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Midkine and pleiotrophin in neural development and cancer

Kenji Kadomatsu*, Takashi Muramatsu

Department of Biochemistry, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan

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Abstract

The midkine (MK) family consists of only two members, namely heparin-binding growth factors MK and pleiotrophin (PTN). During embryogenesis, MK is highly expressed in the mid-gestational period, whereas PTN expression reaches the maximum level around birth. Both proteins are localized in the radial glial processes of the embryonic brain, along which neural stem cells migrate and differentiate. Zebrafish and *Xenopus* MK can induce neural tissues. In addition, deposits of MK and/or PTN are found in neurodegenerative diseases, such as Alzheimer's disease and multiple system atrophy. Both molecules are induced in reactive astrocytes by ischemic insults. In this context, it is interesting that LDL receptor-related protein is a receptor for MK and PTN, and this receptor has been implicated in the pathogenesis of Alzheimer's disease. MK and PTN share receptors, and show similar biological activities that include fibrinolytic, anti-apoptotic, mitogenic, transforming, angiogenic, and chemotactic ones. These activities explain how these molecules are involved in carcinogenesis. MK is detected in human carcinoma specimens from pre-cancerous stages to advanced stages. Strong expression of PTN is also detected in several carcinomas, although, in general, MK is expressed more intensely and in a wide range of carcinomas than PTN. The blood MK level is frequently elevated in advanced human carcinomas, decreases after surgical removal of the tumors, and is correlated with prognostic factors. Thus, it is a good marker for evaluating the progress of carcinomas. Furthermore, antisense oligonucleotides for MK and ribozymes for PTN show anti-tumor activity. Therefore, MK and PTN are candidate molecular targets for therapy for human carcinomas.

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1. Introduction

Extracellular signaling molecules, such as growth factors, cytokines, hormones and matrix proteins, play pivotal roles in intercellular communication, and eventually in tissue building and remodeling that

include development and carcinogenesis. Midkine (MK) and pleiotrophin (PTN; also called HB-GAM) are heparin-binding growth factors, and are the only members of a family distinct from other heparin-binding growth factor families [1–6]. MK was found to be the product of a retinoic acid-responsive gene discovered by screening for induced genes during the differentiation of embryonal carcinoma cells [7,8]. PTN cDNA was cloned [9,10] through searches for neurite outgrowth activity [11] or mitogenic activity

* Corresponding author. Tel.: +81-52-744-2064; fax: +81-52-744-2065.

E-mail address: kkadoma@med.nagoya-u.ac.jp (K. Kadomatsu).

toward fibroblasts [12]. Chicken MK is also called RIHB [13]. PTN is also called OSF-1 [14], HBNF [15], and HARP [16]. MK and PTN share not only neurite outgrowth and mitogenic activities but also other ones; which will be described later. Intriguing features of these two molecules are that they are closely linked to neural development as well as the pathogenesis of neurodegenerative diseases, and that at the same time they are involved in cancer development. In this review, we describe their structures, action mechanisms, and biological significance, especially focusing on neural development and cancer.

2. Protein structure

MK and PTN are cysteine- and basic amino acid-rich proteins. In vertebrates, all 10 cysteine residues of MK and PTN are conserved (Fig. 1). These proteins show approximately 50% identity in amino acid sequence. Both proteins are composed of two domains, i.e., N- and C-terminal half domains [1–3]. The NMR analyses have demonstrated that the three-dimensional structures of these proteins are very similar [17,18] (Figs. 1 and 2). Each domain is formed from three anti-parallel β sheets. The two domains are linked by a flexible linker region. The N- and C-terminal regions are also flexible. Although the precise relationship between structural features and biological activities remains to be elucidated, it is interesting that both the N- and C-terminal half domains of PTN are functional at least in transforming NIH3T3 cells [19], while only the C-terminal half domain of MK retains biological activities, e.g. neurite outgrowth, fibrinolysis and nerve cell migration ones [20–22].

MK and PTN are found in from *Drosophila* to man (Fig. 1). The domain corresponding to the C-terminal

half domain of vertebrate MK and PTN is evolutionally conserved (Fig. 1). This stresses again the importance of the C-terminal half domain structure. From this point of view, it is of note that the N-terminal half domain of Zebrafish MK (*mdk2*) and a part of the N-terminal half domain of human PTN (Fig. 1) have a dominant negative effect on neurogenesis and growth of human breast cancer cells, respectively [23,24]. In the C-terminal half of human and mouse MK, two heparin-binding clusters, namely cluster I (K79, R81 and K102 in human MK) and cluster II (K86, K87 and R89 in human MK), have been identified [17,20]. Cluster I is especially important for the recognition of heparin sulfate as well as chondroitin sulfate proteoglycans, nerve cell migration, and interaction, and interaction with protein tyrosine phosphatase ζ (PTP ζ , see Section 3) [20–22]. The amino acids in cluster I are located on two β sheet strands, βC_2 and βC_3 (Figs. 1 and 2). K79 and R81 are located on βC_2 , and K102 on βC_3 . W69 on βC_1 faces R81, and restricts the orientation of the R81 side chain [17]. Importantly, all these four amino acids are conserved in the MK family from invertebrates to vertebrates (Fig. 1).

