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Platelet-derived growth factor receptor expression and amplification in choroid plexus carcinomas

Nina N Nupponen[1,*], Janna Paulsson[2,*], Astrid Jeibmann[3], Brigitte Wrede[4], Minna Tanner[5], Johannes EA Wolff[6], Werner Paulus[3], Arne Östman[2] and Martin Hasselblatt[3]

[1]*Molecular Cancer Biology Program, University of Helsinki, Helsinki, Finland;* [2]*Department of Oncology–Pathology, Cancer Centrum Karolinska, Karolinska Institutet, Stockholm, Sweden;* [3]*Institute of Neuropathology, University Hospital Münster, Münster, Germany;* [4]*Department of Pediatric Oncology, University of Regensburg, Regensburg, Germany;* [5]*Department of Oncology, Tampere University Hospital, Tampere, Finland and* [6]*Children's Cancer Hospital, MD Anderson Cancer Center, Houston, TX, USA*

Platelet-derived growth factor (PDGF) receptor signaling has been implicated in the development of glial tumors, but not yet been examined in choroid plexus carcinomas, pediatric tumors with dismal prognosis for which novel treatment options would be desirable. Therefore, protein expression of PDGF receptors α and β as well as amplification status of the respective genes, *PDGFRA* and *PDGFRB*, were examined in a series of 22 patients harboring choroid plexus carcinoma using immunohistochemistry and chromogenic *in situ* hybridization (CISH). The majority of choroid plexus carcinomas expressed PDGF receptors with 6 cases (27%) displaying high staining scores for PDGF receptor α and 13 cases (59%) showing high staining scores for PDGF receptor β. Correspondingly, copy-number gains of *PDGFRA* were observed in 8 cases out of 12 cases available for CISH and 1 case displayed amplification (six or more signals per nucleus). The proportion of choroid plexus carcinomas with amplification of *PDGFRB* was even higher (5/12 cases). *PDGFRB* amplification status and PDGF receptor β protein expression scores were significantly correlated ($P = 0.01$, Spearman). Expression status of PDGF receptor α or PDGF receptor β was not significantly associated with progression-free survival. To conclude, expression and amplification of PDGF receptors, particularly PDGF receptor β, are frequent in choroid plexus carcinomas, providing a first rationale for the development of treatments targeting PDGF receptor signaling in these rare malignant pediatric tumors.
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Choroid plexus tumors are rare intraventricular neoplasms that account for only 0.4–0.6% of all intracranial tumors, but represent up to 13% of brain tumors that occur throughout the first year of life.[1] In contrast to benign choroid plexus papillomas, choroid plexus carcinomas have a dismal prognosis[2–4] and are characterized histologically by frank signs of malignancy, that is, brisk mitotic activity, nuclear pleomorphism, increased cellular density, blurring of the papillary growth pattern and necrosis.[1,2,4] Surgery remains the therapeutic mainstay,[3,5] but due to infiltrative growth and high vascularity of these tumors, gross total resection often cannot be achieved. Chemotherapy and cranial irradiation do improve survival,[6,7] but the latter is usually not an option in very young children. Therefore, development of novel therapeutic approaches would be desirable.

Platelet-derived growth factor (PDGF) receptors are expressed in the developing brain.[8,9] *PDGFRA* null mouse embryos display neural tube defects suggesting that PDGF receptor α signaling is crucial for nervous system development.[10] Interestingly, PDGF receptor α as well as the structurally related receptor subtype PDGF receptor β might play a role in the development of the choroid plexus in rodents. Here, PDGF receptor α has been found to be highly expressed in choroid plexus epithelial cells,[8,9]

Correspondence: Dr M Hasselblatt, MD, Institute of Neuropathology, University Hospital Münster, Domagkstrasse 19, Münster 48129, Germany.
E-mail: hasselblatt@uni-muenster.de
*These two authors have contributed equally to this work.
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whereas PDGF receptor β has been described to be expressed in the developing choroid plexus vasculature.[11]

Furthermore, PDGF receptor signaling has been shown to contribute to multiple tumor-associated processes as follows: PDGF receptors are involved in different aspects of tumor growth, including autocrine stimulation of malignant cells, angiogenesis and recruitment of tumor stroma[12,13] and can be inhibited by protein tyrosine kinase inhibitors such as imatinib mesylate (Glivec). Indeed, early clinical studies suggest efficacy of imatinib mesylate in combination with hydroxyurea for the treatment of gliomas.[14,15] Results from pilot trials employing imatinib mesylate for the treatment of children with solid tumors[16] as well as recurrent malignant gliomas[17] point toward a favorable safety profile of imatinib mesylate in that age group.

