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Expression of vascular endothelial growth factor (VEGF) and its receptors VEGFR1 and VEGFR2 in primary and recurrent WHO grade III meningiomas

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Summary. Aims: WHO grade III meningiomas are malignant neoplasms for which new and more targeted treatment strategies are urgently needed. Although clinical trials investigating anti-angiogenic vascular endothelial growth factor (VEGF) targeted therapies are currently recruiting, knowledge about the expression of VEGF and VEGF receptors remains to be determined. Methods: We investigated the expression of VEGF and its receptors VEGFR1 and VEGFR2 in 32 WHO grade III meningioma samples by immunohistochemistry. Furthermore, we performed in-situ hybridisation for VEGF. Results: We found low VEGF expression in tumor and endothelial cells. Highest VEGF expression levels were seen in peri-necrotic tumor cells potentially suffering from hypoxia. VEGFR1 and 2 were virtually absent on tumor cells, although endothelial cells displayed significantly higher levels reaching stronger expression for VEGFR2 than VEGFR1. Conclusions: Our findings showing constant expression levels of VEGFR2 in endothelial cells serve as a first indication that the use of small tyrosine kinase inhibitors such as Sunitinib directly targeting the VEGF-receptors might be worth testing, also in the clinical context in cases of therapy-refractory meningiomas. Further investigations are needed to study the response to drugs targeting the VEGF pathway in relation to the expression profile of VEGF and its receptors in high grade meningiomas.

Key words: Malignant meningioma, Vascular endothelial growth factor, Bevacizumab

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Introduction

The majority of meningiomas are benign tumors which have an excellent prognosis after gross surgical resection and are thus classified as grade I according to the grading system of World Health Organization (WHO) (Louis et al., 2007). However, some meningioma variants, including atypical (WHO grade II) and anaplastic meningiomas (WHO grade III), show a less favourable clinical course. WHO grade III meningiomas are characterized by aggressive biological behaviour and recurrent tumor growth, resulting in a median survival of less than 2 years (Perry et al., 1999; Louis et al., 2007). Radiation therapy is commonly applied after both total and subtotal resection, but well-controlled data about effective adjuvant drug treatment regimens is lacking (Modha and Gutin, 2005). Vascular endothelial growth factor (VEGF) plays a central role in tumor biology and inhibition of VEGF or its receptors can lead to reduced tumor growth in vivo due to diminished vascularization (Folkman, 1971; Kim et al. 1993). VEGF was first recognized as a mediator of vascular permeability but was later also described as a strong mitogen in vascular endothelial cells possessing a key function in promoting angiogenesis in physiological and pathological processes (Senger et al., 1983; Keck et al., 1989; Leung et al., 1989; Plate et al., 1992; Wilting et al., 1992). VEGF is up-regulated upon hypoxia via Hif-1alpha in tumor cells and mediates endothelial cell proliferation by binding to its main receptors, vascular endothelial growth factor receptor 1 (VEGFR1, flt-1) and vascular endothelial growth factor receptor 2 (VEGFR2, flk-1/KDR), leading to an activation of phosphatidylinositol 3-kinase (PI3K/v-akt) and Raf-MAPK kinase pathways (Shweiki et al., 1992; Ryan et

al. 1998; Ferrara et al., 2003). VEGFR1 further acts as promoter of angiogenesis via induction of endothelial cell proteases and growth factor expression (Ferrara et al., 2003; Shibuya, 2006). Although possessing a tyrosine kinase domain, VEGFR1 probably acts as a decoy receptor for VEGF and not as a signal transduction receptor (Koch et al., 2011). In WHO grade I meningiomas, the expression status of VEGF and its receptors, VEGFR1 and VEGFR2, and particularly their role in the formation of peritumoral brain oedema has been extensively studied (Goldman et al., 1997; Lamszus et al., 2000; Pistolesi et al., 2002; Otsuka et al., 2004; Maiuri et al., 2007; Ding et al., 2008; Panagopoulos et al., 2008; Schmid et al., 2010). In contrast, data concerning the expression status in WHO grade III meningiomas, the subgroup for which most urgently new adjuvant drug treatments are needed remain sparse or is mainly limited to single case reports (Lamszus et al., 2000; Panagopoulos et al., 2008). As phase II trials investigating VEGF targeted therapies for the treatment of meningiomas (e.g. NCT01125046 and NCT00561665) are currently recruiting, we aimed to examine the expression status of VEGF and its major receptors in a larger series of primary and recurrent WHO grade III meningiomas (ClinicalTrials.gov; assessed 06.08.2012).

Material and methods

Patients

32 formalin-fixed paraffin-embedded tissue samples of 23 patients with primary or recurrent WHO grade III meningiomas diagnosed between 1989 and 2009 were obtained from the archives of the Institute of Neuropathology Münster (Table 1). Available samples of recurrent tumors of these patients were also included in this series. All samples were neuropathologically reviewed and diagnosed according to the current WHO classification (Louis et al., 2007). MGMT methylation status in this series had been reported earlier (Brokinkel et al., 2010).

Immunohistochemistry

Representative tumor sections (3 μm) were immunohistochemically stained using the following antibodies: mouse IgG2b anti human VEGF dilution 1:100 (clone: MAB293; R&D Systems, Minneapolis, MN, US), mouse IgG1 anti human VEGFR1 dilution 1:50 (clone: ab9540; Abcam, Cambridge, UK) and monoclonal rabbit anti human VEGFR2 dilution 1:100 (clone: 55B11; Cell Signaling, Danvers, MA, US). Tissue labelling for all antigens was performed using DiscoveryXT immunohistochemistry system (Ventana, Strasbourg, France). For all antibodies, a cell conditioning pretreatment was performed for 36 min followed by a 4 min blocking step with inhibitor D for VEGF and VEGFR1 (Ventana, Strasbourg, France). The primary antibodies were applied for either 28 min (VEGF) or 32 min (VEGFR1, VEGFR2). For VEGF and VEGFR1 a 28 min incubation with one drop of Universal Secondary Antibody was added (Ventana). An avidin-biotin blocker was applied to the samples for 4 min. For diaminobenzidine (DAB) visualization, the sections were incubated with one drop of I-View SA-HRP for 16 min and then with DAB/H_2O_2 for an additional 8 min. The sections were finally incubated with a copper enhancer (Ventana) for 4 min. For VEGFR2 primary antibody incubation was followed by 12 min blocking with one drop of Antibody Block (Ventana). One drop of AP-labelled Ultra Map (Ventana) was applied for 16 min. The application of one drop Activator R CM and one drop of Naphtol CM (Ventana) for 4 min was followed by the visualisation using one drop of Fast Red CM (Ventana) for 16 min. Finally, all sections were washed, counterstained with hematoxylin and mounted.

