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LAB. INVESTIGATION-HUMAN/ANIMAL TISSUE

Pleiotrophin expression in astrocytic and oligodendroglial tumors and it's correlation with histological diagnosis, microvascular density, cellular proliferation and overall survival

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Abstract
Background Pleiotrophin (PTN) is a secreted cytokine with several properties related with tumor development, including differentiation, angiogenesis, invasion, apoptosis and metastasis. There is evidence that PTN has also a relevant role in primary brain neoplasms and its inactivation could be important to treatment response. Astrocytic and oligodendroglial tumors are the most frequent primary brain neoplasms. Astrocytic tumors are classified as pilocytic astrocytoma (PA), diffuse astrocytoma (DA), anaplastic astrocytoma (AA) and glioblastoma (GBM). Oligodendroglial tumors are classified as oligodendroglioma (O) and anaplastic oligodendroglioma (AO). The aim of the present study was to compare PTN expression, in astrocytomas and oligodendrogliomas and its association with the histological diagnosis, microvascular density, proliferate potential and clinical outcome.
Methods Seventy-eight central nervous system tumors were analyzed. The histological diagnosis in accordance with WHO classification was: 13PA, 18DA, 8AA, 15GBM, 16O and 8AO. Immunohistochemistry was realized with these specific antibodies: pleiotrophin, CD31 to microvascular density and Ki-67 to cell proliferation.
Results PTN expression was significantly higher in GBM and AA when compared to PA and higher in GBM compared to DA. PTN expression did not differ between O and AO. Proliferate index and microvascular density were evaluated only in high grade tumors (AA, GBM and AO)

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divided in three groups according to PTN expression (low, intermediate and high). These results showed no statistical difference between PTN expression and index of cellular proliferation and neither to PTN expression and microvascular density. Overall survival (OS) analysis (months) showed similar results in high grade gliomas with different levels of PTN expression.

Conclusions Our results suggest that PTN expression is associated with histopathological grade of astrocytomas. Proliferation rate, microvascular density and overall survival do not seem to be associated with PTN expression.

Keywords Pleiotrophin · Glioma · Microvascular density · Cellular proliferation · Overall survival · Immunohistochemistry · Microarray

Introduction

Pleiotrophin (PTN), a heparin-binding growth factor also known as heparin-binding growth-associated molecule (HB-GAM) or heparin affin regulatory peptide (HARP), is a 136 amino acid secreted cytokine related to diverse biological properties, including differentiation of glial progenitor cells, neuritis out-growth, angiogenesis, expansion and metastasis of tumor cells [1–4]. PTN has been reported to interact with the receptor-like protein tyrosine phosphatase zeta/RPTPζ, N-syndecan and the anaplastic lymphoma kinase (ALK) [5]. Several tyrosine kinase receptors and their ligands have been reported to be over expressed in high grade gliomas, suggesting a potential role for an autocrine signaling in these pathways [6]. The expression of PTN is negatively regulated by the tumor suppressor gene PTEN 10, and loss of function of PTEN is an important step in the genetic pathway of glioma progression [7].

PTN is differentially expressed in the neuroepithelium during embryonic and fetal development, but is rarely seen in adult tissues. In early stages of embryogenesis, PTN can be found in the subependymal region and, in the perinatal stage, it can be seen in cells with neuronal and glial origins [2, 8].

Using new proteomic strategies, PTN was reported in conditioned medium of neural stem cells and mRNA which transcripts PTN and its receptors were detected in neurospheres, suggesting that PTN is involved with the differentiation and fate of neural stem cells [9, 10].

Interestingly, elevated PTN expression has been found under pathological conditions, including malignant tumors such as ovarian carcinoma, breast carcinoma, melanocytic tumor, lung cancer, cervical cancer and astrocytomas [8, 11–16].

For lung and gastrointestinal tract cancer patients, high serum levels of PTN may be an indicator of disease and also can be used in monitoring the efficacy of therapy [6, 17]. Study with melanocytic tumors showed significant over expression of PTN in melanocytic cells and an interesting association of its expression with metastatic potential and prognosis of these malignances [16].

