REVIEW ARTICLE

Roles of pleiotrophin in tumor growth and angiogenesis

Evangelia Papadimitriou, Constantinos Mikelis, Evgenia Lampropoulou, Marina Koutsioumpa, Katerina Theochari, Sotiria Tsirmoula, Christina Theodoropoulou, Margarita Lamprou, Evanthia Sfaelou, Dionyssios Vourtsis, Panagiotis Boudouris

Laboratory of Molecular Pharmacology, Department of Pharmacy, University of Patras, Patras, Greece

Correspondence: E. Papadimitriou, Ph.D., Lab. of Molecular Pharmacology, Dept. of Pharmacy, University of Patras, 26504 Greece <epapad@upatras.gr>

Accepted for publication June 12, 2009

ABSTRACT. Pleiotrophin (PTN) is a heparin-binding growth factor with diverse biological activities, the most studied of these being those related to the nervous system, tumor growth and angiogenesis. Although interest in the involvement of PTN in tumor growth is increasing, many questions remain unanswered, particularly concerning the receptors and the signaling pathways involved. In this review, we briefly introduce PTN, and summarize data on its involvement in tumor growth and angiogenesis, and on what is known to date concerning the receptors and pathways involved.

Keywords: angiogenesis, cancer, endothelial cells, pleiotrophin, receptor protein tyrosine phosphatase β/ζ, integrins

Pleiotrophin (PTN) is a secreted heparin-binding growth factor that takes part in many different processes, such as cell growth and survival, cell migration, angiogenesis and neurite outgrowth. It is also known as heparin-binding growth-associated molecule [1, 2], heparin affin regulatory peptide [3], heparin-binding growth factor-8 [4], protein 18 kDa [5], heparin-binding neurotrophic factor [6, 7] and osteoblast-specific factor [8]. PTN is highly homologous to midkine (MK), with which it shares 45-50% sequence identity, forming a family of growth factors [6, 9]. It is highly conserved across different species: more than 90% identity has been observed among the sequences of chicken, rat, mouse, bovine and human [10], while homologues have been also reported in fish, frogs and insects [11].

HUMAN PTN PROTEIN AND GENE/PROMOTER STRUCTURE

PTN consists of 168 amino acids, the mature peptide having 136 amino acids as a result of cleavage of the signal peptide [12, 13]. The calculated mass of the mature protein was determined by plasma desorption time-of-flight mass spectrometry as 15,291 kDa, but in SDS-PAGE it appears as 18 kDa [2], due to the fact that the molecule is rich in cationic amino acids, mainly lysines, that form random coils at both N- and C-terminal ends [12, 14]. The peptide also contains 10 conserved cysteines that participate in the formation of five disulfide bonds [2, 15, 16], and three potential nuclear targeting sequences K-R/K-X-R /K [2]. PTN does not contain any potential sites for N-glycosylation or other post-translational modifications [1, 2] and its binding to heparin is mediated by the two central regions that are homologous to the thrombospondin type I repeat (TSR-1) [14]. More recent data suggest that the carboxyl terminal TSR-1 domain is the main heparin-binding site of PTN [17].
The human *ptn* gene has been identified as being on chromosome 7 band q33a, having a minimum size of 42 kDa, and containing at least seven exons. The open reading frame is located on four exons and the boundaries between introns and extrons seem to be conserved among species in the PTN/MK family [18]. The signal peptide and the first five amino acids of the mature protein are located in exon 2, while the core region is split into exons 2 and 3, containing six and four cysteine residues respectively. Exon 4 comprises the C-terminus of PTN that contains a putative nuclear translocation signal based on its homology with histone Hl [19]. The 5' untranslated region is unique in the human *ptn* gene compared with other species [20], while there may be multiple 5' untranslated regions derived from alternative splicing [21] that may contribute to cell or tissue-specific regulation of PTN expression [19]. From what is known to date, the promoter of the human *ptn* gene contains sequences for the binding of several transcription factors, as shown in *figure 1*.

REGULATION OF PTN EXPRESSION

Although PTN seems to have significant biological functions, little is still known on the regulation of its expres-

doi: 10.1684/ecn.2009.0172

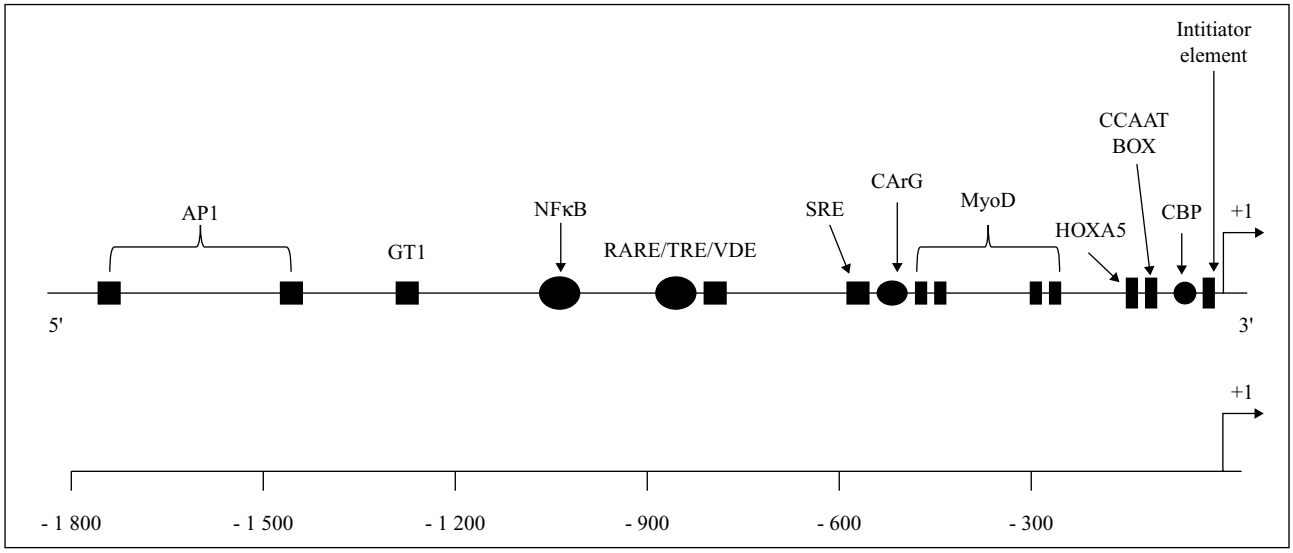

Figure 1

Schematic representation of the transcription factor binding sites of the *ptn* promoter. The human *ptn* promoter contains a CAAT box, four binding sites for myogenic differentiation 1(MyoD), one for nuclear factors called GT (GT1), two for activator protein 1 (AP1), one for homeobox A5 (HOXA5) and a serum response element (SRE) [19, 29, 32, 33, 36], all marked with filled squares. Putative binding sites for nuclear factor kappa B (NFκB), cAMP response element binding protein (CBP), serum response factor (CArG box) and retinoic acid receptor (RARE/TRE/VDE) are marked with filled circles [19] and need further investigation. There is also a binding site of Sox10 at the proximal site of the promoter; however, its exact location has not been identified [22]. Potential binding sites have been also suggested for early growth response factor-1 and specificity protein-1 transcription factors [23].

sion. It is known that *ptn* gene expression is regulated in a cell type- and time-dependent manner [5, 13, 19]. It is also known that it is up-regulated during specific diseases, such as rheumatoid arthritis [24], osteoarthritis [25], after injury [26, 27] or in cancer (see below). Up-regulation of PTN expression has been mentioned for tumor necrosis factor α and epidermal growth factor [24], ciliary neurotrophic factor [28], members of the fibroblast growth factor (FGF) family, such as FGF2 and FGF10 [29, 30], platelet-derived growth factor [31, 32], cAMP [33] hypoxia [32], serum [34], hydrogen peroxide [35] and endothelial nitric oxide synthase [36]. Contradictory results have been published on retinoic acid, which induces PTN expression in some cells [9, 37] or tissues [30], but has no effect on other types of cells [31]. Concerning transcription factors, *ptn* gene expression is directly affected by HOXA5 [38] and AP-1 [35], after direct binding to the corresponding response elements on the *ptn* promoter. Finally, loss of the tumor suppressor gene PTEN leads to up-regulation of PTN expression, mediated by the PTEN-PI3K-AKT pathway [39].

BIOLOGICAL ACTIVITIES OF PTN

The initial reference involving PTN suggested that it plays a role in the maturation and growth of brain [5]. Since then, the importance of PTN in the nervous system has been well described [13, 27, 28, 33, 40-51]. PTN is over-expressed in neurodegenerative diseases [52] and exhibits a protective or/and trophic effect on dopaminergic neurons *in vitro* and *in vivo* [45, 46, 48, 53].

Regarding the muscular system, PTN is expressed in developing muscle *in vivo* [1, 13, 54], is up-regulated during *in vitro* myogenesis and soleus muscle regeneration, and can be found in newly formed myotubes and perfused activated myoblasts [55]. Moreover, PTN mRNA is present in smooth muscle [56] and cardiac muscle cells [57, 58], is down-regulated during postnatal differentiation of the myocardium [58], up-regulated in heart failure [59], and potentiates cardiomyocyte cell death by apoptosis [60].

