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Midkine and Pleiotrophin: Two Related Proteins Involved in Development, Survival, Inflammation and Tumorigenesis

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Midkine (MK) and pleiotrophin (PTN) are low molecular weight proteins with closely related structures. They are mainly composed of two domains held by disulfide bridges, and there are three antiparallel β-sheets in each domain. MK and PTN promote the growth, survival, and migration of various cells, and play roles in neurogenesis and epithelial mesenchymal interactions during organogenesis. A chondroitin sulfate proteoglycan, protein-tyrosine phosphatase ζ (PTPζ), is a receptor for MK and PTN. The downstream signaling system includes ERK and PI3 kinase. MK binds to the chondroitin sulfate portion of PTPζ with high affinity. Among the various chondroitin sulfate structures, the E unit, which has 4,6-disulfated *N*-acetylgalactosamine, provides the strongest binding site. The expression of MK and PTN is increased in various human tumors, making them promising as tumor markers and as targets for tumor therapy. MK and PTN expression also increases upon ischemic injury. MK enhances the migration of inflammatory cells, and is involved in neointima formation and renal injury following ischemia. MK is also interesting from the viewpoints of the treatment of neurodegenerative diseases, increasing the efficiency of *in vitro* development, and the prevention of HIV infection.

Key words: cancer therapy, cell migration, cell survival, protein tyrosine phosphatase, tumor marker.

Recognition at the cell surface regulates differentiation, proliferation, survival, adhesion, migration, and other activities of responding cells (*1, 2*). One class of molecules involved in cell surface recognition is secreted, low molecular weight proteins, which include hormones, growth factors and cytokines. This review focuses on a protein family of such factors consisting of only two proteins, midkine (MK) and pleiotrophin (PTN). MK and PTN are involved in the regulation of development and the etiology of diseases. Recently, they have become promising molecular targets for the treatment of diseases including malignancy (*3–8*). The activities of MK and PTN at the cellular level and their relationship to diseases are summarized in the tables (Tables I and II).

MK was found as the product of a gene whose expression increases during the early differentiation stage in embryonal carcinoma cells (*9*). Chicken MK is also called retinoic acid-inducible heparin binding protein (RIHB) (*10, 11*). PTN was found as a neurite-promoting factor (*12*) or a mitogeneic factor for fibroblasts (*13*). PTN is also called heparin binding growth-associated molecule (HB-GAM) (*14*), OSF-1 (*15*), HARP (*16*), and HBNF (*17*). Molecular cloning of MK (*9, 18*) and PTN (*14, 19*) revealed the close relationship between the two proteins. MK and PTN also have been the subjects of other reviews (*20–28*).

Protein structure

MK and PTN are rich in basic amino acids and cysteine, and share 45% sequence identity with each other (Fig. 1). Both proteins are principally composed of two domains held by disulfide bridges, namely an N-terminally located N-domain and a C-terminally located C-domain (*29, 30*) (Fig. 2). There are short tails at both ends of the molecule. Several activities of MK and some activities of PTN are carried by the C-terminal half molecules with the C-domain (*31, 32*). It is noteworthy that the heparin binding site of MK is mainly in the C-domain (*31*), while that of PTN is equally distributed in both the N- and C-domains (*33*). In agreement with these observations, a heparin binding site, which is conserved in the C-domain of mouse and human MK (*34*), is absent in PTN. Both the N- and C-domains of MK and PTN show weak homology to the type I repeat of thrombospondin; tryptophan is present in each domain 5–7 amino acids downstream from the first cysteine residues (*33*).

Part of the MK activity is enhanced by or requires dimerization of MK (*35, 36*). Both the N- and C-domains are involved in dimerization. A portion of the N-domain has been utilized as a dominant negative inhibitor of MK, since free N-domain inhibits dimerization (*35, 37*). Deletion of the tails at either the N-terminal or C-terminal ends of MK

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Abbreviations: ALK, anaplastic leukemia kinase; FGF, fibroblast growth factor; LRP, low density lipoprotein receptor–related protein; HB-GAM, heparin binding growth-associated molecule; MK, midkine; MPNST, malignant peripheral nerve sheath tumor; NGF, nerve growth factor; PTN, pleiotrophin; PTPζ, protein-tyrosine phosphatase ζ; RIHB, retinoic acid–inducible heparin binding protein.

TABLE I. **Biological activities of MK and PTN at the cellular level.**

Activity	MK	PTN
Cell growth	fibroblasts, keratinocytes, tumor cells	fibroblasts, hepatocytes, endothelial cells, tumor cells
Cell survival	embryonic neurons, tumor cells	
Cell migration	embryonic neurons, inflammatory cells	embryonic neurons
Change in cell shape	neurite outgrowth of embryonic neurons, collagen gel contraction by fibroblasts	neurite outgrowth of embryonic neurons
Fibrinolytic activity	endothelial cells	endothelial cells
Chemokine expression	tubular epithelial cells	
Chondrogenesis	chondrocyte precursors	chondrocyte precursors
Angiogenesis		endothelial cells
Acetylcholine receptor clustering	embryonic myoblasts	embryonic myoblasts

Details are described in the text.

