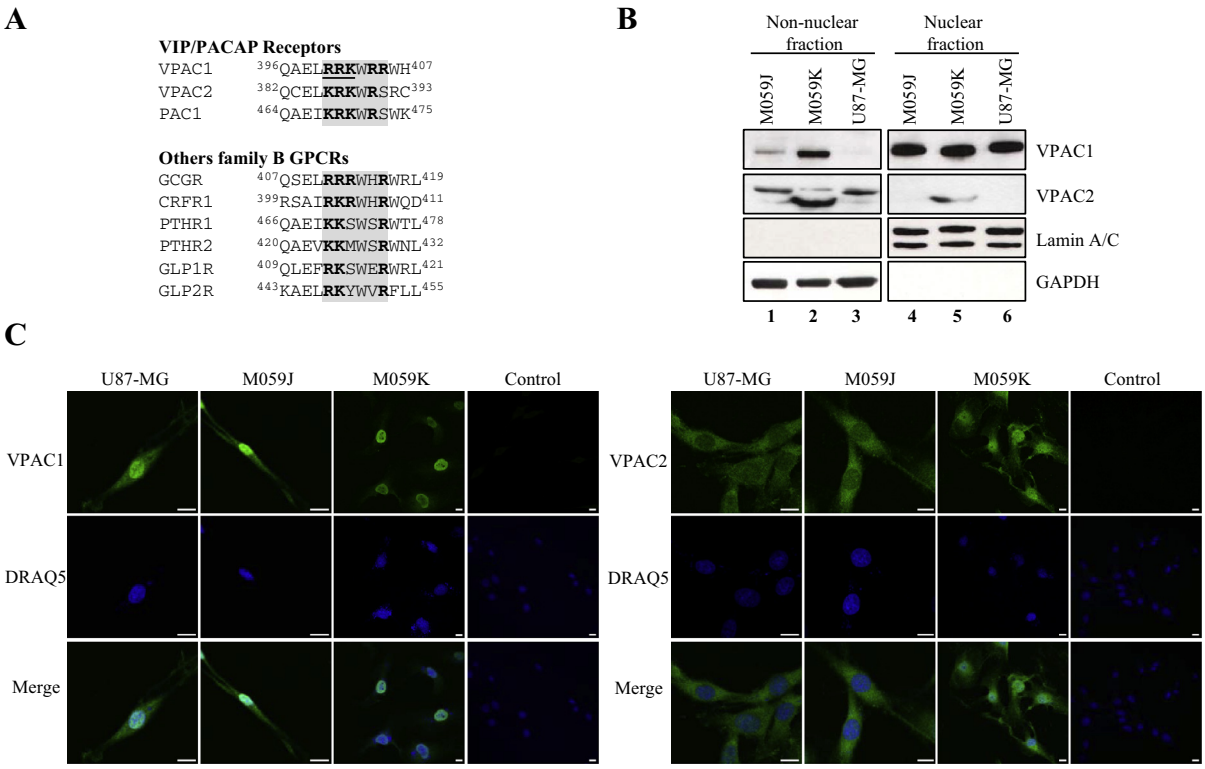

Fig. 1. Nuclear localization of the VPAC1 receptor in GBM cell lines. (A) Localized protein sequence alignments indicate conserved motifs (*gray box*) in the eighth helix for VIP/PACAP receptors (VPAC1, VPAC2 and PAC1) and other type B GPCR family as follows: *GCCR*, glucagon receptor; *CRFR1*, corticotropin-releasing factor receptor 1; *PTHR1*, parathyroid hormone receptor 1; *PTHR2*, parathyroid hormone receptor 2; *GLP1R*, glucagon-like peptide 1 receptor; *GLP2R*, glucagon-like peptide 2 receptor. The putative NLS of the VPAC1 receptor is underlined. (B) Western blotting of VPAC1 and VPAC2 (*upper panels*) in non-nuclear (*lanes 1–3*) and nuclear (*lanes 4–6*) fractions of M059J, M059K and U87-MG human GBM cell lines. Western blots of Lamin A/C and GAPDH (*lower panels*) were used both for control of nuclear protein extraction and for loading control. (C) Confocal imaging of immunofluorescence staining of VPAC1 (*green, left upper panel*), VPAC2 (*green, right upper panel*), the nuclear marker DRAQ5 (*blue, middle panel*) and merge images (*lower panel*). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

residues are conserved in both VPAC1 and VPAC2, although the NucPred software only recognized a NLS signal for the VPAC1 receptor in this region.