Although three glutamine residues in human MK that are responsible for transglutaminase-mediated dimer formation [25] have not been conserved through evolution, dimer formation itself is important for the MK action. MK dimer is formed in both transglutaminase-dependent and independent manners, is enhanced by heparin, and enhances the fibrinolysis activity of MK [25].

3. Receptors and intracellular signals

The heparin-binding activity of MK and PTN postulated that heparin sulfate proteoglycans could

Fig. 1. Sequence homology between members of the MK family. (A) Homology between invertebrate members is shown. Identities (%) at amino acids are shown on the right side. The number of amino acids is shown in parenthesis following each member's name. Note that the middle region, which corresponds to the C-terminal half of vertebrate MK and PTN, shows homology with vertebrate PTN. (B) Homology between vertebrate members is shown. (C) Human MK and PTN are compared. The N- and C-terminal half domains are shown in the upper and lower rows, respectively. The β sheet domains deduced on NMR are indicated. The dominant-negative fragment of PTN, which inhibits the growth of human breast cancer cells, is indicated by a dotted line [24]. The fragment of MK that inhibits transglutaminase-mediated dimer formation and the fibrinolytic activity of MK is also shown by a dotted line [25]. (D) The C-half domain consensus sequence is shown. This sequence is conserved from invertebrates to vertebrates.

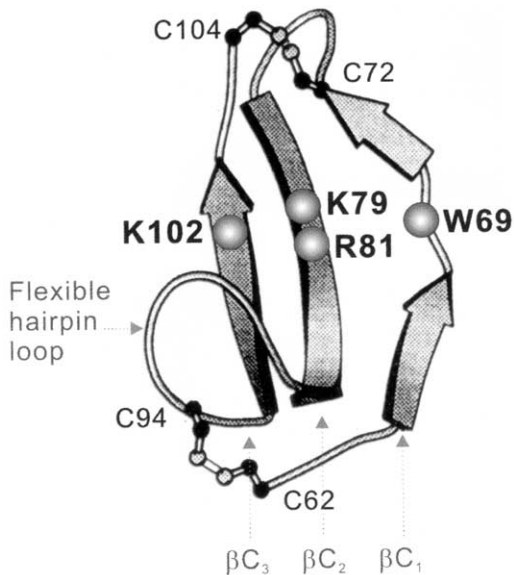


Fig. 2. Ternary structure of the C-terminal half of human MK. The structure of PTN is essentially the same. The three amino acids in heparin-binding cluster I and tryptophan 69 that faces arginine 81 are shown. Note that these amino acids are conserved from invertebrates to vertebrates (Fig. 1).

be members of the receptor complex of MK and PTN. Indeed, the syndecan family members exhibit binding ability as to MK and PTN [26–29]. Although the intracellular domains of syndecans do not exhibit any enzymatic activities, PTN and *N*-syndecan utilize the cortactin-src pathway for the intracellular signaling in neurite outgrowth [30]. However, as described above, the heparin-binding sites are also active in recognition of chondroitin sulfate chains, especially E-type chondroitin sulfate [22,31–34]. Among chondroitin sulfate proteoglycans, PTP ζ is important for the MK family, since it binds to both MK and PTN with high affinity [22,35,36], and its ability to induce nerve cell migration is closely related to its binding activity as to MK, as revealed by analyses involving mutant MKs with mutations in the heparin-binding clusters I and II [22]. PTP ζ is a receptor-type protein tyrosine phosphatase of which the extracellular domain carries chondroitin sulfate chains, and the intracellular domain exhibits protein tyrosine phosphatase activity (Fig. 3). The K_d of PTP ζ as to MK is

0.56 nM, while chondroitinase digestion increases it to 8.8 nM, indicating that the chondroitin sulfate chain is necessary for high affinity [22]. MK and PTP ζ utilize PI3-kinase and Erk for osteoblast cell migration and neuronal survival [33,37,38] (Fig. 3). PTN binds to PTP ζ , inactivates phosphatase activity of PTP ζ , and eventually induces tyrosine phosphorylation of β -catenin [39].

Anaplastic lymphoma kinase (ALK), a receptor-type tyrosine kinase, was found to be a receptor for MK and PTN [40,41]. PI3-kinase and Erk are utilized for intracellular signaling in this system. The combination of PTN and ALK is active in anchorage-independent cell growth [40].