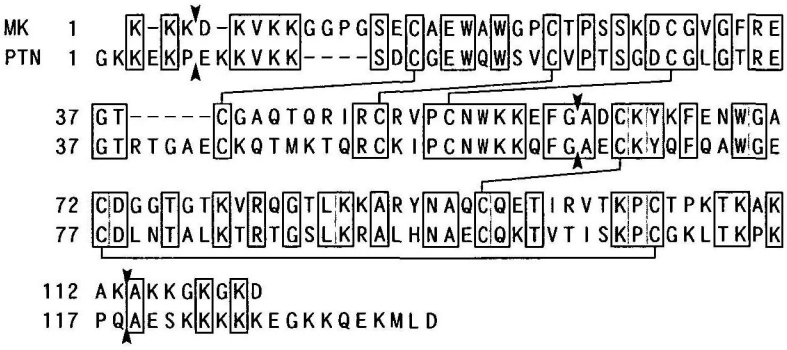

Fig. 1. **Protein sequences of human MK and PTN.** Amino acids conserved between the two proteins are marked by boxes, while those also conserved in *Drosophila* miple are shaded. Marks (▼) indicate exon–intron boundaries. Bars show the location of disulfide bridges.

TABLE II. **Diseases related to MK and/or PTN.**

Disease	Relationship to MK and/or PTN
Carcinomas	MK, PTN
Wilms' tumor	MK
Neuroblastoma	MK, PTN
Malignant peripheral nerve sheath tumor	MK
Melanoma	PTN
Neointima formation upon balloon injury	MK
Ischemic nephritis	MK
Retinal degeneration	MK
Delayed neuronal cell death after ischemia	MK
Alzheimer's disease	MK, PTN
HIV	MK
Osteoporosis	MK, PTN

Diseases related to etiology or cure/prevention are listed. Details are described in the text.

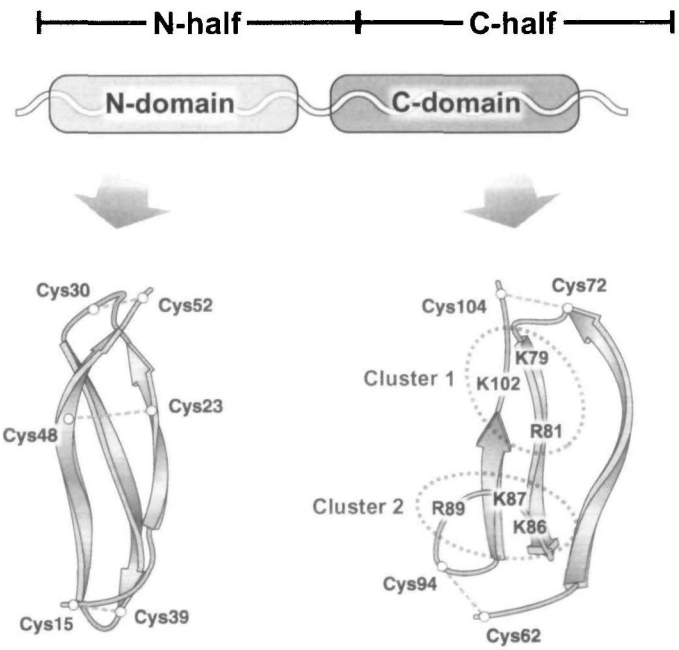

Fig. 2. **Organization of the MK protein and three-dimensional structure of its domains.** Dashed lines indicate two heparin binding sites in the C-domain. The domain figure is based on Ref. *34* and is modified from Ref. *26* (Copyright 2002, John Wiley & Sons) with permission from John Wiley & Sons.

severely reduces the neurite promoting activity (*38*). However, the C-domain lacking the C-terminal tail retains a considerable degree of activity. Therefore, it has been concluded that the role of the tails is an indirect one, probably serving to maintain the two domains in the proper orientation (*38*). Deletion of the C-terminal tail of PTN also hinders the mitogenic activity (*39*). On the other hand, the transforming activity of PTN requires the presence of one of the tails and a portion of the N-domain (*40*); this knowledge was also used to construct a dominant negative mutant (*41*). Surprisingly, the tails of PTN were reported to have some activity (*42*). Due to differences in the processing of the signal peptide, MK with two extended amino acid sequences in the N-terminal exists (*43*). PTN with an extended amino acid sequence has also been found (*39*). The presence of a short form of MK lacking the N-domain and its peripheral sequences encoded by Exon 2 was predicted by mRNA analysis (*44*) and verified using a monoclonal

antibody specific for the short form (*45*).

MK and PTN are broadly distributed in vertebrates; MK from humans to zebra fish, PTN from humans to *Xenopus*. PTN is more conserved between species; human and rat PTNs differ by only one amino acid (*19*), and human PTN shares 83% sequence identity with that of the frog molecule (*46*). Human and mouse MK share 87% sequence identity (*47*). On the other hand, *Drosophila melanogaster* has two molecules, miple and the related miple 2. Miple is named from *mid*kine and *ple*iotrophin. Both miple and miple-2 have twice repeated domains that show a high degree of homology to the C-domains of both MK and PTN, but no significant homology to the N-domains (Fig. 1). The primordial form of the C-domain might be the prototype of MK and PTN.