Few studies have shown over expression of PTN or its ligands in astrocytomas, and they point PTN as a proto-oncogene [13, 14, 18, 19]. Its activity has been related to chemotactic and proliferate effect on microglial cells, cell migration, tumor growth, and apoptosis.

Recently, a new gene therapy approach targeting PTN showed promising results reducing growth factor production in tumors and significantly inhibiting the growth of previous established tumors [20].

Primary brain tumors comprise a wide range of pathological entities, they are classified according to morphology and their presumed original cell. They are basic divided in gliomas, neuronal, embrionary and meningothelial tumors. The most frequent types of gliomas presented are astrocytomas and oligodendrogliomas in different grades of malignancy. Pilocytic Astrocytoma (PA), Diffuse Astrocytoma (DA), Anaplastic Astrocytoma (AA), and Glioblastoma (GBM) are astrocytic tumors classified and graded from I to IV respectively, according to the clinical and pathological criteria of the World Heath Organization (WHO) classification of Nervous System Tumors. Oligodendroglial tumors are classified by WHO as Oligodendroglioma (O), a slow growing grade II tumor and Anaplastic Oligodendroglioma (AO), a grade III tumor that may develop histological features commonly seen in GBM [21].

In order to elucidate the biological function of PTN in astrocytomas and oligodendrogliomas, we compared patterns of PTN expression by immunohistochemistry with histological diagnosis, microvascular density, proliferate index and overall survival of these patients.

Material and methods

The study was approved by our ethical committee.

For microarray investigation, 12 frozen samples of tumors were analyzed. The histological diagnosis in accordance with WHO classification were: 3 Pilocytic Astrocytomas (PA), 3 Diffuse Astrocytomas, and 6 Glioblastomas (GBM). As control were used non-neoplastic tissues comprised by a pool of three samples of white matter from patients subjected to epilepsy surgery refractory to medical treatment.

For immunohistochemistry analysis, 78 central nervous system tumors collected from three university hospitals were analyzed. The histological diagnosis, in accordance with WHO classification were: 13 Pilocytic Astrocytomas

(PA) (WHO grade I), 18 Diffuse Astrocytomas (DA) (WHO grade II), 8 Anaplastic Astrocytomas (AA) (WHO grade III), 15 Glioblastomas (GBM) (WHO grade IV), 16 Oligodendrogliomas (O) (WHO grade II), and 8 Anaplastic Oligodendrogliomas (AO) (WHO grade III).

Patients with PA, DA, and O were submitted to surgery and clinical follow up. Patients with high-grade tumors (GBM, AA and OA) were submitted to surgery, radiation therapy (60Gy) and chemotherapy with carmustine (BCNU) for GBM and PCV (procarbazine, CCNU, and vincristine) for AA and OA. Clinical charts were reviewed to establish the overall survival considering time between diagnosis date and last appointment or death date, until the last follow-up in March 2004.

Microarray

Gene expression profiling was conducted in frozen tumor samples and control.

After RNA extraction, cDNA preparation and hybridization, the analyses were carried out with oligonucleotide micro arrays representing 10,000 human genes (Code Link Bioarrays-Human Uniset I, GE Healthcare, Piscataway, NJ), following the manufacturers protocol.

Independent hybridizations for each tumor sample were carried out in duplicate. The fluorescent images were captured using Gene Pix Pro v.4.1 (Axon Instruments Inc, Sunnyvale, CA) and the light intensities were quantified, corrected for background level and normalized with the Code Link Software v.2.3 (GE Healthcare, Piscataway, NJ).

Differentially expressed genes were identified by calculating the ratio of the mean normalized fluorescence values obtained from each duplicate sample of tumor and non-neoplastic tissue.

