Review

Midkine, a heparin-binding cytokine with multiple roles in development, repair and diseases

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Abstract: Midkine is a heparin-binding cytokine or a growth factor with a molecular weight of 13 kDa. Midkine binds to oversulfated structures in heparan sulfate and chondroitin sulfate. The midkine receptor is a molecular complex containing proteoglycans. Midkine promotes migration, survival and other activities of target cells. Midkine has about 50% sequence identity with pleiotrophin. Mice deficient in both factors exhibit severe abnormalities including female infertility. In adults, midkine is expressed in damaged tissues and involved in the reparative process. It is also involved in inflammatory reactions by promoting the migration of leukocytes, induction of chemokines and suppression of regulatory T cells. Midkine is expressed in a variety of malignant tumors and promotes their growth and invasion. Midkine appears to be helpful for the treatment of injuries in the heart, brain, spinal cord and retina. Midkine inhibitors are expected to be effective in the treatment of malignancies, rheumatoid arthritis, multiple sclerosis, renal diseases, restenosis, hypertension and adhesion after surgery.

Keywords: midkine, pleiotrophin, proteoglycans, cancer, inflammation, repair

Introduction

Midkine (MK) is a cytokine or a growth factor and belongs to the carbohydrate-binding proteins.1–3 Cytokines and growth factors are classified to structurally-related protein families such as the fibroblast growth factor family. MK is the founding member of a family, which is composed of only two members in humans. The other member is pleiotrophin, also called HB-GAM.4,5

MK promotes growth,6 survival,7 migration8 and gene expression9 of various target cells. It is involved in reproduction10 and repair,11 and also plays pathological roles in many diseases.

MK is attracting much attention in relation to the treatment of diseases. MK inhibitors are expected to be useful in treating cancer,12 rheumatoid arthritis,13 multiple sclerosis,14 hypertension,15 renal diseases9 etc., while MK itself is promising for the treatment of ischemic brain injury,11 retinal degeneration16 and heart failure.17

We found MK as a product of a gene, whose expression was induced at the early stages of the retinoic acid-induced differentiation of teratocarcinoma stem cells.18 PTN was found as a protein with neurite-promoting activity19 or as a factor with growth-promoting activity to fibroblasts.20 We reported full protein sequence of MK in 1990,21 and the sequence of PTN was reported subsequently22,23: MK and PTN were revealed to have about 50% sequence identity.

The precisely controlled manner of MK expression during embryogenesis24 and unique features of the protein structure21 were sufficient to convince us of the importance of the molecule, and we initiated systematic studies. Glycosaminoglycan-recognizing activity of MK25 was an additional factor tempted me to study MK extensively, since my long term

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Abbreviations: ALK: anaplastic lymphoma kinase; LRP: low density lipoprotein receptor-related protein; MFP: medial floor plate; MK: midkine; PTN: pleiotrophin; PTPζ: Receptor-like protein tyrosine phosphatase-ζ.

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interest has been the physiological significance of carbohydrate recognition at the cell-surface.  

Here I summarize the information currently available on MK. The details of earlier findings were described in previous reviews.\(^1\),\(^2\)

**Protein and gene**

MK is rich in basic amino acids and cysteine, and has a molecular weight of about 13 kDa\(^{21}\) (Fig. 1). MK is conserved from humans to fish, human MK and mouse MK having 87% sequence identity.\(^1\),\(^2\)\(^7\) Drosophila melanogaster lacks MK, but has miple-1 and miple-2, which have a motif common to MK and PTN\(^{28}\) (Fig. 1).

