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Themed Section: Midkine

REVIEW

Structure and function of midkine as the basis of its pharmacological effects

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Midkine (MK) is a heparin-binding growth factor or cytokine and forms a small protein family, the other member of which is pleiotrophin. MK enhances survival, migration, cytokine expression, differentiation and other activities of target cells. MK is involved in various physiological processes, such as development, reproduction and repair, and also plays important roles in the pathogenesis of inflammatory and malignant diseases. MK is largely composed of two domains, namely a more N-terminally located N-domain and a more C-terminally located C-domain. Both domains are basically composed of three antiparallel β-sheets. In addition, there are short tails in the N-terminal and C-terminal sides and a hinge connecting the two domains. Several membrane proteins have been identified as MK receptors: receptor protein tyrosine phosphatase Z1 (PTPζ), low-density lipoprotein receptor-related protein, integrins, neuroglycan C, anaplastic lymphoma kinase and Notch-2. Among them, the most established one is PTPζ. It is a transmembrane tyrosine phophatase with chondroitin sulfate, which is essential for high-affinity binding with MK. PI3K and MAPK play important roles in the downstream signalling system of MK, while transcription factors affected by MK signalling include NF-κB, Hes-1 and STATs. Because of the involvement of MK in various physiological and pathological processes, MK itself as well as pharmaceuticals targeting MK and its signalling system are expected to be valuable for the treatment of numerous diseases.

LINKED ARTICLES
This article is part of a themed section on Midkine. To view the other articles in this section visit http://dx.doi.org/10.1111/bph.2014.171.issue-4

Abbreviations
ALK, anaplastic lymphoma kinase; Hes-1, hairy and enhancer of split-1; HIF, hypoxia-induced factor; LRP, low-density lipoprotein receptor-related protein; Mdka, Midkine-a; Mdkb, Midkine-b; MK, midkine; PTN, pleiotrophin; PTPζ, receptor protein tyrosine phosphatase Z1

Introduction

Growth factors and/or cytokines play fundamental roles in the regulation of cellular activities and in various pathological processes. One such key factor with increasing importance is midkine (MK), which is a heparin-binding growth factor or cytokine (Erguven *et al.*, 2012). MK was found as the product of a gene up-regulated at an early stage of retinoic

acid-induced differentiation of teratocarcinoma stem cells (Kadomatsu *et al.*, 1988) and became the founding member of a small protein family, the other member of which is pleiotrophin (PTN), also called heparin-binding growth-associated molecule (Milner *et al.*, 1989; Rauvala, 1989; Tomomura *et al.*, 1990; Muramatsu, 2010).

MK is involved in various physiological processes, such as development, reproduction and repair (Erguven *et al.*, 2012). Furthermore, MK plays important roles in the aetiology of inflammatory and malignant diseases (Erguven *et al.*, 2012). Therefore, MK itself and the reagents targeting MK are expected to be valuable for the treatment of diverse diseases, as covered in this special issue. The purpose of this article is to provide concise and up-to-date information on the structure, physiological activities and signalling of MK as a basis to understand the pharmacological effects of MK and its antagonists.

In addition to articles in this special issue, readers are also referred to a book (Erguven *et al.*, 2012) and other review articles on MK (Muramatsu, 2002; 2010; 2011; Kadomatsu and Muramatsu, 2004; Weckbach *et al.*, 2011; Kadomatsu *et al.*, 2013). In particular, there is a recent article on drug development in relation to MK (Muramatsu, 2011) and a comprehensive review on circulating MK in patients with various diseases (Krzystek-Korpacka and Matusiewicz, 2012). Furthermore, it is appropriate to mention that occasionally citations in this introductory article refer to a review article citing the original articles in order to keep the number of references to a moderate level.

MK protein

MK is rich in both basic amino acids and cysteine. Human MK is composed of 121 amino acids with a molecular mass of 13 kDa after cleaving off the signal sequence (Tsutsui *et al.*, 1991) (Figure 1). An isoform with two extended amino acids (valine-alanine or other amino acids) at the N-terminal is present in MK preparations from different species due to a difference in the cleavage of the signal sequence (Raulais *et al.*, 1991; Muramatsu *et al.*, 1993; Novotny *et al.*, 1993; Ohyama *et al.*, 1994). This point is described in more detail at the end of this section in relation to the production of MK for pharmaceutical purposes.

