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RESEARCH

Angiogenesis and Angiogenic Tyrosine Kinase Receptor Expression in Pediatric Brain Tumors

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Abstract Tumor angiogenesis and receptor tyrosine kinases (RTK) are major novel targets in anticancer molecular therapy. Accordingly, we characterized the vascular network and the expression pattern of angiogenic RTK in the most frequent pediatric brain tumors. In a retrospective collection of 44 cases (14 astrocytoma, 16 ependymoma and 14 medulloblastoma), immunohistochemistry for VEGFR1, VEGFR2, PDGFRα, PDGFRβ, and c-Kit as well as microvessel labeling with CD34 and SMA were conducted on surgical specimens. We found a significantly higher vascular density in ependymoma. Glomeruloid formations were abundant in medulloblastoma but rare or almost absent in astrocytoma and ependymoma, respectively. C-Kit and VEGFR2 labeled blood vessels were more abundant in ependymoma than in the other two types of tumors. In contrast, medulloblastoma contained higher number of PDGFRα expressing vessels. In tumor cells, we found no VEGFR2 but VEGFR1 expression in all three tumor types. PDGFRα was strongly expressed on the tumor cells in all three malignancies, while PDGFRβ tumor cell expression was present in the majority of medulloblastoma cases. Interestingly, small populations of c-Kit expressing cancer cells were found in a number of medulloblastoma and ependymoma cases. Our study suggests that different angiogenic mechanisms are present in ependymoma and medulloblastoma. Furthermore ependymoma patients may benefit from anti-angiogenic therapies based on the high vascularization as well as the endothelial expression of c-kit and VEGFR2. The expression pattern of the receptors on tumor cells also suggests the targeting of specific angiogenic tyrosine kinase receptors may have direct antitumor activity. Further preclinical and biomarker driven clinical investigations are needed to establish the application of tyrosine kinase inhibitors in the treatment of pediatric brain tumors.

Keywords Astrocytoma · Ependymoma · Medulloblastoma · Tyrosine kinase receptor · Targeted therapy

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Introduction

The identification of receptor tyrosine kinases that provide growth signals to tumor and endothelial cells as well as the advent of drugs capable of specifically inhibit these receptors has led to the new paradigm of molecularly targeted therapy. With regards to malignancies of the central nervous system the

role of angiogenesis in tumor progression and the underlying molecular mechanisms are rather well described in adult glioblastoma [1–9]. In contrast, there are only few studies investigating angiogenesis in pediatric brain tumors [10–15]. The tumor tissue can acquire vascularization by several distinct mechanisms including neoangiogenesis, postnatal vasculogenesis, vessel cooption, intussusceptive growth or vascular mimicry [16]. Furthermore brain tumors are characterized by another unique form of vessels, the glomeruloid tufts [17]. In glioblastoma vessel cooption was found to be the primary form of vascularization [18] but there is limited data in other brain tumor types concerning their vascularization patterns. Importantly, each of these vascularization mechanisms are associated with a unique angiogenic RTK expression pattern on blood vessels: neoangiogenesis is characterized by VEGFR2 expression, postnatal vasculogenesis is driven by flt3, c-Kit and VEGFR2 expression, vessel cooption is associated with TIE receptors, vascular mimicry can be identified by EphA and FAK expression and glomeruloid vessels are characterized by VEGFR2 and PDGFR expression [16]. Altogether, distinct molecular pathways seem to drive these vascularization mechanisms.

The importance of comprehensive analysis of pediatric brain tumors is highlighted by a recent study that demonstrated that distinct angiogenic patterns are present in glioblastoma and in pediatric pilocytic astrocytoma [11]. Of note, the vascularization and angiogenesis was found to be an important prognostic factor in optic pathway glioma [12]. Furthermore, VEGF expression was found to be a prognostic factor in an ependymoma patient cohort with adult and pediatric cases [13].

Inhibitors of angiogenic receptor tyrosine kinases can be potent antitumor drugs since tumor growth requires the acquisition of appropriate tumor vasculature. Both the binding of active VEGF by therapeutic antibodies and the inhibition of VEGFR by small molecule tyrosine kinase inhibitors are currently approved antitumor treatment options in various malignancies. Bevacizumab is used in combination therapies against adult brain tumors [19, 20]. However, its application in pediatric neuro-oncology is still rather limited [21–24]. Nevertheless, preclinical studies suggest that targeting VEGF and VEGFR is a promising therapeutic option in pediatric brain tumors [25, 26].

Both VEGFR and other angiogenic receptor tyrosine kinases may also be expressed by the tumor cells suggesting that their inhibition may have direct antitumor effects by interfering with tumor cell proliferation and survival. In adult glioblastoma, expression - and amplification - of c-Kit, PDGFRα and VEGFR2 by glioma cells has been described and they represent potential targets [8, 27, 28]. VEGFR2 expression by tumor cells has been studied in medulloblastoma, however the findings are controversial whether the protein is expressed by medulloblastoma tumor cells [29, 30].

The stem cell factor receptor c-Kit (also called CD117) is also often expressed in certain types of tumors. C-Kit expression by endothelial cells has been demonstrated in adult glioblastoma [8] and in a number of pediatric brain tumors [15]. Besides its proangiogenic activity by virtue of its expression on endothelial progenitor cells it is often present in the tumor cells. Currently, imatinib based targeted therapy is approved for certain malignancies such as chronic myelogenous leukemia (CML) and gastrointestinal stromal tumors [31]. However, increased c-Kit expression has been described in other malignant cells including adult glioma cells [32, 33] and pediatric medulloblastoma cells [30, 34, 35]. Thus clinical trials are under way for other malignancies including adult high-grade glioma [36].