PTN is expressed in the fetus liver but its expression gradually decreases [61], although it seems to be involved in liver regeneration [32, 61]. PTN is expressed in the developing kidney mesenchyme [57] and induces formation of branching tubules in an immortalized ureteric bud cell line cultured three-dimensionally in an extracellular matrix gel [62]. PTN is detected during lung development and in embryonic bronchial epithelial cells [57], and regulates lung epithelial cell proliferation and differentiation during fetal lung development [63]. It is also normally expressed in the epithelial ridge of cochlea, suggesting a role in auditory function [64].

In females, PTN is expressed in the uterus, and its expression is dependent on the estrous cycle [56, 65], which it seems to affect [65]. In human mammary gland, PTN is detected in alveolar myoepithelial, epithelial, endothelial and vascular smooth muscle cells [66], and its expression is increased in both terminal end bud and mature ducts in the process of mammary branching morphogenesis [67]. PTN is involved in ectopic endometriosis [68], and female mice deficient in both PTN and MK have shown abnormalities of reproduction [65]. In males, PTN plays a role in normal spermatogenesis. It is expressed in Leydig cells of the testis and is up-regulated in both human Peyronie's and Dupuytren's disease [69, 70]. Dominant negative PTN mutant male mice show sterility, atrophic testes and strikingly apoptotic spermatocytes [71].

PTN promotes proliferation, differentiation and proper attachment of osteoblasts [72, 73], and induces chemotaxis, proliferation and differentiation of human osteoprogenitor cells, as well as both bone and cartilage formation in athymic mice [74]. It is also involved in angiogenesis in the growth plate of mice [75], and regulates the ectopic bone-inducing activity of rhBMP-2 [76]. Furthermore, PTN is a vital signaling molecule in regulating periosteal bone formation and resorption in response to four-point bending of right tibias in C57BL/6J mice [77]. Interestingly, mice that over-express PTN tend to have increased bone growth [78], and although PTN-deficient mice seem to have normal bone formation [79], they show growth retardation in the weight-bearing bones by two months of age, and osteopenia during adulthood [80]. PTN is found in developing [81] and adult [82] nasal cartilage, and participates in the proteoglycan synthesis in the developing matrix of fetal cartilage [83]. It is an autocrine growth factor in cartilage [25], is increasingly expressed in the early stages of osteoarthritis [25, 84] and an increase at its mRNA levels is provoked by sclerotic, subchondrial osteoblasts in osteoarthritic cartilage [85]. Whether PTN improves or deteriorates osteoarthritis is not known to date.

BIOLOGICAL ACTIVITIES RELATED TO CANCERS

Cancer cells in vitro

A role for PTN in human cancers was suggested after its detection in conditioned media of the highly malignant breast cancer cell line MDA-MB231 [86]. Since then, screening of various human cell lines and tumor specimens revealed that PTN is expressed as an autocrine or/and paracrine growth factor by various cancer cells, including human breast [87-89], prostate [29, 87, 90, 91], ovarian [87] and lung [92] cancer, choriocarcinoma [93], melanoma [87, 94], glioblastoma [95-98] and pancreatic carcinoma cells [99]. Multiple myeloma (MM) cell lines and malignant cells from MM patients' bone marrow produced and secreted PTN into the cell culture supernatants and *ptn* gene expression correlated with the patients' disease status. Inhibition of PTN with a polyclonal anti-PTN antibody reduced growth and enhanced apoptosis of MM cell lines and freshly isolated bone marrow tumor cells from MM patients *in vitro* [100]. PTN mRNA is also selectively detected in the meningothelial cells of meningiomas [101], and PTN expression is up-regulated after loss of the tumor suppressor gene PTEN [39]. Interestingly, it has recently been suggested that decrease of PTN expression in U87MG cells induces tetraploidy and aneuploidy, and arrests cells in the G1 phase of the cell cycle, suggesting that PTN signaling may have a critical role in chromosomal segregation and cell cycle progression [102]. Moreover, PTN disrupts cytoskeletal protein complexes, ablates calcium-dependent homophilic cell-cell adhesion, stimulates ubiquitination and degradation of N-cadherin, reorganizes the actin cytoskeleton and induces a morphological epithelial-mesenchymal transition in PTN-stimulated U373 cells [103]. PTN is also involved in hepatocarcinogenesis and has an anti-apoptotic activity against TGFβ1 in hepatoma cell lines [104]. Finally, in line with the notion that PTN may significantly stimulate tumor progression, independently of its effect on the cancer cells themselves, PTN secretion from MCF-7 breast cancer cells stimulates epithelial island formation, activation of stromal fibroblasts, extensive remodeling of the microenvironment and activation of markers of aggressive breast cancer in co-cultures of PTN-expressing MCF-7 and NIH 3T3 cells [105]. It also affects tumor angiogenesis, as discussed more extensively below.

Conversely, there are cases where PTN has been shown to negatively regulate tumor cell growth. For example, PTN mRNA levels are decreased in colorectal cancers compared with those in normal adjacent mucosa [106]. It has been detected in lysates and conditioned medium from contact-arrested NIH 3T3 fibroblasts, but not in cells transformed by the ras oncogene [107], and its expression is up-regulated in confluent compared with actively proliferating cells [108, 109]. Its expression is low or absent in neuroblastomas with a poor prognosis [110], and negatively affects growth and migration of several glioma cells lines [111, 112].

Tumor angiogenesis in vitro

Besides a significant role in the biology of tumor cells themselves, PTN seems also to affect the angiogenic potential of tumor cells. Firstly, PTN stimulates angiogenic functions of endothelial cells *in vitro* [113-117] and induces embryoid body angiogenesis [118] and transdifferentiation of monocytes into functional endothelial cells [119-121]. In the same line, it increases the *in vitro* angiogenic potential of several tumor cells, such as multiple myeloma [120], breast [122] and prostate [91] cancer cells.

In contrast to a positive regulation of *in vitro* angiogenesis by PTN, there are also data that support a negative regulation. First of all, PTN directly binds and inhibits the effect of vascular endothelial growth factor (VEGF) on endothelial cell proliferation, migration and tube formation [123, 124]. It also decreases the expression of the VEGF receptor KDR, another mechanism through which it potentially inhibits VEGF angiogenic activities *in vitro* [125]. Finally, decrease in the expression of endogenous PTN in C6 glioma cells significantly increased the angiogenic potential of these cells *in vitro*, partially due to increased availability and activity of VEGF [111].

Tumor growth and angiogenesis in vivo

Much *in vivo* data suggest that PTN plays a role in angiogenesis of tumors that grow in nude mice. This was initially shown in NIH-3T3 cells that constitutively overexpressed PTN. When these cells were implanted into the flanks of nude mice, they tended to form tumors with significant neovascularization compared with the mock-transfected cells [126]. In the same line, overexpression of PTN in a human adrenal carcinoma cell line SW13 promotes not only *in vivo* tumor growth, but also tumor-induced angiogenesis, suggesting that constitutive PTN signaling fully regulates the angiogenic switch [127]. Expression of PTN in breast cancer MCF-

7 cells stimulates tumor growth, remodeling of the microenvironment and tumor-induced angiogenesis *in vivo* [105, 122].
Ribozyme-mediated depletion of HERV-PTN mRNA in human choriocarcinoma suggests that PTN is an essential and rate-limiting factor for choriocarcinoma growth, invasion, and angiogenesis *in vivo* [93]. Moreover, RNA interference-mediated gene silencing of PTN suppresses glioblastoma growth and angiogenesis *in vivo* [128]. Finally, PTN antisense expression in human prostate cancer LNCaP cells inhibits LNCaP cell-induced angiogenesis *in vivo* in the chicken embryo chorioallantoic membrane [91].
On the other hand, there is also evidence that PTN can act as an angiostatic factor. For example, vascularization was significantly decreased in neuroblastoma xenografts that over-express PTN and that are resistant to the DNA-topoisomerase I inhibitor irinotecan [110]. Similarly, PTN antisense expression in rat glioma C6 cells, increased C6 glioma cell-induced angiogenesis *in vivo* in the chicken embryo chorioallantoic membrane [111]. In both cases, direct binding of PTN to VEGF has been discussed as the possible reason, although other mechanisms may be also involved.