The fourth receptor is LDL receptor-related protein (LRP). LRP is a member of the LDL receptor family that accomplishes endocytosis of a diverse array of ligands [42]. LRP has been identified as a membrane protein, which was purified from the MK-affinity column [43]. LRP is involved in the anti-apoptotic activity of MK [43]. LRP is cross-linked with both MK and PTN through chemical cross-linkers (Kadomatsu et al., unpublished data). The endocytosis of MK is completely dependent on LRP, and initiates nuclear targeting by MK, which is partly needed for the anti-apoptotic activity of MK [44] (Fig. 3). Nuclear targeting by MK is accomplished as a cargo of a nucleo-cytoplasmic shuttle protein, nucleolin, or laminin-binding protein precursor [44,45] (Fig. 3).

LRP recognizes and endocytoses a diverse array of ligands, such as apolipoprotein E, activated $\alpha 2$ macroglobulin ($\alpha 2M^*$), tPA, and rPA:PAI-1 complexes. However, it was recently found that some members of the LDL receptor family also act as cell surface signaling receptors. ApoER2 and VLDLR act as receptors for Reelin, and play a pivotal role in neural stem cell migration [46–48]. LRP5 and 6 act as receptors together with Frizzled, and are crucial for body axis formation and neural development [49,50]. Thus, it is likely that LRP also acts as a signaling receptor for MK and PTN (Fig. 3). Indeed, LRP acts as a co-receptor for PDGF [51,52], and the intracellular domain of LRP recruits several adaptor proteins, such as Disabled and FE65 [53]. LRP is also interesting in terms of the pathogenesis of Alzheimer's disease, since LRP and its ligands, including MK and PTN, are closely related to Alzheimer's disease. This subject will be dealt with later.