MK is mainly composed of two domains held by disulfide linkages.\(^{29}\) The more C-terminally located domain (C-domain) has principal heparin-binding activity and retains some of the physiological activities.\(^{30}\) Each domain of MK has weak homology to the thrombospondin Type I repeat\(^{31}\) (Fig. 1). Both domains are composed of three antiparallel \(\beta\)-sheets as revealed by NMR spectroscopy.\(^{32}\) The C-domain has two clusters of basic amino acids (Cluster-1 and -2), which are required for heparin-binding\(^{32}\)–\(^34\) (Figs. 1, 2). Among them, Cluster-1 is the evolutionarily conserved one (Fig. 1). MK tends to form dimers via spontaneous association, and the dimers are stabilized by crosslinking with transglutaminase.\(^{35}\) Dimerization appears to be required for MK activity.\(^{35}\) After dimerization, Cluster-2 may form a fused and strong binding site.\(^{32}\)

MK is produced in recombinant forms in L cells,\(^{36}\) baculovirus,\(^{37}\) yeast,\(^{38}\) and Escherichia coli,\(^{39}\) and has also been synthesized chemically.\(^{40}\) During

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**Fig. 1.** Primary structure of human MK. *, amino acids conserved between human MK and PTN; O, amino acids conserved among Drosophila miples, human MK and human PTN; ●, tryptophan conserved in the thrombospondin superfamily; ★, key amino acids involved in heparin binding (★, Cluster-1; ★, Cluster-2); \(\beta\)-sheet structure. Lines show the position of disulfide linkages.

**Fig. 2.** Binding of heparan sulfate trisulfated unit and chondroitin sulfate E unit to C-domain of human MK. Two heparin-binding sites in the C-domain are encircled.
the chemical synthesis, MK with aberrant disulfide bridges was rigorously removed. MK is an adhesive protein, and care is needed to avoid absorption to vessels as explained elsewhere.41)

The human MK gene (MDK) is present on chromosome 11 at p11.242) between the diacylglycerol kinase z gene and muscarinic acetylcholine receptor 4 gene,1) while the mouse counterpart (Mdk) is on chromosome 2.43) MDK and Mdk encompass 2 kb and have 4 exons.44),45)

MK expression is induced by retinoic acid, and the promoter region has a functional retinoic acid-responsive element.46) Glucocorticoid suppresses MK expression through its nuclear receptor.47) The promoter region also has a binding site for WT-1, the product of the Wilms’ tumor suppressor gene.48) Furthermore, hypoxia induces MK expression through the binding of hypoxia inducible factor-1α (HIF-1α) to a hypoxia responsive element in the MK promoter.49) MK expression is strictly controlled both spatially and temporally during embryogenesis.24),50) Generally, MK is most intensely expressed during midgestation, while the expression is weak or absent in the majority of adult tissues.1),24)

**In vitro activities and mechanism of action**

MK exhibits various activities in vitro (Table 1). For example, soluble MK promotes the growth of fibroblasts,36) survival of embryonic neurons1),7),51)–53) and expression of chemokines.9),54) The substratum-bound form enhances outgrowth of neurites0),36),37) and migration of neutrophils,55) macrophages,56) embryonic neurons8) and osteoblast-like cells.57) Occasionally, substratum-bound MK shows stronger activity than the soluble form. For example, the growth and survival of neural precursor cells is promoted only by substratum-bound MK.58) Probably, MK in the tissue is delivered to target cells as a matrix-bound form. Inhibition of MK-dependent migration of UMR106 osteoblast-like cells is frequently employed in the screening of MK inhibitors (Matsui et al., unpublished observations).

MK is inhibited by heparin, a sulfated glycosaminoglycan. Therefore it is likely that recognition of glycosaminoglycans in proteoglycans is essential for MK activities. Oligomers of heparan sulfate trisulfated units and those of chondroitin sulfate E units have been identified as structures required for strong binding to MK41),59)–61) (Fig. 2). The affinity of the two structures for MK is considered similar, based on behavior upon MK-affinity chromatography, surface plasmon resonance spectroscopy, and inhibition of neurite outgrowth.41) The presence of these high-affinity binding structures in the embryonic brain of mice has been confirmed by an analysis of glycosaminoglycans synthesized by the cells.51)

Receptor-like protein tyrosine phosphatase-ζ (PTPζ),8) syndecans,50),62),63) glypican-2,64) PG-M/versican65) and neuroglycan C66) are proteoglycans with strong affinity for MK. Among them, PTPζ, a
chondroitin sulfate proteoglycan, is an established component of the MK receptor. PTPζ binds to MK with a Kd of 0.56 nM, which decreases to 8.8 nM after the removal of chondroitin sulfate chains.

