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THE MIDKINE FAMILY IN CANCER, INFLAMMATION AND NEURAL DEVELOPMENT

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ABSTRACT

The midkine (MK) family consists of only two members, namely MK and pleiotrophin (PTN). MK and PTN share receptors and biophysical characteristics, such as a heparin-binding property. MK and PTN exert several biological activities, which include fibrinolytic, anti-apoptotic, mitogenic, transforming, angiogenic, and chemotactic ones. These activities suggest that these growth factors are involved in carcinogenesis. Indeed, strong expression of MK and PTN in human carcinomas, and the anti-tumor activity of antisense oligonucleotides for MK and ribozymes for PTN further support their importance in cancer. In addition, MK plays critical roles in the pathogeneses of various disorders involving inflammation such as reperfusion- and cisplatin-induced renal dysfunction and vascular restenosis after angioplasty. MK antisense oligonucleotide ameliorates these disorders. Zebrafish and Xenopus MK can induce neural tissues. MK and PTN are localized in the radial glial processes of the embryonic brain, and are induced in reactive astrocytes by ischemic insults. I summarize here the biological significance of the MK family in cancer, inflammation and neural development.

Key Words: growth factor, midkine, cancer, inflammation, nervous system

1. INTRODUCTION

Growth factors play pivotal roles in intercellular communication, and eventually in tissue building and remodeling. The midkine (MK) family consists of only two members, namely MK and pleiotrophin (PTN; also called HB-GAM) (1). Intriguing features of these two molecules are that they are closely linked to neural development as well as the pathogeneses of neurodegenerative diseases, and that at the same time they are involved in cancer development and inflammation. MK was found to be the product of a retinoic acid-responsive gene discovered on screening for induced genes during the differentiation of embryonal carcinoma cells (2, 3). PTN cDNA was cloned (4, 5) through searches for neurite outgrowth activity (6) or mitogenic activity toward fibroblasts (7). Chicken MK is also called RIHB (8). PTN is also called OSF-1 (9), HBNF (10), and HARP (11). In this review, I summarize the biological and clinical significance of the MK family.

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2. PROTEIN STRUCTURE, GENE ORGANIZATION, RECEPTORS AND SIGNALING

As the protein structure, gene organization, receptors and signaling for the MK family have been described in detail in a recent review (1), I only briefly describe them here. MK and PTN exhibit approximately 50% identity in amino acid sequence. All 10 cysteine residues are conserved in vertebrates (Fig. 1). Each protein consists of N- and C-terminal half domains (Figs. 1 and 2). The two domains have very similar three- dimensional structures, namely three anti-parallel β-sheets (12). In the C-terminal half domain of MK, there are two clusters of basic amino acids, called clusters I and II (Fig. 2). Cluster I is particularly important as to binding to heparan and chondroitin sulfate (13-15). The C-terminal half domain of MK and PTN is

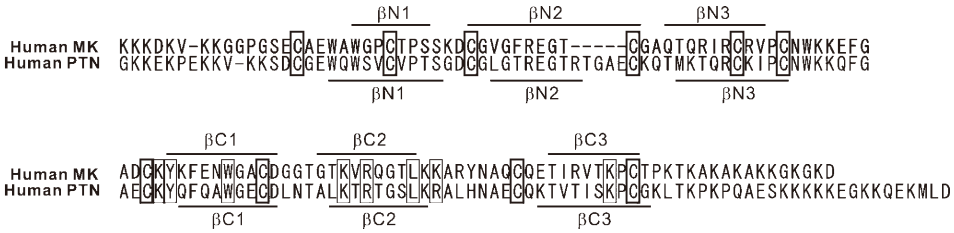

Fig. 1 Homology between human MK and PTN.

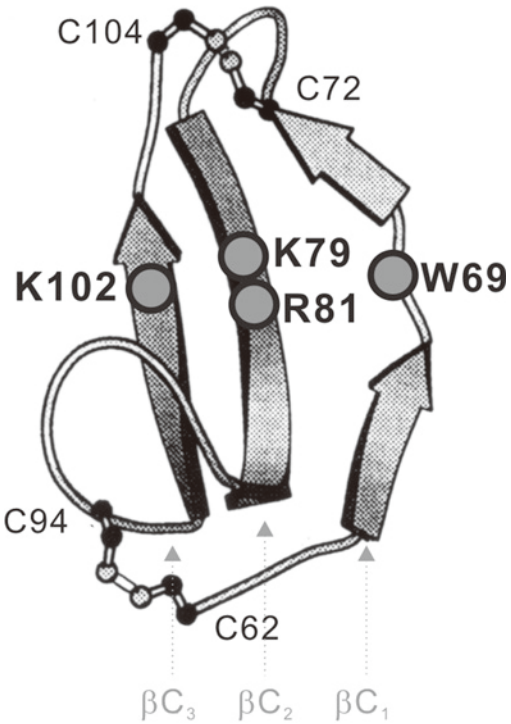

Fig. 2 Three-dimensional structure of the C-terminal half domain of human MK.