Inhibitors of PDGF receptors are also potent anti-angiogenic and anti-tumor agents in certain malignancies. The expression – and often the amplification – of PDGFRα by brain tumor cells was described in certain cases of astrocytoma and glioblastoma [37]. In pediatric glioma, high tumor cell PDGFR expression correlated with lower degree of differentiation of the tumor [38]. In a preclinical study PDGFR targeting has emerged as an effective treatment option in pediatric glioblastoma [39]. A PDGFRA mutation has been identified in a small fraction of pediatric medulloblastoma [40]. In metastatic medulloblastoma an increased expression of PDGFRβ by cancer cells was found when compared to non-metastatic cases [41]. The c-Kit/PDGFR inhibitor imatinib and the VEGFR/PDGFR inhibitor sunitinib were able to inhibit medulloblastoma cell migration and invasion in a PDGFR activation-dependent manner [42, 43]. However, there is only a limited number of studied investigating the potential of these inhibitors in pediatric brain tumors [44, 45]. Nevertheless, some of these inhibitors—often on a compassionate basis—had been employed in pediatric neuro-oncology [21–24].

Accordingly, we studied the microvascular pattern and the expression of the most important and currently druggable angiogenic receptor tyrosine kinases in a panel of pediatric brain tumors. Our findings suggest that targeting angiogenesis and angiogenic receptor tyrosine kinases is a potential therapeutic option that should be further explored in preclinical and clinical studies.

Materials and Methods

Patients

Formalin-fixed paraffin embedded histopathological samples were collected from 44 pediatric patients with ependymoma, astrocytoma or medulloblastoma. Surgical removal of tumors

was performed at the National Institute Neurosurgery, Budapest between 1997 and 2006. Tissue specimen was obtained from the initial surgery. No patients received radiation or chemotherapy prior to the surgery. The average age of the patients was 8.2 year (Table 1). Significant gender bias was only found in the astrocytoma group. Except three ependymoma cases all other tumors were intracranial. Most astrocytoma cases were low-grade astrocytoma except four pediatric glioblastoma. All ependymomas were anaplastic. Among the medulloblastoma cases there were 7 undifferentiated, 4 desmoplastic, and 3 with glial or neuronal differentiation. Altogether this patient population provides a representative cohort for these pediatric brain tumors. This retrospective analysis was approved by the Hungarian Medical Science Council (ETT #84-192/2008).

Immunohistochemistry

Representative paraffin blocks—defined as those with the largest amount of viable tumor cells—for each tumor were selected. The immunohistochemical reactions were performed on 3 μm sections obtained from the FFPE blocks. After the deparaffinization steps, the slides were treated in a microwave oven in Target Retrieval Solution (S1699 from DAKO, Carpenteria, CA, USA) for 30 min for heat-induced epitope retrieval. The immunohistochemical reactions were performed in a Ventana ES immunostainer system (Ventana Medical Systems Inc., Tucson, AZ, USA) with the solutions and steps according to the manufacturer. The list of antibodies and conditions are listed in Supplemental Table 1. Positive controls and negative control tissues (with the omission of the primary antibodies) were included in every run. Scoring for semi-quantitative analysis was evaluated by a pathologist. Three randomly selected areas of each slide were analysed using middle-power field objective (×10). Positive vascular structures (glomeruloid and normal capillaries) on CD34 and SMA stained sections were counted. Glomeruloid vessel counts included small microglomeruloid clusters as described by Goldbrunner et al. previously [46]. For microvascular density or glomeruloid vessels the three fields of view were averaged or summarized, respectively. For the tumor cell specific expression the percentage of labelled malignant cells was estimated.

Statistics

The patient cohorts were statistically analyzed by χ^2-test. The significance of differences between tumor types was determined by Mann–Whitney test. The percentage of positive tumor cells were compared between the tumor types by Kruskal-Wallis test followed by Dunn's multiple comparison test. Statistical significance ($P< 0.05$) was determined using GraphPad Prism 5.0 software (GraphPad Inc., San Diego, CA).

Results

Microvascular Density and Glomeruloid Vessels in Pediatric Brain Tumors

The vasculature of the brain tumor tissue was identified by CD34 immunohistochemistry. Representative images shown in Fig. 1a demonstrate the endothelial lining specific staining of blood vessels. We found a significantly higher density of blood vessels in pediatric ependymoma when compared to pediatric astrocytoma and medulloblastoma (Fig. 1b). While there was no difference in vessel density between the pilocytic astrocytoma and pediatric glioblastoma cases (data not shown). In order to visualise the more mature and stabilized blood vessels, pericytes of tumor-associated vessels were labelled with smooth muscle actin (SMA) (Fig. 1a). In all tumor types CD34 labelled vessels were more frequent than

Table 1 Patient characteristic including age, localization, histological subtype and grade

	Asrocytoma (N= 14)	Ependymoma (N= 16)	Medulloblastoma (N= 14)
Age (year, range)	6.2 (1–14)	8 (1–16)	8.4 (1–20)
Female	2 (14 %)	9 (56 %)	7 (50 %)
Male	12 (86 %)	7 (44 %)	7 (50 %)
Supratentorial	11	7	–
Infratentorial	3	6	14
Spinal	0	3	–
Histology	glioblastoma (4)	–	non-differentiated (7)
	pilocytic (10)	–	differentiated (3)
		–	desmoplastic (4)
Grade	I (10)	II (1)	–
	IV (4)	III (15)	–