The three dimensional structure of MK has been clarified based on the structures of the N-terminal half and C-terminal half molecules determined by NMR (*34*). Each domain is basically composed of three antiparallel β-sheets; in addition, the C-domain has a flexible loop (Fig. 2). The tail portions located outside the domains do not form stable structures, and the two domains move freely with each other. The stability of MK to acid and high temperature is considered to be due to the stabilization of the domain structure by disulfide bridges (*48*). PTN also has a structure similar to that of MK (*33*). In addition, the three dimensional structure of MK serves as the prototype of that of the thrombospondin type 1 repeat.

MK and PTN have been produced as recombinant proteins in mammalian cells, baculovirus, yeast and *Escherichia coli* (*19, 49–54*). Furthermore, both have been chemically synthesized; the N-half and the C-half are formed separately, and then those with correct disulfide bridges are isolated by HPLC and then linked (*55, 56*). There are arguments as to whether baculovirus produced-PTN retains the full range of activities and whether PTN produced by mammalian cells is of a high degree of purity.

Genes

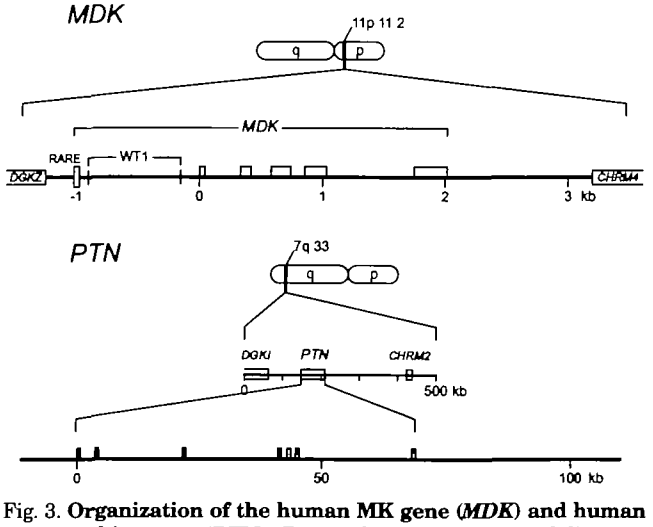

Fig. 3. **Organization of the human MK gene (*MDK*) and human pleiotrophin gene (*PTN*).** Boxes above the horizontal line are exons. RARE, retinoic acid responsible element; WT1, WT1 binding sites; DGK, diacylglycerol kinase; CHRM, muscarinic acetylcholine receptor.

The human MK gene (*MDK*) is located on chromosome 11p.11.2 (*57*) (Fig. 3), while the mouse gene (*Mdk*) is on chromosome 2 near Hox 4.1 (*58*). The human PTN gene (*PTN*) is on chromosome 7q.33, and that of mouse is on chromosome 6 (*59*). *MDK* and *Mdk* encompass 2 kb (*60, 61*), while *PTN* is very large (68 kb) (*62*) (Fig. 3). Despite the difference in genome size, *MDK* and *PTN* share common features. The coding sequences of both proteins are on 4 exons, and the exon–intron boundaries are conserved (*60–62*). Furthermore, the order of arrangement of the diacylglycerol kinase genes, *MDK/PTN* and muscarinic acetylcholine receptor genes (*63*) is conserved in the two human chromosomes (Fig. 3). MK expression is induced by retinoic acid in embryonal carcinoma cells and in certain tissues. In fact, in the promoter region of the MK gene, there is a functional retinoic acid–responsive element (*64, 65*) (Fig. 3). The promoter region also has a binding site for WT1, Wilms' tumor suppressor gene. The expression of PTN is induced by PDGF and progesterone (*66, 67*).

The expression of MK and PTN is developmentally regulated. As an example, in the cerebrum of the mouse or rat, MK expression is strong in the midgestation period, while that of PTN is strong shortly after birth; both are scarcely expressed in the adult cerebrum (*12, 68–71*). Also in other organs, MK expression generally peaks in the midgestation period; strong expression is found in the brain, epithelial tissue in which epithelial–mesenchymal interactions are taking place, and mesenchymal tissues undergoing remodeling (*68, 72*). The expression of MK in adult tissues is restricted. During mouse embryogenesis, strong PTN expression is observed in the brain, digestive and respiratory systems, sense organs, hair whiskers, facial processes and limbs (*72*). In the adult, PTN expression is also restricted.

Biological activities

MK and PTN have diverse activities. MK promotes the growth of fibroblasts, Wilms' tumor cells and keratinocytes (*43, 49, 73*). However, the activity is weaker than that of fibroblast growth factor (FGF). MK has an anti-apoptotic activity to embryonic neurons (*74–77*) and Wilms' tumor cells treated with an anti-cancer drug, cisplatin (*78*). PTN promotes the growth of fibroblasts (*19*), a subpopulation of hepatocytes (*79*), endothelial cells (*16, 80*) and certain carcinoma cells (*3, 4, 6, 81*).