3.2. Subcellular localization of VPAC1 and VPAC2 receptors in human GBM cell lines

To assess the predicted nuclear localization of the VPAC1 receptor, Western blot and immunofluorescence analysis were performed on three human GBM cell lines: U87-MG, M059J and M059K. For Western blot analysis, the nuclear and non-nuclear protein fractions were separated by centrifugation. The quality of the fractionation was further validated by the detection of Lamin A/C only in the nuclear fraction, and GAPDH only in the non-nuclear fraction (Fig. 1B). The VPAC1 (60 kDa) distribution showed a moderate or no signal in the non-nuclear fraction compared to the strong signal observed in the nuclear fraction of the three GBM cell lines tested (Fig. 1B). In contrast, the VPAC2 receptor (50–55 kDa), which was not predicted to be nuclear by the NucPred software, was mainly present in the non-nuclear fraction, and only weakly detected in the nuclear fraction of the M059K cell line (Fig. 1B). VPAC1 and VPAC2 receptors localization were then analyzed by indirect immunofluorescence and confocal imaging. As expected, VPAC1 nuclear localization was clearly confirmed in the three cell lines, as its staining co-localized with the DRAQ5 nuclear marker (Fig. 1C, *right panel*), whereas the VPAC2 receptor was only faintly detected in the nuclei of the M059K cell line (Fig. 1C, *left panel*).

3.3. VPAC1 and VPAC2 localization on human glioma TMAs

Finally, to assess the importance of the nuclear localization of the VPAC1 receptor in tumors, we performed immunohistochemical staining on human glioma TMAs. Seventy-three cores of human glioma tissues from grade I to IV and 13 normal brain cores (see Table 1 for case details) were analyzed for VPAC1 and VPAC2 staining both in nuclear and non-nuclear compartments, using the above antibodies raised against the C-terminal sequence of each receptor [26,27]. Fig. 2A summarizes the results obtained for each group. At the non-nuclear level, the percentage of cases with strong staining for the VPAC1 or the VPAC2 receptor increased with the grade of the disease: from 7% to 63% from grade I to grade IV for the VPAC1 receptor and from no staining in grade I tumors to 46% marked grade IV tumors for the VPAC2 receptor. This observation was as certain by performing the Chi-square test which effectively demonstrated that the level of the staining was significantly different between groups ($p < 0.05$ for both receptors). Furthermore, VPAC1 nuclear staining was found at all stages of the disease, with up to fifty percent (12/24) of stage IV tumors (GBM) displaying strong nuclear staining, whereas VPAC2 localization in the nuclear compartment was marginal and not significantly different between the grades. Chi-square exact test was performed to ascertain the significant level of difference between the groups, which showed significant upregulation of nuclear VPAC1 ($p < 0.05$) with disease progression. VPAC1 and VPAC2 staining could also be observed in normal brain tissue, mainly in neuronal cells, in both nuclear and non-nuclear compartments for VPAC1 and only in the non-nuclear compartment for the VPAC2. Representative examples of VPAC1 (Fig. 2B) and VPAC2 (Fig. 2C) staining, for each stage of glioma progression and in normal brain, illustrate the increased expression of both VPAC1 and VPAC2 during glioma progression and the strong nuclear staining for the VPAC1 receptor in GBM.

4. Discussion

Present results show that the VIP receptor VPAC1, a member of the GPCR family (class B) is located in the nucleus of GBM cell lines and in human GBM tumors of patients. An increasing number of studies are currently reporting such atypical localization of GPCR members in the nucleus of normal and cancer cells in human and other mammal species [2]. Concerning the B family GPCRs, the parathyroid hormone 1 receptor (PTH1R) is the most studied example since a bipartite NLS was found in its sequence in the early 90s [28]. Afterwards, numerous studies have demonstrated the nuclear localization of PTH1R in a variety of cell types (reviewed in [2]). The bipartite NLS of PTH1R is localized between

A

		VPAC1		VPAC2	
Compartment		non-nuclear	nuclear	non-nuclear	nuclear
Grade	*n*				
NB	13	6 (46%)	3 (23%)	2 (15%)	0 (0%)
G1	14	1 (7%)	2 (14%)	0 (0%)	0 (0%)
G2	24	9 (38%)	3 (13%)	11 (46%)	3 (13%)
G3	11	5 (45%)	2 (18%)	3 (27%)	1 (9%)
G4	24	15 (63%)	12 (50%)	11 (46%)	0 (0%)
Chi square test	*p* -value	* 0.0217	* 0.0269	* 0.0129	ns