For quantification, a semi-quantitative score was used. The immunohistochemical staining intensity (low=1, moderate=2, strong=3) was multiplied with the proportion of positive tumor or vascular cells (1-10%=1, 10-25%=2, 25-50%=3, >50%=4).

In-situ hybridization

Human cDNA for VEGF was cloned in pBluescript II (KS+) plasmid (Stratagene, La Jolla, CA, USA). Content of the plasmids were amplified by mini preparation (Qiagen, Hilden, Germany) after transformation of DH5alpha *E.coli*. After restriction with BamHI and EcoRI (VEGF), T3 and T7 polymerases

Table 1. Epidemiological patient data.

ID	Age at operation of WHO grade III meningioma	Sex	Primary (p) or recurrent (r) meningioma	Survival (months) after primary tumor	Dead (1: yes; no: 0)
1	80	m	p	7	1
2	54	m	r	180	0
3	62	f	r	n.d.	n.d.
4	54	f	p (rhabdoid)	6	0
5	66	f	p	n.d.	n.d.
6	69	f	r	70	1
7	63	m	p	98	0
8	72	f	r	21	1
9	52	m	r	n.d.	n.d.
10	78	m	r	n.d.	n.d.
11	65	m	p	20	0
13	61	m	p	18	0
14	70	f	r	n.d.	n.d.
15	78	f	r	n.d.	n.d.
16	67	m	p	n.d.	n.d.
17	75	f	p	n.d.	n.d.
18	66	m	p	36	1
19	92	f	p	n.d.	n.d.
20	65	f	r	62	0
21	76	m	r (rhabdoid)	29	1
22	68	f	r	n.d.	n.d.
23	80	m	p	0	1

n.d.: not determined.

were used for the generation of sense and antisense probes, respectively. Paraffin embedded sections were rehydrated by a descending alcohol series and boiled for 45 min in citrate buffer (pH6.0), then allowed to cool down for 20 min. Endogenous alkaline phosphatase was inactivated with 0.2 M HCL. The slides were washed in PBS and proteins were denatured by proteinase K (10 μg/ml) solved in Tris-EDTA (TE) buffer. For the inactivation of proteinase K, slides were washed in 0.1 M glycin and rinsed in PBS. 625μl acetic anhydride in 250 ml 0.1 M triethanolamine was used for acetylation. For pre-hybridization, slides were incubated in a premix (10 ml stock solutation consists of 5 ml de-ionized formamide, 2 ml 20x standard saline citrate, 1 ml 50% dextransulfate, 1 ml 50x Denhardt's, 0.05 ml 20% SDS, 0.5 ml t-RNA (10 mg/ml) and 0.25 ml Salm Sperm (10 mg/ml)) for 5 h at room temperature. The probes were first diluted in DEPC-water, then in premix to obtain the final concentration of 1ng/μl and denaturized at 95°C. Hybridisation at 56°C (VEGF) was performed overnight. The next day, slides were first rinsed in standard saline citrate at 70°C and then in 2x standard saline citrate at room temperature. Incubation in Dig-1 was followed by blocking in Dig-2 (10% blocking reagent in Dig-1) for 1 h at room temperature. Slides were incubated with anti-Dig antibody diluted (1:500) in Dig-2 for 1 h, then rinsed three times in Dig-1 followed by one rinse in Dig-3. Finally, the slides were incubated in staining solution (10% polyvenyl alcohol, Dig-3, Nitro blue tetrazolium chloride (NBT), 5-Bromo-4-chloro-3-indolyl phosphate (BCIP)) for 4 h at 30°C, and rinsed in PBS. Slides were counterstained by hematoxylin for 20 sec and mounted in Aquatex.

Statistical analysis

Staining scores were used separately for tumor and endothelial cells within our cohort of high-grade meningiomas as ordinal scaled response variable and analysed together with the nominal explanatory variable (factor) using a contingency table followed by likelihood ratio and Pearson tests. Survial analyses were performed using Kaplan-Meier and multivariate analyses, the latter taking age, sex and WHO grade of the meningioma into account. In order to compare the survival curves we used the Wilcoxon test for censored data. A significance level of alpha=0.05 was chosen for all tests. Statistical analysis was performed using JMP 8.0 software (SAS, Cary, NC, USA). Evaluation of the immunohistochemical preparations and photographic documentation was performed using an Olympus BX50 light microscope.

Results

Clinical and epidemiological findings

The median age at first occurrence of WHO grade III meningiomas of the 11 female and 11 male patients was 68.5 years (range: 52-92 years) (Table 1). In 12 patients, a WHO grade III meningioma occurred already as a primary tumor, whereas it emerged from WHO grade II meningiomas in 10 patients. The median follow up period was 25 months in our cohort.

VEGF protein expression in meningioma cells is low in WHO grade III meningiomas and mainly encountered in peri-necrotic areas

Vascular endothelial growth factor (VEGF) expression in meningioma cells was absent in 9/32 (28.1%) of the investigated tumors (Figs. 1A,B, 4A, 5A). In addition, VEGF-expressing tumors showed only low or very moderate expression levels if whole tumor samples are taken into account, with 10/32 (31.3%) reaching score 1, 11/32 (34.4%) score 2 and only 2/32 (6.3%) reaching score 4. The comparison of VEGF expression between tumor and endothelial cells did not reveal significant differences (p=0.29). VEGF was most strongly up-regulated in hypoxic tumor areas surrounding necrotic foci (Fig. 1C,D), whereas vital parts of most meningiomas remained VEGF-negative (Fig. 1E,F). In endothelial cells of WHO grade III meningiomas, the largest amount of samples showed absence of VEGF expression (14/32 (43.8%) score 0; 9/32 (28.1%) score 1; 6/32 (18.8%) score 2; 1/32 (3.1%) score 4 and 2/32 (6.3%) score 6) (Figs. 4B, 5B). VEGF bound or secreted by endothelial cells could only be detected in few tumors to a low or moderate extent compared to the endothelial signal for the VEGF-receptors (Figs. 4B, 5B).

VEGFR1 is moderately expressed on endothelial cells in the majority of WHO grade III meningiomas

Although median VEGFR1 staining scores were low in both tumor (0, range: 0-2) and endothelial cells (2, range: 0-9), endothelial-associated VEGFR1 expression was significantly higher (p<0.0001). 31/32 (96.9%) of the samples showed complete absence of VEGFR1 staining in tumor cells while only 1/32 (3.1%) displayed VEGFR1-negative endothelial cells (Fig. 2A,B). On a cellular level, VEGFR1 was predominantly expressed in endothelial cells surrounding necrotic foci (Fig. 2B,C). In 20/32 (62.5%) of the tumors, a moderate endothelial VEGFR1 staining (score 2-6) was present, whereas 7/32 (21.9%) of the tumors showed high endothelial VEGFR1 expression in the whole tumor area (Fig. 5B). Adjacent brain tissue showed low or moderate levels of VEGFR1 on endothelial cells (Fig. 2E,F).

