Neurobiology

Platelet-Derived Growth Factor Receptor Expression and Activation in Choroid Plexus Tumors

Björn Koos,* Janna Paulsson,[†] Malin Jarvius,[‡]
Betzabe Chavez Sanchez,[†] Brigitte Wrede,[§]
Sonja Mertsch,* Astrid Jeibmann,* Anne Kruse,*
Ove Peters,[§] Johannes E.A. Wolff,[¶]
Hans-Joachim Galla,^{||} Ola Söderberg,[‡]
Werner Paulus,* Arne Östman,[†]
and Martin Hasselblatt*

From the Institute of Neuropathology,* University Hospital Münster, Münster, Germany; the Departments of Oncology-Pathology,[†] Cancer Center Karolinska, Karolinska Institutet, Stockholm, Sweden; the Department of Genetics and Pathology,[‡] Rudbeck Laboratory, University of Uppsala, Uppsala, Sweden; the Department of Pediatric Oncology,[§] University of Regensburg, Regensburg, Germany; the Children's Cancer Hospital,[¶] MD Anderson Cancer Center, Houston, Texas; and the Institute of Biochemistry,^{||} Westfälische Wilhelms Universität, Münster, Germany

Choroid plexus tumors are intraventricular neoplasms predominantly affecting young children. In contrast to choroid plexus papillomas, choroid plexus carcinomas progress frequently, necessitating the development of adjuvant treatment concepts. Platelet derived growth factor (PDGF) signaling has been shown to support growth in a variety of tumors. The finding of PDGF receptor expression in choroid plexus tumors prompted us to elucidate PDGF receptor activation state using a novel method, *in situ* proximity ligation assay, on formalin-fixed, paraffin-embedded, archival samples of 19 choroid plexus tumors. As assessed by *in situ* proximity ligation assay, the proportion of phosphorylated PDGF receptor α was low in choroid plexus papillomas and choroid plexus carcinomas, whereas phosphorylated PDGF receptor β was found to be significantly higher in choroid plexus carcinomas. In the immortalized choroid plexus epithelial cell line Z310 expressing PDGF receptor β , PDGF-BB exhibited a time- and dose-dependent proliferative response, which was significantly attenuated by imatinib (gleevec). In conclusion, our findings suggest that PDGF receptor β is functionally involved in the biology of choroid plexus tumors and may represent a molecular target for therapy. In addition,

this study demonstrates the feasibility and usefulness of *in situ* proximity ligation assay for monitoring receptor tyrosine kinase activation in formalin-fixed, paraffin-embedded, archival tissues. (Am J Pathol 2009; 175:1631–1637; DOI: 10.2353/ajpath.2009.081022)

Choroid plexus tumors are intraventricular neoplasms predominantly affecting very young children, usually under the age of 3 years.¹ In contrast to choroid plexus papillomas (CPP, World Health Organization grade I) and atypical choroid plexus papillomas (aCPP, World Health Organization grade II), choroid plexus carcinomas (CPC, World Health Organization grade III) are malignant neoplasms. Infiltrative growth and high vascularity often impede gross total resection.^{1,2} As radiation is not an option in very young children, the development of novel adjuvant treatment concepts complementing established protocols would be highly desirable.

Platelet derived growth factor (PDGF) has been shown to support growth, angiogenesis, and stroma recruitment in a variety of tumors.^{3,4} These actions are mediated by binding of PDGF isoforms to PDGF receptors α and β , which are members of the tyrosine kinase receptor family.⁵ PDGF receptor signaling can be blocked by imatinib (gleevec), a tyrosine kinase inhibitor showing high specificity for PDGF receptors, c-kit and c-abl⁶ and has been shown to be generally well tolerated in children.⁷ The observation that PDGF receptors α and β are expressed

Supported by Friedrich-Wilhelm Hauss-Lipperheide-Stiftung (M.H.). M.H. and W.P. have support from Deutsche Krebshilfe (Grant 108263). M.J. and O.S. have support from Wallenberg, EU-FP6 (Enlight) and from the Cancer Foundation. A.Ö. and J.P. received support from Swedish Cancer Society (Cancerfonden) and "Cancerföreningen."