No phosphorylation or glycosylation has been detected so far in MK protein produced by baculovirus (Kaneda *et al.*, 1996b) or human hepatoma cells (M. Oda *et al.*, unpubl. data).

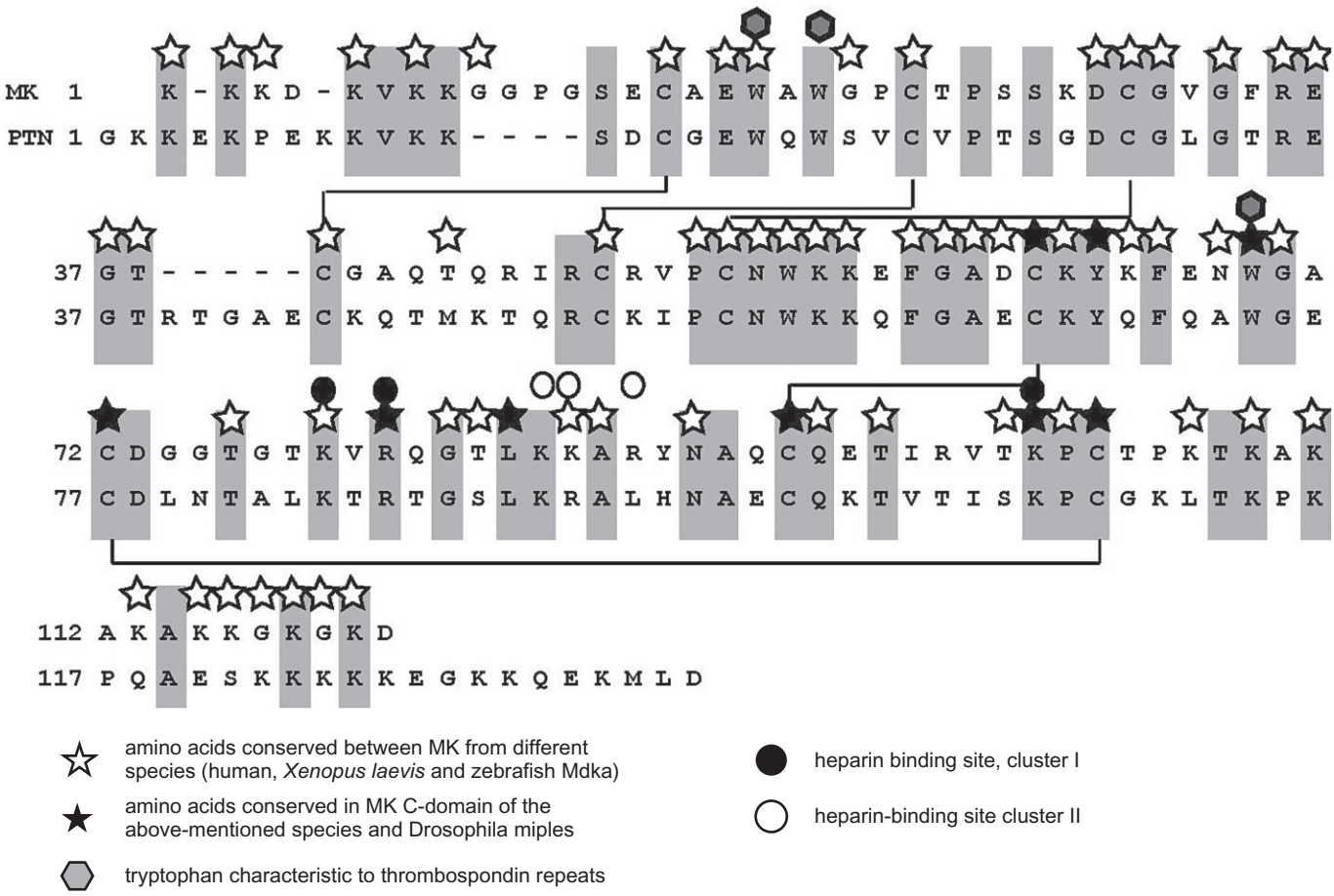

Figure 1

Structure of human MK and PTN. Amino acids shared by them are shaded, and disulfide bridges are shown by solid lines.