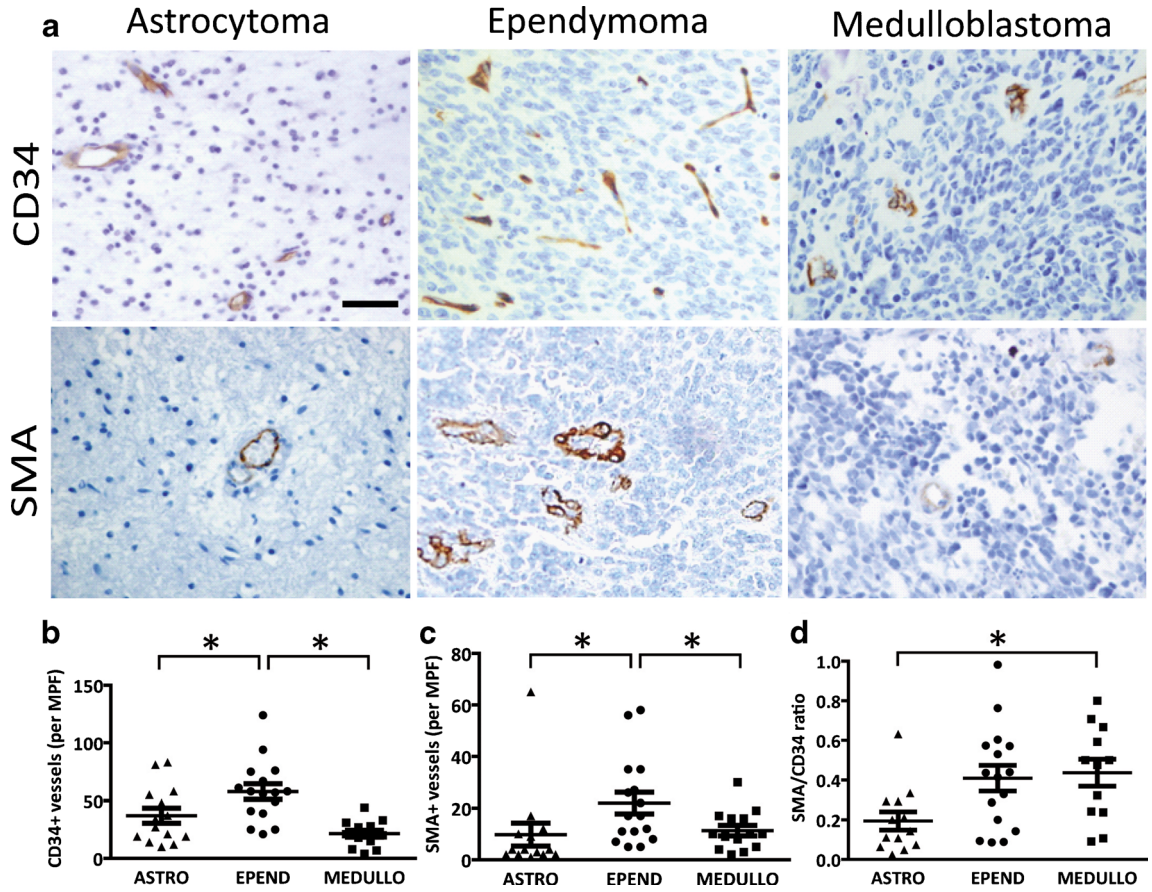

Fig. 1 Microvascular density in pediatric brain tumors. **a** CD34 and smooth muscle actin (SMA) immunohistochemistry readily displays the microvascular network. **b**-**c** Both CD34 and SMA positive blood vessels are significantly more abundant in ependymoma than in glioma or medulloblastoma. Note that in all tumor types CD34 labelled vessels are more frequent than SMA positive structures. **d** The ratio of SMA and CD34 labelled vessels was significantly higher in medulloblastoma than in glioma. (Scale bar is 100 μm, MPF – medium power field of view)

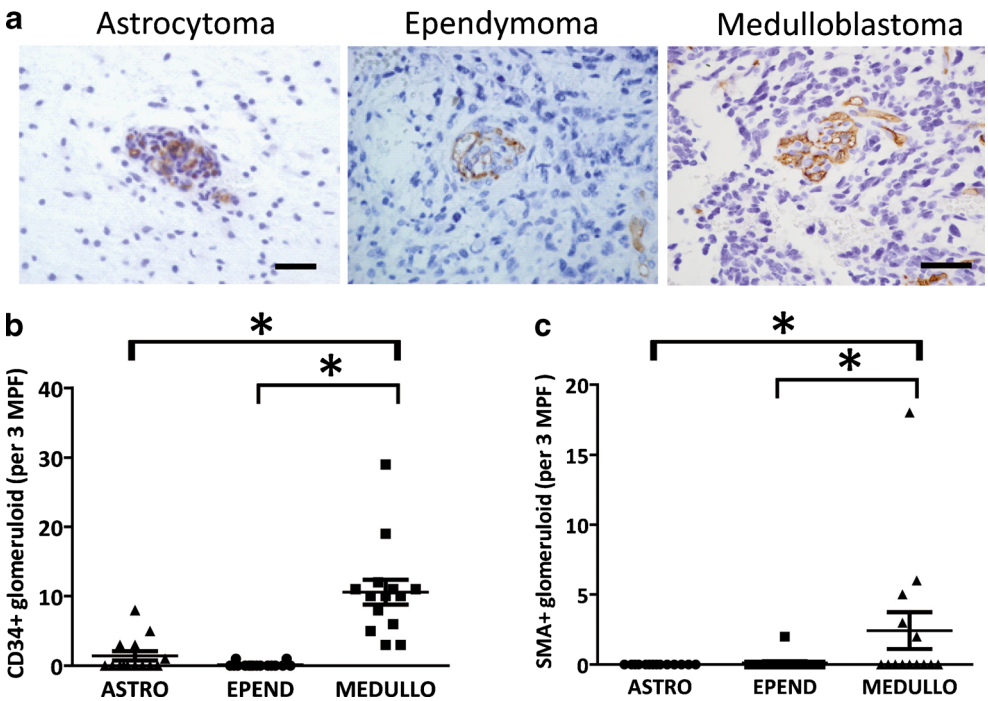

Fig. 2 Glomeruloid formations in pediatric brain tumors. **a** The glomeruloid morphology of blood vessels can readily be identified after CD34 labelling. **b** While medulloblastoma tumors contain a large number of CD34 positive glomeruloid vessels they are almost absent in ependymoma and significantly less frequent in astrocytoma. **c** SMA labelled glomeruloid structures are less frequent than CD34 positive ones. (Scale bar is 100 μm, MPF – medium power field of view)