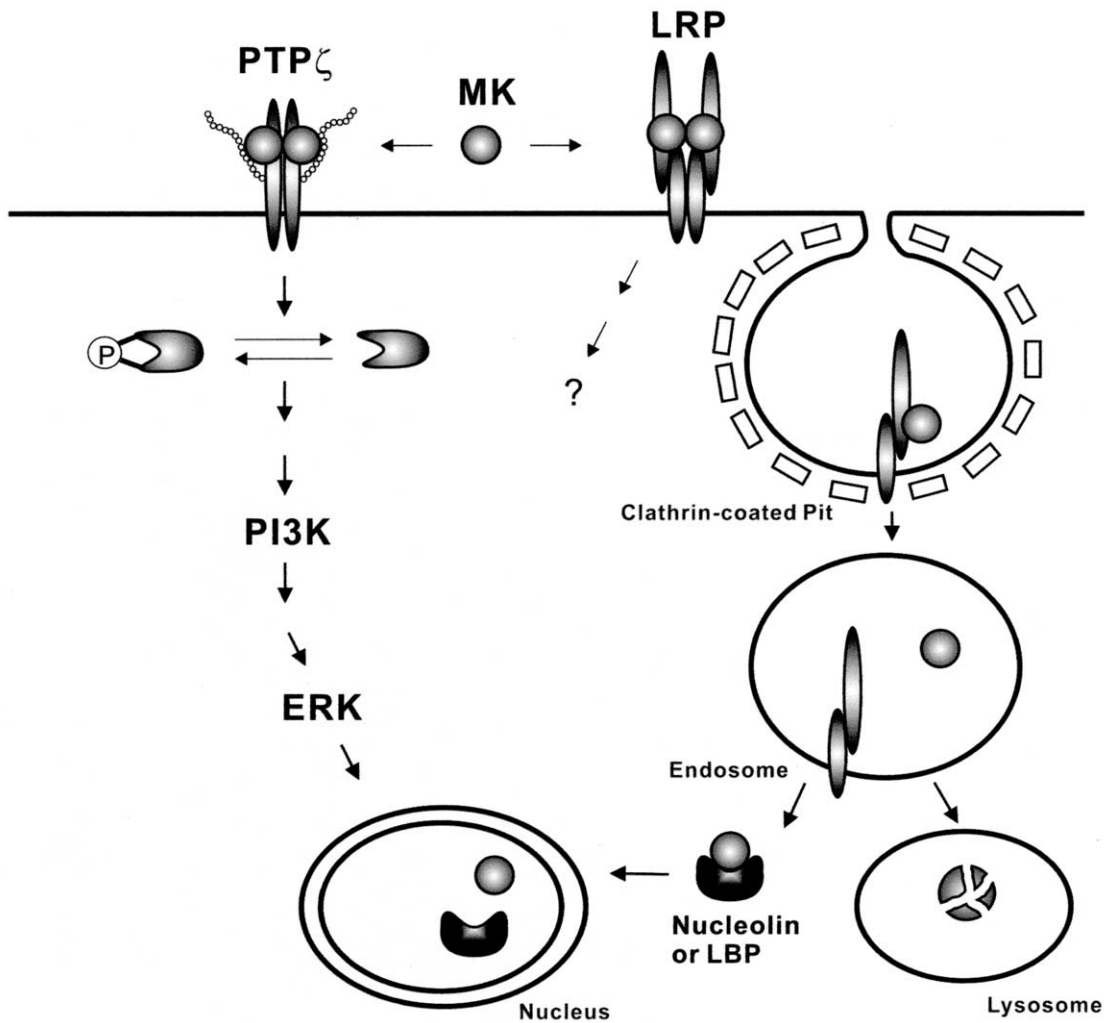


Fig. 3. Mechanisms of action of MK. Two receptors for MK and their signaling cascades are proposed. PTN may use similar mechanisms. In addition to these, ALK and *N*-syndecan pathways may exist. Further studies are needed to clarify the precise mechanism. For example, it is controversial whether the dimerization of PTP ζ activates or inactivates the protein tyrosine phosphatase activity [22,23,39]. LBP, laminin-binding protein precursor.

The four receptors described above, namely *N*-syndecan, PTP ζ , ALK, and LRP, might be differentially utilized for specific biological activities of MK and PTN, or might cooperate with each other to enhance intracellular signals. The functional interaction and complex formation between these receptors remain to be clarified.

In addition, further studies are also needed to clarify whether or not specific signaling pathways that differentiate MK and PTN exist.

4. Gene organization

The human MK, mouse MK, human PTN, and mouse PTN genes are named *MDK*, *Mdk*, *PTN*, and *Ptn*, respectively. MK and PTN have a similar genomic organization. The coding frames of both proteins are divided into four exons, which encode protein elements corresponding to each other [54–56]. Although the genome sizes are different (MK is approximately 3.5 kb, whereas PTN is more

than 60 kb), both genes are surrounded by similar genes, i.e. diacylglycerol kinase (DGK) genes and muscarinic acetylcholine receptor (CHRM) genes: *MDK* is surrounded by *DGK ζ* and *CHRM4*, and *PTN* by *DGK ι* and *CHRM2* [1].

The 5' regulatory region of *MDK* contains one retinoic acid-responsive element and two WT1-responsive elements. The retinoic acid-responsive element is responsible for the MK expression induced by retinoic acid [57]. The WT1-responsive element near the transcription start site is needed for reduction of MK expression by WT1 [58].

The chromosomal location of the human MK family members and their receptors are as follows: MK, 11p11.2; PTN, 7q33-q34; LRP, 12q13-q14; PTP ζ , 7q31.3; ALK, 2p23; and *N*-syndecan, 1pter-p22.3.

5. Biological activities

The biological roles of MK and PTN are diverse. In this section, we will describe their implication in development and some pathological aspects, including neurodegenerative diseases, nephritis, and vascular restenosis. Activities regarding cancer will be described in detail in Section 6.

5.1. Neurogenesis, neural development and neuroprotection

Both MK and PTN promote neurite outgrowth [10,11,59–61] (Fig. 4). Both proteins also promote nerve cell migration [22,36]. Both proteins are localized in radial glial processes in the rat embryonic brain [62,63] (see related phenomenon in Fig. 4). Along radial glial processes, neural stem cells migrate from the ventricular zone to outside, and differentiate. Thus, the localization of MK and PTN on radial glial processes is consistent with their ability to promote the migration of nerve cells. Regarding nerve cell migration, PTP ζ plays a crucial role as a receptor for MK and PTN [22,36].

Xenopus MK starts to be expressed in the neural anlage at the late gastrula stage [64]. From this stage to the tailbud stage, MK expression is detected almost exclusively in the nervous system, i.e. the brain and neural tube. If MK mRNA is injected into ventral

vegetal blastomeres at the 8-cell stage, ventral invagination is inhibited, and aberrantly shaped tadpoles with a short tail and spina bifida are obtained [65]. If MK mRNA is injected into dorsal vegetal blastomeres at the 8-cell stage, the whole invagination is blocked, and a huge mass of head neural tissue appears [65]. Consistent with these results, the animal cap assay demonstrated that MK suppresses activin-mediated mesoderm induction, while MK cooperates with activin to induce anterior neural tissues [65].