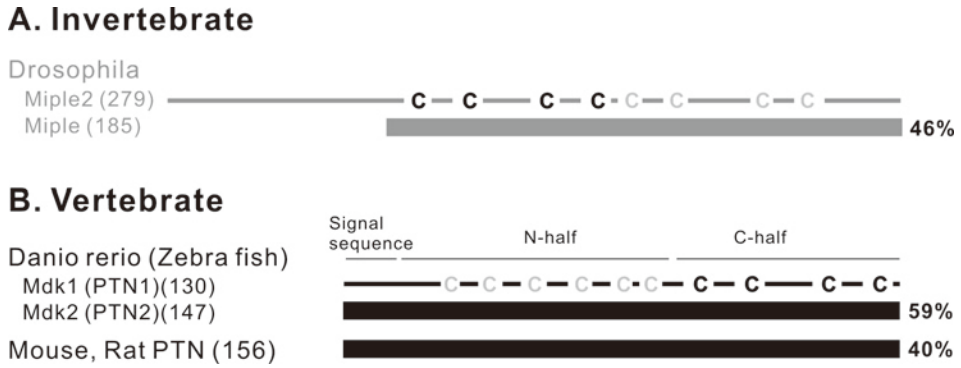

Fig. 3 Evolutional conservation of the MK family.

vertebrates is conserved as the N-terminal half domain of MK/PTN in invertebrates (Fig. 3). This feature is in consistent with the fact that the C-terminal half domain of MK is biologically active in vertebrates (13-15).

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 have a dominant negative effect on neurogenesis and the growth of human breast cancer cells, respectively (16, 17).

Although three glutamine residues in human MK that are responsible for transglutaminase-mediated dimer formation (18) have not been conserved throughout evolution, dimer formation itself is important for the MK action. The MK dimer is formed in both transglutaminase-dependent and independent manners, is enhanced by heparin, and enhances the fibrinolytic activity of MK (18).

As described above, cluster I is vital for binding to heparan and chondroitin sulfate. MK binds to syndecan-1, -3 and -4, and PTN binds to syndecan-3 (19-22). MK and PTN both bind to protein tyrosine phosphatase (PTP) ζ (15, 23, 24). Syndecan is heparan sulfate proteogycan, and PTP ζ is a chondroitin sulfate proteoglycan. In addition to these proteoglycans, MK and PTN also bind to LDL receptor-related protein (25-27), anaplastic lymphoma kinase (28, 29), and integrin $\alpha_4\beta_1$, $\alpha_6\beta_1$ (30). Thus, the signaling of MK and PTN is thought to be mediated through a receptor complex.

The genes for human MK and PTN are named *MDK* and *PTN*, respectively. They are located at 11 p11.2 (*MDK*) and 7q33-q34 (*PTN*). A retinoic acid-responsive element and WT-1 (a suppressor gene product)-responsive elements are found in the 5' region of *MDK* (31, 32).

3. CANCER

MK and PTN exhibit several cancer-related activities (Fig. 4). They transform NIH3T3 cells (33, 34), and enhance the plasminogen activator (PA)/plasmin levels in bovine endothelial cells in dose- and time-dependent manners (35). This fibrinolytic activity can be achieved through upregulation of urokinase-type PA expression and down-regulation of PA inhibitor-1 expression (35), and is not affected by heat or acid treatment of MK and PTN (36). These two factors also promote cell growth (7, 37-39) and cell survival (40, 41). The cell migration-promoting activity of MK and PTN has been demonstrated for neutrophils (42, 43), osteoblastic osteosarcoma cells (44, 45), neural cells (15, 24), macrophages (43, 46), and smooth muscle cells (46). Syndecan-3 and

PTPζ are involved in this activity (15, 24, 44). Chondroitin sulfate chains, especially chondroitin sulfate E, play a crucial role in MK-mediated cell migration (15, 45). Both cell survival and migration mediated by MK involve PI3-kinase and Erk in the intracellular signaling (40, 45). Angiogenic activity can be demonstrated by PTN protein administration or cDNA transfection, while MK exhibits this activity only when its cDNA is transfected (47, 48).

Consistent with these in vitro activities, a variety of data on human carcinomas indicates that MK and PTN are involved in carcinogenesis and tumor development. MK expression is induced in carcinoma tissues as early as at the precancerous stages of human colorectal and prostate carcinomas (49, 50). MK expression is not detected in mild grade dysplasia, but becomes detectable in moderate and severe grade dysplasia of the colon (49). MK expression is apparent in prostative intraepithelial neoplasia (50). Induction of MK expression is also detected in precancerous lesions, i.e., adenomas, of rat lung carcinomas induced with N-nitrosobis(2-hydroxypropyl)amine (51).