Both MK and PTN stimulate the migration of embryonic neurons and osteoblasts (*82–85*). Furthermore, MK promotes the migration of inflammatory cells, namely macrophages and neutrophils (*7, 86*). PTN, as well as MK, promotes neurite outgrowth of embryonic neurons (*12, 43, 49*). MK also promotes fibroblast-mediated contraction of collagen gels (*87*). These activities on MK and PTN are thought to be mediated by their action on the cytoskeleton through intracellular signal transduction.

MK and PTN stimulate the fibrinolytic activity of endothelial cells (*88*). MK also enhances the expression of chemokines in urinary tubular epithelial cells (*8*) and the synthesis of matrix molecules by fibroblasts (*89*). These activities of MK and PTN are thought to be the result of transcriptional activation by these factors.

PTN stimulates chondrogenesis in micromass culture of chicken limb buds (*90*). MK does not have this activity when added to the culture medium, but it exhibits the activity after cDNA transfection into the precursor cells

(*91*). PTN shows angiogenic activity *in vitro* (*92, 93*), while MK lacks this activity. However, transfection with MK as well as PTN cDNA enhances the *in vivo* angiogenic activity of breast carcinoma cells (*94*).

PTN is expressed in the CA1 subregion of the hippocampus in adult rats and mice. High-frequency stimulation leading to the induction of long-term potentiation (LTP) results in increased PTN expression in the CA1 area (*95*). In PTN knockout mice, the hippocampal structure and basal excitatory transmission in the CA1 area appear normal. However, hippocampal slices from deficient mice exhibit a lower threshold for LTP induction, and the application of PTN causes the LTP level to revert to the wild-type level (*96*). Therefore, PTN appears to be an inducible signal to inhibit LTP.

MK and PTN are involved in various processes in development. They have been implicated in the regulation of epithelial–mesenchymal interactions, because of the expression of MK and PTN in tissues undergoing these interactions (*68, 72*). The development of tooth germ *in vitro* is inhibited by the addition of anti-MK antibody (*97*). When the epithelial and mesenchymal tissues from the tooth germ are combined in culture, a translucent zone is formed at the contact site of the mesenchyme; however, the zone is not formed when the tissues are cultured separately. Beads coated with BMP-2 induce the formation of the translucent zone on the mesenchyme. When beads coated with MK are placed together, the action of BMP-2 is inhibited. MK appears to suppress the excessive action of BMP-2. In the *in vitro* differentiation system of the lung germ, MK stimulates the development of mesenchymal tissue (*98*).

Kidney development is a system in which epithelial–mesenchymal interactions are prominent. Anti-MK antibody inhibits the formation of nephrons in *in vitro* kidney development systems (*99*). On the other hand, PTN, which is expressed in the mesenchyme, has been identified as a factor involved in uteric bud branching morphogenesis; PTN added together with glia-derived neurotrophic factor (GDNF) induces the branching of isolated uteric buds in culture (*100*).

A detailed analysis of the involvement of MK in epithelial–mesenchymal interactions has been performed using an artificial blood vessel model in which human umbilical cord vascular endothelial cells are cultured on human aortic smooth muscle cells (*101*). The endothelial cells, but not the smooth muscle cells, produce a small amount of MK. When MK is added to the model, the endothelial cells proliferate and the production of proteoglycans increases. However, MK does not act on these endothelial cells cultured in a monolayer. The direct target of MK has been identified as the smooth muscle cells, which secrete factors acting on endothelial cells. One such factor is IL8. The angiogenic activity of MK *in vivo* (*94*) might also be explained by a similar mechanism; MK may act on cells other than endothelial cells, and the responding cells may secrete angiogenic factors.

MK and PTN play roles in various aspects of neurogenesis. For one, they are considered to be involved in the migration of embryonic neurons. In addition to their migration promoting activity, they are located along the route through which neurons migrate (*70, 102–106*). As an example, both proteins are present in the radial glial processes in the cerebral cortex of day 17 rat embryos (*70*). The radial glial processes are structures along which neurons migrate. Staining with MK can be used as a marker of radial glial processes (*107*). When human fetal brain cells are separated into astrocytes and neurons, the astrocytes have been shown to synthesize MK (*75*). MK and PTN are strongly expressed in the rat cerebellum 7 days after birth, suggesting that they are involved in the migration and neurite outgrowth of granule cells (*108*).

When beads coated with PTN or MK are placed on embryonic myoblasts from *Xenopus*, acetylcholine receptor clustering, a marker of synapse formation, is induced (*109, 110*). PTN is localized on myoblasts and MK on neurons in the synapse; both PTN and MK might be involved in synapse formation; PTN might be regarded as a factor supplied from myoblasts, and MK as a factor supplied from neurons.

MK is also likely to participate in neural development. MK mRNA is expressed strongly in the brain and spinal cord during the development of *Xenopus*. When MK mRNA is injected into the dorsal vegetal region of 8 cell *Xenopus* embryos, neural tissues enlarge abnormally (*111*). Mesoderm is induced in the ectoderm portion of the *Xenopus* embryo when it is cultured with activin. When MK mRNA is pre-injected into the activin-treated embryo, mesoderm induction is suppressed, but neural induction takes place. The induced tissue has been concluded to be the anterior neural tissue based on the expression of neural markers.