B.K. and J.P. contributed equally to this work.

A.Ö. and M.H. contributed equally as supervisors of this work.

Accepted for publication July 9, 2009.

Conflicts of interest: J.P., M.H., W.P., and A.Ö. have received consulting fees and research support grants from Novartis, manufacturer of imatinib.

Supplemental material for this article can be found on <http://ajp.amjpathol.org>.

Address reprint requests to Martin Hasselblatt, M.D., Institute of Neuropathology, University Hospital Münster, Domagkstr. 19, 48129 Münster, Germany. E-mail: hasselblatt@uni-muenster.de.

in choroid plexus carcinomas,⁸ prompted us to examine PDGF receptor activation in choroid plexus tumors as well as its functional consequences. *In situ* proximity ligation assay (*in situ* PLA) is a new sensitive and specific method that has been successfully used for the visualization of PDGF β receptor phosphorylation status in cells and fresh-frozen tissues.⁹

For the purpose of the present study, *in situ* PLA was for the first time also applied on formalin-fixed, paraffin-embedded, archival tissues and successfully used for the detection of phosphorylated PDGF receptors α and β in choroid plexus tumors.

Materials and Methods

Patients and Tumor Material

Paraffin-embedded, formalin-fixed, surgical samples from eight choroid plexus papillomas, four atypical choroid plexus papillomas, and seven choroid plexus carcinomas of 19 children that had been treated according to the Société Internationale d' Oncologie Pédiatrique choroid plexus tumor study (CPT-SIOP-2000) were examined after parental consent for scientific use of archival samples had been obtained. The tumors of the five girls and 14 boys (median age: 1 year) were diagnosed in accordance to criteria of the World Health Organization classification.^{1,10} Furthermore, immunohistochemistry for choroid plexus specific marker Kir7.1¹¹ as well as INI1¹² was performed routinely. The study included only cases that manifested positive immunoreactivity for the choroid plexus-specific marker Kir7.1¹¹ and retention of nuclear immunostaining for INI1 protein.¹² The latter immunostaining was conducted to exclude a histologically similar tumor type, atypical teratoid/rhabdoid tumor.

Immunohistochemistry

Sections were de-paraffinized, rehydrated, and then washed in distilled H₂O. On boiling for antigen retrieval in citrate buffer pH7, sections were incubated with anti-PDGF receptor β [#3169 rabbit monoclonal, 2 μ g/ml (Cell Signaling Technology, Danvers, MA)] or anti-PDGF receptor α [#3164 rabbit polyclonal, 1:50 (Cell Signaling Technology)]. After washes in PBS-Tween 20 (0.1%), slides were incubated with a biotinylated goat anti-rabbit secondary antibody [E0432, 1:500 (Dako, Glostrup, Denmark)] following incubation with the ABC kit (SK6100, Vectastain ABC-HRP, Vector Laboratories, Burlingame, CA). The signal was developed using diaminobenzidine as a substrate (SK4100, Vector Laboratories) and counterstained with hematoxylin. PDGF receptor staining was evaluated semiquantitatively as described previously⁸ by scoring the percentage of stained cells [0 (absent), 1 (<10%), 2 (10 to 50%), 3 (51 to 80%), 4 (81 to 100%)], as well as staining intensity [0 (absent), 1 (weak), 2 (distinct), 3 (strong)] across the whole sections. Both scores were then multiplied to give a maximal staining score of twelve.