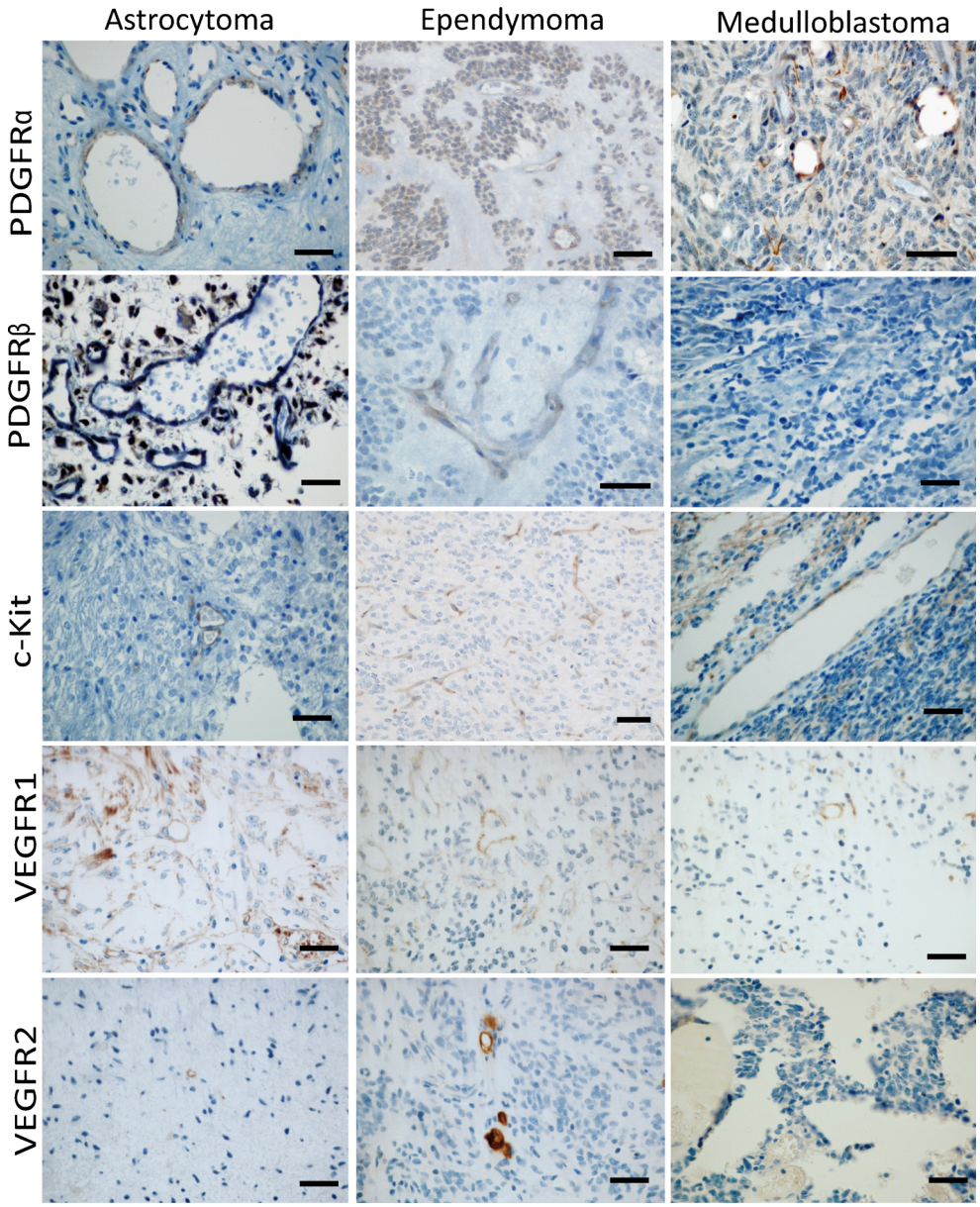

Fig. 3 Immunohistochemical labelling of tyrosine kinase receptors in blood vessels of pediatric brain tumors. The strongest PDGFRα expression is present in medulloblastoma. PDGFRβ demonstrated only low expression in blood vessels. VEGFR1 is expressed on blood vessels irrespective of tumor type. Strong VEGFR2 and c-Kit expression characterizes blood vessels of ependymoma. (Scale bars are 100 μm)

SMA positive ones (Fig. 1b and c). Nevertheless, the SMA positive blood vessels were also significantly more abundant in ependymoma than in astrocytoma or medulloblastoma (Fig. 1c). The ratio of SMA and CD34 labelled blood vessels was significantly lower in astrocytoma then in medulloblastoma (Fig. 1d). Importantly, glomeruloid blood vessels were found in each of the CD34 labelled medulloblastoma specimens (Fig. 2a and b) while significantly lower number of astrocytoma cases contained glomeruloid structures and they were almost absent in ependymoma cases (Fig. 2b). Of note, three out of the four pediatric glioblastoma cases contained glomeruloid capillaries. SMA labelled glomeruloid structures, were observed almost exclusively in medulloblastoma. Only 5 of the 14 medulloblastoma cases contained SMA positive glomeruloid structures and they were less frequent then the CD34 positive glomeruloid vessels (Fig. 2b and c).

Table 2 Number of cases with angiogenic receptor-positive blood vessels in pediatric brain tumors

Receptor	Astrocytoma *N*= 14	Ependymoma *N*= 16	Medulloblastoma *N*= 14
PDGFRα	1	4	13
PDGFRβ	2	6	3
c-Kit	3	10	5
VEGFR1	12	14	13
VEGFR2	3	4	0