VEGFR2 is consistently expressed in endothelial cells of most WHO grade III meningiomas

Compared to VEGFR1, VEGFR2 shows a stronger expression on endothelial cells in WHO grade III meningiomas (p=0.019) (Fig. 4B, 5B). Strong endothelial VEGFR2 expression (score ≥8) was observed in 8/32 (25%) tumor samples (Fig. 3C,D). Moderate or weak endothelial expression (score 1-6) of

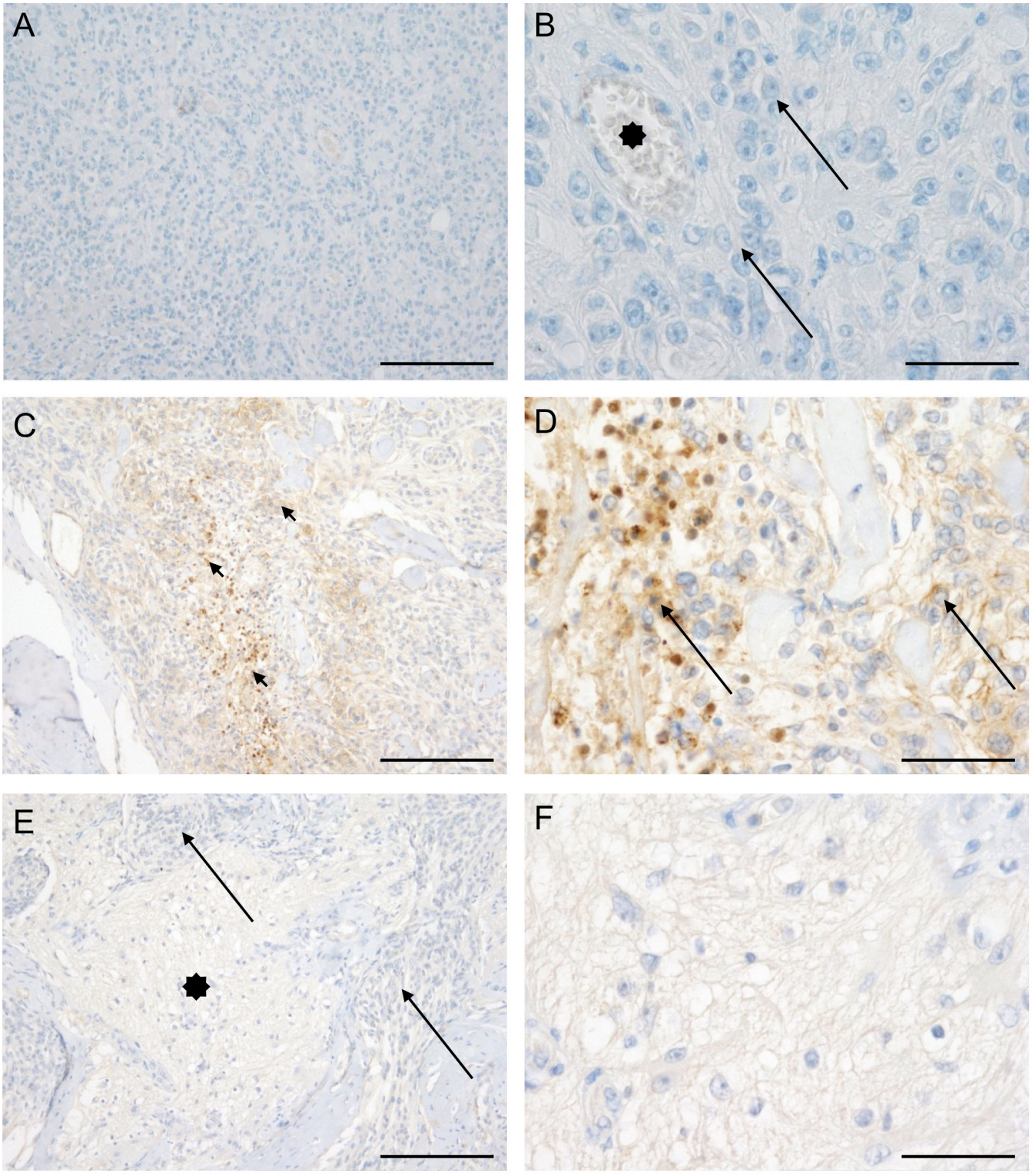

Fig. 1. Immunohistochemistry for VEGF expression in WHO grade III meningiomas. **A-B.** A representative area of a tumor with low VEGF expression is shown with VEGF-negative tumor cells (arrows) and vessels (asterisk). **C-D.** Meningioma cells exhibiting higher VEGF levels (arrows) are mostly located in pre- or peri-necrotic areas (arrow-heads). **E-F.** CNS tissue (asterisk) surrounding meningioma with infiltrating tumor-cones (arrows) is shown. Scale bars: A, C, E, 200 μm; B, D, F, 50 μm.