STRUCTURE-FUNCTION DATA

Many studies have been undertaken in order to determine which regions of PTN are responsible for its diverse functions, in an effort to identify the molecular mechanisms involved and to identify possible therapeutic targets or/and agents. Kilpelainen *et al.* suggested that the two TSR-1 motifs are responsible for the interaction of PTN with heparin, an interaction associated with many of the biological activities of PTN [14]. More recent data suggest the involvement of only the carboxyl terminal TSR-1 motif in heparin binding [17, 129], and the mitogenic, transforming and angiogenic activities of PTN *in vitro* and *in vivo* in nude mice [129].
It has been shown that PTN exists in two naturally occurring forms, PTN15 and PTN18, with differential interactions with its receptors anaplastic lymphoma kinase (ALK) and receptor protein tyrosine phosphatase β/ζ (RPTPβ/ζ), and different activities. PTN18 interacts with RPTPβ/ζ and induces glioma cell migration, while PTN15 interacts with ALK and induces glioma cell proliferation [97]. The two forms of PTN differ in their carboxyl terminus, which is being investigated for its role in tumor growth and angiogenesis. It has long been shown that the C-terminal lysine-rich domain of PTN (amino acids 111-136) is not involved in neurite outgrowth activity, but it seems to play a key role in the mitogenic and tumor formation activities of PTN [130]. A truncated PTN lacking the C-terminal 111-136 portion inhibits tumor development by inhibition of both endothelial and breast cancer cells [131]. The exact mechanism of action of the C-terminal lysine-rich domain of PTN is not known. It has been suggested that it acts through binding to ALK [132], or RPTPβ/ζ [133] and antagonizes PTN binding and activity [132, 133]. Since this domain of PTN seems to play an important role in its biological activities related to tumor growth and angiogenesis, more work is needed to identify the molecule(s) with which it interacts, the result(s) of such interactions, as well as its possible therapeutic potential.

RECEPTORS, MOLECULAR MECHANISMS AND INTERACTIONS WITH OTHER MOLECULES INVOLVED IN PTN ACTIONS RELATED TO ANGIOGENESIS AND CANCER

Syndecans

The first identified receptor for PTN has been N-syndecan [134]. The interaction of PTN with N-syndecan takes place via its heparan sulfate side chains [134, 135] and is mediated by both TSR-1 domains of PTN [136, 137]. Binding of PTN to N-syndecan promotes several PTN-induced actions in the nervous system [135, 138-140].
Besides the nervous system, possible involvement of N-syndecan in PTN activities has been mentioned in osteoblasts [73, 78], and in parenchymal cells in adult and embryonic liver [61]. No involvement of N-syndecan in PTN-induced activities related to angiogenesis and cancer has been mentioned, nor is it known whether other syndecans interact and mediate PTN-induced actions.

Anaplastic lymphoma kinase (ALK)

ALK is a 220-kDa receptor tyrosine kinase (RTK) encoded by the *alk* gene on chromosome 2p23. ALK was first identified as part of the NPM-ALK oncogenic fusion protein, resulting from the (2;5)(p23;q35) translocation that is frequently associated with anaplastic large-cell lymphoma [141]. Full-length ALK has the typical structure of an RTK, with a large extracellular domain, a lipophilic transmembrane segment, a cytoplasmic tyrosine kinase domain, and belongs to the insulin receptor superfamily. It was initially described as an orphan RTK that shows restricted tissue distribution and is regulated during organ development [142, 143]. PTN was initially identified as a potential ligand of ALK, based on a genetic screen by peptide ‘phage display [144]. Different groups have since suggested ALK to be a functional PTN receptor [144-147]. In support of this, the expression pattern of PTN partially overlaps with that of ALK in the rodent developing nervous system [144].
Beyond ALK expression in the nervous system, cultured fibroblasts and endothelial cells, it has also been detected in osteoblastic cells [148] and chondrocytes [25], in pancreatic and breast carcinoma [144, 149], melanoma [150], neuroblastoma [151] glioblastoma [145, 146] and non-Hodgkin’s lymphoma [152].
On the other hand, ALK expression is low in a wide variety of soft tumors [153], and is characterized by limited tissue distribution [142]. Moreover, recent studies performed by different groups argue against PTN as a specific ALK ligand, since binding or activation of ALK by PTN cannot be detected [150, 154-158]. A possible explanation to the confusion in the literature may be

the differential activation of ALK by the two naturally occurring forms of PTN [97], or the indirect PTN-induced ALK activation through PTN-dependent inactivation of the RPTPβ/ζ [159].

Receptor protein tyrosine phosphatase β/ζ (RPTPβ/ζ)

RPTPβ/ζ was initially isolated from neural tissue as a transmembrane protein-tyrosine-phosphatase (PTPase) that consists of a putative signal peptide, a very large extracellular domain containing an N-terminal sequence homologous to carbonic anhydrase, a transmembrane region and a cytoplasmic portion that contains two repeated PTPase-like domains [160]. A shorter transmembrane and two secreted isoforms corresponding to the extracellular portions of the long and short transmembrane isoforms have been described, all considered splice variants of RPTPβ/ζ [161-163]. The short transmembrane isoform lacks 859 amino acids from the extracellular domain [161] and also interacts with PTN. Phosphacan, the secreted isoform that corresponds to the extracellular portion of the long RPTPβ/ζ, is also able to bind PTN [162], and is considered to modulate cell interactions and developmental processes in the nervous system [164]. Changes in chondroitin sulfate on phosphacan are developmentally regulated and regulate phosphacan's affinity for PTN [165]. Phosphacan short isoform that corresponds to the extracellular portion of the short RPTPβ/ζ, is not a proteoglycan [163] and has not been shown to interact with PTN. Apart from the RPTPβ/ζ splicing variants that are normally expressed, under physiological conditions, RPTPβ/ζ is cleaved by matrix metalloproteinase 9, tumor necrosis factor-α converting enzyme, presenilin/γ-secretase [166] and plasmin [167], leading to secreted, transmembrane, or cytoplasmic forms of, not yet, fully identified biological significance.

It has been suggested that PTN binding to RPTPβ/ζ leads to dimerization of the receptor and inhibition of the PTPase activity. The PTN-dependent RPTPβ/ζ inactivation was shown to lead to increased phosphorylation of β-catenin [168], β-adducin [169] and Fyn [170], thus regulating cytoskeletal stability, cell plasticity and cell-cell adhesion mechanisms [169]. In U373 cells, PTN induced increased tyrosine phosphorylation of different RPTPβ/ζ substrates required for epithelial-mesenchymal transition [103]. On the other hand, PTN binding to RPTPβ/ζ in endothelial cells leads to dephosphorylation and thus activation of c-src, focal adhesion kinase, phosphatidylinositol-3-kinase and mitogen-activated protein kinases, all participating in PTN-induced endothelial cell migration and tube formation on matrigel [116]. We have more recently shown that in order for RPTPβ/ζ to induce cell migration, the presence of $\alpha_v\beta_3$ integrin is required. RPTPβ/ζ and $\alpha_v\beta_3$ form a functional complex on the surface of endothelial and glioma cell lines, and RPTPβ/ζ seems to be responsible for β_3 tyrosine phosphorylation through the activation of c-src [112]. PTN inhibits migration of cells that do not express $\alpha_v\beta_3$, even if these cells express RPTPβ/ζ [112], however, the exact mechanism(s) involved are not known.

Other possible (co-)receptors

It has long been shown that PTN interacts with several proteoglycans (PGs) with different affinities [171], interactions that seem to contribute to PTN dimerization [172] or storage into the extracellular matrix [113]. Among PGs, many reports have implicated a role for chondroitin sulfate (CS) PGs in the PTN-mediated signaling pathway. It has been shown by several studies that PTN interacts especially with over-sulfated CSs [162, 165, 173-175], an interaction important for the development of the nervous system [173, 176, 177] and for growth and/or progression of tumors [178]. Versican, a CS-PG with a high content of the E disaccharide units, was found to bind strongly PTN, an interaction abolished by chondroitinase ABC digestion [179]. Similarly, the appican CS chain from rat C6 glioma cells, but not that from SH-SY5Y neuroblastoma cells that contained no E disaccharide, was found to bind specifically PTN [180]. These findings indicate that the E motif is essential for the interaction of the CS chains with PTN. Analysis of the oligosaccharides isolated from embryonic CS/dermatan sulfate (DS) chains revealed that octasaccharide is the minimal size capable of interacting with PTN at a physiological salt concentration, and that PTN binds to multiple sequences in embryonic CS/DS chains with distinct affinity [181].

PTN also binds $\alpha_v\beta_3$, but not $\alpha_5\beta_1$ integrin, an interaction that is responsible for PTN-induced cell migration in both endothelial and glioma cell lines [112]. Integrin $\alpha_v\beta_3$ forms a functional complex with RPTPβ/ζ on the cell surface, both components of which are required for the stimulatory effect of PTN on cell migration. Activation of β_3 through phosphorylation of its cytoplasmic tyrosine 773, is required, but is not sufficient to transduce the stimulatory effect of PTN [112]. Further studies are being conducted to elucidate the signaling pathway involved. Interestingly, $\alpha_v\beta_3$ is not a receptor for MK [112], in contrast to all other PTN receptors discussed to date.

Finally, PTN binds nucleolin [182], a 100 kDa multifunctional protein present in the nucleus, cytoplasm, and on the surface of some types of cells, including endothelial [183] and cancer [184, 185] cells. HB-19 pseudopeptide, a specific antagonist that binds the C-terminal tail of nucleolin, has been shown to suppress the growth of tumor cells and angiogenesis in various *in vitro* and *in vivo* experimental models [184]. Nucleolin is considered to be a low affinity receptor for PTN, and has been suggested to possibly import PTN into the nucleus [182]. PTN binds nucleolin through its C-TSR-1 domain with a Kd value of 1.3-1.4 x 10-6 M [186] in the absence of heparin. This binding is strongly inhibited by heparin even though it has not been clarified whether the inhibition was caused by the binding of heparin to PTN or nucleolin [182]. Following its binding to cell surface nucleolin, PTN is internalized in a temperature-sensitive manner, which is independent of heparin and CS PGs [186]. What is the role of the interaction of PTN with nucleolin and of the subsequent PTN internalization remains unknown, but is under further investigation for its possible implication in the effects of PTN on tumor growth and angiogenesis.