Like other vertebrates, Zebrafish has two members of the MK family, named *mdk1* and *mdk2*. It is hard to determine from the sequence homology, which is the MK or PTN orthologue. *Mdk2* is expressed shortly after the onset of gastrulation in the presumptive neural plate cells of the epiblast [23]. Ectopic expression of a dominant-negative form that corresponds to the N-terminal half domain of *mdk2* results in severe deficiencies of structures posterior to the midbrain–hindbrain boundary. The expression of hindbrain and neural crest markers is strongly reduced, and the formation of posterior primary moto- and sensory neurons is blocked in these embryos. Thus, *mdk2* is involved in posterior neural development in Zebrafish [23]. The difference in induced neural tissue types in Xenopus MK (anterior neural tissue) and Zebrafish *mdk2* (posterior neural tissue) remains to be investigated. In this context, it should be noted that MK and PTN are differentially expressed during early neurogenesis in the mouse. MK expression starts at E5.5 in the whole embryonic ectoderm, while PTN expression starts at E8.5

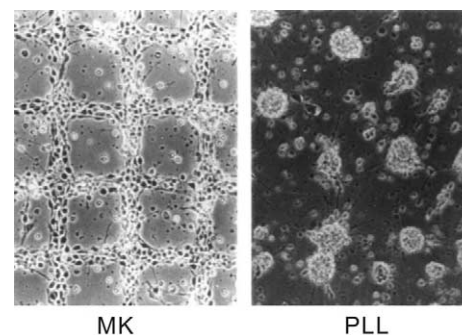


Fig. 4. Neural cell adhesion and neurite outgrowth on a grid of MK. If MK or poly-L-lysine (PLL) is coated in a grid pattern on a culture dish, neuronal cells attach to the grid of MK but not that of PLL. Neurite outgrowth is also promoted on the grid of MK. See for details of the procedures in Refs. [60,61].

exclusively in the neural fold [66]. In the neural tube at 11.5E, MK expression is restricted to the ventricular zone where the neural stem cells reside, whereas PTN expression is restricted to the dorsal half of the ventricular zone [66]. These differential expression profiles might cause the area-specific contributions of MK and PTN to neural development.

MK and PTN are also implicated in neuromuscular junction development. Both proteins induce acetylcholine receptor clustering in *Xenopus*, and are deposited at the intersomitic junction where the neuromuscular junction is located [67,68]. MK is present in spinal cord neurites but PTN in muscle. Both proteins bind to agrin, a heparin sulfate proteoglycan that plays a critical role in neuromuscular junction development. The pattern of acetylcholine receptor clustering caused by MK and PTN changes with the addition of agrin. These results indicate that MK and PTN are neuro- and muscle-derived factors, respectively, that participate in the signal transduction process during neuromuscular junction development.

In adult rats, PTN is expressed in the CA1 region of the hippocampus. Induction of long term potentiation (LTP) increases PTN expression in this area [69]. Hippocampal slices from PTN knockout mice show a lower threshold for LTP induction, and PTN protein administration restores the threshold to the wild-type level [70]. Thus, PTN is an inducible factor that inhibits LTP induction. In MK knockout mice, differentiation of the dentate gyrus cells of the hippocampus is transiently retarded during the neonatal period [71].

Another important activity of MK regarding neural cells is a neuroprotective one [37,72]. This activity is also vital *in vivo*, as shown below. Photoreceptor cells undergo degeneration on constant light exposure. If MK protein is injected into eyes before constant light exposure, MK can prevent the degeneration of photoreceptor cells as effectively as basic FGF, the strongest preventive protein so far reported [73]. Interestingly, MK and PTN are induced in reactive astrocytes by ischemic insults to the brain in animal models and human brain infarction [74–77]. This phenomenon can be observed for LRP, a receptor for MK and PTN [78]. Like the case of photoreceptor cells, intraventricular administration of MK protein ameliorates hippocampal delayed neuronal death

following transient forebrain ischemia in gerbils [79]. In this context, it should be noted that MK is also strongly expressed in neural tumors, including neuroblastomas, astrocytomas and neurofibromas [80–82].

5.2. Neurodegenerative diseases

As described, LRP is a receptor for MK and PTN [43,44; Kadomatsu et al., unpublished data]. An important feature of LRP is its potential roles in Alzheimer's disease [42,83]. In neurons, LRP is the major receptor of amyloid precursor protein (APP), α poE, and α 2M*. These proteins and LRP itself have been genetically linked to Alzheimer's disease [84–88], although the genetic linkage of LRP is controversial [89]. LRP recognizes APP, which contains a Kunitz-type proteinase inhibitor domain, and endocytoses it, and increases amyloid β -protein (A β) production via processing of APP [83,90,91]. By contrast, LRP is also involved in the clearance of A β . Although A β itself is not recognized by LRP, A β forms complexes with apoE, lactoferrin, or α 2M*, and is then endocytosed by LRP [92–94]. LRP thus protects nerve cells from A β -induced cell death [95]. LRP and its ligands, α poE and α 2M*, are deposited at senile plaques in Alzheimer's disease [96–98].

