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On the role of pleiotrophin and its receptors in development and angiogenesis

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ABSTRACT The secreted growth factor pleiotrophin (PTN) is expressed in all species and is evolutionarily highly conserved, suggesting that it plays a significant role in the regulation of important processes. The observation that it is highly expressed at early stages during development and in embryonic progenitor cells highlights a potentially important contribution to development. There is ample evidence of the role of PTN in the development of the nervous system and hematopoiesis, some, albeit inconclusive, evidence of its role in the skeletomuscular system, and limited evidence of its role in the development of other organs. Studies on its role in the cardiovascular system and angiogenesis suggest that PTN has a significant regulatory effect by acting on endothelial cells, while its role in the functions of smooth or cardiac muscle cells has not been studied. This review highlights what is known to date regarding the role of PTN in the development of various organs and in angiogenesis. Wherever possible, evidence on the crosstalk between the receptors that mediate PTN's functions is also quoted, highlighting the complex regulatory pathways that affect development and angiogenesis.

KEYWORDS: angiogenesis, development, growth factors, pleiotrophin, receptors

Introduction

The secreted growth factor pleiotrophin (PTN) was initially isolated from bovine uterus and fetal mouse brain by different groups in 1989. It was given several different names, but the one that dominated and is currently being universally used is PTN (Fig. 1).

PTN is highly conserved among species and shows high amino acid homology with midkine (MK), the other member of the same family of heparin-binding proteins. The mature peptide consists of 136 amino acids, following cleavage of the signal peptide, and contains 24% cationic amino acids mainly at the amino- and carboxy-terminals, as well as 10 cysteines involved in the formation of 5 disulfide bonds. PTN comprises of two β-structures linked by a flexible region. Each β-structure contains three antiparallel β-folds, homologous to the thrombospondin type I repeat (TSR-1) motif. The ends of the molecule have numerous basal amino acids and lack configuration. The carboxy terminus of PTN has been shown to be responsible for its interaction with chondroitin sulfate chains. The human *ptn* gene has been identified on chromosome 7, in arm q33, is at least 42 kb and contains 7 exons. The open reading frame includes 4 exons, mostly exons 3 and 4, and the borders between introns/exons are well maintained. Different PTN isoforms have been suggested but have not been studied. Despite high homology in the coding region, the 3′ and 5′ untranslated regions differ between species. The promoter of the human *ptn* gene contains response elements for myogenic differentiation factor 1, trihelix

Abbreviations used in this paper: ALK, anaplastic lymphoma kinase; CAH, carbonic anhydrase domain; cAMP, Cyclic adenosine monophosphate; c-Src, proto-oncogene tyrosine-protein kinase cellular sarcoma; ERK1/2, extracellular signal-regulated kinases; FNIII, fibronectin type III domain; GR, glycine-rich domain; HARP, heparin affin-regulatory peptide; HB-GAM, heparin binding growth-associated molecule; HBGF-8, heparin binding growth factor-8; HBNF, heparin binding neurotrophic factor; HSCs, hematopoietic stem cells; MAM, meprin A-5, μ domain; MK, midkine; NCL, nucleolin; NK cells, natural killer cells; OSF-1, osteoblast specific factor; PTN, pleiotrophin; PTPRZ1, receptor protein tyrosine phosphatase zeta 1; RAS, rat sarcoma virus pathway; SDC1, syndecan-1; SDC2, syndecan-2; SDC3, syndecan-3; SDC4, syndecan-4; TSR-1, thrombospondin type I repeat; VEGFA, vascular endothelial growth factor A; VEGFR2, vascular endothelial growth factor receptor 2.

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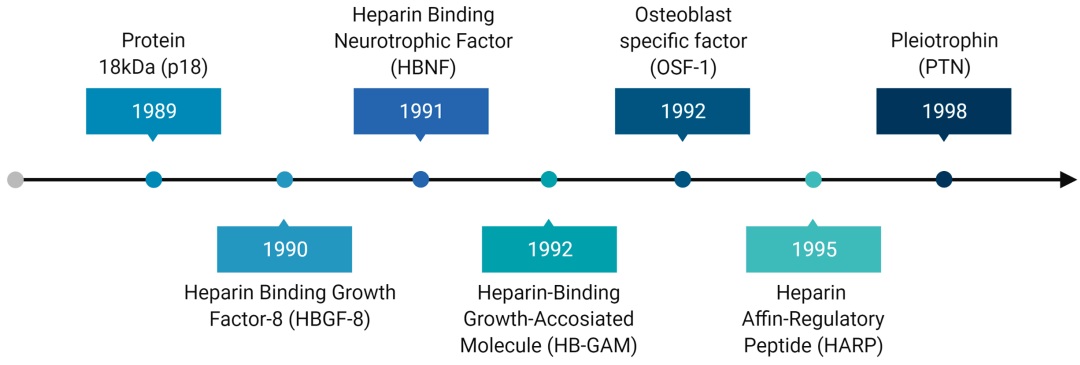

Fig. 1. Timeline representation of PTN discovery. Important time points of the discovery of the molecule and the different names used during the years. The figure was created with BioRender.com.

transcription factor GT-1, activator protein 1, serum, homeobox A5 and Sox10. Possible binding sites for nuclear receptor κB, cAMP binding protein, serum response factor and retinoic acid have been suggested but not proved (revised in Papadimitriou et al., 2009; 2016). In relation to the latter, experimental evidence suggests that PTN expression is not enhanced by retinoic acid (Li *et al*., 1992) and this notion is supported by our data showing that all-trans retinoic acid inhibits PTN expression (Theodorakopoulou *et al*., 2006).