Immunoblotting

For immunoblotting analyses, cells were lysed in ice cold lysis buffer (0.5% Triton X-100, 0.5% deoxycholate, 150 mmol/L NaCl, 20 mmol/L Tris pH 7.5, 10 mmol/L EDTA, 30 mmol/L sodium pyrophosphate pH 7.5) supplemented with 200 μ mol/L Na₃VO₄ and 2 μ g/ml Aprotinin. Insulin-like growth factor (IGF)-1R β and pIGF-1R β were analyzed following immunoprecipitation (1:200, #3027, Cell signaling technology, Danvers, MA). PDGF β R and pPDGF β R were analyzed on total lysates. Proteins were separated on an 8% SDS-polyacrylamide gel and transferred to a polyvinylidene difluoride membrane with semi-dry transfer. The membrane was blocked using 5% bovine serum albumin in Tris-buffered saline before incubated over night with antiphospho-tyrosine antibodies (pY100, #9411, Cell signaling technology) then stripped and reprobed with anti-PDGF β R (#3169, Cell signaling technology) or anti-IGF-1R β (sc-713, Santa Cruz biotechnology, Santa Cruz, CA). Bound antibodies were visualized using enhanced chemiluminescence (ECL, GE Health Care, Uppsala, Sweden) after incubation with horseradish peroxidase conjugated secondary antibodies, and signal captured with a CCD Camera (Fuji).

In Situ PLA

For the detection of receptor phosphorylation, two primary antibodies from different species directed against a receptor domain and a phosphorylated site, respectively, are bound by secondary antibodies, which have been modified by the attachment of DNA strands. Dual recognition of receptor and phosphorylated sites by the primary antibodies in combination with the secondary proximity probes allows for generation of circular DNA strands on ligation of two circularization oligonucleotides.⁹ In brief, freshly cut 2- μ m sections from formalin-fixed, paraffin-embedded, samples were de-paraffinized, rehydrated, and then washed in distilled H₂O. On antigen retrieval (#S3307, Dako, Glostrup, Denmark), slides were incubated with antibodies directed against PDGF receptor α (0.5 ng/ μ l, #3164, Cell Signaling Technology, Danvers, MA) or PDGF receptor β (2 ng/ μ l, #3169, Cell Signaling Technology) in pair with a total phospho-tyrosine antibody (0.9 ng/ μ l, #9411, Cell Signaling Technology). After removal of unbound primary antibodies, proximity probes (donkey anti-mouse, 5 ng/ μ l and donkey anti-rabbit, 2 ng/ μ l, Olink Bioscience, Uppsala, Sweden) were prepared and administered to the tissue. Hybridization of circularization oligonucleotides and ligation of circularization probes was done before rolling circle amplification. Further characterization of the *in situ* PLA included analyses of formalin-fixed paraffin-embedded porcine aortic endothelial cells stably transfected with PDGF receptor α or β , which were stimulated with different concentrations of PDGF (see Supplemental Figures S1 and S2 at <http://ajp.amjpathol.org>).¹³ Other control experiments demonstrated that unspecific activation of tyrosine phosphorylation with pervanadate did not increase the signal (Supplemental Figure S1B at <http://ajp.amjpathol.org>).¹³ Also, activation of

IGF-1 receptors did not increase the PDGF receptor *in situ* PLA signal, as determined by analyses of ethanol-fixed cells (Supplemental Figure S1C at <http://ajp.amjpathol.org>).¹³ Finally, analyses of serial tissue sections revealed that PDGF receptor *in situ* PLA signals were derived from PDGF receptor-positive cells (see Supplemental Figure S3 at <http://ajp.amjpathol.org>).

On hybridization of fluorescence-labeled probes, slides were counterstained with Hoechst 33342 (Sigma-Aldrich, Stockholm, Sweden). Image analysis was conducted as described previously.⁹ Briefly, signals were counted semi-automatically using the freely distributed software BlobFinder, which has been developed by the Centre for Image Analysis at Uppsala University (<http://www.cb.uu.se/~amin/BlobFinder/index.htm>).¹⁴ The BlobFinder program converts the z-stack images to a maximum intensity projection and uses a threshold of intensity as well as size and shape of the objects, to identify the rolling circle products to be counted. Hoechst stained nuclei were counted automatically by the same software. The software also allows for automatically definition of cell borders at a specified distance from the nuclei.¹⁴ However, the exact cell shape and size is not required,

since average signals per nucleus were examined. As automated delineation of nuclei did not always give satisfying results, for example if the software counted two overlapping cell nuclei as one, some had to be corrected manually while being blinded to histopathological diagnosis.