Analyses of MK-binding proteins from embryonic brains have revealed that low density lipoprotein receptor-related protein (LRP), α4β1-integrin, and α6β1-integrin also serve as MK receptors. These proteins and PTPζ form a receptor complex, and MK promotes this process. A model of MK action through the receptor complex is shown in Fig. 3. By the complex formation, PTPζ and the downstream signaling systems of integrins might come to be located close together.

After MK stimulation, intracellular tyrosine phosphorylation increases. Inhibitors of Src family kinases hinder MK activities, indicating the importance of these kinases in signaling. After MK action, PTPζ may act on a tyrosine phosphate residue, which locks Src kinase in an inactive state. Removal of the phosphate residue will lead to the activation of Src, as in the case of PTN signaling. Alternatively, MK stimulates dimerization of PTPζ, leading to inactivation of PTPζ, which might compete with Src. An observation that vanadate, an inhibitor of PTPζ, inhibits MK appears to favor the former view.

Increased tyrosine phosphorylation of paxillin accompanying MK action occurs in case of cell migration of osteoblast-like cells. Upon promotion of survival, activation of P13 kinase and MAP kinase takes place, followed by suppression of caspases.

For promotion of the migration and invasion of human head and neck squamous cell carcinoma cells, MK binds to α6β1-integrin and tetraspanin, and induces tyrosine phosphorylation of FAK followed by activation of paxillin and STAT1α pathway. Phosphorylation of STAT3 by MK stimulates the proliferation of postconfluent 3T3-L1 cells, leading to adipogenesis. Notch2 is a receptor for MK upon epithelial-mesenchymal transition in immortalized keratinocytes, acting through the Jak2/STAT3 system. On the other hand, STAT5 phosphorylation is suppressed by MK.

Anaplastic lymphoma kinase (ALK) has been proposed to be an MK receptor. ALK also forms complex with LRP and integrins, suggesting that it is recruited to the receptor complex and plays roles in MK signaling (Muramatsu, H. et al., unpublished observations). After activation by MK, ALK phosphorylates insulin receptor substrate-1, activates MAP kinase and PI3 kinase and causes transcriptional activation of NFκB.

Neuroglycan C serves as an MK receptor upon process extension in oligodendrocyte precursor-like cells. Neuroglycan C also functions in a receptor complex (Ichihara et al., unpublished observations). In addition to various cell-surface proteins mentioned above, neuropilin-1 was identified in MK-binding proteins from UMR106 osteoblast-like cells (Muramatsu, H. et al., unpublished observations).

MK binds to nucleolin, a nuclear protein which is also located at the cell surface and functions as a shuttle to the nucleus. LRP, a component of the MK receptor, serves to internalize the bound MK. After this internalization, cytoplasmic MK is transferred to the nucleus by nucleolin and by laminin-binding protein precursor, and the transportation is important to cell survival induced by MK. Thus, MK might also act within the nucleus. Indeed, MK transferred to the nucleolus is involved in the synthesis of ribosomal RNA at the place.

Translation initiation factor (eIF3) is also an MK-binding protein in the embryonic brain, the physiological significance of which remains to be established (Muramatsu, H. et al., unpublished observations).

Neurogenesis

During midgestation period, MK expression is intense in neural tissue, epithelial tissue in the pro-
cess of epithelial-mesenchymal interactions and mesoderm undergoing remodeling. In each of these tissues, further studies have revealed the developmental significance of MK activity.