MK is deposited at senile plaques and neurofibrillary tangles in Alzheimer's patients [99]. Deposits of PTN are also found in senile plaques in Alzheimer's disease and Down's syndrome [100]. Furthermore, MK binds to A β and inhibits its cytotoxicity [101]. As already described, MK and PTN bind to LRP. LRP is needed for MK-mediated neural cell survival as well as MK internalization into cells [43,44]. MK, PTN, and LRP are induced in reactive astrocytes [75–79]. Taken together, these data strongly suggest that MK, PTN, and LRP are closely related to the pathogenesis of Alzheimer's disease.

Deposits of MK are also detected in the glial cytoplasmic inclusions in multiple system atrophy [102]. An intriguing feature is that MK is detected only in the glial cytoplasmic inclusions, i.e. not in the neuronal cytoplasmic inclusions. Inclusion formation and degeneration of oligodendrocytes are thought to be primarily involved in the pathogenesis of multiple system atrophy, and the neuronal cells are secondarily affected. The biological significance of deposition of

MK in Alzheimer's disease and multiple system atrophy is an interesting subject to be investigated.

5.3. Development of tooth, lung, kidney and bone

During embryogenesis, MK and PTN are expressed where the epithelial–mesenchymal interaction takes place [27,103]. The teeth, lungs, and kidneys are typical organs formed through the epithelial–mesenchymal interaction. Anti-MK antibody inhibits the development of tooth germ explants [104]. A translucent zone is formed in the mesenchymal tissue in contact with epithelial tissue from the explants or in the mesenchymal tissue surrounding exogenously added beads coated with BMP-2. The formation of a translucent zone is inhibited by beads coated with MK protein [104].

MK is expressed in the embryonic and pseudo-glandular stages of lung development, and decreases during the canalicular and saccular stages (from E15.5 to birth) [27,103]. MK stimulates mesenchymal development of embryonic lung explants [105]. Importantly, MK expression is transiently increased during postnatal development [106]. Since glucocorticoid and retinoic acid regulate postnatal development, the targets of glucocorticoid and retinoic acid are important. MK is upregulated by retinoic acid during the postnatal period [106].

Anti-MK antibody inhibits *in vitro* formation of nephrones [107]. PTN is expressed in the mesenchymal tissue surrounding the ureteric bud. PTN, together with GDNF, induces the branching of ureteric buds *in vitro* [108].

PTN promotes chondrogenesis of chicken limb buds [109]. PTN transgenic mice exhibit bone hyperplasia [110]. MK promotes differentiation of precursor cells into chondrocytes if its cDNA is transfected [111]. MK and PTN induce the migration of osteoblastic cells [33,110].

5.4. Nephritis and vascular restenosis

MK knockout mice show the critical involvement of MK in the pathogenesis of interstitial nephritis and vascular restenosis. Interstitial nephritis can be induced by reperfusion. MK knockout mice are less affected than wild-type mice [112]. In MK knockout mice, neutrophil and macrophage infiltration is lower,

and chemokines, such as MIP2 and MCP1, are less induced. Taking into account that MK can directly recruit inflammatory cells, the direct and indirect effect of MK on recruitment of inflammatory cells is critical in the pathogenesis of interstitial nephritis.

Vascular reconstruction by means of several procedures, e.g. ballooning stenting, and grafting, protects patients from vascular stenosis. However, approximately one-fifth of patients experience this problem, restenosis. Neointima is the basic lesion of the pathology. MK expression is induced during neointima formation. MK knockout mice exhibit much lower neointima formation than wild-type mice [113]. Recruitment of inflammatory cells and smooth muscle cells by MK is critical in this pathogenesis. Furthermore, MK promotes fibroblast-mediated contraction of collagen gel [114], and induces smooth muscle cells to secrete factors, e.g. IL8, that act on endothelial cells [115]. These actions may also change the structure and microenvironment of the blood vessel walls.

6. Cancer

During embryogenesis, MK is highly expressed in the mid-gestational period, and PTN expression reaches the maximum level around birth. These expression profiles postulated the possibility that these proteins could be involved in carcinogenesis and/or be at least markers for carcinomas, like several embryonic antigens, such as CEA and α -fetoprotein. This idea has been examined from early stages of studies on MK and PTN [116,117], and has been proved to be correct by an increasing body of evidence.

6.1. Carcinogenesis

Carcinogenesis processes require sequential molecular events, including mutation of genes, such as the ras and p53 genes, and overexpression of growth factors and their receptors. Therefore, it is important to detect the expression of genes of interest in early stages of carcinogenesis to determine their biological significance in carcinogenesis. There are a few but reliable models that show sequential carcinogenesis in human carcinomas [118]. MK expression is induced

in carcinoma tissues as early as the pre-cancerous stages of human colorectal and prostate carcinomas [119,120]. MK expression is not detected in mild grade dysplasia, but becomes detectable in moderate and severe grade dysplasia of the colon [119]. MK expression is apparent in prostatic intraepithelial neoplasia [120]. Induction of MK expression is also detected in the pre-cancerous lesions, i.e. adenomas, of rat lung carcinomas induced with *N*-nitrosobis(2-hydroxypropyl) amine [121].