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 (52-55). MK expression increases with advancing stages of human astrocytomas and urinary bladder carcinomas, and is significantly linked to the prognosis (56, 57). 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 (53). MK is also highly expressed in all stages of neuroblastomas, while PTN expression is higher in early stages than in advanced ones (58). However, some tumors, such as brain tumors and breast carcinomas, express high levels of PTN (59).

MK is highly expressed in all stages of neuroblastomas, and relatively weakly in ganglioneuromas (benign tumors) (58). By contrast, PTN is highly expressed in favorable stages, i.e., stages I, II, and IVs, but lower in unfavorable ones, III and IV. PTN expression is also high in ganglioneuromas. Importantly, high expression of MK is correlated with a poor prognosis,

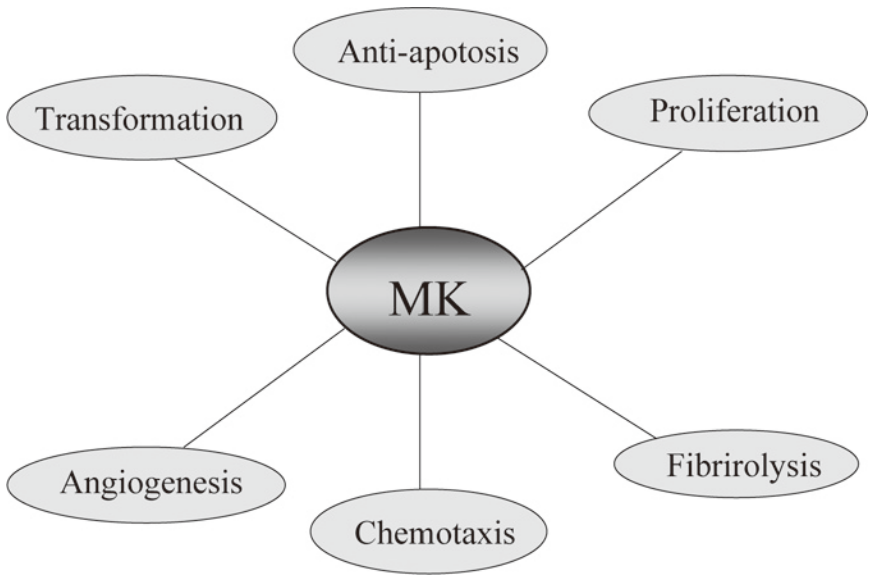

Fig. 4 Cancer-related activities of the MK family.

whereas low expression of PTN is correlated with a poor prognosis (58). 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.

One of the characteristics of MK expression is that it occurs frequently and highly in malignant tumors regardless of the tissue type. 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 (60, 61). The serum MK level decreases on removal of the tumors (61). Furthermore, the blood MK level is significantly correlated with prognostic factors for neuroblastomas, such as *MYCN* amplification, *TrkA* expression, ploidy, and age (62). Therefore, monitoring of the level of blood MK is useful for evaluating the status of carcinomas. The usefulness of the serum PTN level has also been reported for pancreatic and colon carcinomas (63). There is a correlation between the serum PTN level and the prognosis in pancreatic carcinoma patients.

The frequent and high level expression of MK, and the significant correlation of the MK level with the prognosis indicate that MK could be a candidate molecular target for therapy for carcinomas. The cancer-related activities of MK further support this idea. Indeed, antisense MK oligodeoxyribonucleotides exhibit anti-tumor activity toward mouse colorectal carcinoma cells and neurofibroma-derived cells (39, 64-66). Antisense MK oligodeoxyribonucleotides suppress cell growth, anchorage-independent growth, and tumor growth of mouse colorectal carcinoma cells in nude mice (39). Furthermore, they also suppress the growth of pre-grown tumors in nude mice via atelocollagen-mediated gene transfer (39). The aspect of tumor growth most affected by antisense MK oligodeoxyribonucleotides is the mitosis of cancer cells, and angiogenesis is mildly inhibited (39). Thus, abolition of MK production or disruption of its signaling pathway could be a strong means of curing human carcinomas. Ribozymes for PTN can suppress the growth of choriocarcinoma, melanoma, and pancreatic carcinoma cells (67-69). A part of the N-terminal half of PTN acts as a dominant-negative form in the growth of human breast cancer cells (17). Thus, PTN and its signaling molecules could also be candidate molecular targets for therapy for human carcinomas.

The 5' regulatory region of human MK determines its tumor-specific expression. When cytomegalovirus (CMV) and MK promoters were compared, the MK promoter exhibited stronger expression in Wilms' tumor cells than the CMV promoter (70). 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 (70). Therefore, transfer of a suicide gene under the control of the MK promoter is a highly potential strategy for curing carcinomas (70, 71).