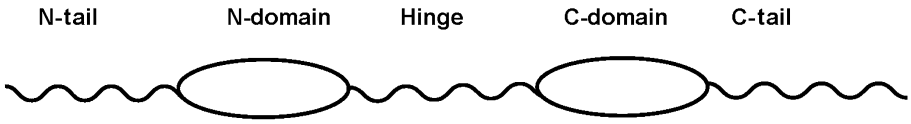

Figure 2

Schematic drawing of MK and its segments.

Disulfide linkages form two domains in the MK molecule, the more N-terminally located N-domain (amino acids 15–52) and the more C-terminally located C-domain (amino acids 62–104) (Fabri *et al*., 1993) (Figure 2). N-domain is held by three disulfide bridges, while C-domain by two disulfide bridges. In addition, there are short tails in the N-terminal region (amino acids 1–14) and the C-terminal region (amino acids 105–121), called N-tail and C-tail respectively. A hinge (amino acids 53–61) connects N-domain and C-domain (Figure 2).

MK and PTN have about 50% sequence identity, cysteine and tryptophan residues being conserved between human MK and PTN (Figure 1). MK and PTN are found in all species of vertebrates so far examined (Muramatsu, 2002; 2010). Zebrafish have two different molecular species of MK called Midkine-a (Mdka) and Midkine-b (Mdkb), which are likely to be generated by gene duplication (Winkler *et al*., 2003). Many amino acid residues in MK are conserved between species. Human and mouse MK have 87% identity (Tsutsui *et al*., 1991), and 55% of amino acids are identical in MK from human, *Xenopus laevis* and zebrafish Mdka (Svensson *et al*., 2010) (Figure 1). Interestingly, a high degree of conservation is observed in the hinge region. *Drosophila melanogaster* lacks MK or PTN but has two molecules called miple (miple-1) and miple-2, which have duplicated domains resembling C-domains of MK and PTN (Englund *et al*., 2006). A mollusc, *Patella caerulea*, also has a related molecule (Vanucci *et al*., 2005). Amino acids characteristically conserved in the C-domain of MK and miples (Figure 1) are mostly conserved in the MK-like protein from *P. caerulea*. An MK-related protein has not been described in *Caenorhabditis elegans*. It is an interesting subject to pinpoint the evolutional event associated with the emergence of an MK-related protein.

The three-dimensional structure of human MK has been studied by NMR spectroscopy (Iwasaki *et al*., 1997). Analysis of the spectrum of the whole molecule suggested that the two domains are relatively independent of each other because no specific interactions are detectable between them. Furthermore, it has been shown that the two tails do not have stable structures. Then, the structure of N-domain and C-domain has been clarified by using the N-terminal half molecule and truncated C-terminal half molecule (Iwasaki *et al*., 1997). The half molecule consists of the tail, domain and a part of the hinge. Both N- and C-domains are basically composed of three antiparallel β-sheets. The structure of C-domain is more complex than that of N-domain. In particular, there is a flexible loop in the middle of the C-domain (amino acids 86–93), and a pocket is created in the domain (Figure 3). Recently, detailed analysis of the whole molecule of zebrafish Mdkb has been performed and has revealed the whole structure of MK, including the hinge region, which has an extended structure (Lim *et al*., 2013).