It is of note that normal tissues of human adults show restricted expression of MK. By contrast, most carcinoma specimens, including ones of esophageal, gastric, gall bladder, pancreas, colorectal, breast, and lung carcinomas, and Wilms' tumors, express MK at a high level in a tissue type-independent manner [116,117,122,123]. MK expression increases with advancing stages of human astrocytomas and urinary bladder carcinomas, and is significantly linked to the prognosis [81,124]. In general, MK is expressed more intensely and in a wider range of human carcinomas than PTN. For example, high expression of MK is detected in most human lung carcinomas, but PTN expression is barely detectable [117]. MK is also highly expressed in all stages of neuroblastomas, while PTN expression is higher in early stages than in advanced ones [80]. However, some tumors, such as brain tumors and breast carcinomas, express high levels of PTN [6].

6.2. Cancer-related activities

Supporting the possible involvement of MK and PTN in carcinogenesis and cancer advancement, these proteins exhibit several cancer-related activities (Fig. 5). They transform NIH3T3 cells [125,126]. MK enhances the plasminogen activator (PA)/plasmin levels in bovine endothelial cells in dose- and time-dependent manner [127]. PTN exhibits the same activity [128]. This fibrinolytic activity can be achieved through upregulation of urokinase-type PA expression and down-regulation of PA inhibitor-1 expression [127], and is not affected by heat or acid treatment of MK and PTN [128]. These two factors also promote cell growth [12,59,129,130] and cell survival [37,131]. Cell migration-promoting activity of MK and PTN has been demonstrated for neutrophils [112,132],

osteoblastic osteosarcoma cells [33,110], neural cells [22,36], macrophages [112,113], and smooth muscle cells [113]. *N*-Syndecan and PTP[?] are involved in this activity [22,36,110]. Chondroitin sulfate chains, especially chondroitin sulfate E, play a crucial role in MK-mediated cell migration [22,33]. Both cell survival and migration mediated by MK involve P13-kinase and Erk in the intracellular signaling [33,37]. Angiogenic activity can be demonstrated by PTN protein administration of cDNA transfection, while MK exhibits this activity only when its cDNA is transfected [133,134].

With regards to biological activities in cancer, we should discuss neuroblastomas again. MK is highly expressed in all stages of neuroblastomas, and relatively weakly in ganglioneuromas (benign tumors) [80]. By contrast, PTN is highly expressed in favorable stages, i.e. stages I, II, and IVs, but lower in unfavorable stages, III and IV. PTN expression is also high in ganglioneuromas. Importantly, high expression of MK is correlated with a poor prognosis, whereas low expression of PTN is correlated with a poor prognosis [80]. These apparent differences between MK and PTN indicate that these molecules may function differentially in the pathogenesis of neuroblastomas. This idea is contrary to the observation that MK and PTN share in vitro biological activities as well as receptors. It is possible that hitherto unknown differential signaling pathways for MK and PTN might explain and shed light on differences in the biological activities as well as expression profiles of MK and PTN, e.g. in carcinomas and during neural development. We need to address the questions of how MK and PTN are

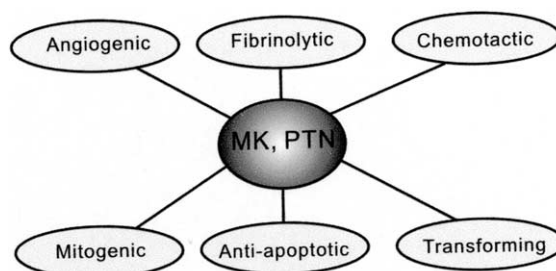


Fig. 5. Cancer-related activities of MK and PTN.

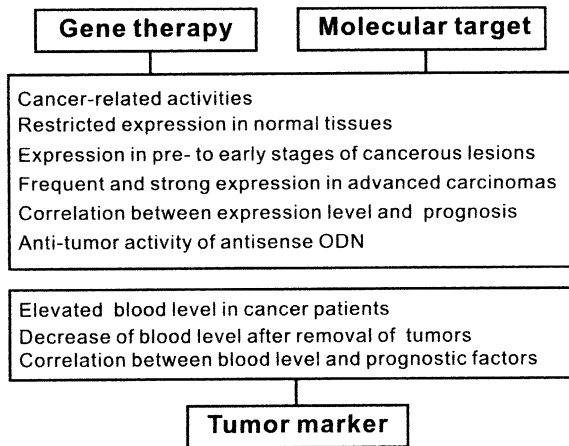


Fig. 6. Evidence-based strategies for cancer therapy and diagnosis. Three important strategies for the application of MK and PTN to cancer and their evidence are listed.

involved in the pathogenesis of neuroblastomas, and whether or not signaling pathways that differentiate MK and PTN exist.