CONCLUSION

Although many aspects remain obscure, PTN seems to be significant for tumor growth and angiogenesis, possibly through diverse mechanisms. Clarification of the receptors, as well as the signaling pathways involved, is of great importance, both for increasing our knowledge concerning cancer growth, and for developing new therapeutic strategies.

REFERENCES

1. Merenmies J, Rauvala H. Molecular cloning of the 18kDa growth-associated protein of developing brain. *J Biol Chem* 1990; 265: 16721.
2. Hampton BS, Marshak DR, Burgess DS. Structural and functional characterization of full-length heparin-binding growth associated molecule. *Mol Biol Cell* 1992; 3: 85.
3. Laaroubi K, Vacherot F, Delbe J, Caruelle D, Barritault D, Courty J. Biochemical and mitogenic properties of the heparin-binding growth factor HARP. *Prog Growth Factor Res* 1995; 6: 25.
4. Milner PG, Li YS, Hoffman RM, Kodner CM, Siegel NR, Deuel TF. A Novel 17 kD Heparin-Binding Growth Factor (HBGF-8) in Bovine Uterus: Purification and N-Terminal Amino Acid Sequence. *Biochem Biophys Res Commun* 1989; 165: 1096.
5. Rauvala H. An 18-kd heparin-binding protein of developing brain that is distinct from fibroblast growth factors. *EMBO J* 1989; 8: 2933.
6. Kovesdi I, Fairhurst JL, Kretschmer PJ, Böhlen P. Heparin-binding neurotrophic factor (HBNF) and MK, members of a new family of homologous, developmentally regulated proteins. *Biochem and Biophys Res Commun* 1990; 172: 850.
7. Böhlen P, Müller T, Gautschi-Sova P, *et al.* Isolation from bovine brain and structural characterization of HBNF, a heparin-binding neurotrophic factor. *Growth Factors* 1991; 4: 97.
8. Tezuka K, Takeshita S, Hakeda Y, Kumegawa M, Kikuno R, Hashimoto-Gotoh T. Isolation of mouse and human cDNA clones encoding a protein expressed specifically in osteoblasts and brain tissues. *Biochem Biophys Res Commun* 1990; 173: 246.
9. Kretschmer PJ, Fairhurst JL, Decker MM, *et al.* Cloning, characterization and developmental regulation of two members of a novel human gene family of neurite outgrowth-promoting proteins. *Growth Factors* 1991; 5: 99.
10. Tsutsui J, Uehara K, Kadomatsu K, Matsubara S, Muramatsu T. A new family of heparin-binding factors: strong conservation of midkine (MK) sequences between the human and the mouse. *Biochem Biophys Res Commun* 1991; 176: 792.
11. Englund C, Birve A, Falileeva L, Grabbe C, Palmer RH. Miple1 and miple2 encode a family of MK/PTN homologues in Drosophila melanogaster. *Dev Genes Evol* 2005; 216: 10.
12. Kuo MD, Oda Y, Huang JS, Huang SS. Amino acid sequence and characterization of a heparin binding neurite-promoting factor (p18) from bovine brain. *J Biol Chem* 1990; 265: 18749.
13. Li YS, Milner PG, Chauhan AK, *et al.* Cloning and expression of a developmentally regulated protein that induces mitogenic and neurite outgrowth activity. *Science* 1990; 250: 1690.
14. Kilpelainen I, Kaksonen M, Kinnunen T, *et al.* Heparin-binding growth-associated molecule contains two heparin-binding beta-sheet domains that are homologous to the thrombospondin type I repeat. *J Biol Chem* 2000; 275: 13564.
15. Fabri L, Nice EC, Ward LD, Maruta H, Burgess AW, Simpson RG. Characterization of bovine heparin-binding neurotrophic factor (HBNF): assignment of disulfide bonds. *Biochem Int* 1992; 28: 1.
16. Inui T, Nakao M, Nishio H, *et al.* Solution synthesis and biological activity of human pleiotrophin, a novel heparin-binding neurotrophic factor consisting of 136 amino acid residues with five disulfide bonds. *J Pept Res* 2000; 55: 384.
17. Ori A, Free P, Courty J, Wilkinson MC, Fernig DG. Identification of heparin binding sites in proteins by selective labelling. *Mol Cell Proteomics* 2009 (Epub ahead of print).
18. Li YS, Hoffman RM, Le Beauin MM, *et al.* Characterization of the human pleiotrophin gene. *J Biol Chem* 1992; 267: 26011.
19. Kretschmer PJ, Fairhurst JL, Hulmes JD, Popjes ML, Böhlen P, Kovesdi I. Genomic structure of the human HBNF gene and characterization of the HBNF variant protein as a splice mutant. *Biochem Biophys Res Commun* 1993; 192: 420.
20. Lai S, Czubayko F, Riegel AT, Wellstein A. Structure of the human heparin-binding growth factor gene pleiotrophin. *Biochem Biophys Res Commun* 1992; 187: 1113.
21. Lai S, Schulte AM, Wellstein A, Riegel AT. An additional 5′-upstream exon exists in the human pleiotrophin-encoding gene. *Gene* 1995; 153: 301.
22. Lee KE, Nam S, Cho EA, *et al.* Identification of direct regulatory targets of the transcription factor Sox10 based on function and conservation. *BMC Genomics* 2008; 9: 408.
23. Liedert A, Kassem M, Claes L, Ignatius A. Mechanosensitive promoter region in the human HB-GAM gene. *Biochem Biophys Res Commun* 2009; 387: 289.
24. Pufe T, Bartscher M, Petersen W, Tillmann B, Mentlein R. Expression of pleiotrophin, an embryonic growth and differentiation factor, in rheumatoid arthritis. *Arthritis Rheum* 2003; 48: 660.
25. Pufe T, Grothy G, Goldring MB, Tillmann B, Mentlein R. Effects of pleiotrophin, a heparin-binding growth factor, on human primary and immortalized chondrocytes. *Osteoarthritis Cartilage* 2007; 15: 155.
26. Li G, Bunn JR, Mushipe MT, He Q, Chen X. Effects of pleiotrophin (PTN) over-expression on mouse long bone development, fracture healing and bone repair. *Calcif Tissue Int* 2005; 76: 299.
27. Ezquerra L, Alguacil LF, Nguyen T, Deuel TF, Santiago IS, Herradon G. Different pattern of pleiotrophin and midkine expression in neuropathic pain: Correlation between changes in pleiotrophin gene expression and rat strain differences in neuropathic pain. *Growth Factors* 2008; 26: 44.
28. Roger J, Brajeul V, Thomasseau S, *et al.* Involvement of Pleiotrophin in CNTF-mediated differentiation of the late retinal progenitor cells. *Dev Biol* 2006; 298: 527.
29. Hatziapostolou M, Polytarchou C, Katsoris P, Courty J, Papadimitriou E. Heparin affin regulatory peptide/ pleiotrophin mediates fibroblast growth factor 2 stimulatory effects on human prostate cancer cells. *J Biol Chem* 2006; 281: 32217.
30. Mitsiadis TA, Caton J, De Bari C, Bluteau G. The large functional spectrum of the heparin-binding cytokines MK and HB-GAM in continuously growing organs: the rodent incisor as a model. *Dev Biol* 2008; 320: 256.
31. Li YS, Gurrieri M, Deuel TF. Pleiotrophin gene expression is highly restricted and is regulated by platelet-derived growth factor. *Biochem Biophys Res Commun* 1992; 184: 427.
32. Antoine M, Tag CG, Wirz W, *et al.* Upregulation of pleiotrophin expression in rat hepatic stellate cells by PDGF and hypoxia: Implications for its role in experimental biliary liver fibrogenesis. *Biochem Biophys Res Commun* 2005; 337: 1153.

33. Mourlevat S, Debeir T, Ferrario JE, *et al.* Pleiotrophin mediates the neurotrophic effect of cyclic AMP on dopaminergic neurons: Analysis of suppression-subtracted cDNA libraries and confirmation in vitro. *Exp Neurol* 2005; 194: 243.

34. Poimenidi E, Hatziapostolou M, Papadimitriou E. Serum stimulates Pleiotrophin gene expression in an AP-1-dependent manner in human endothelial and glioblastoma cells. *Anticancer Res* 2009; 29: 349.

35. Polytarchou C, Hatziapostolou M, Papadimitriou E. Hydrogen peroxide stimulates proliferation and migration of human prostate cancer cells through activation of activator protein-1 and up-regulation of the heparin affin regulatory peptide gene. *J Biol Chem* 2005; 280: 40428.

36. Polytarchou C, Hatziapostolou M, Poimenidi E, *et al.* Nitric oxide stimulates migration of human endothelial and prostate cancer cells through up-regulation of pleiotrophin expression and its receptor protein tyrosine phosphatase beta/zeta. *Int J Cancer* 2009; 124: 1785.