An MK-related protein, Mdk-2, cloned in zebra fish, is first expressed in the neural plate during development. When Mdk2 mRNA is injected into the embryo, the development of posterior neural tissue is enhanced (*37*). Injection of dominant negative MdK-2 mRNA, which lacks the portion encoding the C-domain, suppresses the development of the posterior nervous tissue. Although the activity of Mdk2 is related to that of MK, the target of Mdk2 is posterior neurons. It has been reported that zebrafish have another MK-related protein (*37*). Two MK related proteins might act on neurons at different locations. It has also been reported that an MK antisense oligonucleotide inhibits the conversion of mesenchyme to neuroectoderm in the cultured tail bud of chicken embryos (*112*).

Despite the expected important roles MK and PTN, no significant defect in development has been reported in mice deficient in MK or PTN, except for a delay in postnatal hippocampal development in MK-deficient mice (*96, 113*). However, closer analysis has revealed that when heterozygotes are crossbred, the numbers of *Mdk* (–/–) and *PTN* (–/–) mice born are significantly smaller than wild-type born (Maruyama, S. and Muramatsu, H., unpublished).

Mechanism of action

The MK receptor is thought to be a molecular complex containing proteoglycans including protein-tyrosine phosphatase ζ (PTPζ) (*83*) and members of the low density lipoprotein receptor-related protein (LRP) family (*114*) (Fig. 4). On the other hand, the PTN receptor has been proposed to be syndecan-3 (*115*), PTPζ (*82, 116*), ALK tyrosine kinase (*117*), or their complexes (Fig. 4). The consensus reached is that PTPζ plays significant roles in the signaling of both MK and PTN (*28*).

Syndecan-3 has been proposed to be the receptor of PTN in PTN-induced neurite outgrowth of embryonic neurons (*115*). Syndecan-3 is a member of the syndecan family,

which comprises 4 transmembrane heparan sulfate proteoglycans. PTN-induced neurite outgrowth is inhibited by anti–syndecan-3 antibody and the digestion of neurons by heparitinase, which cleaves heparan sulfate chains. Src is found to be attached to the cytoplasmic tail of syndecan-3, and the binding of PTN to syndecan-3 might alter Src activity and its interaction with cortactin (*118*). The binding constant (K_d) of PTN to syndecan-3 is 0.6 nM.

MK also binds strongly to members of the syndecan family, namely syndecan-1, -3, and -4 (*72, 119, 120*). The binding constant between MK and syndecan-4 is 0.3 nM. Digestion of target cells with heparitinase reduces MK-induced neurite outgrowth and the enhancement of fibrinolytic activity, indicating that heparan sulfate proteoglycans are also involved in these MK activities (*38, 50*).

The binding of PTN and MK to syndecans is mediated by the heparan sulfate chains. Heparan sulfate has variable structures; the building unit starts from glucuronyl-*N*-acetylglucosamine, and progressive sulfation and conversion of D-glucuronic acid to L-iduronic acid yields a heparin-like domain. The strong binding to PTN is suggested to require *N*-sulfated glucosamine and 2-sulfated uronic acid (*121*), while that to MK requires the trisulfated structure of *N*-and 6-*O*-sulfated glucosamine and 2-sulfated uronic acid, namely the heparin-like domain (*122, 123*).

The binding of MK to heparin is mainly mediated by the C-domain (*31*). On the surface of the C-domain are two clusters of basic amino acids, Cluster 1 and Cluster 2 (Fig. 2). That both are involved in MK binding has been confirmed by NMR (*34*) and site-directed mutagenesis (*124*). Among the basic amino acids, an arginine residue in Cluster 1 is the most important; mutation of this amino acid leads to decreased activity in heparin binding and neurite outgrowth (*124*). This arginine residue is conserved in MK and PTN of all species and in *Drosophila* miples.

Receptor-type protein tyrosine phosphatase ζ (PTPζ) is a transmembrane protein with chondroitin sulfate chains and an intracellular tyrosine phosphatase domain. PTPζ has been assigned as the receptor of PTN and MK in the migration of neurons (*82, 83*) and osteoblasts (*85*) and in the survival of embryonic neurons (Sakaguchi *et al.*, submitted). In these systems, anti-PTPζ antibody prevents the activity of PTN and MK. Digestion of target cells with chondroitinase also abolishes the responsiveness to PTN and MK. Furthermore, the binding activities of various MK mutants to PTPζ correlate with the biological activities of the mutants. As an example, in a mutant, in which the arginine in Cluster 1 is changed to glutamine, the affinity for PTPζ is decreased with a concomitant decrease in the migration promoting activity (*83*).