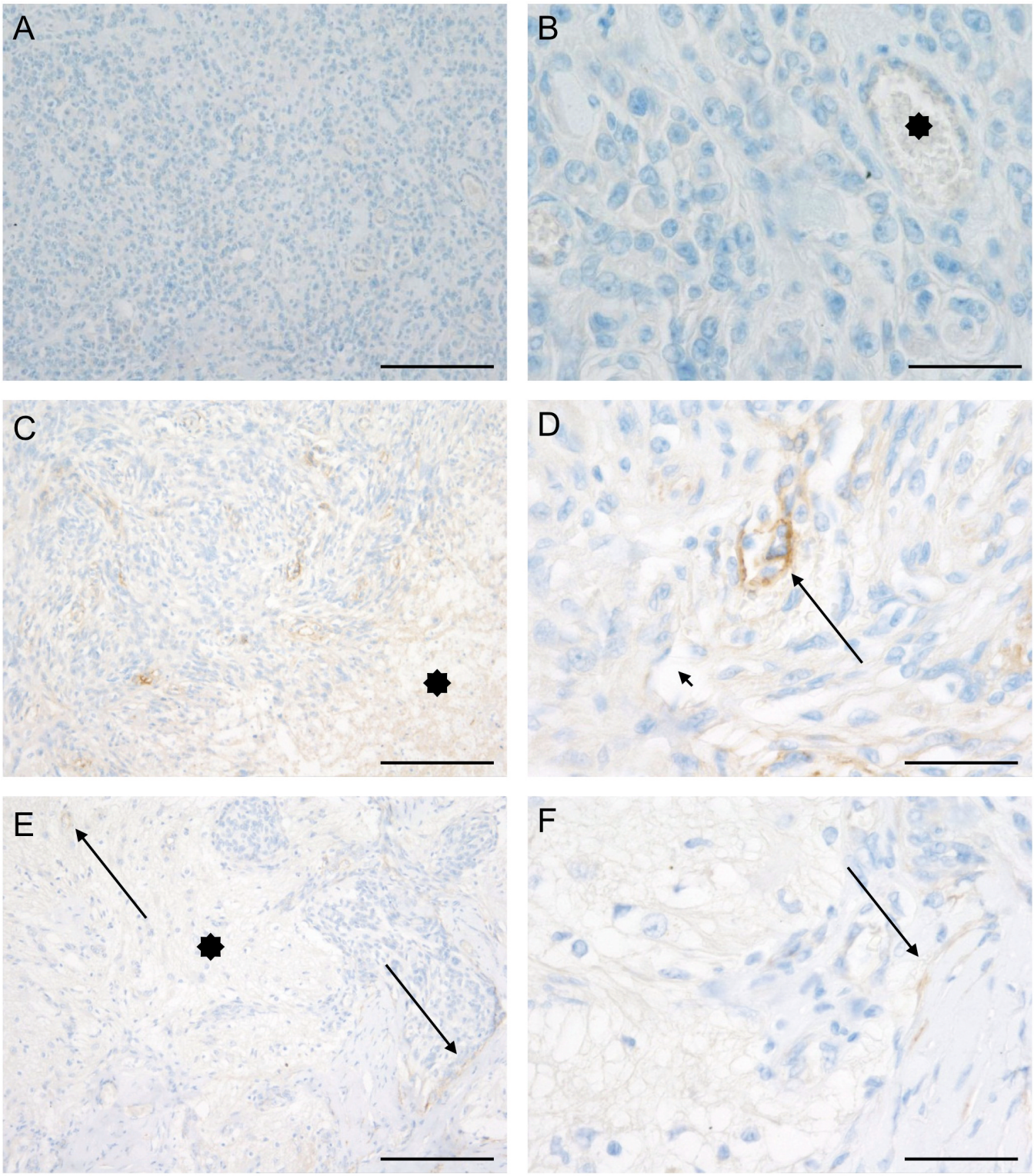

Fig. 2. Immunohistochemistry for VEGFR1 expression in WHO grade III meningiomas. **A-B.** A representative area of a tumor with low VEGFR1 expression is shown with negative endothelial cells in all blood vessels (asterisk). **C-D.** Vessels with high VEGFR1 levels (arrow) are found in close vicinity to necrotic areas (asterisk) also accompanied by negative vessels (arrow-head). **E-F.** The CNS tissue (asterisk) surrounding meningioma tissue exhibits few vessels with low VEGFR1 expression within the tumor-brain interface (arrow). Scale bars: A, C, E, 200 μm; B, D, F, 50 μm.

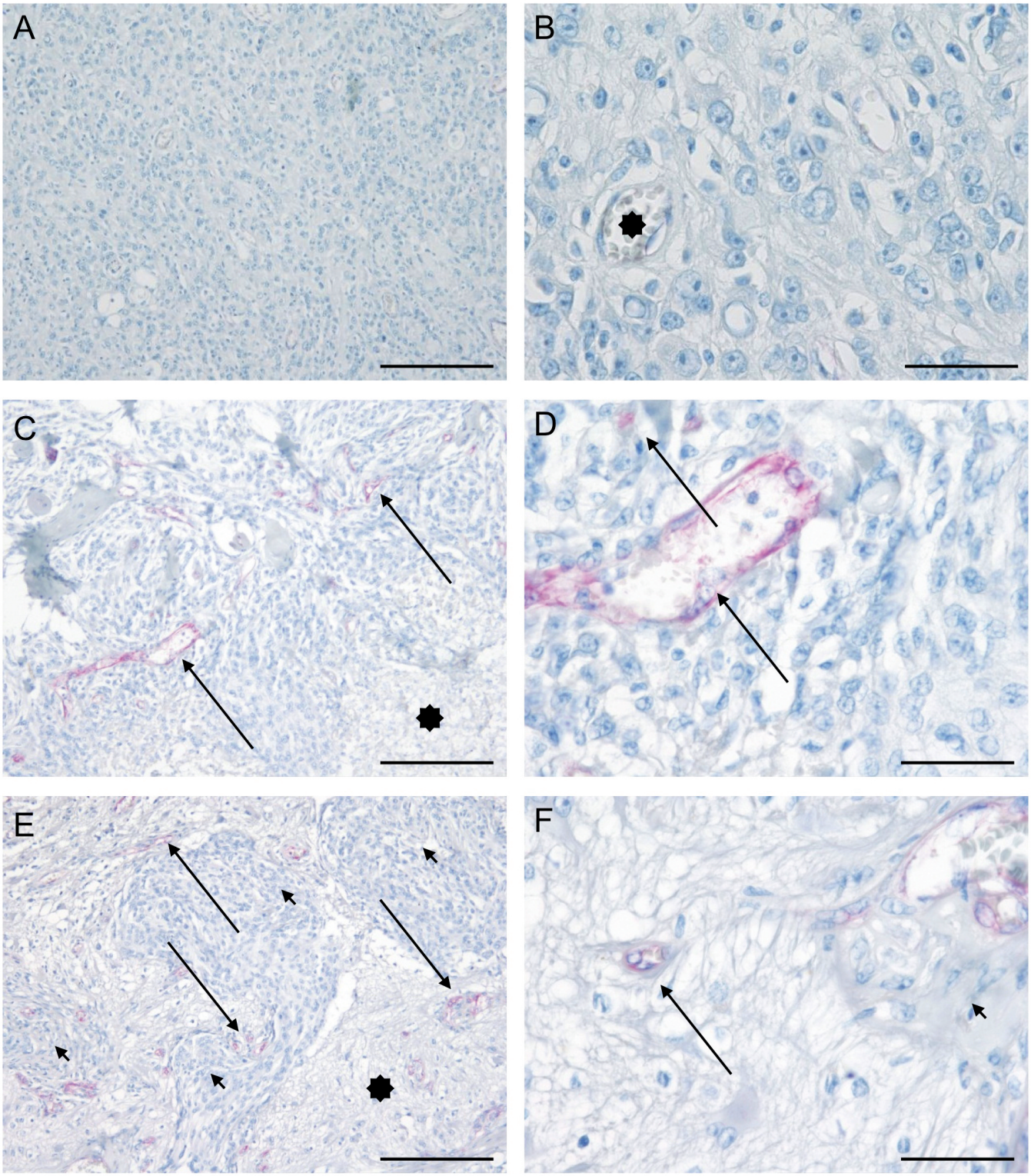

Fig. 3. Immunohistochemistry for VEGFR2 expression in WHO grade III meningiomas. **A-B.** Representative area of a meningioma showing low VEGFR2 expression in tumor and endothelial cells (asterisk). **C-D.** Endothelial cells showing high VEGFR2 levels (arrows) are found in tumor areas surrounding necrotic tumor foci (asterisks). **E-F.** Both tumor surrounding brain tissue (asterisk) and infiltrating tumor cones (arrow-heads) show vessels with moderate VEGFR2 expression (arrows). Scale bars: A, C, E, 200 μm; B, D, F, 50 μm.