During the development of *Xenopus laevis*, MK is found in the neural anlagen and strongly expressed in the brain and spinal cord. When MK mRNA is injected into the dorsal vegetal region of 8-cell embryos, neural tissues enlarge abnormally. The excised ectoderm from this embryo is enhanced in development of anterior neural tissue after activin treatment, and is suppressed in mesoderm induction as compared to the normal ectoderm.

The role of MK in the initial stages of neurogenesis has been elucidated in the zebrafish, which has two MK molecules (Mdkα and Mdkβ) as a result of gene duplication. Mdkα is expressed in the paraxial mesoderm and is involved in the formation of the medial floor plate (MFP) in the adjacent neural tube. MFP organizes the specification of neurons and outgrowth of axons in the ventral spinal cord. Overexpression of Mdkα results in an enlargement of the MFP and reduction in the size of the notochord, and downregulation of Mdkα expression results in a defective MFP and in increased cell density of the notochord. Mdkα appears to promote the growth and survival of MFP progenitors, and the increased number of MFP cells probably explains the reduction in size of the notochord. On the other hand, overexpression and knockdown experiments have indicated that Mdkβ expressed in the neural plate is required for the earliest steps of cell specification at the neural plate border and is essential for the development of neural crest cells and sensory neurons.

In the developing cortex of mice and rats, MK expression is intense in the basal layer, which is composed of proliferating neural precursor cells including neural stem cells. Radial glial processes are extended structures derived from neural stem cells, and differentiated neurons migrate externally along these processes. MK is also strongly expressed in radial glial processes, and is a good immunohistochemical marker of the processes. An *in vitro* study has shown that MK enhances the growth and survival of neural precursor cells without inhibiting their differentiation capability. This finding provides a cell biological basis to MK activities found in *Xenopus laevis*. In addition, MK secreted by the neurites of embryonic neurons induces clustering of acetylcholine receptors on myoblasts, suggesting that MK is involved in the formation of synapses.

In spite of important roles of MK in neurogenesis, mice deficient in *Mdk* exhibit normal phenotypes in overall neural functions. However, in depth analysis of the deficient mice revealed deficits in specific neural functions. These mice are in a hypodopaminergic state in terms of levels of dopamine and its receptors, with defects related to dopamine function (prepulse inhibition). Furthermore, mice deficient in *Mdk* or *Ptn* exhibit a moderate auditory deficit, while mice deficient in both show a severer phenotype. Although the defect in the single knockout mice may be due to deficit in sensory neurons, the severe defect in the double knockout could principally be caused by abnormalities in the cochlea, including a drastic decrease in β-pectin expression. In any event, it is likely that the loss of MK is usually compensated for by other molecules during mouse neurogenesis, but in certain regions it is not compensated leading to expression of phenotypes. Detailed analyses of MK deficient mice are expected to reveal more neurological phenotypes.

**Epithelial-mesenchymal interactions**

The role of MK in epithelial-mesenchymal interaction has been studied using an artificial blood vessel model, in which vascular endothelial cells from the human umbilical cord are cultured on human aortic smooth muscle cells. The endothelial cells secrete MK, which acts on smooth muscle cells and induces production of factors including IL8. IL8 then acts on the endothelial cells and promotes their growth. Thus, MK plays a key role in the interaction between epithelial cells and mesenchymal cells. In the *in vitro* differentiation system of the lung germ, MK produced by epithelial tissue stimulates the development of mesenchymal tissue. The development of tooth germ *in vitro* is inhibited by anti-MK antibody. In this system, MK appears to suppress excessive activity of BMP-2. Furthermore, MK which is expressed in lung epithelial cells causes vascular remodeling in the organ. MK is also involved in the epithelial-mesenchymal transition of tumor cells.