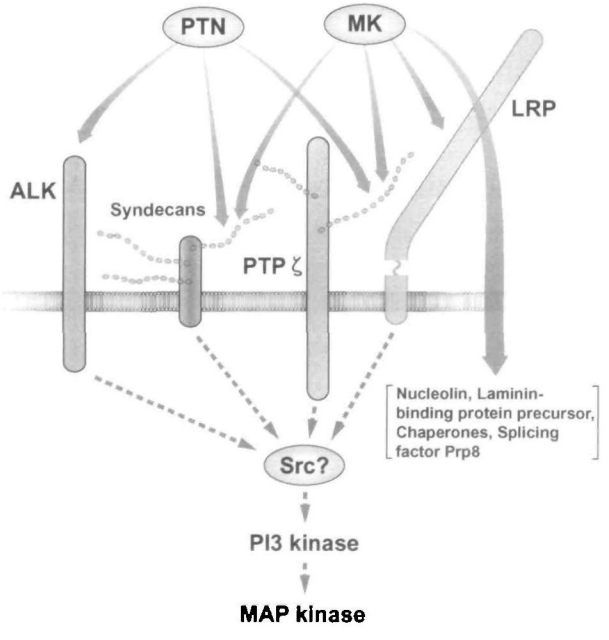

Fig. 4. **Molecules proposed to be components of the signaling system of MK and PTN.** Intracellular molecules with the possibility of involvement are shown in brackets.

MK and PTN bind to the chondroitin sulfate portion of PTPζ with high affinity and to the protein portion with low affinity (*83, 116*). The K_d of PTPζ to MK is 0.56 nM, while digestion by chondroitinase causes it to decrease to 8.8 nM. Among the various structures of chondroitin sulfates, the E unit, which has 4,6-disulfated *N*-acetylgalactosamine, binds to MK with high affinity (*125*). Although the authentic chondroitin sulfate E used for binding studies has 3-sulfated glucuronic acid, the fact that this structure is not essential for strong binding was verified using artificial chondroitin sulfate E, which was formed by transferring a sulfate to the 6-position of chondroitin-4 sulfate (Zou *et al.*, submitted). Furthermore, the susceptibility of the MK binding structure to chondroitinase B suggests that the E structure has a dermatan sulfate domain (*85, 126*).

After binding of the ligand, PTPζ is thought to dimerize, leading to the inactivation of the phosphatase domain and an increase in intracellular tyrosine phosphate. CIT1/Cat-1 (*127*) and β-catenin (*128*) have been identified as the substrates of PTPζ. Although PTPζ has been well characterized as the receptor of MK and PTN, the distribution of PTPζ does not completely overlap with the action spectra of the factors. As an example, the migration of macrophages is increased by MK, and chondroitinase treatment of macrophages reduces the MK responsiveness even though PTPζ was not detected in macrophages (*129*).

To identify transmembrane proteins other than proteoglycans in the MK receptor, MK-binding glycoproteins were isolated from day-13 mouse embryos, and their identities were determined by protein microsequencing after separation by SDS-PAGE. Consequently, the low density lipoprotein receptor–related protein (LRP) has been identified as an MK-binding protein (*114*). LRP is a member of the LDL receptor family and is a typical endocytosis receptor. However, recently, LDL receptor family members have been identified as components of the signaling receptor complex. First, the reelin receptor was shown to be a complex of cadherin-related neural receptor and Apo E receptor 2 or very low density lipoprotein receptor (*130, 131*). Then, the Wnt receptor was revealed to be a complex of frizzled and low density lipoprotein receptor-related proteins (LRP5/6) (*132*). MK binds to LRP with a K_d of 3 nM. When RAP (receptor-associated protein), which is a chaperone involved in the synthesis of LRP and inhibits the function of LRP, is added to the culture medium, MK-dependent survival of embryonic neurons is inhibited. Thus, LRP has been concluded to be involved in MK signaling (*114*). LRP6 also binds to MK with a similar affinity (Sakaguchi *et al.*, submitted). Because of the difference in the cytoplasmic

domains, LRP and LRP6 can interact with different signaling systems, and the choice of the expression of LRP or LRP6 in target cells can change the cellular response to the MK signal. Although the possibility is not excluded that LRP is involved in the internalization of MK, and the internalized MK also acts directly in cells as described below, currently it is regarded that LRP is part of the MK receptor complex, which is composed of proteoglycans such as PTPζ or syndecans and LRP.

In the stimulation of fibrinolysis by MK, the active form is the dimer of MK, which is stabilized by an isopeptide linkage formed by transglutaminase (*35*). However, in the case of growth stimulation of Wilms' tumor cells, the MK monomer is concluded to be the active form, and MK is thought to bind to a 200 kDa cell surface receptor (*133*). Anaplastic leukemia kinase (ALK), which is a transmembrane tyrosine kinase with a molecular mass of 200 kDa, is proposed to be a receptor of PTN (*117*).

The downstream signaling system of MK and PTN is generally agreed to include PI3 kinase and MAP kinase, with MAP kinase downstream of PI3 kinase (*77, 85, 134*). The signaling from the receptor to PI3 kinase is not well understood, although it is likely that Src is involved in the process, since an inhibitor of Src, PP1, inhibits many MK and PTN activities.