We therefore investigated protein expression of PDGF receptors α and β, as well as amplification status of the respective genes, *PDGFRA* and *PDGFRB*, in a large retrospective series of choroid plexus carcinomas.

Materials and methods

Patients

A total of 22 primary choroid plexus carcinomas were retrieved from the archives of the Institute of Neuropathology, University Hospital Münster. All cases were sporadic. Some of the children had been examined as part of the Société Internationale d' Oncologie Pédiatrique choroid plexus tumor study (CPT-SIOP-2000).[18] All cases were reevaluated neuropathologically according to the current WHO criteria[4] including immunohistochemistry for INI1[19,20] as well as choroid plexus-specific markers.[20] Data on tumor location, extent of surgical resection, adjuvant treatment and postoperative course were compiled by reviewing patient records. Moreover, general practitioners, pediatricians and neurosurgeons were contacted to provide follow-up information on recurrence and survival.

Immunohistochemistry

Sections were deparaffinized, rehydrated and then washed in distilled H_2O. For antigen retrieval, slides were microwaved for 2×7 min at 650 W in antigen retrieval buffer, high pH (S3307, Dako, Glostrup, Denmark), for PDGF receptor β, or in 1 mM EDTA pH 8 for PDGF receptor α. Sections were then left to cool for at least 30 min before being washed in PBS with 0.1% Tween 20 (PBT). Endogenous peroxidase activity was quenched by incubation in PBT containing 3% H_2O_2 for 10 min, then was washed in PBT for 3×5 min. Slides were blocked in 20% goat serum diluted in PBT for 30 min and then incubated with anti-PDGF receptor β (no. 3169 rabbit monoclonal, 2 μg/ml (Cell Signaling Technology, Danvers, MA, USA)) or anti-PDGF receptor α (no. 3164 rabbit polyclonal, 1:50 (Cell Signaling Technology)). After washes in PBT, slides were incubated with a biotinylated goat anti-rabbit secondary antibody (E0432, 1:500 (Dako)) for 45 min at room temperature following incubation with the ABC kit (SK6100, Vectastain ABC-HRP, Vector Laboratories, Burlingame, CA, USA) for 45 min after washing in PBT. The signal was developed using the DAB substrate kit, (SK4100, Vector Laboratories) and sections were counterstained with hematoxylin. PDGF receptor staining was evaluated semiquantitatively by scoring the percentage of stained cells (0 (absent), 1 ($<$10%), 2 (10–50%), 3 (51–80%), 4 (81–100%)) as well staining intensity (0 (absent), 1 (weak), 2 (distinct), 3 (strong)). Both scores were then multiplied to give a maximal staining score of 12.

Chromogenic *In Situ* Hybridization

Freshly cut paraffin-sections for chromogenic *in situ* hybridization (CISH) were available for 12 of the choroid plexus carcinomas. CISH methodology is described elsewhere in detail.[21] Briefly, digoxigenin-labeled (DIG-Nick mix Roche, Mannheim, Germany) BAC-probes were used for *PDGFRA* (a mixture of clones, IDs RP11-117E8 and RP11-626H4) and for *PDGFRB* (clone ID RP11-754J8, Invitrogen Ltd, Paisley, UK). Gene copy numbers were evaluated using a Leica DM2000 (Leica Microsystems GmbH, Germany) with $\times 63$ and $\times 100$ magnification. Gained gene copy number (low-level amplification) was defined as 3–5 signals per nucleus. Amplification was defined as six or more signals per nucleus.

Statistics

Comparison of patient characteristics was carried out by Fisher's exact test or Mann–Whitney *U*-test. Probabilities of survival were estimated using the Kaplan–Meier method. The effect of PDGF receptor expression status on the probability of survival was investigated on univariate analysis using the log-rank test. Correlations were tested using the Spearman's test. All statistical analyses were performed using SPSS version 13.0 (SPSS Inc., Chicago, IL, USA).

Results

Patients

The median age of the patients was 2 years (quartiles: 1–4 years). Even though the vast majority of tumors was located supratentorially (95%), gross total resection of the tumors could only be achieved in 50% of the children. Further patient characteristics have been compiled in Table 1.

Table 1 Patient characteristics

Age (median, quartiles)	2 (1–4) years
Sex (male:female)	11:11
Location	
Lateral ventricles	21
Third ventricle	0
Fourth ventricle	1
Treatment	
Gross total resection	11 (50%)
Radiotherapy	4 (18%)
Chemotherapy	11 (50%)

Age, sex, tumor location and treatment of the 22 choroid plexus carcinoma patients.