VEGFR2 was observed in 22/32 (68.8%) tumor samples while only 2/32 (6.3%) of all samples were completely VEGFR2-negative (Fig. 3A,B). Many cases showed stronger VEGFR2 expression in endothelial cells at the tumor margins. In adjacent brain tissue, expression of VEGFR2 on endothelial cells was also observed (Fig. 3E, F). VEGFR2 was virtually absent on tumor cells in high-grade meningiomas (31/32 of the samples showed negative staining results for VEGFR2 on tumor cells; $p<0.0001$ as compared to the levels in endothelial cells) (Figs. 4A, 5A).

Spatial VEGF protein levels meet VEGF mRNA expression

Since VEGF acts as a soluble factor, we further assessed the cell of origin producing VEGF in WHO grade III meningiomas. We therefore performed in-situ hybridisation for VEGF (Fig. 6) showing that both tumor and endothelial cells are the source of VEGF production (Fig. 6B). Furthermore, the spatial VEGF mRNA expression is similar to the VEGF protein expression (Fig. 6A) and most strongly induced in perinecrotic/hypoxic areas.

Patient survival in WHO grade III meningiomas is only significantly associated with age at operation

In general, expression of VEGF and its receptors VEGFR1 and VEGFR2 was not associated with patient survival. Multivariate analysis including age, gender and WHO grade of the initial tumor showed only a significant association of patient survival with age at first operation ($p=0.021$), but not for WHO grade ($p=0.28$) and gender ($p=0.31$). Since VEGF and VEGFR1 and –R2 expression did not reach the level of significance ($p<0.05$) in the univariate analyses, these factors were not included in the multivariate analyses.

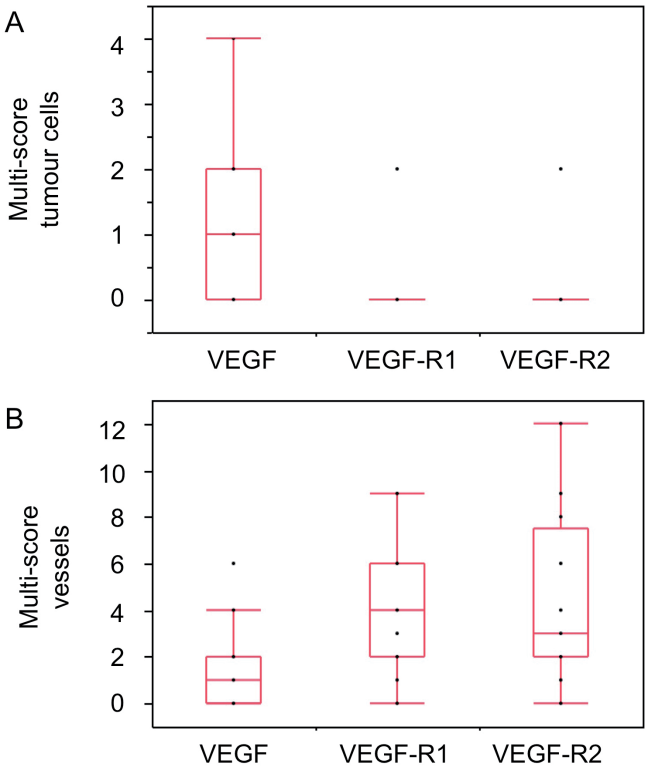

Fig. 4. Box-and-Whisker plot for VEGF and VEGFR expression in WHO grade III meningiomas in **(A)** tumor cells and **(B)** endothelial cells.

Discussion

In our studies investigating the expression of VEGF and its receptors in WHO grade III meningiomas, we found low VEGF levels in both tumor and endothelial cells reaching strongest expression in perinecrotic/hypoxic areas. Its receptors were constantly expressed by tumor endothelial cells showing higher VEGFR2 than VEGFR1 levels. VEGFR1 was most prominently upregulated on endothelial cells in close vicinity to necrotic foci while in contrast, VEGFR2 did not show a hypoxia-related expression pattern.

Since angiogenesis goes along with malignant progression in many different tumors and inhibition of

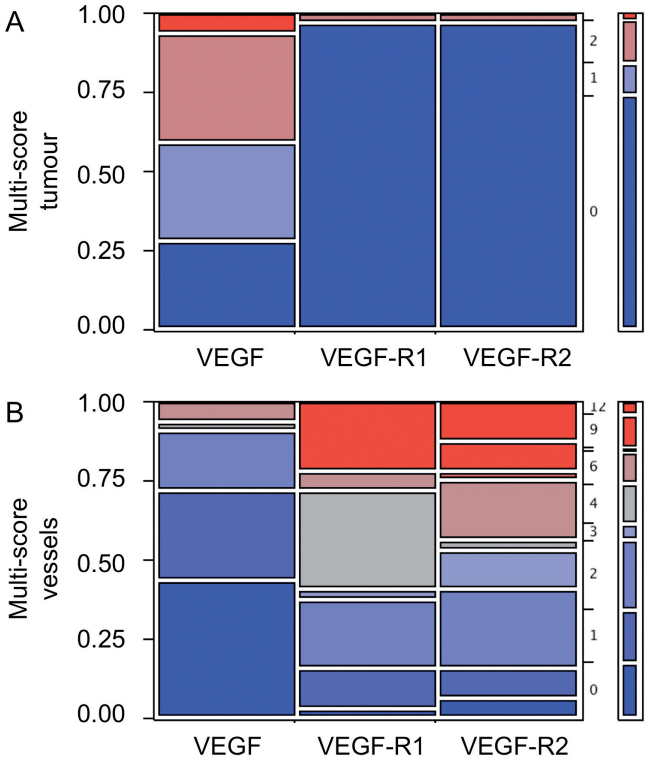

Figure 5: Contingency analysis for **(A)** tumor cells and **(B)** endothelial cells. The multiplied score was used as ordinal scaled response variable and analysed together with the nominal explanatory variable (factor) using a contingency table followed by Pearson test. (For tumor cells **(A)**: VEGF vs. VEGFR1: $p<0.0001$; VEGF vs. VEGFR2: $p<0.0001$; VEGFR1 vs. VEGFR2: not significant; For endothelial cells **(B)**: VEGF vs. VEGFR1: $p=0.0049$; VEGF vs. VEGFR2: $p=0.0009$; VEGFR1 vs. VEGFR2: $p=0.033$).