PTN receptors

PTN has been shown to act through numerous receptors (Fig. 2). The first molecule identified as a functional PTN receptor in brain neurons was N-syndecan or syndecan-3, SDC3), which was found to interact with PTN in a solid phase binding assay. Using a similar assay, PTN was also found to interact with SDC1 in a heparan sulfate-dependent manner. It was later shown that the chondroitin sulphate chains on SDC1 and SDC4 are involved in their interaction with PTN, while the core protein of SDC1 may also play a role. A cooperative action of both TSR-1 domains of PTN is required for its interaction with the heparan sulphate chains of SDC3 and the regulation of synaptic plasticity. Despite these studies showing interaction of PTN with different syndecans, only interaction with SDC3 has been linked to PTN functions, as discussed later (revised in Papadimitriou *et al*., 2009; 2016; Pantazaka and Papadimitriou, 2014).

Another promptly identified PTN receptor in the nervous system is receptor protein tyrosine phosphatase zeta 1 (PTPRZ1). PTN binds

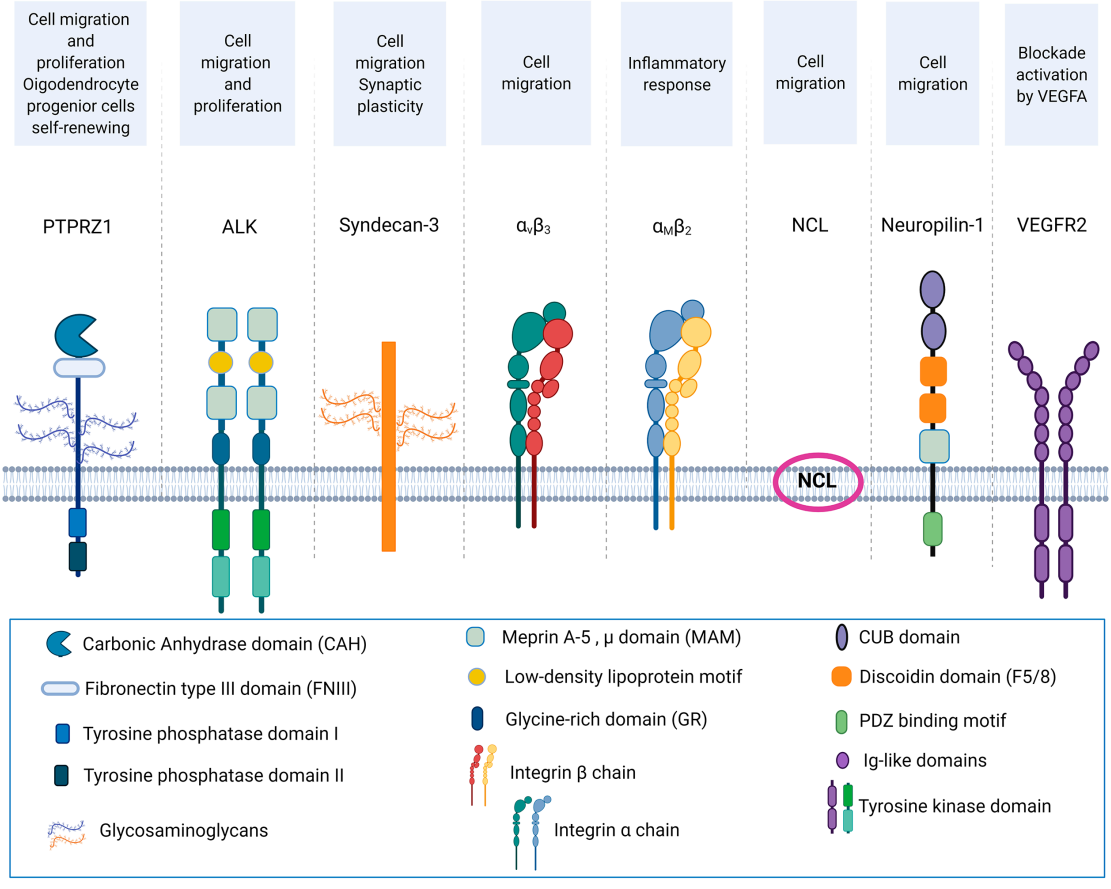

Fig. 2. Schematic representation of the known PTN receptors and their proven biological effects upon PTN binding. All shown receptors are functional PTN receptors that mainly affect the PTN-mediated regulation of migration of different types of cells. SDC1 and SDC4 have been shown to interact with PTN but there are no data on their involvement in any PTN actions; therefore, they are not presented in this figure. Data on ALK as a PTN receptor are conflicting (see text), but PTN may activate ALK through PTPRZ1 and it seems that ALK mediates some of PTN's actions. NCL conformation and exact mode of localization on the cell surface is not known. The figure was created with BioRender.com.