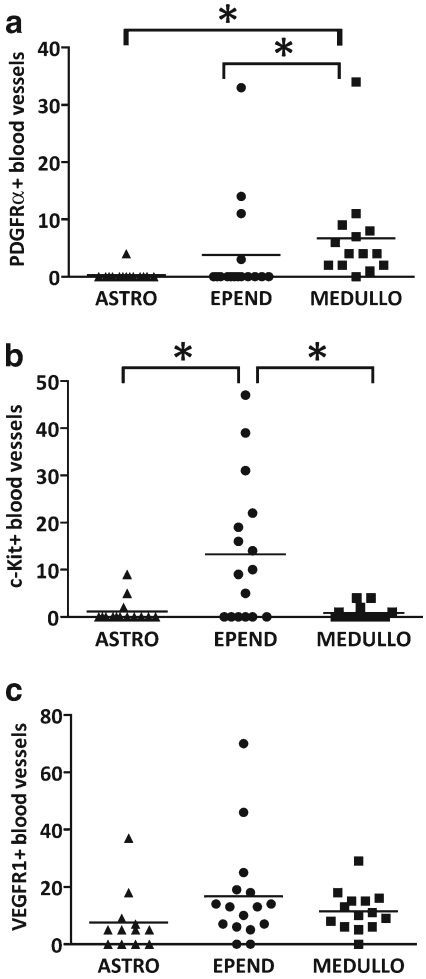

Fig. 4 Angiogenic tyrosine kinase receptor expression in blood vessels of pediatric brain tumors. **a** PDGFRα labelled blood vessels are significantly more abundant in medulloblastoma than in glioma or in ependymoma. **b** The majority of ependymoma cases contain high number c-Kit expressing blood vessels. In contrast, blood vessels from glioma and medulloblastoma only occasionally expressed c-Kit. PDGFRβ demonstrated only low expression in blood vessels. **c** VEGFR1 is expressed on blood vessels irrespective of tumor type

Tyrosine Kinase Receptor Expression in Tumor Associated Blood Vessels

In order to investigate whether the different vascular density and the distinct pattern of glomeruloid formations in the pediatric brain tumors are related to the angiogenic receptor tyrosine kinase expression we performed immunohistochemical labelling of these receptors. Images—taken at areas where low tumor cell derived staining is present—clearly indicate the blood vessel specific staining for each type of the tyrosine kinase receptors (Fig. 3). Almost all medulloblastoma specimens contained PDGFRα expressing blood vessels (Table 2). Both the strongest labelling and the highest number of PDGFRα-positive vessels were present in medulloblastoma (Figs. 3 and 4). PDGFRβ demonstrated only weak staining and thus low vascular expression in either type of brain tumors (Fig. 3). VEGFR1 was strongly expressed on blood vessels in most of the specimens irrespective of the pediatric brain tumor type (Table 2). Strong VEGFR2 and c-Kit expression was found only on blood vessels of certain ependymoma cases (Figs. 3 and 4) while we found no VEGFR2 positive blood vessels in medulloblastoma.

Tyrosine Kinase Receptor Expression of Pediatric Brain Tumor Cells

The tumor cell specific angiogenic RTK expression was also analyzed in a semi-quantitative manner. Strong tumor cell PDGFRα expression was found in all three pediatric brain tumor types (Fig. 5, Table 3). PDGFRβ was expressed by tumor cells in the majority of medulloblastoma cases (10 out of 14) but it was less frequent in astrocytoma and significantly less abundant in ependymoma (Table 3, Figs. 5 and 6). Low intensity tumor cell specific c-Kit expression was found in only two astrocytoma and five ependymoma cases. In contrast we found three cases of medulloblastoma with intense c-Kit staining on the plasma membrane of tumor cells (Fig. 5). VEGFR1 positivity was observed in all three types of tumors while there was no VEGFR2 expression present on the pediatric brain tumor cells (Table 3).

Discussion

In the past decade molecularly targeted drugs had been developed against various angiogenic tyrosine kinase receptors including growth factor receptors that play pivotal role in tumor induced angiogenesis. However, their application in pediatric neuro-oncology is still rather limited. On one hand our understanding of tumor induced angiogenesis in these cancers is still insufficient to be able to predict the potential efficacy of these modalities. On the other hand, since these agents may have direct anti-tumor activity, it is necessary to the tumor cell tyrosine kinase receptor expression pattern in these rather frequent pediatric brain tumors. One of the major findings of the present study is that pediatric ependymoma have the highest microvascular density and that these vessels are covered by pericytes indicating either a full-fledged neoangiogenesis process or efficient vessel cooption that seems to be very efficient as compared to the pediatric astrocytoma and medulloblastoma. Similar to previous studies we have shown that ependymoma-associated blood vessels expressed three types of angiogenic TKs (VEGFR1>VEGFR2, c-kit) on endothelial cells [9, 15].