PDGF Receptor Protein Expression

The majority of choroid plexus carcinomas expressed PDGF receptor subtypes, particularly PDGF receptor β. As shown in Figure 1a, PDGF receptor α staining scores were high in 6 cases (27%); 3 cases displayed intermediate staining scores (5–8), whereas low staining scores (0–4) were observed in 13 cases (59%). The proportion of tumors displaying high staining scores for PDGF receptor β was higher: 13 cases (59%) showed high staining scores for PDGF receptor β, whereas 6 cases displayed intermediate staining scores (5–8) and only 3 cases (14%) displayed low staining scores (0–4; Figure 1b). Staining scores for both receptor subtypes were not correlated ($P = 0.56$); only three cases displayed high staining scores for both receptor subtypes.

PDGF Receptor Amplification

Gained gene copy numbers of *PDGFRA* were observed in 8 out of the 12 cases in which *PDGFRA* amplification status could be examined (Figure 1e). One single case displayed *PDGFRA* amplification. This supratentorial choroid plexus carcinoma in a 12-year-old boy also displayed a high staining score for PDGF receptor α. In two cases, CISH for *PDGFRA* yielded no results. Gained gene copy numbers or amplification of *PDGFRB* were encountered in five cases each (Figure 1f). *PDGFRB* amplification status and PDGF receptor β protein expression scores were significantly correlated ($P = 0.01$, Spearman). All cases with *PDGFRB* amplification displayed high PDGFR receptor β protein staining scores. Simultaneous amplification of both *PDGFRA* and *PDGFRB* was not observed.

PDGF Receptor Status and Prognosis

Prognosis was poor in the 20 patients for whom information on follow-up could be obtained: median recurrence-free survival accounted for only 22 months. On log-rank test, protein expression status of PDGF receptors α or β was not significantly associated with progression-free survival. Because the number of cases available for CISH was even smaller, the effect of amplification status on progression-free survival was not statistically evaluated.

Discussion

As PDGF receptor signaling is strongly linked to central nervous system and choroid plexus development and has been shown to contribute to multiple tumor-associated processes, we conducted analyses of protein expression status and gene copy number of PDGF receptors in choroid plexus carcinomas. To our knowledge, our series is among the largest published to date and the first to report protein expression of PDGF receptors α and β as well as copy number increases of *PDGFRA* or *PDGFRB* in the majority of choroid plexus carcinomas examined. In addition, *PDGRB* amplification significantly correlated to PDGF receptor β protein expression, suggesting that increased copy number may trigger abnormal protein overexpression, potentially stimulating malignant tumor growth.

PDGF receptors and their ligands have an established role in the development and progression of human gliomas: gene amplification and overexpression of *PDGFRA* are found in up to 33% of malignant gliomas[22,23] and might stimulate tumor growth by autocrine PDGF receptor signaling.[12,13] In a recent study on pediatric gliomas, *PDGFRA* was found to be amplified in 2 out of 14 malignant gliomas but not in any of the low-grade tumors examined.[24] Expression of PDGF receptors α and β has also been described in ependymomas[25] as well as primitive neuroectodermal tumors and medulloblastomas,[26] but gene amplification has not yet been described in these pediatric tumors. Interestingly, however, PDGF receptors have been shown to be preferentially expressed in medulloblastomas showing aggressive biological behavior with metastatic spread,[27,28] suggesting not only a role in the promotion of tumor growth but also prognostic significance of PDGF receptor expression. In the present retrospective series, the proportion of choroid plexus carcinomas expressing PDGF receptors was high and treatments employed were heterogeneous. Therefore, the absent prognostic value of PDGF receptor expression status is not too discouraging. Determination of PDGF receptor status will certainly be of value in future therapeutic trials assessing the efficiency of treatments targeting PDGF receptor signaling in choroid plexus carcinomas.