Immunohistochemistry

Briefly, after deparafinization and rehydration in graded alcohol, 4-μm-thick sections were incubated at room temperature for 30 min in 99.7% methanol containing 0.3% hydrogen peroxide to inhibit endogenous peroxidase. Slides were washed with Tris-buffered saline solution and blocked in diluted normal goat serum. Antigen epitopes were retrieved by wet heat.

Polyclonal antibodies against PTN, CD-31, and Ki-67 were used as primary antibodies, which dilutions and antigen retrieval buffers are shown in Table 1.

Tissue slides were incubated with primary antibodies overnight, at room temperature and staining was performed using the avidin-biotin peroxidase method. The slides were developed in ABC chromogen, counterstained with hematoxylin, dehydrated, cover slipped and examined under a light microscope.

PTN staining intensity was evaluated in all tumors and considered using an arbitrary five-tiered scale (0, +, ++, +++ and ++++) in all tumors.

Microvascular density (CD-31) and proliferate index (Ki-67) were analyzed only in high-grade tumors (GBM, AA, and OA). Microvascular density was analyzed considering the number of vessels or stained cells in 0.24 mm^2 using 400× magnification and proliferate index was the percentage of positive cells considering 1,000 cells using 400× magnifications in hot spots areas. To analyze the relationship between PTN expression, microvascular density and proliferate index, the high grade tumors (GBM, AA and OA) were divided in three groups according to PTN staining: PTN low expression (0 or +), PTN intermediate expression (++) and PTN high expression (+++ or ++++).

Statistical analysis

Kruskal–Wallis test and Dunn's Multiple Comparison Test were conducted to assess PTN staining intensity, microvascular density and proliferate index. Survival curve was analyzed by Kaplan-Meyer method. Significance of PTN differential expression in the DNA microarray experiments was determined by Student *T*-test.

Results

Microarray

Previous microarray analysis (data not shown) with astrocytomas (PA, DA and GBM) and control non-neoplastic tissue indicated that the pleiotrophin gene is hyper-expressed in astrocytomas, mainly in GBM (Fig. 1A). The normalized microarray expression data for pleiotrophin was 1.9 ± 0.7 fold higher in PA ($P < 0.05$), 1.3 ± 0.5 fold

Table 1 Primary antibodies dilution, antigen retrieval methods, and characteristics

Antibody	Dilution	Antigen retrieval	Buffer	Time (min)	Clone	Company
Ki-67	1:200	Wet heat	Tri-sodium citrate 10 mM (pH 6.0)	40	NCL-Ki67-MM1	Novo Castra
CD31	1:75	Wet heat	Tris-EDTA 0.1 M (pH 8.0)	40	JC/70A	Dako
Pleiotrophin	1:80	Wet heat	Tri-sodium citrate 10 mM (pH 7.4)	20	A5-252-PB	RD systems

higher in A ($P = 0.44$) and 2.85 ± 1.2 fold higher in GBM ($P < 0.005$), compared to controls.

Immunohistochemistry

The semi-quantitative staining intensity results of PTN for different malignant grade gliomas are shown in Fig. 1B.

For PA, all but one tumor were PTN negative, the unique case of PA showed weak PTN staining (Figs. 1B, 2F), for DA the staining varied from 0 to ++, for AA varied from 0 to +++ (Figs. 1B, 2D, 2E) and only in GBM the higher intensity (++++) was found (Figs. 1B, 2C).

The staining was significantly higher in GBM and AA when compared to PA ($P < 0.05$). There was also a significantly greater PTN expression in GBM compared to DA ($P < 0.05$) (Fig. 1B).

PTN expression in oligodendrogliomas varied from 0 to +++ for grade II (O) and from 0 to ++++ for grade III (AO), but there was no significant difference showed between them. An elevated PTN expression (+++ and ++++) was found only in two out of eight anaplastic oligodendrogliomas samples (Fig. 1B).