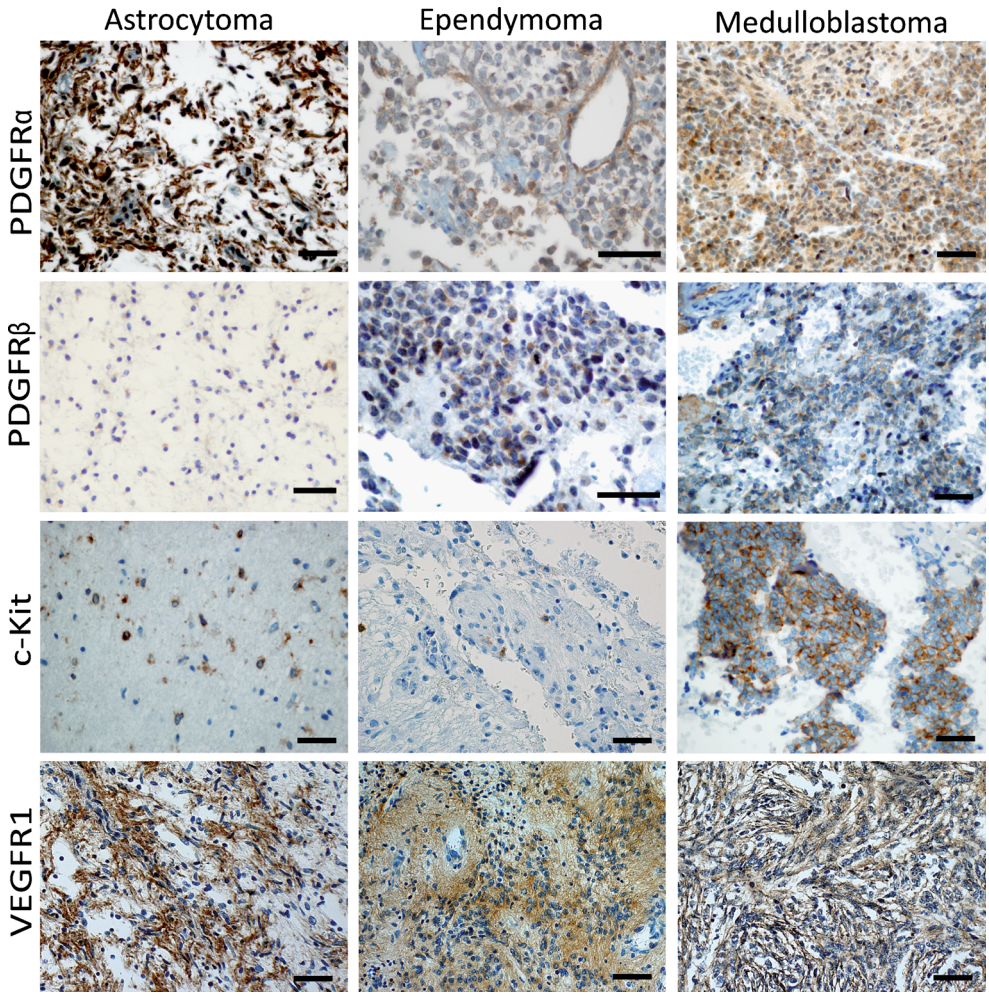

Fig. 5 PDGFR, c-Kit and VEGFR expression on tumor cells in pediatric brain tumors. Note the abundant staining of PDGFRα in all three types of tumor cells. PDGFRβ is only expressed by ependymoma and medulloblastoma cells. Robust c-Kit expression was only found in medulloblastoma cells. The c-Kit positive cells in the sections of astrocytoma and ependymoma are endothelial cells of the capillary network. (Scale bars are 100 μm)

In the light of these observations, the subset of pediatric ependymomas with high vascular density and with an angiogenic vessel expression profile is a promising candidate for anti-angiogenic therapies. Supporting this notion, Wagemakers et al. demonstrated that pediatric ependymomas have the same microvascular density and VEGF-A expression level as adult glioblastoma where anti-angiogenic therapy is becoming a standard modality [14]. On the other hand, we have demonstrated that medulloblastoma rely on other mechanism since glomeruloid vessels were more abundant in this tumor. Our observation is in line with a previous study [46]. These glomeruloid structures have been described in glioblastoma as an early step in VEGF induced neoangiogenesis [47]. Another important finding of our investigation is the abundant expression of VEGFR1 on tumor associated microvessels of the pediatric brain tumors. It suggests that VEGFR1 may be a primary anti-angiogenic drug target. While VEGFR2 is the primary mitogen receptor for endothelial cells the role of VEGFR1 is more complex. This later VEGF receptor has a weak kinase activity and is expressed as a membrane receptor as well as in a soluble form serving as VEGF-eliminator [48].

There is very limited information about the alternative vascularization mechanisms in the malignancies of the central nervous system and pediatric forms in particular

Table 3 Number of cases with angiogenic receptor-positive tumor cells in pediatric brain tumors

Receptor	Astrocytoma *N*= 14	Ependymoma *N*= 16	Medulloblastoma *N*= 14
PDGFRα	13	16	16
PDGFRβ	6	4	10
c-Kit	0	3	3
VEGFR1	7	10	12
VEGFR2	0	0	0

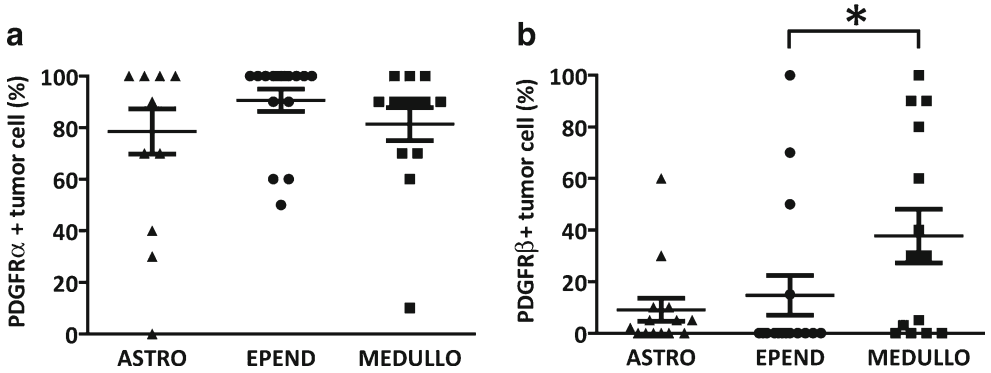

Fig. 6 The frequency of PDGFRα (**a**) and PDGFRβ (**b**) expressing tumor cells in all three types of pediatric brain tumors. Almost all cases displayed abundant expression of PDGFRα. In contrast, there was a significant difference in PDGFRβ expression. Medulloblastoma had significantly higher proportion of PDGFRβ labelled tumor cells then ependymoma

[16]. Intussusceptive angiogenesis and vasculogenic mimicry have been demonstrated in adult glioblastoma [49–52]. In the present study the large number of glomeruloid vessel formations in medulloblastoma suggests that this tumor type may acquire the blood supply through alternative vascularization mechanisms and thus may be less sensitive to therapeutic approaches targeting primarily tumor induced neoangiogenesis.

In our study we found that all medulloblastoma cases displayed abundant tumor cell expression of PDGFR. This finding confirms previous studies that indicated the importance of PDGFR signaling [30, 41, 42] and found oncogenic mutations in the PDGFRA gene in pediatric medulloblastoma [40]. Furthermore, we found PDGFR expression by ependymoma tumor cells similar to a very recent study by Moreno et al. Importantly, in their investigation the angiogenic characteristics of the tumors were independent of tumor cell PDGFR expression [53]. Altogether, these findings support the notion that targeting PDGFR may be a promising approach in the PDGFR overexpressing subset of medulloblastoma and ependymoma.