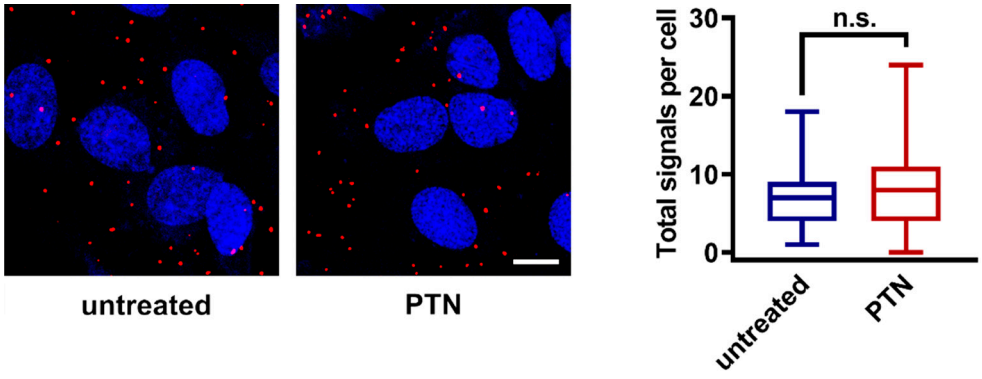

Fig. 3. PTPRZ1 interacts with $\alpha_v\beta_3$ in endothelial cells independently of PTN. Serum-starved human umbilical vein endothelial cells were incubated with PTN (100 ng/ml) for 10 min. Formation of β_3-PTPRZ1 complexes as evidenced by *in situ* proximity ligation assay are shown on the left. The box plots shown on the right indicate the median, mean and range of the detected signals (n = 10-12 image fields with 4-5 cells per image per sample type, each sample run in duplicate). Details on the methodology used can be found at Koutsioumpa *et al.*, 2015. Scale bar corresponds to 10 μm.

to high and low-affinity sites that involve both the chondroitin sulphate chains and the protein core of PTPRZ1. The carboxy-terminal PTN domain seems to be involved in the interaction with the PTPRZ1 chondroitin sulphate structures, which differ during development or in pathologies (revised in Pantazaka and Papadimitriou, 2014; Papadimitriou *et al*., 2016). Binding of PTN to PTPRZ1 increases tyrosine phosphorylation of numerous downstream molecules, such as c-Src and Fyn kinases, β-catenin, β-adducin, $\alpha_v\beta_3$ integrin, focal adhesion kinase, phosphoinositide 3-kinase and activation of protein kinase C alpha, beta or delta, ERK1/2 kinases (reviewed in Papadimitriou *et al*., 2009; 2016) and cyclin dependent kinase 5 (Lampropoulou *et al*., 2018). It was initially suggested that binding of PTN to PTPRZ1 inhibits the phosphatase activity of the receptor through dimerization/oligomerization (revised in Papadimitriou *et al*., 2016), and in response to the doubts with regard to how the highly negatively charged chondroitin sulphate moieties on PTPRZ1 could allow dimerization, it has been suggested more recently that the positively charged ligand PTN may neutralize electrostatic repulsion between chondroitin sulphate chains, thus inducing PTPRZ1 clustering, an effect also observed after removal of the PTPRZ1 chondroitin sulphate chains (Kuboyama *et al*., 2016). However, this point requires further study and clarification, since it is also possible that the PTPRZ1 downstream signalling results from the regulation of tyrosine kinase receptors autophosphorylation by PTPRZ1. Such a mechanism has been described for the neuregulin receptor ErbB4 that interacts with PTPRZ1 through postsynaptic density-95 and results in decreased tyrosine phosphorylation of ErbB4 (Fujikawa *et al*., 2007). Similarly, PTPRZ1 has been shown to dephosphorylate anaplastic lymphoma kinase (ALK) autophosphorylation sites, an effect that may be inhibited upon PTN binding to PTPRZ1 or following PTPRZ1 deletion or tyrosine phosphatase inhibition, leading to ALK activation (Xia *et al*., 2019; Ntenekou *et al*., 2020).

ALK has been suggested as a PTN receptor in ligand-receptor binding studies performed in cell-free assays and in intact cells, in which PTN was shown to bind to the ALK extracellular domain. Although direct PTN binding to ALK has been questioned by numerous studies, ALK seems to be activated by PTN and to be involved in PTN's signaling and actions. It has been suggested that PTN may activate ALK through PTPRZ1 (Papadimitriou *et al*., 2016; Xia *et al*., 2019) but the exact pathway requires further study.

Another cell surface protein that was identified at an early stage and has been discussed as a low affinity PTN receptor is nucleolin (NCL). NCL is a ubiquitous nucleolar protein that regulates several aspects of the DNA and RNA metabolism, ribosome assembly and the development of various tissues and organs. NCL is present on the surface of activated endothelial and cancer cells following $\alpha_v\beta_3$ integrin activation by PTN. Cell surface NCL interacts with $\alpha_v\beta_3$ and PTPRZ1 and seems to affect both PTN signaling and its translocation to the nucleus (Koutsioumpa and Papadimitriou, 2014; Papadimitriou *et al*., 2016) through mechanisms that remain unclear.