Proliferate index (Fig. 3A) and microvascular density (Figs. 2A, 2B, 3B) were evaluated only in high grade tumors (AA, GBM and OA) by immunohistochemical analysis of Ki-67 and CD-31, respectively. Proliferate index results (ranging from 1 to 61%) were analyzed considering intensity of PTN with was divided in three groups (low, intermediate and high), as described before. Statistical analysis showed no correlation between proliferate index and pleiotrophin expression in high grade gliomas (Fig. 3A). Microvascular density results (ranging from 20 to 169 vessels/stained cells in 0.24 mm^2) were compared with the same groups of PTN expression and there was no statistical significance between them (Fig. 3B).

Survival curve analysis showed comparable results in tumors with different levels of PTN expression, with no statistically difference among groups (Fig. 3C).

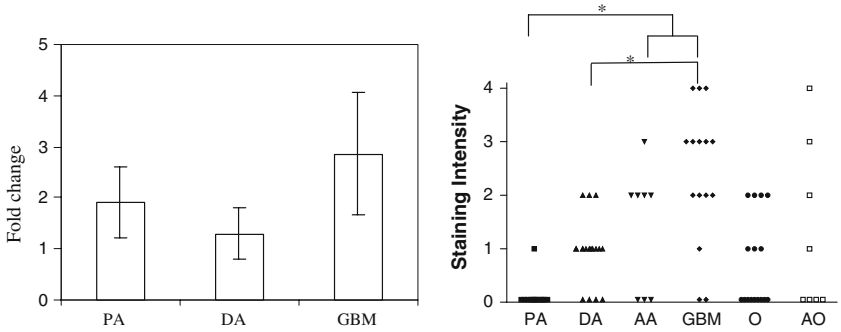

Fig. 1 (**A**) Microarray results. Differential expression of pleiotrophin in astrocytomas compared to correspond non-neoplastic tissue. Normalized expression data was acquired by microarray hybridization and plotted as mean fold change (tumor/normal). (**B**) PTN staining intensity according to the histological diagnosis. (*$P < 0.05$) Legend: PA, pilocytic astrocytoma; DA, diffuse astrocytoma; AA, anaplastic astrocytoma; GBM, glioblastoma; O, oligodendroglioma; and AO, anaplastic oligodendroglioma

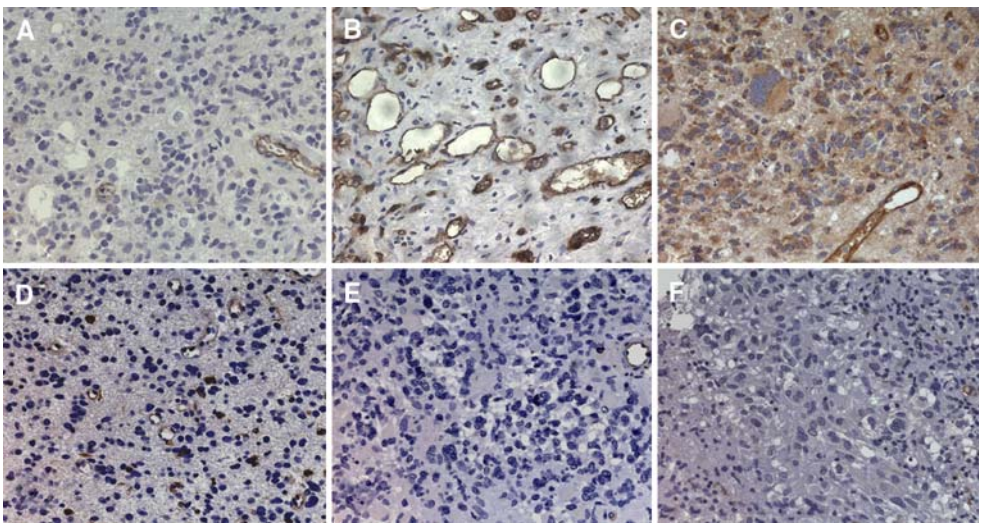

Fig. 2 Microphotography (400×). (**A**) and (**B**) CD 31 staining. (**A**) Glioblastoma showing low microvascular density. (**B**) Glioblastoma showing high microvascular density. (**C–F**) Pleiotrophin staining. (**C**) Glioblastoma with high expression. (**D**) Intermediate pleiotrophin expression in Anaplastic Astrocytoma, staining of endothelium cells can also be observed (**E**) and (**F**) negative staining for Anaplastic astrocytoma (**E**) and Pilocytic astrocytoma (**F**)