In terms of the remodeling of mesenchyme, MK has been identified as an autocrine factor that induces adipocyte formation from 3T3-L1 cells. Furthermore, transfection of MKcDNA to chondrogenic cells enhances chondrogenesis.
Reproduction

In spite of various roles of MK in developmental processes, MK-deficient mice are born without major defects.\(^\text{86}\) The same is true for mice deficient in PTN. However, mice deficient in both were born with 1/3 of the frequency expected by Mendelian segregation,\(^\text{10}\) were small in size\(^\text{10}\) and about 50% died before 4 weeks (Muramatsu, H. et al., unpublished observations). Thus, MK and PTN play important roles in development, and compensate for each other. Furthermore, the double knockout mice exhibited female infertility: after repeated mating with wild-type males, 79% of the double-deficient females remained sterile.\(^\text{10}\) Defects in follicular maturation, an altered estrous cycle and vaginal malformation have been found to be the basis of the infertility.

Among them, the defects in follicular maturation appear to be the principal cause; MK and PTN are concluded to be important in follicular maturation.\(^\text{10}\)

The efficiency with which in vitro fertilized bovine embryos develop to the blastocyst stage is increased by adding MK to the culture medium.\(^\text{92}\) This effect of MK is mediated by cumulus cells surrounding oocytes. MK acts on cumulus cells to help them survive and secrete factors acting on oocytes.\(^\text{93}\) This MK activity has practical importance. Furthermore, a similar mechanism can be considered for the actions of MK and PTN to promote follicular maturation.

Cancer

MK is overexpressed in many malignant tumors of humans (Table 2),\(^\text{1,154}\) including hepatocellular

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carcinoma,95) gastric carcinoma,95) colon carcinoma,95) lung carcinoma,96) urinary bladder carcinoma,97) prostate carcinoma,98) neuroblastoma99) and astrocytoma.100) Overexpression is observed in about 80% cases in respective tumors.11 Furthermore, patients with high MK expression in the tumor frequently have a worse prognosis than those with low MK expression (Table 2).1,102)–106) MK is expected to contribute to tumor invasion by promoting growth, survival and migration of tumors and by promoting angiogenesis. Indeed, transfection with MK cDNA results in malignant transformation of NIH 3T3 cells.101) Furthermore, MDK, MK has been also shown to be a mediator of chemotherapy resistance to neighboring cells.103) AcylCoA synthetase 5 is frequently overexpressed in malignant gliomas and contributes to cell survival under extracellular acidosis. Overexpression of the enzyme leads to increased expression of MK, which is partly responsible for the effect of the enzyme.104)

The induction of MK in various tumors is probably mediated by hypoxic conditions in the tumor.49) Loss of function of WT1, Wilms’ tumor suppressor gene, appears to be the cause of high levels of MK expression in Wilms’ tumor,94) since a functional WT1 binding site is present in the MK promoter.48) Oncogenesis due to loss of function of WT1 is likely to involve the induction of MK expression. Furthermore, MK is overexpressed in malignant peripheral nerve sheath tumor, based on a lack of function of NF1, another tumor suppressor gene.105)

Serum MK levels can be measured with a sandwich enzyme-linked immunosassay.106),107) The levels are increased in patients with cancer,106)–108) rheumatoid arthritis13) and Alzheimer’s disease.109) Because of the distinct clinical features of these diseases, MK is expected to be helpful in the screening of cancer.106),107),110)–113) MK levels are not increased in the cases of viral hepatitis,110) with a few exceptions, in which hepatocellular carcinoma developed later (Salama et al., unpublished observations). Patients with high MK serum levels had a poor prognosis in cases of esophageal carcinoma111) and neuroblastoma.114),115) For large scale screening, a combination of two monoclonal antibodies and usage of beads and an automated system appear to be especially helpful.116) Because of its elevated levels even early on, irrespective of α-fetoprotein levels, MK is considered to be helpful in detection of α-fetoprotein-negative hepatocellular carcinoma.112) Further information is provided in a previous review.108)