37. Brunet-de Carvalho N, Raulais D, Rauvala H, Souttou B, Vigny M. HB-GAM/Pleiotrophin and midkine are differently expressed and distributed during retinoic acid-induced neural differentiation of P19 cells. *Growth Factors* 2003; 21: 139.

38. Chen H, Rubin E, Zhang H, *et al.* Identification of transcriptional targets of HOXA5. *J Biol Chem* 2005; 280: 19373.

39. Li G, Hu Y, Huo Y, *et al.* PTEN deletion leads to up-regulation of a secreted growth factor pleiotrophin. *J Biol Chem* 2006; 281: 10663.

40. Rauvala H, Vanhala A, Castren E, *et al.* Expression of HB-GAM (heparin-binding growth-associated molecules) in the pathways of developing axonal processes in vivo and neurite outgrowth in vitro induced by HB-GAM. *Brain Res Dev Brain Res* 1994; 79: 157.

41. Takeda A, Onodera H, Sugimoto A, *et al.* Induction of heparin-binding growth-associated molecule expression in reactive astrocytes following hippocampal neuronal injury. *Neuroscience* 1995; 68: 57.

42. Silos-Santiago I, Yeh HJ, Gurrieri MA, *et al.* Localization of pleiotrophin and its mRNA in subpopulations of neurons and their corresponding axonal tracts suggests important roles in neural-glial interactions during development and in maturity. *J Neurobiol* 1996; 31: 283.

43. Yeh HJ, He YY, Xu J, Hsu CY, Deuel TF. Upregulation of pleiotrophin gene expression in developing microvasculature, macrophages, and astrocytes after acute ischemic brain injury. *J Neurosci* 1998; 18: 3699.

44. Pavlov I, Voikar V, Kaksonen M, *et al.* Role of heparin-binding growth-associated molecule (HB-GAM) in hippocampal LTP and spatial learning revealed by studies on overexpressing and knockout mice. *Mol Cell Neurosci* 2002; 20: 330.

45. Hida H, Jung CG, Wu CZ, *et al.* Pleiotrophin exhibits a trophic effect on survival of dopaminergic neurons in vitro. *Eur J Neurosci* 2003; 17: 2127.

46. Hida H, Masuda T, Sato T, Kim TS, Misumi S, Nishino H. Pleiotrophin promotes functional recovery after neural transplantation in rats. *Neuroreport* 2007; 18: 179.

47. Hienola A, Pekkanen M, Raulo E, Vanttola P, Rauvala H. HB-GAM inhibits proliferation and enhances differentiation of neural stem cells. *Mol Cell Neurosci* 2004; 26: 75.

48. Jung CG, Hida H, Nakahira K, Ikenaka K, Kim HJ, Nishino H. Pleiotrophin mRNA is highly expressed in neural stem (progenitor) cells of mouse ventral mesencephalon and the product promotes production of dopaminergic neurons from embryonic stem cell-derived nestin-positive cells. *FASEB J* 2004; 18: 1237.

49. Blondet B, Carpentier G, Lafdil F, Courty J. Pleiotrophin cellular localization in nerve regeneration after peripheral nerve injury. *J Histochem Cytochem* 2005; 53: 971.

50. Gramage E, Alguacil LF, Herradon G. Pleiotrophin prevents cocaine-induced toxicity in vitro. *Eur J Pharmacol* 2008; 595: 35.

51. del Olmo N, Gramage E, Alguacil LF, Pérez-Pinera P, Deuel TF, Herradón G. Pleiotrophin inhibits hippocampal long-term potentiation: a role of pleiotrophin in learning and memory. *Growth Factors* 2009; 27: 189.

52. Wisniewski T, Lalowski M, Baumann M, *et al.* HB-GAM is a cytokine present in Alzheimer's and Down's syndrome lesions. *Neuroreport* 1996; 7: 667.

53. Vazin T, Becker KG, Chen J, *et al.* A novel combination of factors, termed SPIE, which promotes dopaminergic neuron differentiation from human embryonic stem cells. *PLoS One* 2009; 4: e6606.

54. Peng HB, Ali AA, Dai Z, Daggett DF, Raulo E, Rauvala H. The role of heparin-binding growth-associated molecule (HB-GAM) in the postsynaptic induction in cultured muscle cells. *J Neurosci* 1995; 5: 3027.

55. Caruelle D, Mazouzi Z, Husmann I, *et al.* Upregulation of HARP during in vitro myogenesis and rat soleus muscle regeneration. *J Muscle Res Cell Motil* 2004; 25: 45.

56. Milhiet PE, Vacherot F, Caruelle JP, Barritault D, Caruelle D, Courty J. Upregulation of the angiogenic factor heparin affin regulatory peptide by progesterone in rat uterus. *J Endocrinol* 1998; 158: 389.

57. Mitsiadis TA, Salmivirta M, Muramatsu T, *et al.* Expression of the heparin-binding cytokines, midkine (MK) and HB-GAM (pleiotrophin) is associated with epithelial-mesenchymal interactions during fetal development and organogenesis. *Development* 1995; 121: 37.

58. Chen HW, Yu SL, Chen WJ, *et al.* Dynamic changes of gene expression profiles during postnatal development of the heart in mice. *Heart* 2004; 90: 927.

59. Asakura M, Kitakaze M. Global gene expression profiling in the failing myocardium. *Circ J* 2009; 73: 1568.

60. Li J, Wei H, Chesley A, *et al.* The pro-angiogenic cytokine pleiotrophin potentiates cardiomyocyte apoptosis through inhibition of endogenous AKT/PKB activity. *J Biol Chem* 2007; 282: 34984.

61. Asahina K, Sato H, Yamasaki C, *et al.* Pleiotrophin/Heparin-Binding Growth-Associated Molecule as a mitogen of rat hepatocytes and its role in regeneration and development of liver. *Am J Pathol* 2002; 160: 2191.

62. Sakurai H, Bush KT, Nigam SK. Identification of pleiotrophin as a mesenchymal factor involved in ureteric bud branching morphogenesis. *Development* 2001; 128: 3283.

63. Weng T, Gao L, Bhaskaran M, *et al.* Pleiotrophin regulates lung epithelial cell proliferation and differentiation during fetal lung development via {beta}-catenin and Dlk1. *J Biol Chem* 2009 (Epub ahead of print).

64. Zou P, Muramatsu H, Sone M, Hayashi H, Nakashima T, Muramatsu T. Mice doubly deficient in the midkine and pleiotrophin genes exhibit deficits in the expression of beta-tectorin gene and in auditory response. *Lab Invest* 2006; 86: 645.

65. Muramatsu H, Zou P, Kurosawa N, *et al.* Female infertility in mice deficient in midkine and pleiotrophin, which form a distinct family of growth factors. *Genes Cells* 2006; 11: 1405.

66. Ledoux D, Caruelle D, Sabourin JC, *et al.* Cellular distribution of the angiogenic factor heparin affin regulatory peptide (HARP) mRNA and protein in the human mammary gland. *J Histochem Cytochem* 1997; 45: 1239.

67. Kouros-Mehr H, Werb Z. Candidate regulators of mammary branching morphogenesis identified by genome-wide transcript analysis. *Dev Dyn* 2006; 235: 3404.

68. Chung HW, Wen Y, Choi EA, Hao-Li *et al.* Pleiotrophin (PTN) and midkine (MK) mRNA expression in eutopic and ectopic endometrium in advanced stage endometriosis. *Mol Hum Reprod* 2002; 8: 350.

69. Qian A, Meals RA, Rajfer J, Gonzales-Cadavid NF. Comparison of gene expression profiles between Peuronie's disease and Dupuytren's contracture. *Urology* 2004; 64: 99.

70. Vernet D, Nolazco G, Cantini L, *et al.* Evidence that osteogenic progenitor cells in the human tunica albuginea may originate from stem cells: implications for Peyronie disease. *Biol Reprod* 2005; 73: 1199.

71. Zhang N, Yeh HJ, Zhong R, Li YS, Deuel TF. A dominant-negative pleiotrophin mutant introduced by homologous recombination leads to germ-cell apoptosis in male mice. *Proc Natl Acad Sci U S A* 1999; 96: 6734.

72. Zhou H-Y, Ohnuma Y, Takita H, Fujisawa R, Mizuno M, Kuboki Y. Effects of a bone lysine-rich 18KDa protein on osteoblast-like MC3T3-E1 cells. *Biochem Biophys Res Commun* 1992; 186: 1288.

73. Imai S, Kaksonen M, Raulo E, *et al.* Osteoblast recruitment and bone formation enhanced by cell matrix-associated heparin-binding growth-associated molecule (HB-GAM). *J Cell Biol* 1998; 143: 1113.

74. Yang X, Tare RS, Partridge KA, *et al.* Induction of human osteoprogenitor chemotaxis, proliferation, differentiation, and bone formation by osteoblast stimulating factor-1/pleiotrophin: osteoconductive biomimetic scaffolds for tissue engineering. *J Bone Miner Res* 2003; 18: 47.

75. Petersen W, Rafii M. Immunolocalization of the angiogenetic factor pleiotrophin (PTN) in the growth plate of mice. *Arch Orthop Trauma Surg* 2001; 121: 414.