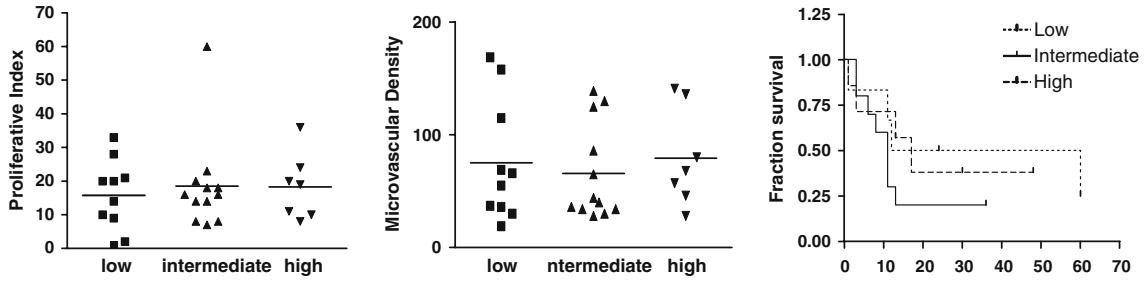

Fig. 3 (**A**) Proliferative index in different groups of high grade tumors divided according to PTN staining intensity ($P > 0.05$). (**B**) Microvascular density in different groups of high grade tumors divided according to PTN staining intensity ($P > 0.05$). (**C**) Survival curves of patients with high grade tumors divided according to PTN staining intensity. ($P = 0.48$) Legend: low, PTN low expression; intermediate, PTN intermediate expression; and high, PTN high expression

Discussion

Previous studies have shown over expression of PTN and its ligands in glioblastomas [13, 14, 18, 19], thus corroborating the importance of this tyrosine kinase pathway in such tumors. Although the biological role of PTN is probably linked to tumor growth and migration [11], the clinical relevance of PTN expression patterns has not been fully elucidated.

To add new data in this field, our approach was to study the expression of PTN by immunohistochemistry, in comparison with the histopathological grade (malignancy potential) in high and low grade astrocytomas and oligodendrogliomas and compare this expression only in high grade gliomas with the rate of cell proliferation, microvessel density (angiogenesis) and outcome (global survival). In a previous study, PTN evaluation by immunohistochemistry showed significant correlation with ELISA analysis [15], therefore immunohistochemistry can be considered an adequate method to detect this protein with advantage to be used in paraffin fixed tissue.

In order to assess the correlation between PTN and malignancy, we analyzed its expression in astrocytomas and oligodendrogliomas of different grades. In pilocytic astrocytoma, a tumor with only rare cases of malignant progression, PTN expression was detectable by DNA microarray but undetectable by immunohistochemistry in 12 out of 13 cases, and slightly positive in the remaining case. Immunohistochemistry has the advantage to analyze protein expression exclusively in tumor cells in contrary to microarray technique that even using microdissected tumor samples can not avoid to include endothelium cells in its evaluation. Interestingly, AA and GBM presented a large variation in PTN expression levels, showing that these levels are not a specific marker of the malignant phenotype. Higher PTN expression in GBM (grade IV) compared to DA (grade II) was verified by both microarray and immunohistochemistry. Ulbricht et al. [19] reported an increase of PTN and its tyrosine phosphatase receptor in astrocytomas. Positive correlation with malignancy was found for the receptor but not for PTN. Our data support Ulbricht et al. [19] hypothesis that PTN expression is an early event in glioma development. However, we also showed that PTN levels are positively correlated to astrocytoma grade. The wide range of PTN expression in all diffusely infiltrating astrocytomas, including the high grade ones, corroborate results seen in glioma cell lines or glioma cells from tumors, measured by immunoreactive PTN in culture [13, 14]. It is important to point that previous study in normal brain showed PTN expression on blood vessels; weak diffuse staining in cortex and white matter parenchyma and in some cortical neurons [19]. Zhang et al. revealed, using a Western blot analysis, that pleiotrophin contents in glioma cell lines and glioblastoma tissues were higher than those in normal brain tissues and that the treatment of these glioblastoma cells with an anti-PDGF antibody did not affect the pleiotrophin production, concluding that this protein may be involved in tumor growth [22].