Tumor cell expression of the c-Kit has been previously described in pediatric brain tumors mostly in medulloblastoma [30, 34, 35] and in ependymoma [54]. In line with our observations, Enguita-German et al. found that transcription level overexpression of c-Kit was present in 25 % (3 out of 12 cases) of medulloblastoma tumors [35]. Importantly, Zahalvia et al. found an incidence (25 %) similar to our study (19 %) in c-Kit positive ependymoma. These findings together strongly suggest that c-Kit may be an oncogenic factor in a subset of pediatric ependymoma and medulloblastoma and these patients may eventually benefit from a therapy with selective inhibitors of c-Kit.

While VEGFR2 is the major receptor that mediates the angiogenic functions of VEGF-A, VEGFR1 - by virtue of its tyrosine kinase activity—can function as a proto-oncogene [55, 56]. Tumor cell expression of VEGFR1 has not been previously demonstrated in ependymoma. Importantly, we found a significant number of ependymoma cases (10 out of 16) that expressed VEGFR1. In preclinical studies, antibodies capable of inhibiting VEGFR1 activation can interfere with tumor cell growth in vitro and in vivo [55]. These observations together suggest that this subset of ependymoma may respond to anti-VEGFR1 therapy. In a previous study VEGFR2 expression by medulloblastoma tumor cells was found to be almost absent—one single positive case—in a study with a rather large cohort of 41 medulloblastoma cases [30]. In contrast, Slongo et al. found that all 13 medulloblastoma cases from their cohort contained with VEGFR2 bearing tumor cells [29]. In our study there was no tumor cell specific VEGFR2 expression in any of the 14 medulloblastoma cases.

Despite the relatively small number of cases in our series of pediatric brain tumors, subsets of these tumors were identified with a distinct receptor tyrosine kinase expression pattern suggesting that there are subgroups that may carry different sensitivity to specific TKI drugs. Whether the receptor tyrosine kinase expression pattern is related to the molecular subgroups of ependymoma and medulloblastoma remains to be explored. Altogether, our study indicates that certain pediatric malignancies may display angiogenic activity and thus identifying them as potential targets for anti-angiogenic therapy. Furthermore, the targeting of specific angiogenic tyrosine kinase receptors in distinct subsets of pediatric malignancies may have direct antitumor activity and thus potential therapeutic role. Nevertheless, further preclinical and biomarker driven clinical investigations are urgently needed to investigate the application of tyrosine kinase inhibitors in the treatment of common pediatric brain tumors.