In terms of cancer detection, a truncated MK in which exon 3 is skipped by alternative splicing may also be helpful.117) In certain carcinomas such as colon carcinoma, a truncated MK protein is expressed only in tumor tissue, especially in metastasized lymph nodes.117)–119) Although the truncated form was initially detected as mRNA, subsequent study revealed the truncated protein by using a specific antibody.120) Truncated MK can also transform fibroblasts.121)

The involvement of MK in tumor progression implies that inhibition of the synthesis or action of MK will contribute to cancer therapy. Indeed, an antisense oligoDNA to MK inhibits the growth of mouse colorectal carcinoma cells in culture,12 most probably by down-regulating intracellular tyrosine phosphorylation as mentioned in the previous section. Furthermore the antisense oligoDNA suppresses the growth of the tumor in nude mice. The delivery of antisense oligoDNA into pregrown tumors has been achieved with the aid of atellocollagen.12) siRNA to human MK enhances the growth inhibitory effect of pacitaxel on human prostate carcinoma cells grown in nude mice.122) It is noted that inhibition of the synthesis of human MK had less of an effect on tumor growth than that of mouse MK. This is probably because even after inhibition of the synthesis of human MK, mouse MK provided by the host supports the growth of the xenografted tumor. Indeed, lung metastasis of an MK-negative tumor is less severe in MK-deficient mice than in wild-type mice.123) The growth of hepatocellular carcinoma in mice is inhibited by antisense oligoDNA to MK delivered by nanoparticles.124)

Although polyclonal anti-MK antibodies inhibit the growth of tumor cells in vitro,6),125) many monoclonal antibodies to MK do not have a strong effect, probably because not only extracellular MK, but also intracellular MK contributes to tumor growth, and only a population of anti-MK is able to inhibit intracellular MK. Aptamers to MK did not exhibit significant growth inhibitory effects to tumor cells either. A promising candidate to inhibit MK is a low molecular weight compound. After in silico screening we found that some trifluoro compounds inhibited MK-dependent migration of osteoblast-like cells without significant cytotoxic effects (Muramatsu, T. et al.,
Inflammation and repair

MK expression is induced in damaged tissues, especially after ischemia in blood vessels,\textsuperscript{56} the brain cortex,\textsuperscript{131} and the myocardium,\textsuperscript{17} and exhibits two effects, an enhancement of inflammation and a promotion of survival and repair. Thus, MK is either beneficial or harmful to the injured tissue, depending on its origin. Phenotypes of MK-deficient mice have revealed the role of MK in a pathological state. As an example, upon partial hepatectomy, the remaining liver of MK-deficient mice exhibits less inflammation than that of wild-type mice. However, the growth of the liver is also less extensive in the deficient mice, and as a whole MK-deficiency hinders liver regeneration.\textsuperscript{132}

MK enhances inflammation by promoting the migration of inflammatory leukocytes,\textsuperscript{9,55,56} inducing synthesis of chemokines\textsuperscript{9} and suppressing the increase of regulatory T cells.\textsuperscript{14} Upon ischemic injury to blood vessels, MK recruits inflammatory leukocytes, which secrete factors promoting the migration of smooth muscle cells and trigger the formation of neointima.\textsuperscript{56} This neointima formation, which is a model of restenosis after balloon therapy of infarcted coronary vessels, is less extensive in MK-deficient mice than in wild-type mice.\textsuperscript{56} MK deficiency also has beneficial effects on antibody-induced arthritis (a model of rheumatoid arthritis),\textsuperscript{13} experimental autoimmune encephalitis (a model of multiple sclerosis),\textsuperscript{14} adhesion after surgery\textsuperscript{133} and nephritis, which is caused either by ischemia,\textsuperscript{9} exposure to chemotherapeutics\textsuperscript{134} or diabetes.\textsuperscript{135} The MK-deficient mice used in these experiments were repeatedly backcrossed to C57BL/6 mice so that both mice were in a syngenic state. That the genes flanking Mdk were not altered in the deficient mice was confirmed by sequencing (unpublished observations).