The relation between PTN expression and proliferate index of the tumors was analyzed considering only malignant astrocytomas (AA and GBM) and AO. This strategy of considering only high grade tumors was used because proliferation rate in gliomas is not uniform, ranging from 1 to 60% of in our series and no correlation was demonstrated. An experiment using cells cultivated from solid gliomas and glioma cell lines has shown that proliferating cells were mostly PTN positive. In the same study, the addition of PTN into glioma cell medium did not change their proliferation rate as measured by thymidine incorporation [14].

Angiogenesis has been pointed out as a major biological effect of PTN in several experimental studies. However, it does not seem to be in our cases of gliomas and oligodendrogliomas. In all groups of tumors with differential expression of PTN, the microvascular density was very similar, with a large range of variation. Our data are in accordance with Ulbricht et al. [19]. Zhang et al., using a

nude mouse model, demonstrated the possibility that mutations which activate PTN in premalignant cells are sufficient to stimulate an angiogenic switch in vivo [23].

An exciting report of PTN biological importance was made by Powers et al. [14]. The authors studied the influence of the inhibition of ALK, a PTN receptor, in glioma cells and their results suggest that tumor cells submitted to this inhibition became more sensitive to cisplatinum, a cytotoxic agent. Therefore, tumors with inhibition of PTN/ALK pathway could have a better clinical course after chemotherapy.

In order to test this hypothesis, we analyzed survival curves of patients with different levels of PTN expression treated based in standard international guidelines. The results were very similar, showing that tumors with different levels of PTN expression present comparable results, proving that this protein should not be considered as a prognostic marker for gliomas.

Different patterns of tumor invasion have been described for malignant gliomas based on clinical studies (mainly imaging analysis). These tumors vary from round localized ones to bilateral tumors infiltrating the corpus calosum, periventricular region and perivascular region. These different patterns of invasion have not yet been related to pathological and molecular data. Here we used an indirect strategy for analyzing the relation between PTN expression and tumor invasion by comparing protein expression in localized tumors (PA) versus very invasive tumors (glioblastoma). As demonstrated here, the difference in PTN expression levels was quite important. It is worth to mention that a previous study reported that PTN induces chemotaxis in microglial cells [18], supporting the idea that PTN can be related to tumor invasion. Muller et al., analyzing tyrosine phosphatase zeta (RPTPzeta) that is one of pleiotrophin ligands, established a functional role of RPTPzeta in glioblastoma cell migration, suggesting a novel function for RPTPzeta in regulating glioblastoma cell motility and pointing the therapeutic utility of RPTPzeta as a target for antibody-mediated therapy of brain tumors [13].

Some other studies have been developed investigating the relation between pleiotrophin, tumor growth and invasion, looking for a use to this protein and its receptors in novel molecular target and gene therapies. Foehr et al. using the recombinant extra cellular domain of human RPTPbeta, generated monoclonal antibodies that recognized this target in tumor cells, killing glioma cells ''in vitro'' when coupled to a cytotoxin. Studies ''in vivo'' significantly delayed human U87 glioma tumors in a mouse xenograft model [24]. Other distinguished result was demonstrated by Ulbricht et al. when he transfected the human glioblastoma cell line U251—MG with small interfering RNA directed against tyrosine phosphatase zeta/receptor-type protein tyrosine phosphatase beta (PTPzeta/RPTPbeta), followed by a strong down-regulation of PTPzeta/RPTPbeta expression. These clones when injected subcutaneously into nude mice, tumors growth were almost completely abrogated. All these findings confirm the potential of this protein and its pathways to the future treatment strategy [25].