76. Sato Y, Takita H, Ohata N, Tamura M, Kuboki Y. Pleiotrophin regulates bone morphogenetic protein (bmp)-induced ectopic osteogenesis. *J Biochem* 2002; 131: 877.

77. Xing W, Baylink D, Kesavan C, *et al.* Global gene expression analysis in the bones reveals involvement of several novel genes and pathways in mediating an anabolic response of mechanical loading in mice. *J Cell Biochem* 2005; 96: 1049.

78. Tare RS, Oreffo ROC, Sato K, Rauvala H, Clarke NMP, Roach HI. Effects of targeted overexpression of pleiotrophin on postnatal bone development. *Biochem Biophys Res Commun* 2002; 298: 324.

79. Lehmann W, Schinke T, Schilling AF, *et al.* Absence of mouse pleiotrophin does not affect bone formation in vivo. *Bone* 2004; 35: 1247.

80. Imai S, Heino TJ, Hienola A, *et al.* Osteocyte-derived HB-GAM (pleiotrophin) is associated with bone formation and mechanical loading. *Bone* 2009; 44: 785.

81. Dreyfus J, Brunet-de Carvalho N, Duprez D, Raulais D, Vigny M. HB-GAM/pleiotrophin: localization of mRNA and protein in the chicken developing leg. *Int J Dev Biol* 1998; 42: 189.

82. Neame PJ, Young CN, Brock CW, *et al.* Pleiotrophin is an abundant protein in dissociative extracts of bovine fetal epiphyseal cartilage and nasal cartilage from newborns. *J Orthop Res* 1993; 11: 479.

83. Tapp H, Hernandez DJ, Neame PJ, Koob TJ. Pleiotrophin inhibits chondrocyte proliferation and stimulates proteoglycan synthesis in mature bovine cartilage. *Matrix Biol* 1999; 18: 543.

84. Pufe T, Bartscher M, Petersen W, Tillmann B, Mentlein R. Pleiotrophin, an embryonic differentiation and growth factor, is expressed in osteoarthritis. *Osteoarthritis Cartilage* 2003; 11: 260.

85. Sanchez C, Deberg MA, Piccardi N, Msika P, Reginster JY, Henrotin YE. Subchondral bone osteoblasts induce phenotypic changes in human osteoarthritic chondrocytes. *Osteoarthritis Cartilage* 2005; 13: 988.

86. Lupu R, Wellstein A, Sheridan J, *et al.* Purification and characterization of a novel growth factor from human breast cancer cells. *Biochemistry* 1992; 31: 7330.

87. Fang W, Hartmann N, Chow DT, Riegel AT, Wellstein A. Pleiotrophin stimulates fibroblasts and endothelial and epithelial cells and is expressed in human cancer. *J Biol Chem* 1992; 267: 25889.

88. Wellstein A, Fang WJ, Khatri A, *et al.* A heparin-binding growth factor secreted from breast cancer cells homologous to a developmentally regulated cytokine. *J Biol Chem* 1992; 267: 2582.

89. Zhang N, Zhong R, Wang ZY, Deuel TF. Human breast cancer growth inhibited in vivo by a dominant negative pleiotrophin mutant. *J Biol Chem* 1997; 272: 16733.

90. Vacherot F, Caruelle D, Chopin D, *et al.* Involvement of heparin affin regulatory peptide in human prostate cancer. *Prostate* 1999; 38: 126.

91. Hatziapostolou M, Delbe J, Katsoris P, Polytarchou C, Courty J, Papadimitriou E. Heparin affin regulatory peptide is a key player in prostate cancer cell growth and angiogenicity. *Prostate* 2005; 65: 151.

92. Jager R, Noll K, Havemann K, *et al.* Differential expression and biological activity of the Heparin-Binding Growth-Associated Molecule (HB-GAM) in lung cancer cell lines. *Int J Cancer* 1997; 73: 537.

93. Schulte AM, Lai S, Kurtz A, Czubayko F, Riegel AT, Wellstein A. Human trophoblast and choriocarcinoma expression of the growth factor pleiotrophin attributable to germ-line insertion of an endogenous retrovirus. *Proc Natl Acad Sci U S A* 1996; 93: 14759.

94. Czubayko F, Schulte AM, Berchem GJ, Wellstein A. Melanoma angiogenesis and metastasis modulated by ribozyme targeting of the secreted growth factor pleiotrophin. *Proc Natl Acad Sci U S A* 1996; 93: 14753.

95. Mentlein R, Held-Feindt J. Pleiotrophin, an angiogenic and mitogenic growth factor, is expressed in human gliomas. *J Neurochem* 2002; 83: 747.

96. Zhang L, Mabuchi T, Satoh E, Maeda S, Nukui H, Naganuma H. Overexpression of heparin-binding growth-associated molecule in malignant glioma cells. *Neurol Med Chir (Tokyo)* 2004; 44: 637.

97. Lu KV, Jong KA, Kim GY, *et al.* Differential induction of glioblastoma migration and growth by two forms of Pleiotrophin. *J Biol Chem* 2005; 280: 26953.

98. Grzelinski M, Bader N, Czubayko F, Aigner F. Ribozyme-targeting reveals the rate-limiting role of pleiotrophin in glioblastoma. *Int J Cancer* 2005; 117: 942.

99. Weber D, Klomp HJ, Czubayko F, Wellstein A, Juhl H. Pleiotrophin can be rate-limiting for pancreatic cancer cell growth. *Cancer Res* 2000; 60: 5284.

100. Chen H, Gordon M, Campbell R, *et al.* Pleiotrophin is highly expressed by myeloma cells and promotes myeloma tumor growth. *Blood* 2007; 110: 287.

101. Tong Y, Mentlein R, Buhl R, *et al.* Overexpression of midkine contributes to anti-apoptotic effects in human meningiomas. *J Neurochem* 2007; 100: 1097.

102. Chang Y, Berenson J, Wang Z, Deuel TF. Dominant negative pleiotrophin induces tetraploidy and aneuploidy in U87MG human glioblastoma cells. *Biochem Biophys Res Commun* 2006; 351: 336.

103. Perez-Pinera P, Alcantara S, Dimitrov T, Vega JA, Deuel TF. Pleiotrophin disrupts calcium-dependent homophilic cell-cell adhesion and initiates an epithelial-mesenchymal transition. *Proc Natl Acad Sci U S A* 2006; 103: 17795.

104. Park TJ, Jeong BR, Tateno C, *et al.* Pleiotrophin inhibits transforming growth factor beta1-induced apoptosis in hepatoma cell lines. *Mol Carcinog* 2008; 47: 784.

105. Chang Y, Zuka M, Perez-Pinera P, *et al.* Secretion of pleiotrophin stimulates breast cancer progression through remodeling of the tumor microenvironment. *Proc Natl Acad Sci U S A* 2007; 104: 10888.

106. Yamakawa T, Kurosawa N, Kadomatsu K, *et al.* Levels of expression of pleiotrophin and protein tyrosine phosphatase zeta are decreased in human colorectal cancers. *Cancer Lett* 1999; 135: 91.

107. Corbley MJ. Transformation by Ras suppresses expression of the neurotrophic growth factor pleiotrophin. *J Biol Chem* 1997; 272: 24696.

108. Merenmies J. Cell density-dependent expression of heparin-binding growth-associated molecule (HB-GAM, p18) and its down-regulation by fibroblast growth factors. *FEBS Letters* 1992; 307: 297.

109. Papadimitriou E, Polykratis A, Hatziapostolou M, Parthymou A, Polytarchou C, Mikelis C. Heparin affin regulatory peptide: a new target for tumour therapy? *Curr Cancer Drug Targets* 2004; 4: 471.

110. Calvet L, Geoerger B, Regairaz M, *et al.* Pleiotrophin, a candidate gene for poor tumor vasculature and in vivo neuroblastoma sensitivity to irinotecan. *Oncogene* 2006; 25: 3150.

111. Parthymou A, Lampropoulou E, Mikelis C, Drosou G, Papadimitriou E. Heparin affin regulatory peptide/pleiotrophin negatively affects diverse biological activities in C6 glioma cells. *Eur J Cell Biol* 2008; 87: 17.

112. Mikelis C, Sfaelou E, Koutsioumpa M, Kieffer N, Papadimitriou E. Integrin alpha(v)beta(3) is a pleiotrophin receptor required for pleiotrophin-induced endothelial cell migration through receptor protein tyrosine phosphatase beta/zeta. *FASEB J* 2009; 23: 1459.

113. Papadimitriou E, Heroult M, Courty J, Polykratis A, Stergiou C, Katsoris P. Endothelial cell proliferation induced by HARP: Implication of N or C terminal peptides. *Biochem Biophys Res Commun* 2000; 274: 242.

114. Papadimitriou E, Polykratis A, Courty J, Koolwijk P, Heroult M, Katsoris P. HARP Induces Angiogenesis in Vivo and in Vitro: Implication of N or C terminal peptides. *Biochem Biophys Res Commun* 2001; 282: 306.