In the above-mentioned diseases, MK inhibitors are candidates for therapeutics. siRNAs, antisense oligoDNAs and aptamers to MK have already proved effective in experimental models (Table 3). Notable examples are the treatment of nephritis with an antisense oligoDNA to MK,\textsuperscript{136} of neointima formation with an siRNA to MK\textsuperscript{137} and of experimental autoimmune encephalitis using an RNA aptamer to MK.\textsuperscript{14} MK inhibitors might be also effective in the treatment of endometriosis, because of the possible involvement of MK in the disease.\textsuperscript{138}

MK is a regulator of the renin-angiotensin system.\textsuperscript{15,139} Hypertension is induced by 5/6 nephrectomy in wild-type mice, but not significantly in MK-deficient mice.\textsuperscript{15} Further studies have revealed that MK is expressed in the lung after 5/6 nephrectomy, and induces the expression of angiotensin-converting enzyme in microvascular endothelial cells.\textsuperscript{15} MK appears to be a key molecule causing hypertension upon chronic nephritis. Thus, there is a possibility that MK inhibitors become useful for the treatment of hypertension. MK is also known to suppress catecholamine biosynthesis in the aorta, but not in other tissues.\textsuperscript{140}

The role of MK in the reparative process was first demonstrated in the retina. Light-induced retinal degeneration is suppressed by the injection of MK into the subretinal region.\textsuperscript{16} Injection of MK into the brain ventricle delays the onset of the death of hippocampal neurons after ischemia.\textsuperscript{11} Transfection and expression of MK is also effective.\textsuperscript{141} Both degeneration and regeneration of injured peripheral nerve are delayed in Mdk-deficient mice compared to wild-type mice.\textsuperscript{142} After ischemia and reperfusion of the heart, more Mdk-deficient mice die due to heart failure than wild-type mice.\textsuperscript{17} Delivery of MK into the heart substantially enhances the survival of the mice.\textsuperscript{17} MK also improves heart function upon chronic heart failure after ischemic injury.\textsuperscript{143,144} Thus, in these diseases, MK itself is expected to be a potent therapeutic by promoting survival of damaged tissues (Table 3). MK produced by the yeast system is best suited for this purpose, since large scale production is possible and the yield of
properly folded protein is high compared to production in bacteria.

MK is expressed in senile plaques in the brain of the patients. Senile plaques are hallmark of the disease, and the primary deposit is amyloid β-peptide. In the brain of aged MK-deficient mice, plaque formation of amyloid β-peptide is more than in the brain of age-matched wild-type mice. Thus, MK expression in Alzheimer’s disease might be induced to counteract the progression of the disease (Muramatsu, H. et al., unpublished observations). Indeed, MK inhibits cytotoxicity of amyloid β-peptide and its fibril formation. MK expression is also markedly increased in the prefrontal cortex of chronic alcoholics, concomitant with shrinkage of the neural tissue. MK is probably synthesized to counteract the loss of neurons also in this case. In the rat hippocampus, chronic administration of morphine and yohimbine also upregulates MK expression.

Conclusions and perspectives

MK is involved in a variety of physiological and pathological processes. I expect that significant roles of MK will be further revealed even in fields not mentioned in this review. In this context, it is proper to mention that MK may be involved in protection against HIV infection, since the binding of HIV to nucleolin appears to be required for viral entry, and MK competes with HIV for nucleolin binding.

Our studies have shown that the MK receptor is a molecular complex containing proteoglycans. More studies are required to clarify all the components of the receptor complex and their mutual interactions. Essential roles of the MK family in development and reproduction have been established by severe phenotypes of mice doubly deficient in both MK and PTN. In depth analysis of MK single-knockout mice is expected to reveal new functions of MK, unshared with PTN.

Clinical application of MK inhibitors is much needed, especially for treatment of malignancy. The improvement in delivery methods of oligonucleotide reagents and development of low molecular weight inhibitors are among the subjects on which further basic research is required for the purpose.

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Profile

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