Recently, growth factors have been found to work directly in cells, in addition to their action through signaling receptors. Thus, it should be kept in mind that MK and PTN also act directly within cells. Indeed, MK and PTN bind to nucleolin, a shuttle protein between the cytoplasm and nucleus (*52*). Furthermore, LRP is required for MK internalization (Shibata *et al.*, submitted), and internalized MK has been found to be translocated to nuclei after binding with nucleolin (Shibata *et al.*, submitted) or laminin-binding protein precursor (*135*). MK is known to bind to some chaperones (Muramatsu, H. *et al.*, unpublished) and a splicing factor Prp8 (*136*). Some of this binding may well have physiological importance. In addition, possible differences in intracellular binding sites might explain differences in the activities of MK and PTN described before.

Medical significance

Both MK and PTN are correlated with tumorigenesis and tumor progression. MK expression is increased in a number of malignant tumors compared to the adjacent non-cancerous tissue (Fig. 5) including esophageal, stomach, colon, hepatocellular, breast, thyroid, lung, prostate, and urinary bladder carcinomas, Hodgkin's disease, cholangiocarcinoma, Wilms' tumor, neuroblastoma, glioblastoma and other brain tumors (*137–149*). The frequency of overexpression depends on the type of tumor, while in case of gastrointestinal carcinomas, MK is overexpressed in about 80% of cases. In prostate carcinoma, MK is already detectable at the early stage (*143*). In colon carcinogenesis, MK expression becomes increased at the adenoma stage and the intensity increases progressively during tumor progression (*150*). Similarly, MK expression starts in hyperplasia and increases progressively during lung carcinogenesis in the rat (*151*). In neuroblastoma (*147*), glioblastoma (*148*) and urinary bladder carcinoma (*144*), strong expression of MK correlates with a poorer prognosis for patients.

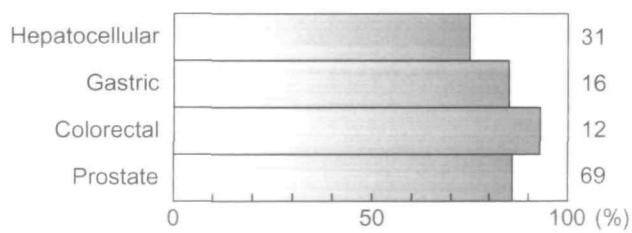

Fig. 5. **Increased expression of MK in human carcinomas compared to non-cancerous tissue.** Shaded regions show the percent of positive cases. Numbers on the right show the number of cases. The data are taken from Refs. *140*, *141*, and *143*.

The product of the Wilms' tumor suppressor gene, WT1, whose deletion leads to Wilms' tumor, is known to bind to the promoter region of the MK gene, and inhibits its expression (*152*). Since all specimens of Wilms' tumor overexpress MK (*137*), and MK is involved in the growth of these cells (*43*), it is believed that MK is an autocrine tumor growth factor whose expression is suppressed by WT1.

MK is also overexpressed in malignant peripheral nerve sheath tumor (MPNST), which occurs in patients deficient in the NF1 tumor suppressor gene (*153*). Since an MK antisense oligonucleotide inhibits colony formation of MPNST in soft agar, MK expression is also considered to be a cause of MPNST. The product of the NF1 tumor suppressor gene is the ras GTPase activating protein, whose deletion results in an increased level of activated ras. In many human cancers, an increase in activated ras is observed; the result raises the possibility that one of the routes by which the increase in activated ras leads to tumor phenotype is through the expression of MK.

PTN expression in human carcinomas is relatively restricted; overexpression has been observed in pancreatic carcinoma (*6*), choliocarcinoma (*4*), neuroblastoma (*147*) and melanoma (*3*). Strong expression of PTN in neuroblastoma correlates with a better prognosis for patients (*147*).

In experimental systems, both MK and PTN are correlated with tumorigenesis. First, transfection with PTN or MK cDNA results in oncogenic transformation of NIH 3T3 cells (*154, 155*). Also, ribozyme targeting of PTN and antisense MK oligonucleotides suppresses the growth of some tumor cells (*3–6*). Probably, the promotion of growth, survival, migration and angiogenesis by MK or PTN contributes to the growth and progression of various tumors. There is also an interesting report describing the inhibition of melanoma growth in soft agar, but not in a monolayer, by antisense PTN (*156*).

The increased expression of MK in many carcinomas and that of PTN in some indicates that MK and PTN can be applied to the diagnosis and treatment of malignancy. Enzyme-linked immunoassay of MK has shown that the serum MK level in normal human subjects is usually less than 0.6 ng/ml or 0.5 ng/ml (*51, 157*). The serum MK level is elevated in patients with various malignancies such as hepatocellular carcinoma and lung carcinoma: the percent of positive cases in patients was about 50% in one study and 80% in another (*51, 157, 158*). The urinary MK level is also increased in cancer patients (Ikematsu *et al.*, unpublished). Enzyme-linked immunoassay of PTN has revealed that the serum PTN level is increased in patients with pancreatic and colon carcinomas (*159*). Furthermore, in the case of pancreatic carcinoma, patients with higher serum PTN levels exhibit a poorer prognosis.