Cell Culture

The SV40-immortalized rat choroid plexus epithelial cell line Z310¹⁵ was kindly provided by Dr. Wei Zheng (Purdue University School of Health Sciences). Z310 cells were grown in Dulbecco's modified Eagle's minimal essential medium, supplemented with 10% fetal calf serum, 100 U/ml penicillin, 100 μ g/ml streptomycin, gentamicin (40 μ g/ml) and 1% epidermal growth factor (10 ng/ml) at 37°C in 5% CO₂. All cell culture reagents were purchased from PAA (Linz, Austria).

For analyses of dose-dependent activation of PDGF receptors, cells (PAE/PDGFR α R and PAE/PDGFR β R) were starved in medium containing 1% fetal calf serum overnight and then incubated with or without PDGF-BB (1, 10, 30 ng/ml) for 5 minutes at 37°C.

Figure 1. Immunohistochemistry. Expression of PDGF receptor α (A,C,E) and β (B,D,F) in choroid plexus papilloma (CPP, World Health Organization grade I; A,B), atypical choroid plexus papilloma (aCPP, World Health Organization grade II; C,D) and choroid plexus carcinoma (CPC, World Health Organization grade III; E,F). Quantification of protein expression shows no significant difference in mean expression scores according to tumor grade for PDGF receptor α (G), but significantly lower expression scores in choroid plexus carcinomas for PDGF receptor β (H). * P < 0.05. Note differences in staining intensity for PDGF receptor β between panels B (CPP), D (aCPP), and F (CPC).

Proliferation

Cellular growth was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Briefly, cells were seeded at a concentration of 1×10^4 cells per well in a 96-well plate and incubated in the presence of varying concentrations of PDGF-BB (Pepro-Tech GmbH, Hamburg, Germany) and/or imatinib-mesylate (kindly provided by Novartis, Basel, Switzerland). For each plate, additional controls (exponential dilution series of cells with 1×10^5 cells per well as a starting point) were used to ensure that cells used for experiments were in the exponential growth phase. For quantification of mitochondrial activity, incubation medium was replaced with 200 μ l MTT solution (0.5 mg/ml) and incubated for 3 hours at 37°C and 5% CO₂. MTT solution was then discarded and 200 μ l of propan-2-ol was added to lyse the cells and dissolve the released formazan crystals. The absorption was measured at 570 nm using an enzyme-linked immunosorbent assay reader. Experiments were independently performed in triplicates and for each condition a number of 12 wells were used.

Statistics

Studies that involved multiple comparisons were evaluated by Kruskal-Wallis test followed by Mann-Whitney *U*-test or analysis of variance followed by LSD-test. Statistical analyses were performed using SPSS version 15.0 (SPSS Inc., Chicago, IL).

Results

PDGF Receptor Subtypes α and β Are Both Expressed in Choroid Plexus Papillomas, Atypical Choroid Papillomas and Choroid Plexus Carcinomas

As shown in Figure 1A–H, PDGF receptor subtypes α and β were highly expressed in the majority of choroid plexus tumors. Only one out of 19 tumors showed negative staining for PDGF receptor α , whereas all samples showed positive immunoreactivity for PDGF receptor β . While in choroid

Figure 2. In situ proximity ligation assay (*in situ* PLA). Representative images of phosphorylated PDGF receptor α (A,C,E) and β (B,D,F) (red dots) in choroid plexus papilloma (CPP, World Health Organization grade I; A,B), atypical choroid plexus papilloma (aCPP, World Health Organization grade II; C,D) and choroid plexus carcinoma (CPC, World Health Organization grade III; E,F), respectively. Quantification of PDGF receptor phosphorylation shows no significant difference in mean scores according to tumor grade for PDGF receptor α (G), but significantly increased scores in choroid plexus carcinomas for PDGF receptor β (H). **P* < 0.05.