Integrins are heterodimeric cell-membrane receptors that mediate cell-cell interactions and adhesion between the extracellular matrix and the cytoskeleton and play important roles during development (Maartens and Brown, 2015). Integrins that have been characterized as PTN receptors are $\alpha_v\beta_3$ on endothelial cells (Mikelis *et al*., 2009) and $\alpha_M\beta_2$ on leukocytes (Shen *et al*., 2017), mediating functions of PTN related to angiogenesis and inflammatory responses, respectively. PTN interacts with the extracellular domain of the β_3 integrin subunit through its carboxy-terminal domain (Mikelis *et al*., 2011), and with the α_5-β_5 loop of the α_MI-domain through its amino-terminal domain (Feng *et al*., 2021). In endothelial cells, $\alpha_v\beta_3$ interacts with PTPRZ1 even in the absence of PTN, and this interaction is not affected by PTN (Mikelis *et al*., 2009 and Fig. 3), although PTN induces PTPRZ1-dependent β_3 Tyr774 phosphorylation (Mikelis *et al*., 2009).

Neuropilin-1, a receptor for semaphorins that has significant role(s) in embryonic development of the nervous and the vascular system (Jubb *et al*., 2012) has been shown to bind PTN. This interaction requires the TSR-1 domains of PTN and leads to ERK1/2 activation and enhancement of endothelial cell migration (Elahouel *et al*., 2015), but has not been studied further.

More recently, we have shown that PTN selectively binds to vascular endothelial growth factor receptor 2 (VEGFR2) in endothelial and other types of cells, and inhibits vascular endothelial growth factor A (VEGFA)-induced VEGFR2 phosphorylation at Tyr1175 (Lamprou *et al*., 2020).

Expression and functions of PTN and its receptors during development

Without a doubt, the highest PTN expression is observed in the nervous system, the pituitary, the heart, the eye, the placenta, the seminal tissue and the testis, the bladder, and the stomach. PTPRZ1 is significantly expressed in the nervous system, the skin, and the eye. They are both highly expressed in induced pluripotent stem cells and embryonic stem cells (Papadimitriou *et al*., 2016), suggesting important role(s) in early development. However, only in few cases has a causal relationship between PTN and its receptors been linked to specific functions.

Nervous system

Since its discovery, PTN has been implicated in brain development and maturation. During development, it is highly expressed in the nervous system at sites of active mitogenesis, initially in the developing neuroepithelium and later in both glial cells and neurons in developing axonal tracts. In zebrafish embryos, overexpression of PTN induces extensive neurite outgrowth with complicated branching patterns. In the perinatal period, it is expressed by neurons, astrocytes and oligodendrocytes and has been characterized as a potent stimulator of neurite outgrowth in central nervous system neurons (revised in Winkler and Yao, 2004; González-Castillo *et al*., 2015) and in spiral ganglion neurons (Bertram *et al*., 2019). A recent study showed that neural stem cells secrete PTN into the niche and that this contributes to the newborn neurons' maturation (Tang *et al*., 2019). In adults, PTN expression is limited to specific neuronal subpopulations in the brain cortex, the hippocampus, the cerebellum, and the olfactory bulb (González-Castillo *et al*., 2015).

A specific role of PTN has been described in relation to dopaminergic neurons. It is highly expressed in neural stem cells of mouse ventral mesencephalon and promotes the production of dopaminergic neurons (González-Castillo *et al*., 2015). It has also been shown to induce the differentiation of human umbilical cord mesenchymal stem cells into dopaminergic neuron-like cells (Yang *et al*., 2013), and in combination with stromal cell-derived factor 1, insulin-like growth factor 2, and ephrin B1, to promote the differentiation of human embryonic stem cells to functional tyrosine hydroxylase-positive neurons (Vazin *et al*., 2009). PTN is overexpressed in neurodegenerative diseases and confers a protective and/or nourishing effect on dopaminergic neurons *in vivo* and *in vitro* (Mourlevat *et al*., 2005). The effects of PTN on the development of the nigrostriatal dopaminergic pathway are shown to be mediated by SDC3 and PTPRZ1 (González-Castillo *et al*., 2015).

PTPRZ1 during brain development is expressed mainly to the ventricular and subventricular zone (Levy *et al.,* 1993), on both neuronal and glial cells (Canoll *et al.,* 1993). *Ptprz1*-knockout mice show neurological abnormalities and differences in behavior and learning, including increased exploratory activities to novelty, deficits in spatial and contextual learning, and reduced responses to methamphetamine (Tanga *et al.,* 2019). PTN also maintains the self-renewing phenotype of oligodendrocyte progenitor cells through PTPRZ1 (McClain *et al*., 2012), which has made a significant contribution to the survival and recovery of oligodendrocytes from demyelinating disease (Harroch *et al*., 2002). More recently, PTPRZ1 has been shown to be important for the perineuronal net structures that are important for both development and plasticity of the brain in the adult (Eill *et al*., 2020).