angiogenesis leads to a reduction of tumor growth in vivo, anti-angiogenic therapies using the anti-VEGF-antibody bevacizumab recently entered clinical trials in different tumor entities, partially leading to better patient survival in combination with chemotherapy than chemotherapy alone (Kim et al., 1993; Hurwitz et al., 2004; Sandler et al., 2006; Escudier et al., 2007; Miller et al., 2007). These findings could also have a socio-economic impact since health insurances frequently ask for the expression status of VEGF and its receptor before bevacizumab treatment. Recently, it has been shown that high VEGF levels in low-grade meningiomas were significantly related to the development of peritumoral brain edema leading to the conclusion that Bevacizumab could also be used to decrease brain edema in meningioma patients (Schmid et al., 2010; Nassehi et al., 2011).

In meningioma cells, VEGF expression could be induced upon hypoxic conditions in vitro and was associated with increased HIF-1alpha expression levels, pointing to a VEGF-mediated hypoxia-response axis also operative in neoplastic meningeal cells (Jensen et al. 2002; Sakuma et al., 2008). Such a hypoxia-dependent VEGF expression pattern which we also observed in our cohort of WHO grade III meningiomas *in vivo* has previously been described in other tumors such as glioblastomas (Plate et al., 1992; Shweiki et al., 1992). Besides its regulation by HIF-1alpha mediated hypoxia-sensing, the signal transducer and activator of transcription 3 (STAT3), one of the major players in human cancer development and progression, is also able to increase VEGF expression in neoplastic meningeal cells (Zhang et al., 2010a,b). Most interestingly, VEGF blood plasma levels of meningioma patients were even higher as compared to patients with high-grade gliomas and CNS metastases, pointing to a very strong pro-angiogenic milieu in tumors of meningeal origin (Ilhan et al., 2009). VEGF expression does not only seem to have a clinical relevance in high-grade meningiomas but also in their low-grade counterparts, although low grade meningiomas (WHO grade I-II) which usually show less necrotic changes only display very low or absent VEGF expression in 58% of WHO grade I and 83% of WHO grade II meningiomas (Pietsch et al., 1997).

Our findings of upregulated VEGFR1 on vessels in close vicinity to necrotic areas, similarly to the investigated VEGF-expression in tumor cells, is most probably related to the previously described direct hypoxia-dependent regulation of VEGFR1 via a hypoxia-inducible enhancer element located at positions -976 to -937 in the VEGFR-1 promotor (Gerber et al., 1997). The different expression-pattern of VEGFR2 compared to VEGFR1 around necrotic areas could be explained by the distinct regulation of both receptors with VEGFR2 not primarily being regulated or even being down-regulated upon hypoxia (Gerber et al., 1997; Ulyatt et al., 2011). In endothelial cells of human squamous-cell carcinomas, a differential inverse and

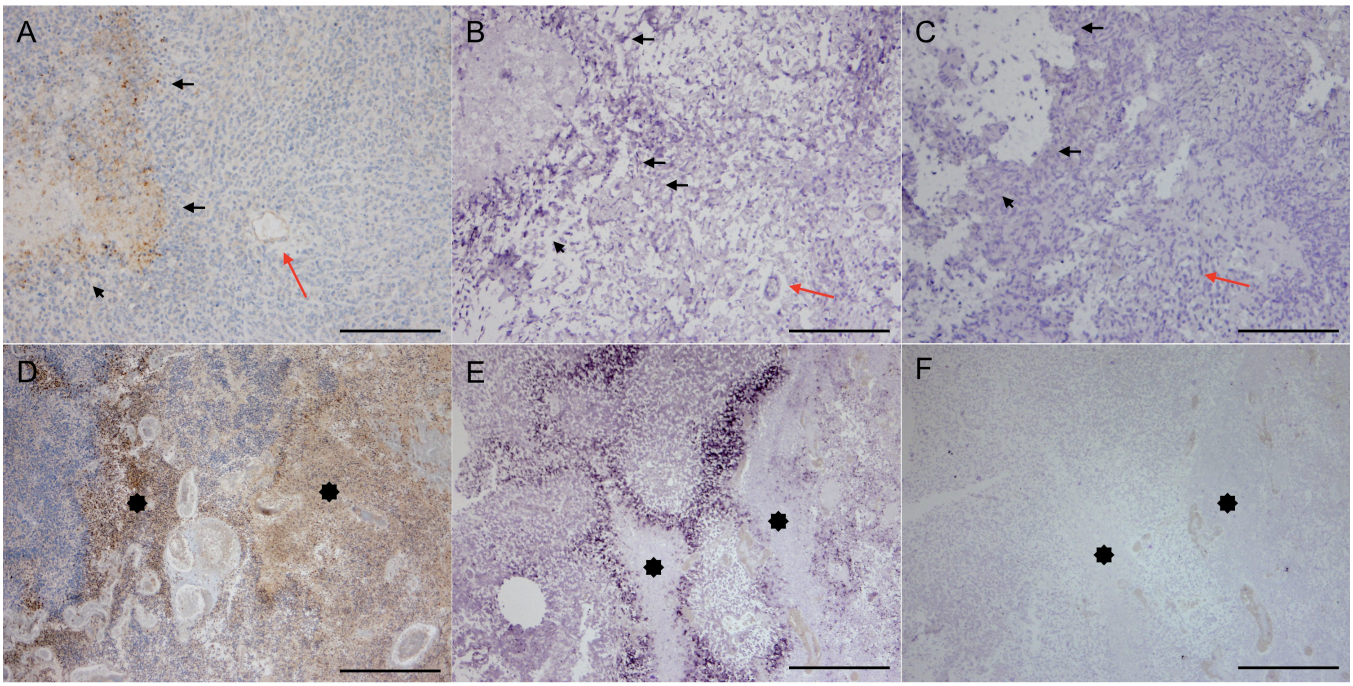

Fig. 6.VEGF protein and mRNA expression show similar spatial distribution. **A-C.** WHO grade III meningiomas most prominently exhibit **(A)** VEGF protein expression as detected by immunohistochemistry and **(B)** mRNA expression as detected by in-situ hybridisation in both tumor cells (black arrows) and endothelial cells (red arrows) in perinecrotic/hypoxic areas (**C** in-situ hybridisation with VEGF sense probe serving as negative control for **B**. **D-F.** As a control, glioblastoma samples were assessed. Immunohistochemistry for VEGF **(D)**; VEGF in-situ hybridisation (anti-sense probe) **(E)**; in-situ hybridisation using VEGF sense probe **(F)** serving as negative control for **E**. Asterisks: necrotic foci. Scale bars: A-C, 200 μm; D-F, 500 μm.