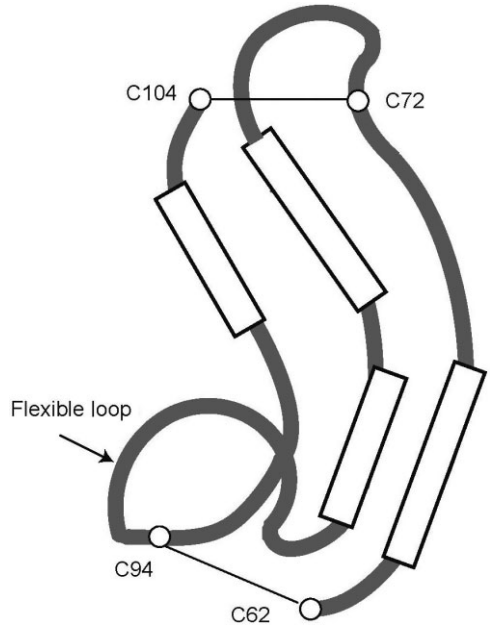

Figure 3

Schematic illustration of the steric structure of MK C-domain. Long boxes are emphasized portions of β-sheets as illustrated in the protein structure database (http://www.ncbi.nlm.nih.gov/structure/mn).

Both domains of MK and the corresponding domains of PTN have weak homology to thrombospondin type-1 repeats, which are present in thrombospondin-1, F-spondin and many other proteins, including circumsporozoite protein from malaria paracytes (Kilpelainen *et al*., 2000). Thrombospondin type-1 repeats are composed of three antiparallel β-sheets, which are generally held by three disulfide bridges (Tan *et al*., 2002). In this respect, N-domains of MK and PTN are more closely related to thrombospondin type-1 repeats as compared with the C-domains, which are held by two disulfide bridges. Furthermore, domains of MK and PTN, especially N-domains, are truncated. Consequently, domains of MK and PTN lack the core structure of thrombospondin repeats, namely a CWR-layered structure, which has repeated tryptophan and arginine arrangements sandwiched by cysteine, and contributes to the stability of thrombospondin repeats (Tan *et al*., 2002). Two tryptophan residues, which are located in the first β-sheets of thrombospondin type-1 repeats, are present in the N-domain of human MK and PTN (closed hexagon, Figure 1). However, W20 in human MK is not conserved throughout MK of different species. Furthermore, only one such tryptophan residue is present in the

C-domain of MK and PTN (Figure 1). As above, MK and PTN domains are only distantly related to thrombospondin type-1 repeats.

The evolutional origin of the two domains in MK remains to be clarified. Considering the structural resemblance of the N- and C-domains, it is reasonable to consider that a primordial molecule common to MK and PTN emerged by intra chromosome duplication of a primordial gene encoding the primordial domain, and then the two proteins, MK and PTN, emerged as the result of duplication of a segment of a chromosome. From the homology to thrombospondin type-1 repeats, N- and C-domains can be considered to have evolved independently from the primordial domain related to thrombospondin repeats, while the presence of *Drosophila* miples is consistent with the view that the C-domain is more related to the primordial domain.

Among the two domains, C-domain has been considered to play more important roles in MK function as neurite-promoting activity of MK is observed in the C-terminal half molecule, and to a lesser extent, also by the C-domain (Muramatsu *et al.*, 1994; Akhter *et al.*, 1998). Furthermore, an MK receptor, receptor protein tyrosine phosphatase Z1 (PTPζ), binds to the C-terminal half but not to the N-terminal half (Maeda *et al.*, 1999). Two heparin-binding sites are present in the C-domain of human MK. The site present in β-sheets called cluster 1 (K79, R81, K102) (Iwasaki *et al.*, 1997) is conserved in MK of different species and also in PTN (closed circle, Figure 1). The other site in the flexible loop called cluster 2 (K86, K87, R89) is conserved only partly (open circle, Figure 1). However, in zebrafish Mdkb, which has only two basic amino acids in cluster 2, the cluster still functions as a heparin-binding site (Lim *et al.*, 2013). Cluster 1 appears to be important not only to heparin binding but also to MK function because a mutation in cluster 1 reduces neurite-promoting activity (Asai *et al.*, 1997) and binding to PTPζ (Maeda *et al.*, 1999).