PTN has been shown to mediate the neurite stimulating activity of the peptide Y-P30 by binding to SCD2 and SDC3 (Landgraf *et al*., 2008). Phosphacan, the alternatively spliced extracellular PTPRZ1 domain, inhibits neurite outgrowth (Margolis *et al*., 1996), and PTN has been shown to convert the neurite growth inhibitory effect of chondroitin sulfate proteoglycans into an activating effect (Paveliev *et al*., 2016). The expression pattern of PTN, PTPRZ1 and SDC3 in the postnatal mouse cerebellum appears to confirm their involvement in the morphogenesis of Purkinje cells, as well as in the control of the granule cell migration (Basille-Dugay *et al*., 2013), while disruption of PTN distribution extracellularly alters the development and function of the neuronal circuits of the cerebellum (Hamza *et al*., 2016). Besides the central nervous system, PTN is highly expressed in the peripheral nervous system during development and seems to promote the repair of neurons after injury of peripheral nerves. It may also have a significant role in muscle innervation, based on the observations that it is present at the neuromuscular junction and contributes to acetylcholine receptors clustering (revised in Jin *et al*., 2009).

The effects of PTN on neural development and neurite outgrowth have also been shown to be mediated by ALK (Yanagisawa *et al*., 2010). ALK has been implicated in the nervous system development, signaling during neuromuscular junction development in *C. elegans*, affecting the establishment of iridophores and normal pigmentation patterns in zebrafish, and neurogenesis in mice. It is highly expressed during embryonic mouse and chick development, in both the central and peripheral nervous system, while ALK expression levels are decreased after birth (revised in Kalamatianos *et al*., 2018). Whether any of these functions are related to PTN has not been studied.

Muscle

Outside the nervous system, soon after its discovery, PTN was found to be expressed in human intestinal smooth muscle cells (Li *et al*., 1992) and in satellite cells (Wanaka *et al*., 1993). However, although a potential role of PTN in myogenesis was suspected at an early stage, the first evidence came from studies showing that PTN mRNA and protein expression are increased during myogenesis and regeneration after crushing, in newly formed myotubes and in activated myoblasts before fusion *in vivo*. *In vitro*, PTN expression increases during the differentiation process, being maximal on fusion of myoblasts into myotubes (Caruelle *et al*., 2004).

PTN expression is found to increase during postnatal heart development and PTN increases postnatal cardiomyocytes' DNA synthesis (Chen *et al*., 2004), suggesting that PTN may have a role in myocardial development and regeneration. PTN overexpression in slow-twitch soleus muscle leads to an increased number of pure type 1 fibers and decreased number of pure type 2A and 2X fibers, as well as increased sulfonylurea receptor 1, citrate synthase and cycloxygenase IV gene expression and increased vascularization, suggesting a shift toward a more oxidative metabolism and improved muscle performance (Camerino *et al*., 2013). The role of PTN receptors in heart development has not been studied. We have unpublished evidence using two different animal models, as well as human data showing that PTPRZ1 is expressed in the embryonic but not in the adult heart and plays a role in heart morphogenesis and subsequent function (Katraki-Pavlou *et al.,* unpublished).

As is also mentioned above, PTN is a muscle protein present at the neuromuscular junction in close contact with the acetylcholine receptors and may have a significant role in muscle innervation (Jin *et al*., 2009).

Skeletal system

A role of PTN in bone development was suggested at an early stage on the basis of studies showing expression of its mRNA during bone growth in ancestral cartilage and pulp. PTN has initially been considered important for new bone formation on the basis of observations that it induces osteoblast attachment to the extracellular bone material through binding to a specific receptor, which may be SDC3. It was also shown to induce hypertrophy during the chondrogenetic differentiation of human bone marrow progenitor cells, an effect that has been associated with both ALK and PTPRZ1.

PTN enhances the release of heparin-binding epidermal growth factor, which then activates its receptor in precursor osteoblasts and osteoblast-type cells, increases alkaline phosphatase activity and inhibits dexamethasone-induced cell death. PTN overexpressing transgenic mice develop a phenotype characterized by increased bone growth and higher salt and bone density. When PTN is overexpressed solely in the mouse skeletal system, increased bone length and maturation are observed at the early stages of bone development, a balanced phenomenon in adult life. PTN-deficient mice appear to have bone growth retardation at two months, and osteopenia and resistance to bone remodeling in adult life (revised in Lamprou *et al*., 2014). In transgenic mice that overexpress PTN, bone loss due to estrogen deficiency (Masuda *et al*., 1997) or almost zero gravity (Tavella *et al*., 2012) is compensated, in line with the observation that estrogen enhances PTN expression (Xi *et al*., 2020). It has been suggested that the increased expression of PTN and PTPRZ1 in osteoblasts depleted of insulin-like growth factor binding protein-2 helps maintain, partially at least, normal bone mass in female mice (Xi *et al*., 2020).

PTN is also involved in odontogenesis. It was initially shown that PTN is expressed during initiation, morphogenesis and cytodifferentiation stages of incisor development at structures that are important for tooth morphogenesis (Mitsiadis *et al*., 2008). PTN is also expressed in epithelial and mesenchymal dental cell lines and its expression is regulated by bone morphogenetic proteins. During maturation of the ameloblasts and odontoblasts, PTN is expressed in the inner enamel epithelium; PTN is also expressed in the terminally differentiated and enamel matrix-secreting ameloblasts and odontoblasts of the adult mouse incisors and molars (Erlandsen *et al*., 2012). More recently, it has been shown that PTN positively regulates dental pulp stem cell proliferation and potential to differentiate (Jin *et al*., 2020), protecting them from senescence (Zhang *et al*., 2021).