reciprocal regulation of VEGFR1 and -R2 is required for tumor angiogenesis (Zhang et al., 2010a,b). These findings have been mechanistically linked to a rapid endothelial VEGFR1 upregulation upon VEGF-mediated activation of the Akt/ERK pathway leading to increased survival of endothelial cells, whereas VEGFR2 regulates capillary tube formation (Zhang et al., 2010a,b). Whether these findings might also play a similar role in high-grade meningiomas requires further investigation. The fact that our study provides evidence for a constant expression of VEGF receptors while only very few cases display a complete absence of these receptors on their tumor vessels might serve as a principle rationale for the currently performed clinical off-label use of drugs targeting the VEGF pathway (Nayak et al., 2012).

Concerning the prognostic relevance of the VEGF pathway in high-grade meningiomas, we did not find a significant association of patient survival and the epxression of VEGF and its receptors, although these findings have to be considered with caution due to the small sample size. As one cannot clearly distinguish whether VEGF protein located at endothelial cells is released from the endothelium itself or by tumor cells subsequently bound to endothelial VEGF receptors, we performed in-situ hybridisation. Our results show that spatial VEGF mRNA expression nicely meets VEGF protein expression in tumor and endothelial cells predominantly in perinecrotic/hypoxic areas, thereby presenting both cell types as a source of VEGF in WHO grade III meningiomas. These findings at least partly suggest an involvement of a vessel-associated VEGF-driven pathway in malignant progression in meningiomas. A trend for VEGF being associated with worse prognosis ($p=0.01$) was previously described for a population of canine meningiomas, and so VEGF expression therefore was proposed as a prognostic marker (Platt et al., 2006). In contrast, in a human cohort, VEGF could not be linked to an increased frequency of recurrences in WHO grade I meningiomas (Maiuri et al., 2007). However, if anti-angiogenic molecules such as semaphorin3A are also taken into account in the analyses as potential prognostic factors, it could be shown that a VEGF to semaphorin3A expression ratio in meningiomas is significantly associated with histological grade, proliferation index and microvascular density (Barresi and Tuccari, 2010). The latter findings point to a complex angiogenic interplay in meningiomas and suggest that not only the VEGF-VEGF-R system, but its anti-angiogenic counterparts should be assessed for a better understanding of meningioma biology and the development of targeted-therapy approaches.

In summary, our findings suggest that VEGF expression in high grade meningiomas is also hypoxia-driven. The expression of VEGF receptors may serve as evidence for testing the application of drugs targeting the VEGF pathway in high-grade meningiomas. Especially, due to the constant VEGFR2 expression levels in endothelial cells of high grade meningiomas, the use of small tyrosine kinase inhibitors such as Sunitinib directly targeting the VEGF-receptors in cases of therapy-refractory tumors needs further investigation. Concerning the therapeutic relevance of these expression profiles, it would be of great interest to obtain data about the expression of VEGF and its receptors from currently performed clinical studies targeting the VEGF pathway in high-grade meningiomas and to correlate the findings with the response rates.

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References

Barresi V. and Tuccari G. (2010). Increased ratio of vascular endothelial growth factor to semaphorin3A is a negative prognostic factor in human meningiomas. Neuropathology 30, 537-546.

Brokinkel B., Fischer B.R., Peetz-Dienhart S., Ebel H., Sepehrnia A., Rama B., Albert F.K., Stummer W., Paulus W. and Hasselblatt M. (2010). MGMT promoter methylation status in anaplastic meningiomas. J. Neurooncol. 100, 489-490.

Ding Y.S., Wang H.D., Tang K., Hu Z.G., Jin W. and Yan W. (2008). Expression of vascular endothelial growth factor in human meningiomas and peritumoral brain areas. Ann. Clin. Lab. Sci. 38, 344-351.

Escudier B., Pluzanska A., Koralewski P., Ravaud A., Bracarda S., Szczylik C., Chevreau C., Filipek M., Melichar B., Bajetta E., Gorbunova V., Bay J.O., Bodrogi I., Jagiello-Gruszfeld A., Moore N.; AVOREN Trial investigators. (2007). Bevacizumab plus interferon alfa-2a for treatment of metastatic renal cell carcinoma: a randomised, double-blind phase III trial. Lancet 370, 2103-2111.

Ferrara N., Gerber H.P. and LeCouter J. (2003). The biology of VEGF and its receptors. Nat. Med. 9, 669-676.

Folkman J. (1971). Tumor angiogenesis: therapeutic implications. N. Engl. J. Med. 285, 1182-1186.

Gerber H.P., Condorelli F., Park J. and Ferrara N. (1997). Differential transcriptional regulation of the two vascular endothelial growth factor receptor genes. Flt-1, but not Flk-1/KDR, is up-regulated by hypoxia. J. Biol. Chem. 272, 23659-23667.

Goldman C.K., Bharara S., Palmer C.A., Vitek J., Tsai J.C., Weiss H.L. and Gillespie G.Y. (1997). Brain edema in meningiomas is associated with increased vascular endothelial growth factor expression. Neurosurgery 40, 1269-1277.

Hurwitz H., Fehrenbacher L., Novotny W., Cartwright T., Hainsworth J., Heim W., Berlin J., Baron A., Griffing S., Holmgren E., Ferrara N., Fyfe G., Rogers B., Ross R. and Kabbinavar F. (2004). Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N. Engl. J. Med. 350, 2335-2342.

Ilhan A., Gartner W., Neziri D., Czech T., Base W., Hörl W.H. and Wagner L. (2009). Angiogenic factors in plasma of brain tumour patients. Anticancer Res. 29, 731-736.

Jensen R.L., Soleau S., Bhayani M.K. and Christiansen D. (2002). Expression of hypoxia inducible factor-1 alpha and correlation with preoperative embolization of meningiomas. J. Neurosurg. 97, 658-667.

Keck P.J., Hauser S.D., Krivi G., Sanzo K., Warren T., Feder J. and Connolly D.T. (1989). Vascular permeability factor, an endothelial cell mitogen related to PDGF. Science 246, 1309-1312.