115. Souttou B, Raulais D, Vigny M. Pleiotrophin induces angiogenesis: involvement of the phosphoinositide-3 kinase but not the nitric oxide synthase pathways. *J Cell Physiol* 2001; 187: 59.

116. Polykratis A, Katsoris P, Courty J, Papadimitriou E. Characterization of heparin affin regulatory peptide signaling in human endothelial cells. *J Biol Chem* 2005; 280: 22454.

117. Heiss C, Wong ML, Block VI, *et al.* Pleiotrophin induces nitric oxide dependent migration of endothelial progenitor cells. *J Cell Physiol* 2008; 215: 366.

118. Magnusson PU, Dimberg A, Mellberg S, Lukinius A, Claesson-Welsh L. FGFR-1 regulates angiogenesis through cytokines interleukin-4 and pleiotrophin. *Blood* 2007; 110: 4214.

119. Sharifi BG, Zeng Z, Wang L, *et al.* Pleiotrophin induces transdifferentiation of monocytes into functional endothelial cells. *Arterioscler Thromb Vasc Biol* 2006; 26: 1273.

120. Chen H, Campbell RA, Chang Y, *et al.* Pleiotrophin produced by multiple myeloma induces transdifferentiation of monocytes into vascular endothelial cells: a novel mechanism of tumor-induced vasculogenesis. *Blood* 2009; 113: 1992.

121. Collino F, Revelli A, Massobrio M, *et al.* Epithelial-mesenchymal transition of ovarian tumor cells induces an angiogenic monocyte cell population. *Exp Cell Res* 2009 (Epub ahead of print).

122. Choudhuri R, Zhang HT, Donnini S, Ziche M, Bicknell A. An Angiogenic Role for the neurokines midkine and pleiotrophin in tumorigenesis. *Cancer Res* 1997; 57: 1814.

123. Heroult M, Bernard-Pierrot I, Delbe J, *et al.* Heparin affin regulatory peptide binds to vascular endothelial growth factor (VEGF) and inhibits VEGF-induced angiogenesis. *Oncogene* 2004; 23: 1745.

124. Polykratis A, Delbe J, Courty J, Papadimitriou E, Katsoris P. Identification of heparin affin regulatory peptide domains with potential role on angiogenesis. *Int J Biochem Cell Biol* 2004; 36: 1954.

125. Kokolakis G, Mikelis C, Papadimitriou E, Courty J, Karetsou E, Katsoris P. Effect of heparin affin regulatory peptide on the expression of vascular endothelial growth factor receptors in endothelial cells. *In Vivo* 2006; 20: 629.

126. Chauhan AK, Li YS, Deuel TF. Pleiotrophin transforms NIH 3T3 cells and induces tumors in nude mice. *Proc Natl Acad Sci U S A* 1993; 90: 679.

127. Zhang N, Zhong R, Perez-Pinera P, *et al.* Identification of the angiogenesis signaling domain in pleiotrophin defines a mechanism of the angiogenic switch. *Biochem Biophys Res Commun* 2006; 343: 653.

128. Grzelinski M, Urban-Klein B, Martens T, *et al.* RNA interference-mediated gene silencing of pleiotrophin through polyethylenimine-complexed small interfering RNAs in vivo exerts antitumoral effects in glioblastoma xenografts. *Hum Gene Ther* 2006; 17: 751.

129. Hamma-Kourbali Y, Bernard-Pierrot I, Heroult M, *et al.* Inhibition of the mitogenic, angiogenic and tumorigenic activities of pleiotrophin by a synthetic peptide corresponding to its C-thrombospondin repeat-I domain. *J Cell Physiol* 2008; 214: 250.

130. Bernard-Pierrot I, Delbe J, Caruelle D, Barritault D, Courty J, Milhiet PE. The lysine-rich c-terminal tail of heparin affin regulatory peptide is required for mitogenic and tumor formation activities. *J Biol Chem* 2001; 276: 12228.

131. Ducès A, Karaky R, Martel-Renoir D, *et al.* 16-kDa fragment of pleiotrophin acts on endothelial and breast tumor cells and inhibits tumor development. *Mol Cancer Ther* 2008; 7: 2817.

132. Bernard-Pierrot I, Delbe J, Rouet V, *et al.* Dominant negative effectors of heparin affin regulatory peptide (HARP) angiogenic and transforming activities. *J Biol Chem* 2002; 277: 32071.

133. Bermek O, Diamantopoulou Z, Polykratis A, *et al.* A basic peptide derived from the HARP C-terminus inhibits anchorage-independent growth of DU145 prostate cancer cells. *Exp Cell Res* 2007; 313: 4041.

134. Raulo E, Chernousov MA, Carey DJ, Nolo R, Rauvala H. Isolation of a neuronal cell surface receptor of heparin binding growth-associated molecule (HB-GAM). *J Biol Chem* 1994; 269: 12999.

135. Kinnunen T, Kaksonen M, Saarinen J, Kalkkinen N, Peng HB, Rauvala H. Cortactin-Src kinase signaling pathway is involved in N-syndecan-dependent neurite outgrowth. *J Biol Chem* 1998; 273: 10702.

136. Raulo E, Tumova S, Pavlov I, *et al.* The two thrombospondin type I repeat domains of the heparin-binding growth-associated molecule bind to heparin/heparan sulfate and regulate neurite extension and plasticity in hippocampal neurons. *J Biol Chem* 2005; 280: 41576.

137. Raulo E, Tumova S, Pavlov I, *et al.* The Two Thrombospondin type I repeat domains of HB-GAM display a cooperative function in N-syndecan binding and regulation of synaptic plasticity. *ScientificWorldJournal* 2006; 6: 406.

138. Nolo R, Kaksonen M, Raulo E, Rauvala H. Co-expression of heparin-binding growth-associated molecule (HB-GAM) and N-syndecan (syndecan-3) in developing rat brain. *Neurosci Lett* 1995; 19: 39.

139. Iseki K, Hagino S, Mori T, *et al.* Increased syndecan expression by pleiotrophin and FGF receptor- expressing astrocytes in injured brain tissue. *Glia* 2002; 39: 1.

140. Landgraf P, Wahle P, Pape HC, Gundelfinger ED, Kreutz MR. The Survival-promoting Peptide Y-P30 Enhances Binding of Pleiotrophin to Syndecan-2 and -3 and Supports Its Neuritogenic Activity. *J Biol Chem* 2008; 283: 25036.

141. Morris SW, Kirstein MN, Valentine MB, *et al.* Fusion of a kinase gene ALK, to a nucleolar protein gene NPM, in non-Hodgkin's lymphoma. *Science* 1994; 263: 1281.

142. Iwahara T, Fujimoto J, Wen D, *et al.* Molecular characterization of ALK, a receptor tyrosine kinase expressed specifically in the nervous system. *Oncogene* 1997; 14: 439.

143. Morris SW, Naeve C, Mathew P, *et al.* ALK, the chromosome 2 gene locus altered by the t(2;5) in non-Hodgkin's lymphoma, encodes a novel neural receptor tyrosine kinase that is highly related to leukocyte tyrosine kinase (LTK). *Oncogene* 1997; 14: 2175.

144. Stoica GE, Kuo A, Aigner A, *et al.* Identification of anaplastic lymphoma kinase as a receptor for the growth factor pleiotrophin. *J Biol Chem* 2001; 276: 16772.

145. Powers C, Aigner A, Stoica GE, McDonnell K, Wellstein A. Pleiotrophin signaling through anaplastic lymphoma kinase is rate-limiting for glioblastoma growth. *J Biol Chem* 2002; 277: 14153.

146. Bowden ET, Stoica GE, Wellstein A. Anti-apoptotic signaling of pleiotrophin through its receptor, anaplastic lymphoma kinase. *J Biol Chem* 2002; 277: 35862.

147. Stylianou DC, Auf der Maur A, Kodack DP, *et al.* Effect of single-chain antibody targeting of the ligand-binding domain in the anaplastic lymphoma kinase receptor. *Oncogene* 2009 (Epub ahead of print).

148. Hausser HJ, Brenner RE. Phenotypic instability of Saos-2 cells in long-term culture. *Biochem Biophys Res Commun* 2005; 333: 216.

149. Perez-Pinera P, Chang Y, Astudillo A, Mortimer J, Deuel TF. Anaplastic lymphoma kinase is expressed in different subtypes of human breast cancer. *Biochem Biophys Res Commun* 2007; 358: 399.

150. Dirks WG, Fahnrich S, Lis Y, Becker E, Macleodand RAF, Drexler HG. Expression and functional analysis of the anaplastic lymphoma kinase (ALK) gene in tumor cell lines. *Int J Cancer* 2002; 100: 49.

151. Lamant L, Pulford K, Bischof D, *et al.* Expression of the ALK tyrosine kinase gene in neuroblastoma. *Am J Pathol* 2000; 156: 1711.

152. Delsol G, Lamant L, Mariame B, Pulford K, Dastugue N, Brousset P. A new subtype of large B-cell lymphoma expressing the ALK kinase and lacking the 2; 5 translocation. *Blood* 1997; 89: 1483.