In low and high grade primary brain tumors have been isolated and characterized cancer stem cells. These stem cells expressed neural markers and had neural ''stem cell like'' behavior ''in vitro'' and ''in vivo'' and represent only a small fraction of the total tumor cell population [26]. The mRNA transcripts PTN and its receptors were detected in neurospheres, suggesting that pleiotrophin signaling systems are present in the neural stem cell and are involved in the modulation of fate of these cells fate [9]. Identification of the brain tumor stem cell has important implications for understanding the mechanisms of brain tumorigenesis suggesting that therapy which spares this cell should explain the tumor recurrence. Considering PTN as a proto-oncogene it seems to be an interesting target for specifics molecular therapies acting by blocking these stem cells which conventional chemotherapy can not destroy.

Studies with brain tumor stem cells will lead to further insight which of these cells will develop a brain tumor.

In summary, our data suggest the association between PTN expression and histopathological grade of astrocytomas. Proliferation rate, angiogenesis and overall survival do not seem to be associated to its expression.

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References

1. Bao X, Mikami T, Yamada S, Faissner A, Muramatsu T, Sugahara S (2005) Heparin-binding growth factor, pleiotrophin, mediates neuritogenic activity of embryonic pig brain-derived chondroitin sulfate/dermatan sulfate hybrid chains. J Biol Chem 280:9180–9191
2. Deuel TF, Zhang N, Yeh HJ, Silos-Santiago I, Wang ZY (2002) Pleiotrophin: a cytokine with diverse functions and a novel signaling pathway. Arch Biochem Biophys 397:162–171
3. Laaroubi K, Delbe J, Vacherot F, Desgrandes P, Tardieu M, Jaye M, Barritault D, Courty J (1994) Mitogenic and in vitro angiogenic activity of human recombinant heparin affin regulatory peptide. Growth Factors 10:89–98
4. Merenmies J, Rauvula H (1990) Molecular cloning of the 18-Kda growth-associated protein of developing brain. J Biol Chem 285:16721–16724
5. Bowden ET, Stoica GE, Wellstein A (2002) Anti-apoptotic signaling of pleiotrophin through its receptor, anaplastic lymphoma kinase. J Biol Chem 277:3582–3586
6. Jager R, List B, Knabbe C, Souttou B, Raulais D, Zeiler T, Wellstein A, Aigner A, Neubauer A, Zugmaier G (2002) Serum