In contrast to myeloproliferative disease,[29] constitutive activating mutations of PDGF receptors seem to be rare events in central nervous system tumors.[30] Therefore, further functional studies on the role of PDGF receptor signaling in the tumorigenesis of choroid plexus carcinomas are clearly

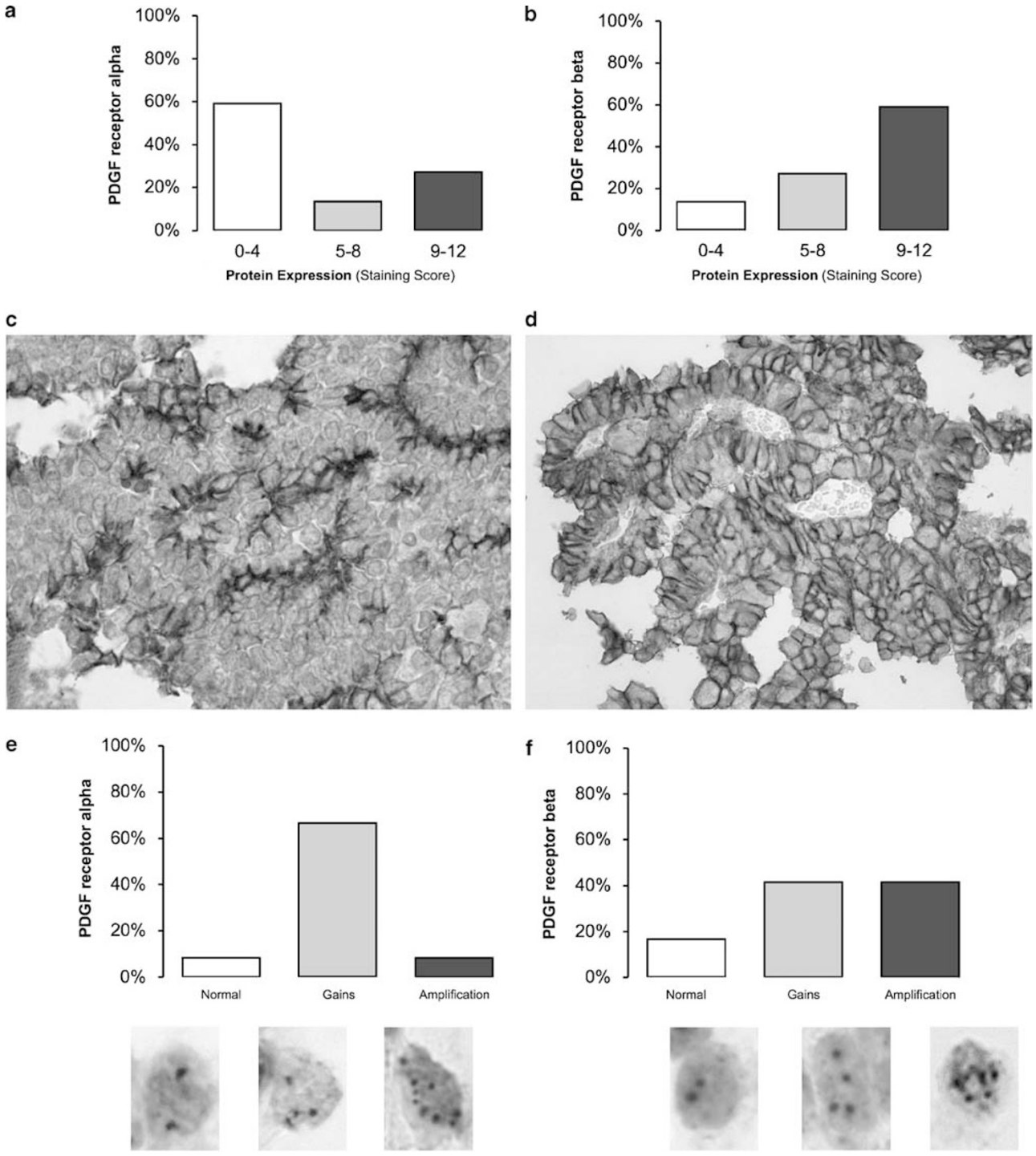

Figure 1 Platelet-derived growth factor (PDGF) receptor protein expression and amplification. Proportion of choroid plexus carcinomas displaying low (0–4), intermediate (5–8) or high (9–12) staining scores as well as representative images depicting high immunohistochemistry staining scores for PDGF receptor α (**a** and **c**) and PDGF receptor β (**b** and **d**) as well as proportion of tumors with copy number gains or amplification as well as representative CISH images for *PDGFRA* (**e**) and *PDGFRB* (**f**).

needed before definite conclusions on the functional role of PDGF receptors in the development of choroid plexus carcinoma can be drawn. However, such studies are hampered by the fact that besides immortalized rat choroid plexus epithelial cells,[31] true choroid plexus carcinoma cell lines have not yet been established.

To conclude, expression and amplification of PDGF receptors, particularly PDGF receptor β, is frequent in choroid plexus carcinomas providing a

first rationale for the development of treatments targeting PDGF receptor signaling in choroid plexus carcinomas.

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Disclosure/conflict of interest

The authors state no conflict of interest to declare.

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