plexus papillomas and atypical choroid plexus papillomas membranous staining for both receptor subtypes was fairly homogeneous, in choroid plexus carcinomas a more heterogeneous staining pattern was observed. PDGF receptor α expression scores accounted for 9.3 ± 2.4 (mean $\pm$ SD) in choroid plexus papillomas and 9.0 ± 6.0 in atypical choroid plexus papillomas, but tended to be lower in choroid plexus carcinomas (6.3 ± 2.6 ; $P = 0.18$). With regards to PDGF receptor β , expression scores accounted for 8.8 ± 2.8 in choroid plexus papillomas and 10.5 ± 3.0 in atypical choroid plexus papillomas, whereas PDGF receptor β expression scores were significantly lower in choroid plexus carcinomas (3.6 ± 2.6 ; $P < 0.05$).

Choroid Plexus Carcinomas Show Significantly Increased PDGF Receptor β Phosphorylation as Compared with Choroid Plexus Papillomas

To investigate the extent of activated PDGF receptors, *in situ* PLA was used. Before applying this procedure to formalin-fixed, paraffin-embedded tissues a set of control experiments were performed on formalin-fixed, paraffin-embedded cells with known PDGF receptor phosphorylation status. These demonstrated specific and dose-dependent detection of phosphorylated PDGF receptor α and β (see Supplemental Figures S1 and S2 at <http://ajp.amjpathol.org>).¹³ Furthermore, immunofluorescence analyses and *in situ* PLA for PDGF receptor α and β was performed on serial sections confirming that *in situ* PLA signals were only detected in cells expressing the corresponding receptors (see Supplemental Figure S3 at <http://ajp.amjpathol.org>).

As shown in Figure 2, A–H, the amount of phosphorylated PDGF receptor α (signals/nucleus) was low in choroid plexus papillomas (6.6 ± 7.9 , mean $\pm$ SD), atypical choroid plexus papillomas (1.6 ± 2.1) and choroid plexus carcinomas (3.0 ± 2.4), not differing significantly. In contrast, the amount of phosphorylated PDGF receptor β was significantly higher in choroid plexus carcinomas (World Health Organization grade III) as compared with choroid plexus papillomas (World Health Organization grade I) 9.7 ± 11.1 vs. 2.0 ± 2.5 , $P = 0.049$).

PDGF Promotes Proliferation in Immortalized Choroid Plexus Epithelial Cells

To evaluate the effect of PDGF receptor activation on proliferation, the immortalized choroid plexus epithelial cell line Z310 was used. As shown in Figure 3A, Z310 cells showed distinct membranous expression of PDGF receptor β in the majority of cells, whereas PDGF receptor α immunoreactivity was absent (data not shown). On incubation with PDGF-BB, Z310 cells exhibited a time and dose dependent proliferative response, reaching a maximum increase of 185% as compared with control on 27 hours in the presence of 20 ng/ml (Figure 3B). Absorption was 0.807 ± 0.031 (mean $\pm$ SD) as compared with 0.437 ± 0.050 in controls ($P < 0.001$). As shown in Figure 3C, this effect could be attenuated

Figure 3. Cell culture experiments: Immunohistochemistry for PDGF receptor β in Z310 immortalized rat choroid plexus epithelial cells (A), dose-dependent response of Z310 cells on incubation with PDGF-BB for 4 hours and 27 hours (B) and dose-dependent inhibition of the PDGF-induced proliferative response by imatinib (C). *** $P < 0.001$ in comparison with control (B) or PDGF 20 ng/ml (C).

dose-dependently by imatinib. At a concentration of 3 μ mol/L, imatinib significantly reduced the PDGF-BB induced proliferative response (0.776 ± 0.037 vs. 0.904 ± 0.044 ; $P < 0.001$).

Discussion

Extending previous observations,⁸ the majority of choroid plexus tumors showed protein expression of PDGF re-

ceptor subtypes α and β . Whereas PDGF receptor subtypes α and β were both expressed in choroid plexus papillomas, atypical choroid plexus papillomas, and choroid plexus carcinomas, increased receptor phosphorylation was restricted to PDGF receptor β and only observed in choroid plexus carcinomas, suggesting a role of PDGF receptor β activation in the biology of choroid plexus carcinomas.