Hematopoiesis and immune system

PTN promotes hematopoietic stem cell (HSC) expansion *in vitro* and HSC regeneration *in vivo* (Himburg *et al*., 2010) via PTPRZ1-mediated activation of the RAS pathway (Himburg *et al*., 2014), supporting a role of the PTN/PTPRZ1 axis in hemopoiesis. The main source of PTN during steady-state hematopoiesis is the leptin receptor expressing bone marrow stromal cells, but regeneration of HSCs depends on endothelial cell-derived PTN (Himburg *et al*., 2018). In the same line, PTN secreted into the human tonsil mesenchymal stem cells conditioned medium increases HSC transplantation efficacy (Kim *et al*., 2020). It has been also shown that loss of stromal PTN results in changes in expression of genes that lead to myeloid engraftment dominance, suggesting that PTN plays a role in maintaining the balance of myeloid and lymphoid potential of regenerating HSCs (Istvanffy *et al*., 2011).

PTN is mitogenic for human peripheral blood mononuclear cells, and this initial observation suggested that it may be involved in the growth regulation of cell mediated immunity (Achour *et al*., 2001), further supported by a subsequent study showing enhanced expression of inflammatory cytokines, such as tumor necrosis factor α and interleukins 1β and 6 by PTN (Achour *et al*., 2008). A role of PTN in adaptive immunity was corroborated by the observation that interferon γ induces PTN expression by macrophages (Li *et al*., 2010) and a role in innate immunity has been suggested by its bactericidal properties (Svensson *et al*., 2010; Guyot *et al*., 2016). PTN has also been suggested as a potent regulator of neuroinflammation through PTPRZ1 (Herradon *et al*., 2019), and a regulator of the levels of functional cytokines in both M1 and M2 type microglia cells, strengthening M1/M2 transformation (Miao *et al*., 2019).

Mice that are knockout for PTPRZ1 have a higher number and proportion of mature B cells, and signaling of MK through PTPRZ1 leads to B cell survival (Cohen *et al*., 2012). Whether PTN has such an effect on B cells has not been studied. It has been only shown that PTN expression is significantly increased in B cells from both chronic and acute leukemia patients compared to healthy controls and suppression of PTN activity induced apoptosis of B cells from both leukemia patients and cell lines (Du *et al*., 2014).

Urogenital system

In the human mammary gland, PTN is expressed in epithelial and endothelial cells (Ledoux *et al*., 1997) and helps maintain the mammary epithelial cells in a progenitor phenotype, thus delaying mammary gland maturation (Rosenfield *et al*., 2012). Its expression in the uterus depends on the estrous cycle and is upregulated by progesterone (Milhiet *et al*., 1998). In the endometrial stromal cells, it is upregulated during decidualization (Bany and Schultz, 2001), apparently having a vital role in the progesterone-induced decidualization pathway (Yu *et al*., 2018). High mobility group box 3 regulates uterine stromal cells proliferation and differentiation by targeting PTN (Wang *et al*., 2019), further supporting a physiological role of PTN in uterine decidualization. $CD49a^+$ $Eomes^+$ natural killer (NK) cells in the human and mouse uterus secrete PTN and enhance fetal growth during early stages of fetal development (Fu *et al*., 2017). The $CD49a^+$ PBX homeobox 1 enhances PTN transcription in decidual NK cells, thus promoting fetal growth (Zhou *et al*., 2020). Female mice that are deficient in both PTN and MK show reproductive abnormalities (Muramatsu *et al*., 2006).

PTN is expressed in testicular Leydig cells and plays a normal role in spermatogenesis. Male mice deficient in PTN are characterized by infertility, atrophic testes and apoptotic sperm cells (Zhang *et al*., 1999). PTN mRNA and protein are expressed in ventral mesenchymal pad and prostatic mesenchyme surrounding ductal epithelial tips that undergo branching morphogenesis *in vivo*. *In vitro*, PTN is upregulated by androgens and stimulates both stromal and epithelial cell proliferation, as well as branching morphogenesis (Orr *et al*, 2011).

In the embryonic kidney, PTN is expressed in the developing kidney mesenchyme (Mitsiadis *et al*., 1995) and localizes onto the basement membrane of the developing ureteric bud (Sakurai *et al*., 2001).

Respiratory system

PTN and PTPRZ1 have been shown to be highly expressed in mesenchymal and epithelial cells of fetal lungs, respectively. PTN promotes fetal type II cell proliferation and inhibits their trans-differentiation into alveolar epithelial type I cells, through multiple signaling pathways that include β-catenin and Notch (Weng *et al*., 2009).

Eye

PTN is mitogenic for bovine lens epithelial cells (Delbé *et al*., 1995), and blocks or promotes rod or bipolar cell differentiation respectively (Roger *et al*., 2006). In Drosophila, overexpression of the MK/PTN orthologue miple leads to defective eye patterning

through Ptp99A, the Drosophila ortholog of PTPRZ1 (Muñoz-Soriano *et al*., 2013). During embryonic development, PTN is expressed in the iris mesenchyme, the optic nerve, and the neural retina, suggesting that it may have a role in epithelial-mesenchymal interactions and affect optic nerve and retinal development. Among PTN receptors, SDC1, and SDC3 are expressed in the cornea, whereas all receptors including RPTPZ1 and ALK are expressed in the retina (Cui and Lwigale, 2019) but a causal relation to the PTN effects on the eye has not been studied.