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References

1. Goudar RK et al (2005) Combination therapy of inhibitors of epidermal growth factor receptor/vascular endothelial growth factor receptor 2 (AEE788) and the mammalian target of rapamycin (RAD001) offers improved glioblastoma tumor growth inhibition. Mol Cancer Ther 4(1):101–112
2. Goldman CK et al (1993) Epidermal growth factor stimulates vascular endothelial growth factor production by human malignant glioma cells: a model of glioblastoma multiforme pathophysiology. Mol Biol Cell 4(1):121–133
3. Jain RK et al (2007) Angiogenesis in brain tumours. Nat Rev Neurosci 8(8):610–622
4. Lopes MB (2003) Angiogenesis in brain tumors. Microsc Res Tech 60(2):225–230
5. Zagzag D et al (2000) Molecular events implicated in brain tumor angiogenesis and invasion. Pediatr Neurosurg 33(1):49–55
6. Zagzag D et al (2000) Vascular apoptosis and involution in gliomas precede neovascularization: a novel concept for glioma growth and angiogenesis. Lab Invest 80(6):837–849
7. Zagzag D et al (2000) Expression of hypoxia-inducible factor 1alpha in brain tumors: association with angiogenesis, invasion, and progression. Cancer 88(11):2606–2618
8. Haberler C et al (2006) Immunohistochemical analysis of platelet-derived growth factor receptor-alpha, -beta, c-kit, c-abl, and arg proteins in glioblastoma: possible implications for patient selection for imatinib mesylate therapy. J Neurooncol 76(2):105–109
9. Chan AS et al (1998) Expression of vascular endothelial growth factor and its receptors in the anaplastic progression of astrocytoma, oligodendroglioma, and ependymoma. Am J Surg Pathol 22(7):816–826
10. Gilhuis HJ et al (2006) Three-dimensional (3D) reconstruction and quantitative analysis of the microvasculature in medulloblastoma and ependymoma subtypes. Angiogenesis 9(4):201–208
11. Sie M et al (2010) Tumour vasculature and angiogenic profile of paediatric pilocytic astrocytoma; is it much different from glioblastoma? Neuropathol Appl Neurobiol 36(7):636–647
12. Bartels U et al (2006) Vascularity and angiogenesis as predictors of growth in optic pathway/hypothalamic gliomas. J Neurosurg 104(5 Suppl):314–320
13. Korshunov A, Golanov A, Timirgaz V (2002) Immunohistochemical markers for prognosis of ependymal neoplasms. J Neurooncol 58(3): 255–270
14. Wagemakers M et al (2010) Tumor vessel biology in pediatric intracranial ependymoma. J Neurosurg Pediatr 5(4):335–341
15. Puputti M et al (2010) Expression of KIT receptor tyrosine kinase in endothelial cells of juvenile brain tumors. Brain Pathol 20(4):763–770
16. Dome B et al (2007) Alternative vascularization mechanisms in cancer: Pathology and therapeutic implications. Am J Pathol 170(1):1–15
17. Selby DM et al (1997) Are endothelial cell patterns of astrocytomas indicative of grade? In Vivo 11(5):371–375
18. Holash J et al (1999) Vessel cooption, regression, and growth in tumors mediated by angiopoietins and VEGF. Science 284(5422): 1994–1998
19. Vredenburgh JJ et al (2007) Bevacizumab plus irinotecan in recurrent glioblastoma multiforme. J Clin Oncol 25(30):4722–4729
20. Green RM et al (2009) Bevacizumab for recurrent ependymoma. Neurology 73(20):1677–1680
21. Aguilera DG, Goldman S, Fangusaro J (2011) Bevacizumab and irinotecan in the treatment of children with recurrent/refractory medulloblastoma. Pediatr Blood Cancer 56(3):491–494
22. Benesch M et al (2008) Compassionate use of bevacizumab (Avastin) in children and young adults with refractory or recurrent solid tumors. Ann Oncol 19(4):807–813
23. Couec ML et al (2012) Bevacizumab and irinotecan in children with recurrent or refractory brain tumors: toxicity and efficacy trends. Pediatr Blood Cancer 59(1):34–38
24. Peyrl A et al (2012) Antiangiogenic metronomic therapy for children with recurrent embryonal brain tumors. Pediatr Blood Cancer 59(3): 511–517
25. Maris JM et al (2008) Initial testing of the VEGFR inhibitor AZD2171 by the pediatric preclinical testing program. Pediatr Blood Cancer 50(3):581–587
26. Meco D et al (2010) Dual inhibitor AEE788 reduces tumor growth in preclinical models of medulloblastoma. Transl Oncol 3(5):326–335
27. Joensuu H et al (2005) Amplification of genes encoding KIT, PDGFRalpha and VEGFR2 receptor tyrosine kinases is frequent in glioblastoma multiforme. J Pathol 207(2):224–231
28. de Groot JF, Gilbert MR (2007) New molecular targets in malignant gliomas. Curr Opin Neurol 20(6):712–718
29. Slongo ML et al (2007) Functional VEGF and VEGF receptors are expressed in human medulloblastomas. Neuro Oncol 9(4):384–392
30. Blom T et al (2010) Amplification and overexpression of KIT, PDGFRA, and VEGFR2 in medulloblastomas and primitive neuroectodermal tumors. J Neurooncol 97(2):217–224
31. Shiba K, Matsumoto T, Hirota S (2009) Small-molecule inhibitors against KIT and PDGFRs especially in GISTs. Gan To Kagaku Ryoho 36(7):1080–1085
32. Cetin N, Dienel G, Gokden M (2005) CD117 expression in glial tumors. J Neurooncol 75(2):195–202
33. Gomes AL et al (2007) Molecular alterations of KIT oncogene in gliomas. Cell Oncol 29(5):399–408
34. Chilton-Macneill S et al (2004) C-kit expression and mutational analysis in medulloblastoma. Pediatr Dev Pathol 7(5):493–498
35. Enguita-German M et al (2011) KIT expression and methylation in medulloblastoma and PNET cell lines and tumors. J Neurooncol 103(2):247–253
36. Razis E et al (2009) Phase II study of neoadjuvant imatinib in glioblastoma: evaluation of clinical and molecular effects of the treatment. Clin Cancer Res 15(19):6258–6266
37. Puputti M et al (2006) Amplification of KIT, PDGFRA, VEGFR2, and EGFR in gliomas. Mol Cancer Res 4(12):927–934
38. Thorarinsdottir HK et al (2008) Protein expression of platelet-derived growth factor receptor correlates with malignant histology and PTEN with survival in childhood gliomas. Clin Cancer Res 14(11):3386–3394
39. Bielen A et al (2011) Enhanced efficacy of IGF1R inhibition in pediatric glioblastoma by combinatorial targeting of PDGFRalpha/beta. Mol Cancer Ther 10(8):1407–1418
40. Gilbertson RJ et al (2006) Mutational analysis of PDGFR-RAS/MAPK pathway activation in childhood medulloblastoma. Eur J Cancer 42(5):646–649
41. Gilbertson RJ, Clifford SC (2003) PDGFRB is overexpressed in metastatic medulloblastoma. Nat Genet 35(3):197–198
42. Abouantoun TJ, MacDonald TJ (2009) Imatinib blocks migration and invasion of medulloblastoma cells by concurrently inhibiting activation of platelet-derived growth factor receptor and transactivation of epidermal growth factor receptor. Mol Cancer Ther 8(5):1137–1147
43. Abouantoun TJ, Castellino RC, MacDonald TJ (2011) Sunitinib induces PTEN expression and inhibits PDGFR signaling and migration of medulloblastoma cells. J Neurooncol 101(2):215–226
44. Herrington B, Kieran MW (2009) Small molecule inhibitors in children with malignant gliomas. Pediatr Blood Cancer 53(3):312–317
45. Goumnerova LC (1996) Growth factor receptors and medulloblastoma. J Neurooncol 29(1):85–89
46. Goldbrunner RH et al (1999) Different vascular patterns of medulloblastoma and supratentorial primitive neuroectodermal tumors. Int J Dev Neurosci 17(5–6):593–599
47. Sundberg C et al (2001) Glomeruloid microvascular proliferation follows adenoviral vascular permeability factor/vascular endothelial growth factor-164 gene delivery. Am J Pathol 158(3):1145–1160

48. Jain S et al (2012) Incremental increase in VEGFR1(+) hematopoietic progenitor cells and VEGFR2(+) endothelial progenitor cells predicts relapse and lack of tumor response in breast cancer patients. Breast Cancer Res Treat 132(1):235–242
49. Scully S et al (2012) Transdifferentiation of glioblastoma stem-like cells into mural cells drives vasculogenic mimicry in glioblastomas. J Neurosci 32(37):12950–12960
50. Francescone R et al (2012) Glioblastoma-derived tumor cells induce vasculogenic mimicry through Flk-1 protein activation. J Biol Chem 287(29):24821–24831
51. El Hallani S et al (2010) A new alternative mechanism in glioblastoma vascularization: tubular vasculogenic mimicry. Brain 133(Pt 4):973–982
52. Nico B et al (2010) Intussusceptive microvascular growth in human glioma. Clin Exp Med 10(2):93–98
53. Moreno L, et al. (2012) Role of platelet derived growth factor receptor (PDGFR) over-expression and angiogenesis in ependymoma. J Neurooncol
54. Zavalhia LS et al (2012) Evaluation of the expression of C-kit (CD117) in ependymomas and oligodendrogliomas. Dis Markers 33(2):61–68
55. Yao J et al (2011) Expression of a functional VEGFR-1 in tumor cells is a major determinant of anti-PlGF antibodies efficacy. Proc Natl Acad Sci U S A 108(28):11590–11595
56. Lichtenberger BM et al (2010) Autocrine VEGF signaling synergizes with EGFR in tumor cells to promote epithelial cancer development. Cell 140(2):268–279