As compared with previously published data,⁸ mean PDGF receptor β protein expression scores in choroid plexus carcinomas of the current series were lower. This finding could be explained by the intertumoral heterogeneity of choroid plexus carcinomas. The fact that PDGF receptor β protein expression scores were lower in choroid plexus carcinomas as compared with choroid plexus papillomas and atypical choroid plexus papillomas, however, could well be related to down-regulation and/or receptor internalization following receptor activation in choroid plexus carcinomas.

While in unfixed, fresh-frozen tissues, receptor phosphorylation can be conveniently determined using Western blot and phospho-antibodies, methods for the examination of receptor phosphorylation in formalin-fixed, paraffin-embedded archival tissues have thus far been unreliable. *In situ* PLA has previously been used for the detection of phosphorylated PDGF receptor β in cultured cells and fresh frozen tissues.⁹ Even though fresh-frozen samples were not available to perform side-by-side comparisons, we here show that PDGF receptor *in situ* PLA can be successfully used on formalin-fixed, paraffin-embedded archival tissues, suggesting that this method will be a valuable tool for the examination of phosphorylation status of PDGF receptors in archival samples of other tumors, for which a role of PDGF receptor signaling has been suggested, such as malignant gliomas,¹⁶ ependyomas, and medulloblastomas.¹⁷

As *in situ* PLA is performed on fixed material, only a fraction of epitopes is available for antibody binding. In addition to multiple binding events (primary antibodies, PLA-probes, and circularization oligonucleotides), the subsequent enzymatic steps will also not be 100% efficient, adding up to only a fraction of the targeted interactions or modifications giving detectable signals. Thus this method does not allow for absolute quantification, but rather gives a relative estimate of the number of molecules of interest.

Nevertheless, the ability for localized detection of endogenous protein interactions and modifications by *in situ* PLA enables studies in clinical specimens and makes it a possible tool for future diagnostics. It is obvious that the *in situ* PLA method could also be adopted for analyses of the activation of other tyrosine kinase receptors in archival samples. Furthermore, it can be envisioned that the method could be adopted for *in situ* monitoring of other signaling-relevant post-translational modifications such as acetylation, ubiquitination, or sumoylation.

PDGF receptor β , found to be activated in choroid plexus carcinomas was also expressed in the immortalized choroid plexus epithelial cell line Z310, making these cells a suitable model to study the functional effects of PDGF receptor activation in neoplastic choroid plexus

tumor cells. Here, PDGF-BB induced a dose- and time-dependent proliferative response, suggesting that auto-crine or paracrine PDGF receptor β signaling might well contribute to the excessive proliferative activity observed in choroid plexus carcinomas. Since proliferation could be blocked by the tyrosine kinase inhibitor imatinib in concentrations that have been reported to be in a physiologically acceptable range,¹⁸ imatinib might potentially have a role in the treatment of choroid plexus carcinomas. It would have been interesting to perform survival analyses to assess the potential prognostic impact of PDGF receptor activation in choroid plexus tumors. Because of the relatively short follow-up in the ongoing CPT-SIOP study, such analyses were not yet deemed feasible.

In conclusion, the present findings demonstrate the feasibility and usefulness of *in situ* PLA for tissue analyses and suggest an involvement of PDGF receptor β signaling in choroid plexus carcinomas that might eventually be possible to exploit for therapeutic purposes.