N-domain has functions different from those of C-domain. Firstly, another heparin-binding site has been identified in the N-domain of Mdkb (Lim *et al.*, 2013). The key residue of the new binding site is R36, corresponding to R35 in human MK (Lim *et al.*, 2013). Secondly, K46/K48 in Mdkb, which corresponds to R45/R47 in human MK, is required for perturbation of zebrafish embryogenesis upon Mdkb injection into the embryos at the blastocyst stage (Lim *et al.*, 2013).

Furthermore, N-domain appears to be important for the stability of MK as the C-terminal half of MK is more susceptible to chymotrypsin digestion than intact MK (Matsuda *et al.*, 1996). N-domain is also involved in dimerization. MK forms a dimer by spontaneous association, and the dimer is stabilized via cross linking by transglutaminase (Kojima *et al.*, 1997). Dimerization is essential for some MK activity, such as activation of fibrinolytic activity by endothelial cells. Q42 or Q44 in N-domain and Q95 in C-domain serve as amine acceptors in the reaction. A peptide fragment, A41-P51, containing the amine acceptor site inhibits MK activity for activation of fibrinolysis (Kojima *et al.*, 1997).

N-tail and C-tail appear to function to keep the two domains independent based on the following observation. Although removal of either tail from intact MK strongly suppresses neurite-promoting activity, isolated C-domain retains a moderate level of activity (Akhter *et al.*, 1998). However, bacteriocidal activity is observed in a peptide containing C-tail and another peptide containing part of the hinge and N-domain (Svensson *et al.*, 2010).

The conserved hinge is likely to have functions other than domain orientation as a mutant Mdkb called MdkbG, in which the hinge is exchanged with oligo-glycine, exhibits the overall structure indistinguishable from that of wild-type Mdkb (Lim *et al.*, 2013). Indeed, another new heparin-binding site has been found in the hinge. The binding site consists of K56 and K57, corresponding to K55 and K56 in human MK (Lim *et al.*, 2013). Furthermore, upon injection into zebrafish embryos, MdkbG mutant exhibits abnormality different from that observed upon injection with wild-type Mdkb (Lim *et al.*, 2013).

MK proteins have been produced by various means and even chemically synthesized (Muramatsu, 2010; 2011). The choice of organisms to produce recombinant MK has been reviewed, and the most important aspect is to avoid the formation of aberrant disulfide linkages (Muramatsu, 2011). In addition, practical information for handling MK protein has been described (Muramatsu *et al.*, 2003; Muramatsu, 2011). Notably, it is important to prevent from MK sticking to the vessel.

Finally, to produce MK with a longer half-life, the extended form, which has a VA extended sequence on the N-terminal side due to differential cleavage of the signal sequence, is of significant interest. In mouse MK produced by L cells and purified by heparin-agarose column chromatography, 5% is the VA extended isoform, and the rest is the conventional form (Muramatsu *et al.*, 1993), while heparin-released MK in human plasma is the VA extended form (Novotny *et al.*, 1993). In MK purified from bovine follicular fluid, 30% is the extended form, and the rest is the conventional form (Ohyama *et al.*, 1994). Chicken MK called RI-HB (retinoic acid-induced heparin-binding protein) purified from chicken embryo is the corresponding alanine-lysine extended form (Raulais *et al.*, 1991).

Oda *et al.* separated the two isoforms produced by human hepatoma cells using a poly-sulfoethyl A column (M. Oda *et al.*, unpublished results). About 60% was the extended form, and the rest was the conventional form. The extended form bound to heparin more weakly and was more resistant to chymotrypsin digestion, while no difference was found in neurite-promoting activity. They also developed a specific immunoassay procedure to detect the extended form and found that the extended form was more readily released into blood by heparin administration and stayed longer in blood, probably due to less affinity to heparin and relative resistance to protease. Their result is consistent with the finding of Novotny *et al.* (1993). In sera from cancer patients, the conventional form was always detected, and the extended form was detected in restricted cases, while in amniotic fluid, the extended form was found in most cases (M. Oda *et al.*, unpubl. data). Thus, the VA extended form constitutes a significant portion of naturally occurring MK in humans. Although chymotrypsin cleaves MK only at the hinge region (Matsuda *et al.*, 1996), alteration of interactions of the N- and C-domains due to VA extension is expected to result in chymotrypsin resistance. The same consideration is applicable to

the weaker heparin-binding activity of the extended form. The relative resistance of the extended form to proteolysis is of significant interest, and further alteration in the N-terminal sequence might be attempted to create MK with a longer half-life.