Kim K.J., Li B., Winer J., Armanini M., Gillett N., Phillips H.S. and

Ferrara N. (1993). Inhibition of vascular endothelial growth factor-induced angiogenesis suppresses tumour growth in vivo. Nature 362, 841-844.

Koch S., Tugues S., Li X., Gualandi L. and Claesson-Welsh L. (2011). Signal transduction by vascular endothelial growth factor receptors. Biochem. J. 437, 169-183.

Lamszus K., Lengler U., Schmidt N.O., Stavrou D., Ergun S. and Westphal M. (2000). Vascular endothelial growth factor, hepatocyte growth factor/scatter factor, basic fibroblast growth factor, and placenta growth factor in human meningiomas and their relation to angiogenesis and malignancy. Neurosurgery 46, 938-947.

Leung D.W., Cachianes G., Kuang W.J., Goeddel D.V. and Ferrara N. (1989). Vascular endothelial growth factor is a secreted angiogenic mitogen. Science 246, 1306-1309.

Louis D.N., Ohgaki H., Wiestler O.D., Cavenee W.K., Burger P.C., Jouvet A., Scheithauer B.W. and Kleihues P. (2007). The 2007 WHO classification of tumours of the central nervous system. Acta Neuropathol. 114, 97-109.

Maiuri F., De Caro M.B., Esposito F., Cappabianca P., Strazzullo V., Pettinato G. and de Divitiis E. (2007). Recurrences of meningiomas: predictive value of pathological features and hormonal and growth factors. J. Neurooncol. 82, 63-68.

Miller K., Wang M., Gralow J., Dickler M., Cobleigh M., Perez E.A., Shenkier T., Cella D. and Davidson N.E. (2007). Paclitaxel plus bevacizumab versus paclitaxel alone for metastatic breast cancer. N. Engl. J. Med. 357, 2666-2676.

Modha A. and Gutin P.H. (2005). Diagnosis and treatment of atypical and anaplastic meningiomas: a review. Neurosurgery 57, 538-550.

Nassehi D., Dyrbye H., Andresen M., Thomsen C., Juhler M., Laursen H. and Broholm H. (2011). Vascular endothelial growth factor A protein level and gene expression in intracranial meningiomas with brain edema. APMIS. 119, 831-843.

Nayak L., Iwamoto F.M., Rudnick J.D., Norden A.D., Lee E.Q., Drappatz J., Omuro A. and Kaley T.J. (2012) Atypical and anaplastic meningiomas treated with bevacizumab. J. Neurooncol. 109, 187-193.

Otsuka S., Tamiya T., Ono Y., Michiue H., Kurozumi K., Daido S., Kambara H., Date I. and Ohmoto T. (2004). The relationship between peritumoral brain edema and the expression of vascular endothelial growth factor and its receptors in intracranial meningiomas. J. Neurooncol. 70, 349-357.

Panagopoulos A.T., Lancellotti C.L., Veiga J.C., de Aguiar P.H. and Colquhoun A. (2008). Expression of cell adhesion proteins and proteins related to angiogenesis and fatty acid metabolism in benign, atypical, and anaplastic meningiomas. J. Neurooncol. 89, 73-87.

Perry A., Scheithauer B.W., Stafford S.L., Lohse C.M. and Wollan P.C. (1999). "Malignancy" in meningiomas: a clinicopathologic study of 116 patients, with grading implications. Cancer 85, 2046-2056.

Pietsch T., Valter M.M., Wolf H.K., von Deimling A., Huang H.J., Cavenee W.K. and Wiestler O.D. (1997). Expression and distribution of vascular endothelial growth factor protein in human brain tumors. Acta Neuropathol. 93, 109-117.

Pistolesi S., Fontanini G., Camacci T., De Ieso K., Boldrini L., Lupi G., Padolecchia R., Pingitore R. and Parenti G. (2002). Meningioma-associated brain oedema: the role of angiogenic factors and pial blood supply. J. Neurooncol. 60, 159-164.

Plate K.H., Breier G., Weich H.A. and Risau W. (1992). Vascular endothelial growth factor is a potential tumour angiogenesis factor in human gliomas in vivo. Nature 359, 845-848.

Platt S.R., Scase T.J., Adams V., Wieczorek L., Miller J., Adamo F. and Long S. (2006). Vascular endothelial growth factor expression in canine intracranial meningiomas and association with patient survival. J. Vet. Intern. Med. 20, 663-668.

Ryan H.E., Lo J. and Johnson R.S. (1998). HIF-1 alpha is required for solid tumor formation and embryonic vascularization. EMBO J. 17, 3005-3015.

Sakuma T., Nakagawa T., Ido K., Takeuchi H., Sato K. and Kubota T. (2008). Expression of vascular endothelial growth factor-A and mRNA stability factor HuR in human meningiomas. J. Neurooncol. 88, 143-155.

Sandler A., Gray R., Perry M.C., Brahmer J., Schiller J.H., Dowlati A., Lilenbaum R. and Johnson D.H. (2006). Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer. N. Engl. J. Med. 355, 2542-2550.

Schmid S., Aboul-Enein F., Pfisterer W., Birkner T., Stadek C. and Knosp E. (2010). Vascular endothelial growth factor: the major factor for tumor neovascularization and edema formation in meningioma patients. Neurosurgery 67, 1703-1708.

Senger D.R., Galli S.J., Dvorak A.M., Perruzzi C.A., Harvey V.S. and Dvorak H.F. (1983). Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science 219, 983-985.

Shibuya M. (2006). Vascular endothelial growth factor receptor-1 (VEGFR-1/Flt-1): a dual regulator for angiogenesis. Angiogenesis 9, 225-230.

Shweiki D., Itin A., Soffer D. and Keshet E. (1992). Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. Nature 359, 843-845.

Ulyatt C., Walker J. and Ponnambalam S. (2011). Hypoxia differentially regulates VEGFR1 and VEGFR2 levels and alters intracellular signaling and cell migration in endothelial cells. Biochem. Biophys. Res. Commun. 404, 774-779.

Wilting J., Christ B. and Weich H.A. (1992). The effects of growth factors on the day 13 chorioallantoic membrane (CAM): a study of VEGF165 and PDGF-BB. Anat. Embryol. 186, 251-257.

Zhang Z., Neiva K.G., Lingen M.W., Ellis L.M. and Nör J.E. (2010a) VEGF-dependent tumor angiogenesis requires inverse and reciprocal regulation of VEGFR1 and VEGFR2. Cell. Death. Differ. 17, 499-512.

Zhang M.X., Zhao X., Wang Z.G., Zhao W.M. and Wang Y.S. (2010b). Constitutive activation of signal transducer and activator of transcription 3 regulates expression of vascular endothelial growth factor in human meningioma differentiation. J. Cancer Res. Clin. Oncol. 136, 981-988.

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