4. INFLAMMATION

MK knockout mice show the critical involvement of MK in the pathogeneses of vascular restenosis, interstitial nephritis, cisplatin-induced renal injury, and rheumatoid arthritis (43, 46, 72, 73). 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 restenosis. Neointima is the basic lesion in the pathology. MK expression is induced during neointima formation. MK knockout mice exhibit much lower neointima formation than wild-type ones (46). Recruitment of inflammatory cells and smooth muscle cells by MK is critical for this pathogenesis. Furthermore, MK promotes fibroblast-mediated contraction of a collagen gel (74), and causes smooth muscle cells to secrete factors, e.g., IL8, that act on endothelial cells (75). These actions may also change the structure and microenvironment of the blood vessel walls.

Interstitial nephritis can be induced by reperfusion. MK knockout mice are less affected than wild-type ones (43). 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 effects of MK on recruitment of inflammatory cells are critical in the pathogenesis of interstitial nephritis.

Cisplatin is frequently used as an anti-cancer drug. However, an adverse effect, i.e., proximal tubule injury, limits its clinical use. Although the primary effect of cisplatin is injury to the proximal tubules, the subsequent inflammation strikingly worsens the renal injury. It has been proven that MK is critically involved in the pathogenesis of cisplatin-indnced renal injury (72).

MK antisense oligonucleotide efficiently ameliorates these inflammation-related disorders, i.e., vascular restenosis, interstitial nephritis, and cisplatin-induced renal injury (72, 76, 77).

5. THE NERVOUS SYSTEM

Xenopus MK starts to be expressed in the neural anlage at the late gastrula stage (78). From this stage to the tailbud stage, MK expression is detected almost exclusively in the nervous system, i.e., the brain and neural tube. MK mRNA injection into ventral vegetal blastomeres at the 8-cell stage inhibits ventral invagination, and produces aberrantly shaped tadpoles with a short tail and spina bifida (79). MK mRNA injection into dorsal vegetal blastomeres at the 8-cell stage completely blocks invagination, and produces a huge mass of head neural tissue (79). 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 (79).

Zebrafish has two members of the MK family, named mdk1 and mdk2. Mdk2 is expressed shortly after the onset of gastrulation in the presumptive neural plate cells of the epiblast (16). 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 the embryos. Thus, mdk2 is involved in posterior neural development in Zebrafish (16). The difference in the induced neural tissue type between Xenopus MK (anterior neural tissue) and Zebrafish mdk2 (posterior neural tissue) remains to be investigated.

Both MK and PTN promote neurite outgrowth (5, 6, 37, 80, 81) and nerve cell migration (15, 24). Both proteins are localized in radial glial processes in the rat embryonic brain (82, 83). However, 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 exclusively in the neural fold (84). 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 (84). These differential expression profiles might cause the area-specific contributions of MK and PTN to neural 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 (85). 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 (86). 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 (87).

Another important activity of MK regarding neural cells is a neuroprotective one (40, 88). 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 (89). Interestingly, MK and PTN are induced in reactive astrocytes by ischemic insults to the brain in animal models and human brain infarction (90-93). This phenomenon has been observed for LRP, a receptor for MK and PTN (94). As in the case of photoreceptor cells, intraventricular administration of MK protein ameliorates hippocampal delayed neuronal death following transient forebrain ischemia in gerbils (95). MK-expressing adenovirus can also ameliorate ischemia-iduced neuronal death in rats. In this context, it should be noted that MK is also strongly expressed in neural tumors, including neuroblastomas, astrocytomas, and neurofibromas (56, 58, 64).

MK is deposited in senile plaques and neurofibrillary tangles in Alzheimer's patients (97). Deposits of PTN are also found in senile plaques in Alzheimer's disease and Down's syndrome (98). Furthermore, MK binds to Aβ and inhibits its cytotoxicity (99). As already mentioned, MK and PTN bind to LRP. LRP is needed for MK-mediated neural cell survival as well as MK internalization into cells (25-27). MK, PTN and LRP are induced in reactive astrocytes (91-95). Taken together, these data 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 (100). An intriguing feature is that MK is only detected 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.

6. CONCLUSION

In this review, I described the diverse array of biological activities of MK and PTN and their clinical implication. Based on the results of experimental MK antisense therapy, it is expected that MK is a vital molecular target for several diseases, such as cancer and inflammation-related diseases. In addition, the use of serum MK as a marker for monitoring the cancer status could be the closest clinical application. On the other hand, there are many issues to be resolved. The molecular mechanism that differentiates MK and PTN signaling remains unclear. The precise structure of the receptor complex of MK and PTN has also got to be verified.

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