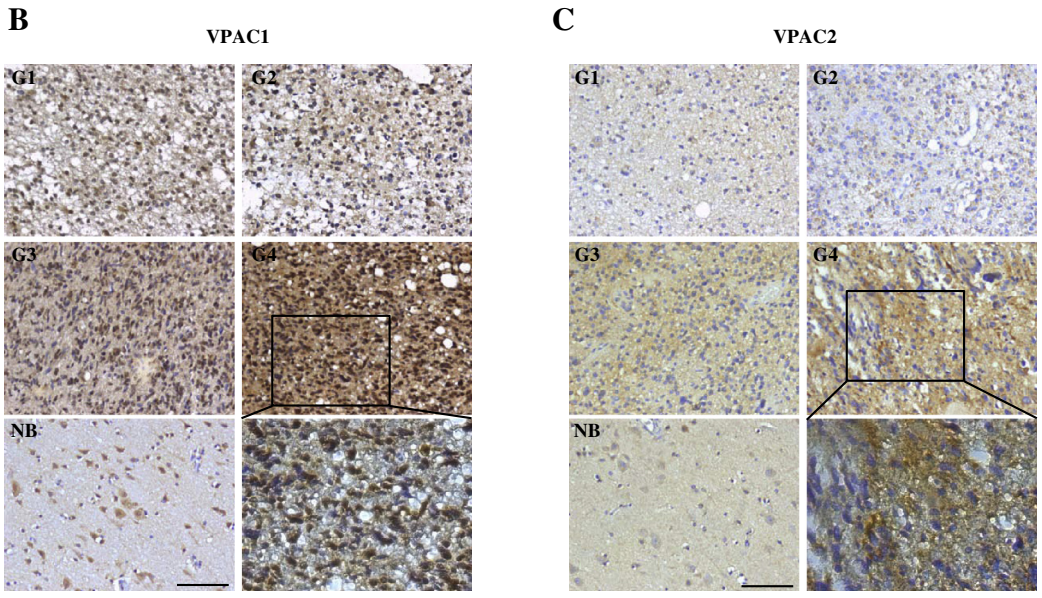

Fig. 2. Immunohistochemical staining of VPAC1 and VPAC2 in glioma and normal brain tissues using TMAs. (A) TMAs from glioma tumors and normal brain were stained by immunohistochemistry using antibodies against VPAC1 or VPAC2. Staining intensity in the non-nuclear and nuclear compartments was assessed by two independent biologists (*see* Section 2 *for details*). Number (%) of cases with strong VPAC1 or VPAC2 staining in the non-nuclear or the nuclear compartments are presented in the table. A Chi square test was used, *p-value <0.05, *ns*: non-significant. (B and C) Representative sections from TMAs stained with anti-VPAC1 (B) or anti-VPAC2 (C) are shown for grade I (*G1*), grade II (*G2*), grade III (*G3*), grade IV (*G4*) glioma and normal brain (*NB, as defined in* Section 2) tissues. All images were obtained at the magnification of 20X (scale bar 100 μm) except for the lower right images, which are enlargements of the grade 4 images and were obtained at the magnification of 40X.

amino-acid 471 and 488, and the first part of this NLS matched with the putative NLS identified by the NucPred software for the VPAC1 receptor. However, in the VPAC1 sequence, no second part of NLS was identified. Thus, it may be that the NLS of VPAC1 resembles more those of GPCRs from the class A Rhodopsin like, particularly those with peptide ligands. Indeed, three of these receptors, the apelin receptor, the angiotensin 1 receptor and the bradykinin receptor, can be nuclear and display five basic conserved amino acids (RKRRR or similar), located in the eighth alpha helix of the C-terminal intracytoplasmic domain [8], like the putative NLS sequence we identified for the VPAC1 receptor. In the VPAC1 putative NLS, the involvement of the R^{405} is particularly intriguing, since this is the only basic residue that differs with the VPAC2 sequence in this region, and the VPAC2 receptor was not found to be nuclear in our study. Further studies are needed to determine whether this putative NLS is responsible for the localization of the VPAC1 receptor in the nucleus. Differences were observed for VPAC2 molecular weight between the cell lines (Fig. 1B), likely due to post-translational modifications or splice variants [29,30].

On the second part of our study, we showed that VPAC1 and VPAC2 protein expression increases with the grade of glioma. VIP receptors are expressed in a variety of tumors [31]. VIP and PACAP can exert pro- or antitumoral effects depending on the cancer type [14,26,27,32–35]. This differential response is also observed in glioma cell lines, where VIP and PACAP can either increase, decrease or have no effect on cell proliferation [16–18,20–22]. These neuropeptides also negatively regulate migration in GBM cells [19]. The present study is the first to document the increasing expression of the VPAC1 and VPAC2 receptors during human glioma progression. This pattern of expression suggests that the VIP-receptor system could exert a protumoral activity in glioma.

To date, only four studies have taken into account the subcellular localization of VIP receptors. The first report in the late 80s, identified VIP binding sites in the nucleus of colon adenocarcinoma HT-29 cells [12]. More recently, the presence of VPAC1 was observed in the nucleus of human renal cell carcinoma xenografts [19] and of both hormone-dependent and -independent breast cancers [13]. In this latter study, the VPAC2 receptor was not detected in the nucleus, which is very consistent with our results in GBM tumors. Finally, nuclear VPAC receptors were also found in normal T cells with a different role than VPAC receptors localized to the plasma membrane. In this report, VPAC receptors at the plasma membrane could trigger a transient regulation of adhesion and migration in response to secreted VIP while the nuclear receptors stimulated by endogenous VIP could mediate long-lasting effects on cell survival and differentiation [15]. In the astrocytoma cells, differential functions of VPAC receptors depending on their subcellular localization could be responsible for glioma progression leading to GBM development. GBM prognosis remains dismal and new therapies are needed. Deciphering the mechanisms of glioma progression could allow the emergence of new therapies for these tumors.

Disclosure statement

The authors have nothing to disclose.

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