153. Li X-Q, Hisaoka M, Shi DR, Zhu XZ, Hashimoto H. Expression of anaplastic lymphoma kinase in soft tissue tumors: An immunohistochemical and molecular study of 249 cases. *Hum Pathol* 2004; 35: 711.

154. Miyake I, Hakomori Y, Shinohara A, *et al.* Activation of anaplastic lymphoma kinase is responsible for hyperphosphorylation of ShcC in neuroblastoma cell lines. *Oncogene* 2002; 21: 5823.

155. Moog-Lutz C, Degoutin J, Gouzi JY, *et al.* Activation and inhibition of anaplastic lymphoma kinase receptor tyrosine kinase by monoclonal antibodies and absence of agonist activity of pleiotrophin. *J Biol Chem* 2005; 280: 26039.

156. Motegi A, Fujimoto J, Kotani M, Sakuraba H, Yamamoto T. ALK receptor tyrosine kinase promotes cell growth and neurite outgrowth. *J Cell Sci* 2004; 117: 3319.

157. Mourali J, Bénard A, Lourenço FC, *et al.* Anaplastic lymphoma kinase is a dependence receptor whose proapoptotic functions are activated by caspase cleavage. *Mol Cell Biol* 2006; 26: 6209.

158. Mathivet T, Mazot P, Vigny M. In contrast to agonist monoclonal antibodies, both C-terminal truncated form and full length form of pleiotrophin failed to activate vertebrate ALK (anaplastic lymphoma kinase). *Cell Signal* 2007; 19: 2434.

159. Perez-Pinera P, Zhang W, Chang Y, Vega JA, Deuel TF. Anaplastic lymphoma kinase is activated through the pleiotrophin/receptor protein-tyrosine phosphatase beta/zeta signaling pathway: an alternative mechanism of receptor tyrosine kinase activation. *J Biol Chem* 2007; 282: 28683.

160. Krueger NX, Saito H. A human transmembrane protein-tyrosine-phosphatase, PTP zeta, is expressed in brain and has an N-terminal receptor domain homologous to carbonic anhydrases. *Proc Natl Acad Sci U S A* 1992; 89: 7417.

161. Levy JB, Canoll PD, Silvennoinen O, *et al.* The cloning of a receptor-type protein tyrosine phosphatase expressed in the central nervous system. *J Biol Chem* 1993; 268: 10573.

162. Maeda N, Nishiwaki T, Shintani T, Hamanaka H, Noda M. 6B4 Proteoglycan/Phosphacan, an Extracellular Variant of Receptor-like Protein-tyrosine Phosphatase/RPTP, Binds Pleiotrophin/Heparin-binding Growth-associated Molecule (HB-GAM). *J Biol Chem* 1996; 271: 21446.

163. Garwood J, Heck N, Reichardt F, Faissner A. Phosphacan short isoform, a novel non-proteoglycan variant of phosphacan/receptor protein tyrosine phosphatase-beta, interacts with neuronal receptors and promotes neurite outgrowth. *J Biol Chem* 2003; 278: 24164.

164. Maurel P, Rauch U, Flad M, Margolis RK, Margolis RU. Phosphacan, a chondroitin sulfate proteoglycan of brain that interacts with neurons and neural cell-adhesion molecules, is an extracellular variant of a receptor-type protein tyrosine phosphatase. *Proc Natl Acad Sci U S A* 1994; 91: 2512.

165. Maeda N, He J, Yajima Y, Mikami T, Sugahara K, Yabe T. Heterogeneity of the Chondroitin Sulfate Portion of Phosphacan/6B4 Proteoglycan Regulates Its Binding Affinity for Pleiotrophin/Heparin Binding Growth-associated Molecule. *J Biol Chem* 2003; 278: 35805.

166. Chow JP, Fujikawa A, Shimizu H, Suzuki R, Noda M. Metalloproteinase and gamma-secretase mediated cleavage of protein tyrosine phosphatase receptor type Z. *J Biol Chem* 2008; 283: 30879.

167. Chow JP, Fujikawa A, Shimizu H, Noda M. Plasmin-mediated processing of protein tyrosine phosphatase receptor type Z in the mouse brain. *Neurosci Lett* 2008; 442: 208.

168. Meng K, Rodriguez-Pena A, Dimitrov T, *et al.* Pleiotrophin signals increased tyrosine phosphorylation of beta beta-catenin through inactivation of the intrinsic catalytic activity of the receptor-type protein tyrosine phosphatase beta/zeta. *Proc Natl Acad Sci U S A* 2000; 97: 2603.

169. Pariser H, Herradon G, Ezquerra L, Perez-Pinera P, Deuel TF. Pleiotrophin regulates serine phosphorylation and the cellular distribution of beta-adducin through activation of protein kinase C. *Proc Natl Acad Sci U S A* 2005; 102: 12407.

170. Pariser H, Ezquerra L, Herradon G, Perez-Pinera P, Deuel TF. Fyn is a downstream target of the pleiotrophin/receptor protein tyrosine phosphatase beta/zeta-signaling pathway: regulation of tyrosine phosphorylation of Fyn by pleiotrophin. *Biochem Biophys Res Commun* 2005; 332: 664.

171. Vacherot F, Delbe J, Heroult M, Barritault D, Fernig DG, Courty J. Glycosaminoglycans differentially bind HARP and modulate its biological activity. *J Biol Chem* 1999; 274: 7741.

172. Bernard-Pierrot I, Heroult M, Lemaitre G, Barritault D, Courty J, Milhiet PE. Glycosaminoglycans promote HARP/PTN dimerization. *Biochem Biophys Res Commun* 1999; 266: 437.

173. Maeda N, Fukazawa N, Hata T. The binding of chondroitin sulfate to pleiotrophin/heparin-binding growth-associated molecule is regulated by chain length and oversulfated structures. *J Biol Chem* 2006; 281: 4894.

174. Deepa SS, Umehara Y, Higashiyama S, Itoh N, Sugahara K. Specific molecular interactions of oversulfated chondroitin sulfate E with various heparin-binding growth factors. Implications as a physiological binding partner in the brain and other tissues. *J Biol Chem* 2002; 277: 43707.

175. Xingfeng B, Nishimura S, Mikami T, Yamada S, Itoh N, Sugahara K. Chondroitin sulfate/dermatan sulfate hybrid chains from embryonic pig brain, which contain a higher proportion of l-iduronic acid than those from adult pig brain, exhibit neuritogenic and growth factor binding activities. *J Biol Chem* 2004; 279: 9765.

176. Bao X, Mikami T, Yamada S, Faissner A, Muramatsu T, Sugahara K. Heparin-binding growth factor, pleiotrophin, mediates neuritogenic activity of embryonic pig brain-derived chondroitin sulfate/dermatan sulfate hybrid chains. *J Biol Chem* 2005; 280: 9180.

177. Li F, Shetty AK, Sugahara K. Neuritogenic activity of chondroitin/dermatan sulfate hybrid chains of embryonic pig brain and their mimicry from shark liver. Involvement of the pleiotrophin and hepatocyte growth factor signaling pathways. *J Biol Chem* 2007; 282: 2956.

178. Malavaki C, Mizumoto S, Karamanos N, Sugahara K. Recent advances in the structural study of functional chondroitin sulfate and dermatan sulfate in health and disease. *Connect Tissue Res* 2008; 49: 133.

179. Zou K, Muramatsu H, Ikematsu S, *et al.* A heparin-binding growth factor, midkine, binds to a chondroitin sulfate proteoglycan, PG-M/versican. *Eur J Biochem* 2000; 267: 4046.

180. Umehara Y, Yamada S, Nishimura S, Shioi J, Robakis NK, Sugahara K. Chondroitin sulfate of appican, the proteoglycan form of amyloid precursor protein, produced by C6 glioma cells interacts with heparin-binding neuroregulatory factors. *FEBS Lett* 2004; 557: 233.

181. Bao X, Muramatsu T, Sugahara K. Demonstration of the pleiotrophin-binding oligosaccharide sequences isolated from chondroitin sulfate/dermatan sulfate hybrid chains of embryonic pig brains. *J Biol Chem* 2005; 280: 35318.

182. Take M, Tsutsui J, Obama M, *et al.* Identification of nucleolin as a binding protein for midkine (MK) and heparin-binding growth associated molecule (HB-GAM). *J Biochem* 1994; 116: 1063.

183. Christian S, Pilch J, Akerman ME, Porkka K, Laakkonen P, Ruoslahti E. Nucleolin expressed at the cell surface is a marker of endothelial cells in angiogenic blood vessels. *J Cell Biol* 2003; 163: 871.

184. Destouches D, Khoury D, Hamma-Kourbali Y, *et al.* Suppression of tumor growth and angiogenesis by a specific antagonist of the cell-surface expressed nucleolin. *PLoS ONE* 2008; 3: e2518.

185. Joo EJ, ten Dam GB, van Kuppevelt TH, Toida T, Linhardt RJ, Kim YS. Nucleolin: acharan sulfate-binding protein on the surface of cancer cells. *Glycobiology* 2005; 15: 1.

186. Said EA, Courty J, Svab J, Delbe J, Krust B, Hovanessian AG. Pleiotrophin inhibits HIV infection by binding the cell surface-expressed nucleolin. *FEBS J* 2005; 272: 4646.