References

1. Paulus W, Brander S: Choroid plexus tumours. In: WHO Classification of central Nervous System Tumours. Edited by Louis D, Ohgaki H, Wiestler O, Cavenee WK. Lyon, IARC Press, 2007, pp. 82–85
2. Jeibmann A, Hasselblatt M, Gerss J, Wrede B, Egensperger R, Beschoner R, Hans VH, Rickert CH, Wolff JE, Paulus W: Prognostic implications of atypical histologic features in choroid plexus papilloma. *J Neuropathol Exp Neurol* 2006, 65:1069–1073
3. Östman A, Heldin CH: PDGF receptors as targets in tumor treatment. *Adv Cancer Res* 2007, 97:247–274
4. Andrae J, Gallini R, Betsholtz C: Role of platelet-derived growth factors in physiology and medicine. *Genes Dev* 2008, 22:1276–1312
5. Fredriksson L, Li H, Eriksson U: The PDGF family: four gene products form five dimeric isoforms. *Cytokine Growth Factor Rev* 2004, 15:197–204
6. Capdeville R, Buchdunger E, Zimmermann J, Matter A: Glivec (STI571, imatinib), a rationally developed, targeted anticancer drug. *Nat Rev Drug Discov* 2002, 1:493–502
7. Champagne MA, Capdeville R, Krailo M, Qu W, Peng B, Rosamilia M, Therrien M, Zoellner U, Blaney SM, Bernstein M: Imatinib mesylate (STI571) for treatment of children with Philadelphia chromosome-positive leukemia: results from a Children's Oncology Group phase 1 study. *Blood* 2004, 104:2655–2660
8. Nupponen NN, Paulsson J, Jeibmann A, Wrede B, Tanner M, Wolff JE, Paulus W, Östman A, Hasselblatt M: Platelet-derived growth factor receptor expression and amplification in choroid plexus carcinomas. *Mod Pathol* 2008, 21:265–270
9. Jarvius M, Paulsson J, Weibrecht I, Leuchowius KJ, Andersson AC, Wahlby C, Gullberg M, Botling J, Sjöblom T, Markova B, Östman A, Landegren U, Söderberg O: In situ detection of phosphorylated platelet-derived growth factor receptor beta using a generalized proximity ligation method. *Mol Cell Proteomics* 2007, 6:1500–1509
10. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK, Burger PC, Jouvet A, Scheithauer BW, Kleihues P: The 2007 WHO classification of tumours of the central nervous system. *Acta Neuropathol* 2007, 114:97–109
11. Hasselblatt M, Böhm C, Tatenhorst L, Dinh V, Newrzella D, Keyvani K, Jeibmann A, Buerger H, Rickert CH, Paulus W: Identification of novel diagnostic markers for choroid plexus tumors: a microarray-based approach. *Am J Surg Pathol* 2006, 30:66–74
12. Judkins AR, Burger PC, Hamilton RL, Kleinschmidt-DeMasters B, Perry A, Pomeroy SL, Rosenblum MK, Yachnis AT, Zhou H, Rorke LB, Biegel JA: INI1 protein expression distinguishes atypical teratoid/rhabdoid tumor from choroid plexus carcinoma. *J Neuropathol Exp Neurol* 2005, 64:391–397
13. Eriksson A, Siegbahn A, Westermarck B, Heldin CH, Claesson-Welsh

- L: PDGF alpha- and beta-receptors activate unique and common signal transduction pathways. *EMBO J* 1992, 11:543–550
14. Allalou A, Wahlby C: BlobFinder, a tool for fluorescence microscopy image cytometry. *Comput Methods Programs Biomed* 2009, 94:58–65
 15. Zheng W, Zhao Q: Establishment and characterization of an immortalized Z310 choroidal epithelial cell line from murine choroid plexus. *Brain Res* 2002, 958:371–380
 16. Haberler C, Gelpi E, Marosi C, Rössler K, Birner P, Budka H, Hainfellner JA: 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* 2006, 76:105–109
 17. MacDonald TJ, Brown KM, LaFleur B, Peterson K, Lawlor C, Chen Y, Packer RJ, Cogen P, Stephan DA: Expression profiling of medulloblastoma: pDGFRα and the RAS/MAPK pathway as therapeutic targets for metastatic disease. *Nat Genet* 2001, 29:143–152
 18. Zhang P, Gao WY, Turner S, Ducatman BS: Gleevec (STI-571) inhibits lung cancer cell growth (A549) and potentiates the cisplatin effect in vitro. *Mol Cancer* 2003, 2:1