Truncated MK, in which Exon 2 is lacking, has been found at the mRNA level and the protein level in cancer specimens, but so far not in normal specimens (*44, 45*). Since the frequency of the expression of the truncated form increases during tumor progression (*153, 160*), this can also be utilized as a tumor marker, especially to detect metastatic nests.

Ribozymes targeted to PTN mRNA prevent the growth of choliocarcinoma, melanoma and pancreatic carcinoma in nude mice (*3, 4, 6*). An antisense oligonucleotide to MK mRNA inhibits the growth of colon carcinoma cells (*5*) and MPNST cells (*153*). More interestingly, the MK antisense oligonucleotide mixed with atelocollagen retards the growth of colon carcinoma cells pre-grown in nude mice (*5*). It is hoped that these strategies can be applied clinically to cure human malignancy.

Equally promising is the use of the human MK promoter to express the thymidine kinase gene preferentially in tumor cells (*161–163*). The MK promoter activity is comparable to that of the SV40 promoter in certain tumor cells. The MK promoter fused with the thymidine kinase gene is especially helpful in adenovirus-mediated gene delivery to carcinomas followed by ganciclovir treatment (*161*), since the major problem in the regime is liver toxicity and the MK promoter does not act in the liver.

MK is also correlated with other diseases. MK is involved in the migration of inflammatory leukocytes; this activity is probably important in tissue repair and defense against infection. However, excessive recruitment of inflammatory cells leads to pathological conditions. Neointima formation in the aortic wall is a hallmark of restenosis after balloon therapy for a blocked carotid artery. This is formed by migrating smooth muscle cells and precursors in response to cytokines released by inflammatory leukocytes and injured endothelial cells. In the mouse model of neointima formation, mice deficient in the MK gene develop a lesser neointima region compared to wild-type mice; the number of migrating leukocytes is also less compared with wild-type mice (*7*).

Nephritis after ischemia is also less severe in MK-deficient mice than in wild-type controls, and the recruitment of inflammatory leukocytes is also suppressed in knockout mice (*8*). In addition to the direct action enhancing the migration of inflammatory cells, MK enhances the synthesis of chemokines by tubular epithelial cells (*8*). The strong expression of MK in the synovial membranes of patients with inflammatory arthritis has also been noted (*86*).

The anti-apoptotic activity of MK can be utilized to promote the survival of nerve cells. When rat eye is exposed to constant light, retinal photoreceptor cells degenerate. Administration of MK beneath the retinal layer helps the survival of the photoreceptor cells (*164*) with an activity as strong as that of FGF. After ischemic injury, MK is expressed by astrocytes in the surviving region of the cerebral cortex (*71, 165*) and the hippocampal CA1 subregion (*166*). This finding suggests that MK might contribute to the survival of injured neurons. Indeed, the delayed neuronal death in the hippocampus of gerbils after ischemia can be temporarily prevented by an injection of MK into the brain ventricle (*167*). The MK activity is stronger than that of nerve growth factor (NGF). After ischemic injury, MK expression also increases in the heart (*168*) and retina (*169*). It remains to be elucidated whether the increased MK expression contributes to the protection of tissue or the augmentation of injury as mentioned before. PTN is also upregulated after ischemic injury (*170, 171*).

MK has been found to be deposited in the senile plaques of Alzheimer's disease patients (*172*). MK binds strongly to amyloid β-peptide, and neutralizes the cytotoxic activity (*173*). Most probably, MK is produced to counteract the toxicity of amyloid β-peptide. From all these findings, there exists the possibility that MK can be applied to prevent neural degeneration in neurodegenerative diseases. MK is also strongly detected in glial cytoplasmic inclusions found in the brain of multiple system atropy (*174*). PTN is also deposited in senile plaques in both Alzheimer's disease and Down's syndrome (*175*).

The addition of MK to cultures of preimplantation bovine embryos promotes the recovery of the embryos (*176*). MK acts on granulosa cells around the embryos to enhance the synthesis of factors that promote the survival of embryos. Thus, MK might be used for the efficient *in vitro* development of bovine embryos. Since the follicular fluid contains large amounts of MK (*177*), the above activity appears to be physiologically important.

A receptor involved in the intracellular invasion of HIV has been found as a cell surface-located nucleolin. MK, which binds strongly to nucleolin, when added to a culture of target cells, inhibits HIV infection (*178*). Since MK is expressed in peripheral lymphocytes and its expression increases after lymphocyte activation, MK may be regarded as a cytokine that prevents infection of certain viruses including HIV (*178*). The amniotic fluid contains large amounts of MK (*179*). Among its functions, it is possible that MK prevents infection by pathogens.

The stimulation of osteoblast migration by PTN or MK is also interesting from the viewpoint of osteogenesis to prevent osteoporosis (*84, 85*). Indeed, transgenic mice with overexpressed PTN show compensation for the bone mass loss caused by estrogen deficiency (*180*). MK is also known to be expressed during the reparative stage of bone fractures (*91*). Given their activities to enhance chondrogenesis (*90, 91*), PTN and MK might be beneficial for the healing of bone fractures.

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