Liver

PTN was initially identified as a mitogen for adult hepatocytes (Sato *et al*., 1999) and parenchymal cells in both adult and embryonic liver (Asahina *et al*., 2002). It is expressed by mesenchymal cells in fetal liver; its expression is gradually reduced during development (Asahina *et al*., 2002; Ito *et al*., 2014) and seems to play a role in liver regeneration (Asahina *et al*., 2002; Michelotti *et al*., 2016) through its receptor PTPRZ1 (Michelotti *et al*., 2016). It was recently shown that in myofibroblasts, there is decreased expression of several growth factors, including PTN, compared to fibroblasts, and this leads to reduced hepatoblast proliferation and induced maturation of the differentiated cholangiocytes (Wang *et al*., 2020).

Other organs

PTN expression has been identified during all embryonic stages in the neurohypophysis primordium. It is expressed only in neurohypophyseal cells and might be involved in pituitary development (Fujiwara *et al*., 2014).

PTN mRNA is strongly expressed in the mouse cochlea one week after birth and gradually decreases, being undetectable by week 8 after birth. PTN knockout mice exhibit low to moderate auditory deficits and have higher hearing thresholds compared to the wild-type mice, with an almost normal appearance of the stria vascularis but weak expression of the Kir4.1 potassium channel (Zou et al., 2006). Mice knockout for both PTN and MK also appears with severe vacuolar degeneration in the intermediate cells (Sone *et al*., 2011).

PTN and angiogenesis

The vascular system is very important for fetal development and there is evidence that PTN has a significant regulatory role in vascular homeostasis, although the exact pathways involved are still being explored.

The initial evidence came from *in vitro* studies showing that PTN is mitogenic for endothelial cells. The mitogenic effect of PTN was questioned in subsequent studies and it seems that it may be cell-type and cell-context specific or depend on the origin of the recombinant protein used (revised in Papadimitriou et al., 2004; 2009). PTN induces endothelial cell migration and differentiation into tubes in various substrates *in vitro*, through its receptor PTPRZ1, which forms a functional complex with $\alpha_v\beta_3$ integrin on the surface of endothelial cells and leads to cell surface translocation of NCL (revised in Papadimitriou et al., 2009; 2016). PTN has also been shown to enhance VEGFA expression (Koutsioumpa *et al*., 2012; Palmieri *et al*., 2015) and this may contribute to its