levels of the angiogenic factor pleiotrophin in relation to disease stage in lung cancer patients. Br J Cancer 86:858–863
7. Li G, Hu Y, Huo Y, Liu M, Freeman D, Gao J, Liu X, Wu D, Wu H (2006) PTEN deletion leads to up-regulation of a secreted growth factor pleiotrophin. J Biol Chem 281:10663–10668
8. Kadomatsu K, Muramatsu T (2004) Midkine and pleiotrophin in neural development and cancer. Cancer Lett 204:127–143
9. Furuta M, Shiraishi T, Okamoto H, Mineta T, Tabuchi K, Shiwa M (2004) Identification of pleiotrophin in conditioned secreted from neural stem cells by SELDI-TOF and SELDI-tandem mass spectrometry. Dev Brain Res 132:189–197
10. Jung CG, Hida H, Nakahira K, Ikenaka K, Kim HJ, Nishino N (2004) Pleiotrophin mRNA is highly expresses in neural stem (progenitor) cells of mouse ventral mesencephalon and the product promotes production of dopaminergic neurons from embryonic stem cell-derivated nestin-positive cells. FASEB J 18:1237–1239
11. Lu KV, Jong KA, Kim GY, Singh J, Dia EQ, Yoshimoto K, Wang MY, Cloughesy TF, Nelson SF, Mischel PS (2005) Differential induction of glioblastoma migration and growth by two forms of pleiotrophin. J Biol Chem 280:26953–26964
12. Moon HS, Park WI, Sung SH, Choi EA, Chung HW, Woo BH (2003) Immunohistochemical and quantitative competitive PCR analyses of midkine and pleiotrophin expression in cervical cancer. Gynecol Oncol 88:289–297
13. Muller S, Kunkel P, Lamszus K, Ulbricht U, Lorente GA, Nelson AM, von Schack D, Chin D, Lohr SC, Westphal M, Melcher T (2003) A role for tyrosine phospatase ζ in glioma cell migration. Oncogene 22:6661–6668
14. Powers C, Aigner A, Stoica GE, McDonnell K, Wellstein A (2002) Pleitrophin signaling through anaplastic lymphoma kinase is rate-limiting for glioblastoma growth. J Biol Chem 277:14153–14158
15. Sanson M, Thillet J, Hoang-Xuan K (2004) Molecular changes in gliomas. Curr Opin Oncol 16:607–613
16. Wu H, Barusevicius A, Babb J, Klein-Szanto AK, Godwin A, Elentsas R, Gelfand JM, Lessin S, Seykora JT (2005) Pleiotrophin expression correlates with melanocytic tumor progression and metastatic potential. J Cutan Pathol 32:125–130
17. Souttou B, Juhl H, Hackenbruck J, Rockseisen M, Klomp HJ, Raulais D, Vigny M, Wellstein A (1998) Relationship between serum concentrations of the growth factor pleiotrophin and pleiotrophin-positive tumors. J Nat Cancer Inst 90:1468–1473
18. Mentlein M, Held-Feindt J (2002) Pleiotrophin, an angiogenic and mitogenic growth factor, is expressed in human gliomas. J Neurochem 83:747–753
19. Ulbricht U, Brockmann MA, Aigner A, Eckerich C, Muller S, Fillbrandt R, Westphal M, Lamszus K (2003) Expression and function of the receptor of the protein tyrosine phosphatase ζ and its ligand pleiotrophin in human astrocytomas. J Neuropathol Exp Neurol 62:1265–1275
20. Malerczyk C, Schulte AM, Czubayko F, Bellon L, Macejak D, Riegel AT, Wellstein A (2005) Ribozyme targeting of the growth factor pleiotrophin in established tumors: a gene therapy approach. Gene Ther 12:339–346
21. Kleihues P, Louis DN, Scheithauer BW, Rorke LB, Reifenberger G, Burger PC, Cavenee WK (2002) The WHO classification of tumors of the nervous system. J Neuropathol Exp Neurol 61:215–225
22. Zhang L, Mabuchi T, Satoh E, Maeda S, Nukui H, Naganuma H (2004) Over expression of heparin-binding growth-associated molecule in malignant glioma cells. Neurol Med Chir 44:637–645
23. Zhang N, Zhong R, Perez-Pinera P, Herradon G, Ezquerra L, Wang ZY, Deuel TF (2006) Identification of the angiogenesis signaling domain in pleiotrophin defines a mechanism of the angiogenic switch. Biochem Biophys Res Commun 343:653–658
24. Foehr ED, Lorente G, Kuo J, Ram R, Nikolich K, Urfer R (2006) Targeting of the receptor protein tyrosine phosphatase β with a monclonal antibody delays tumor growth in a glioblastoma model. Cancer Res 66:2271–2278
25. Ulbricht U, Eckerich C, Fillbrandt R, Westphal M, Lamszus K (2006) RNA interference targeting protein tyrosine phosphatase ζ/receptor-type protein tyrosine phosphatase β supresses glioblastoma growth in vitro and in vivo. J Neurochem 98:1497–1506
26. Singh SK, Clarke ID, Terasaki M, Bonn VE, Hawkins C, Squire J, Dirks PB (2003) Identification of a cancer stem cell in human brain tumors. Cancer Res 63:5821–5828