6.3. Tumor marker

One of the characteristics of MK expression is that it occurs frequently and highly in malignant tumors regardless of the tissue type (Fig. 6). This phenomenon is reminiscent of mutations in the p53 gene. In contrast to p53, the blood MK level can be monitored, since MK is a secretory protein. An elevated serum MK level is detected in more than 60% of human adult carcinomas [135–137]. The serum MK level decreases on the removal of tumors [136,137]. Furthermore, the blood MK level is significantly correlated with prognostic factors of neuroblastomas, such as *MYCN* amplification, *TrkA* expression, ploidy, and age (Ikematsu et al., submitted). Therefore, monitoring of the level of blood MK is useful for evaluating the status of carcinomas (Fig. 6). The usefulness of the serum PTN level has also been reported for pancreatic and colon carcinomas [138]. There is a correlation between the serum PTN level and the prognosis in pancreatic carcinoma patients.

MK has an alternative splicing variant that lacks a fragment of exon 3 corresponding to most of the N-terminal half [139]. Interestingly, this variant is only detected in carcinoma tissues, i.e. not in normal ones, and is frequently found in metastatic lymph nodes of

gastrointestinal carcinomas [140]. As a monoclonal antibody is available, it would be interesting to monitor the level of this variant in the blood [141].

6.4. Gene therapy

The 5' regulatory region of human MK determines its tumor-specific expression. When cytomegalovirus (CMV) and MK promoters are compared, the MK promoter exhibits stronger expression in Wilms' tumor cells than the CMV promoter [142]. By contrast, the MK promoter is weak in all normal tissues examined, whereas the CMV promoter is very active in the liver. Accordingly, the CMV promoter has a severe side effect, namely liver damage, if adenovirus containing the CMV promoter-thymidine kinase gene is systemically administered. But the MK promoter-thymidine kinase gene does not have such a side effect [142]. Therefore, of a suicide gene under the control of the MK promoter is a highly potential strategy for curing carcinomas [142,143] (Fig. 6).

6.5. Molecular target

The frequent and high level expression of MK, and significant correlation of the MK level with the prognosis indicate that MK could be a candidate molecular target for therapy for carcinomas (Fig. 6). The cancer-related activities of MK further support this idea (Fig. 6). Indeed, antisense MK oligodeoxyribonucleotides exhibit anti-tumor activity towards mouse colorectal carcinoma cells and neurofibroma-derived cells [82,130,144] (Fig. 6). Antisense MK oligodeoxyribonucleotides suppress cell growth, anchorage-independent growth, and tumor growth of mouse colorectal carcinoma cells in nude mice [130]. Furthermore, they also suppress the growth of pre-grown tumors in nude mice via atelocollagen-mediated gene transfer [130]. The most affected aspect of tumor growth caused by antisense MK oligodeoxyribonucleotides is the mitosis of cancer cells, and angiogenesis is mildly inhibited [130]. Thus, abolition of MK production or disruption of its signaling pathway could be a strong means of curing human carcinomas (Fig. 6). Ribozymes for PTN can suppress the growth of choriocarcinoma, melanoma, and pancreatic carcinoma cells [145–147]. A part of the N-terminal half of PTN acts as

a dominant-negative form in the growth of human breast cancer cells [24]. Thus, PTN and its signaling molecules could also be candidate molecular targets for therapy for human carcinomas.

7. Conclusion

In the review, we described that MK and PTN show approximately 50% identity in amino acid sequence, and have very similar three-dimensional structures. They show distinct but specific expression patterns in neuronal development, and are involved in neurogenesis, as revealed by analyses using *Xenopus* and Zebrafish. In terms of clinical significance, their relation to neurodegenerative diseases, e.g. Alzheimer's disease, is a very intriguing subject to be investigated. They are deposited in senile plaques, and MK blocks A β 's cytotoxicity. Mounting evidence indicating that LRP, a receptor of MK and PTN, is involved in the pathogenesis of Alzheimer's disease supports the idea that MK and PTN are vital in the pathogenesis.

MK and PTN exhibit the same biological activities to similar extents, including mitogenic, anti-apoptotic, transforming, fibrinolytic, chemotactic, and angiogenic ones. They also share receptors. Further analyses of their action mechanisms should shed light on therapies for neurodegenerative diseases and cancer. As to employing MK and PTN for gene therapy and as molecular targets for cancer, translational research for clinical application of these strategies is currently being carried out in several laboratories, and should provide a new avenue for cancer therapy. To further establish blood MK and PTN as tumor markers, prospective studies on blood levels and prognosis will provide useful information.

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