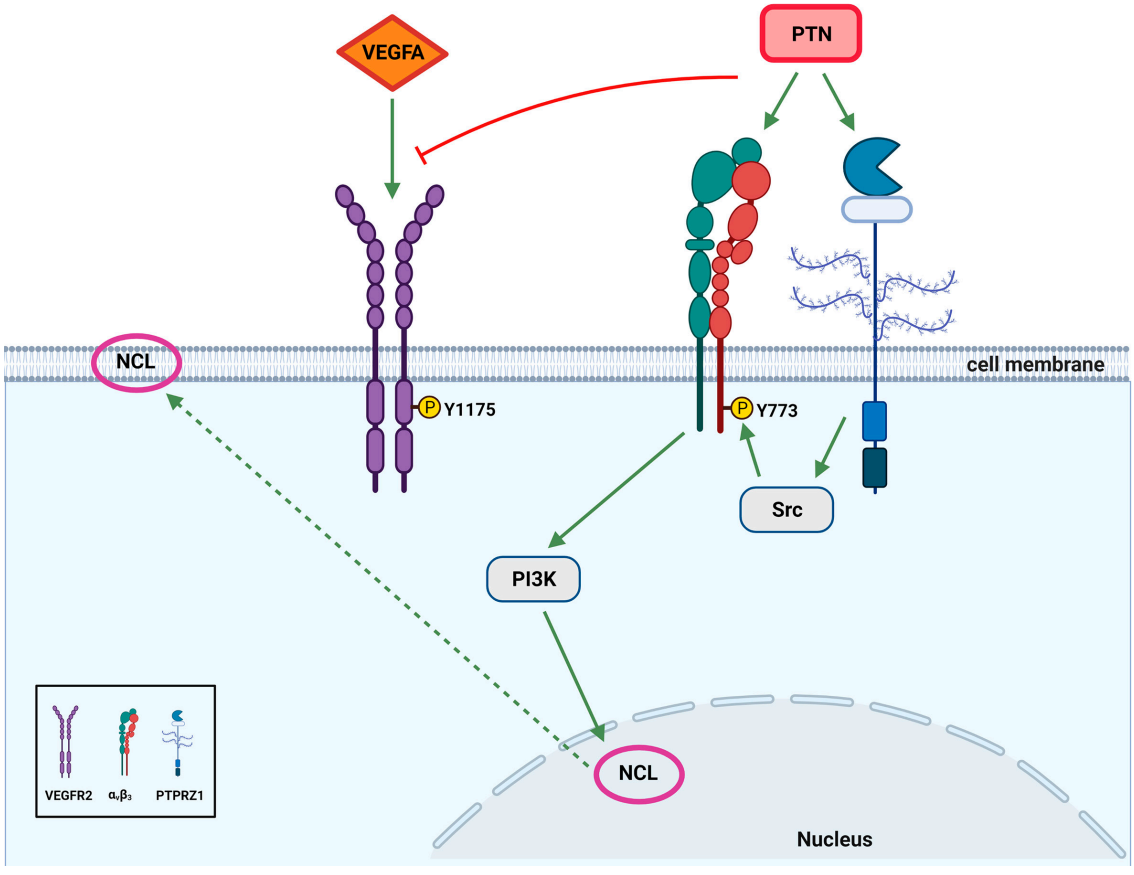

Fig. 4. Schematic representation of the up to date known PTN-induced pathway in endothelial cells. PTN has a moderate stimulatory effect on endothelial cell migration via PTPRZ1/$\alpha_v\beta_3$/cell surface NCL (Mikelis *et al*., 2009; Koutsioumpa *et al*., 2013) but an inhibitory effect through VEGFR2 (Lamprou *et al*., 2020). The figure was created with BioRender.com.

angiogenic activities, although the receptors/pathways involved in this action have not been studied. In contrast to the initial characterization of PTN as an angiogenesis-promoting growth factor, later studies suggested that PTN may also limit the angiogenic effect of VEGFA (Héroult *et al*., 2004; Koutsioumpa *et al*., 2015). This possibility was further supported by data showing that VEGFA competes with PTN for binding to PTPRZ1 (Koutsioumpa *et al*., 2015). Binding of VEGFA to PTPRZ1 inhibits endogenous PTN levels (Poimenidi *et al*., 2016) that limit the angiogenic effects of VEGFA, thus enhancing VEGA-induced endothelial cell migration (Koutsioumpa *et al*., 2015; Poimenidi *et al*., 2016). More recently, we have shown that PTN selectively binds VEGFR2 and inhibits its activation by VEGFA, highlighting another mechanism through which PTN limits the angiogenic effect of VEGFA (Lamprou *et al*., 2020). Although the cross talk between PTPRZ1, $\alpha_v\beta_3$ and VEGFR2 is still being studied, our up-to-date data support a model through which PTN has a moderate stimulatory effect on endothelial cells via PTPRZ1/$\alpha_v\beta_3$/cell surface NCL but limits the angiogenic effects of VEGFA through VEGFR2 (Fig. 4).

In line with a balancing effect on angiogenesis,, PTN has had moderate effects on *in vivo* animal models of angiogenesis, such as the rabbit cornea, the chorioallantoic membrane of the chick embryo, the ischemic myocardium in rats, a model of acute posterior muscle ischemia, slow-twitch soleus and fast-twitch extensor digitorum longus muscles, and numerous tumor models (revised in Papadimitriou *et al*., 2009; 2016). When expressed by monocytes, it has been shown to lead to a decrease in the expression of monocyte markers and an increase in endothelial cell markers, inducing differentiation of monocytes into functional endothelial cells (Sharifi *et al*., 2006). A role played by PTN in angiogenesis is also supported by the expression of PTN and its receptors PTPRZ1, ALK, SDC1 and SDC3 in mesenchymal cells, fetal macrophages, and fetal vessels, in line with increasing levels of PTN in maternal bloodstream as pregnancy progresses (Ball *et al*., 2009). PTN has been also suggested as a promising factor for inducing angiogenesis during aging based on the observation that it restores the age-related attenuation of angiogenesis in the aortic ring model (Besse *et al*., 2013).

Most discussion of the role of PTPRZ1 in angiogenesis has been based on its function as a PTN and VEGFA receptor, as mentioned above. We have recently shown that PTPRZ1 knockout mice show enhanced angiogenesis in several organs, such as lung, heart and the retina, and microvascular endothelial cells isolated from the lungs of these animals have enhanced angiogenic properties (Ntenekou *et al*., 2020; Katraki-Pavlou *et al.,* unpublished).

Conclusions

PTN is a highly conserved, ubiquitously expressed protein that seems to play a notable role in the development and maturation of the nervous system. Data from several other organs show that it is also involved in epithelial-mesenchymal interactions during development and is expressed by endothelial cells, regulating angiogenesis. Due to the high degree of homology with MK, the one compensates for the other in several but not all cases, and this adds to the complexity of studying PTN. Another point of complexity is the increasing number of receptors that have been identified as mediating the effects of PTN in several different types of cells and organs, forcing researchers to try to clarify the interactions and the crosstalk of the different receptors/pathways involved in each case. Moreover, the PTN receptors identified to date are not specific for PTN and mediate the effects of several other molecules besides PTN and MK (revised in Pantazaka and Papadimitriou, 2014; Papadimitriou *et al*., 2016), suggesting that PTN may regulate the effects of other growth factors, as has been shown for VEGFA in endothelial cells (Koutsioumpa *et al*., 2015; Poimenidi *et al*., 2016). Although PTN does not seem to be indispensable for survival, it seems to play important roles in several pathological conditions, affecting pathways that are deranged in pathological settings, and this is still an open field to study. Meticulous *in vitro* work combined with high throughput assays and loss- or gain-of-function *in vivo* studies will shed light on many unidentified aspects related to the role of PTN in development, angiogenesis, and pathologies.

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