MK gene

The human MK gene (*MDK*) is located on chromosome 11 at p11.2 (Kaname *et al.*, 1993) and is flanked by the diacylglycerol kinase ζ gene and the muscarinic cholinergic receptor 4 gene (Muramatsu, 2002). *MDK* has four coding exons (Figure 4). Due to differential splicing and differences in the transcription initiation site, there are seven isoforms in MK mRNA. Five different non-coding sequences are present in the 5′ ends of the isoforms. Two isoforms are generated by skipping a coding exon and yield truncated MK. A truncated MK derived from mRNA without the second coding exon is apparently tumour-specific and might be of diagnostic value (Kaname *et al.*, 1996; Paul *et al.*, 2001; Muramatsu, 2010).

In the promoter region of *MDK*, there are functional binding sites for retinoic acid receptor (Pedraza *et al.*, 1995) (closed star, Figure 4) and for the product of Wilms' tumour suppressor gene (Adachi *et al.*, 1996) (open hexagon, Figure 4). They are regarded to be important for retinoic acid-induced expression of MK and up-regulation of MK in Wilms' tumour cells respectively. There is also a hypoxia responsible element in the promoter region; this element is expected to be involved in the induction of MK upon ischaemia and possibly in the increased expression of MK in various tumours (Reynolds *et al.*, 2004). A binding site for NF-κB in the promoter region (Uehara *et al.*, 1992) (open star, Figure 4) might participate in the induction of MK upon inflammation and tumourigenesis.

Glucocorticoid receptor plays a role in the down-regulation of MK expression (Kaplan *et al.*, 2003). In mice deficient in the glucocorticoid receptor, robust expression of MK in the lung is continued in the neonatal stage. Further studies have revealed that glucocorticoids indeed down-regulate MK expression in fetal lung cells (Kaplan *et al.*, 2003). This activity of glucocorticoids is interesting as they are potent anti-inflammatory compounds, and MK is involved in inflammation; MK gene might be a target of glucocorticoids.

Concerning SNP, a variant in an intron is correlated with about a fivefold increase in the risk of colorectal carcinoma (Ahmed *et al.*, 2002). To the best of my knowledge, this is the only SNP with known physiological or pathological impacts. However, it is quite possible that other SNPs, which serve as valuable biomarkers in other diseases, remain to be reported.

Finally, the human PTN gene is very large and is located at a location unrelated to *MDK*, namely 7q33. However, the number of coding exons, sequences around the intron/exon boundaries and gene families flanking *PTN* are shared with *MDK* (Muramatsu, 2002), consistent with the view that both genes evolved from a common ancestral gene (Winkler *et al.*, 2003).

MK in development and reproduction

MK is involved in development, reproduction, repair, inflammation, innate immunity, control of blood pressure and angiogenesis (Erguven *et al.*, 2012). The mode of MK expression is consistent with the view that MK plays a role in both development and repair. Thus, MK is strongly expressed during embryogenesis, especially in the mid-gestation period (Kadomatsu *et al.*, 1990; Mitsiadis *et al.*, 1995b). In adults, significant MK expression is observed only in restricted sites such as the kidney (Kadomatsu *et al.*, 1990), gut (Aridome *et al.*, 1995), epidermis (Inazumi *et al.*, 1997), bronchial epithelium (Nordin *et al.*, 2013), lymphocytes (Cohen *et al.*, 2012) and macrophages (Inoh *et al.*, 2004), while MK expression is induced in many tissues after injury (Yoshida *et al.*, 1995; Obama *et al.*, 1998; Horiba *et al.*, 2000; Sato *et al.*, 2001).

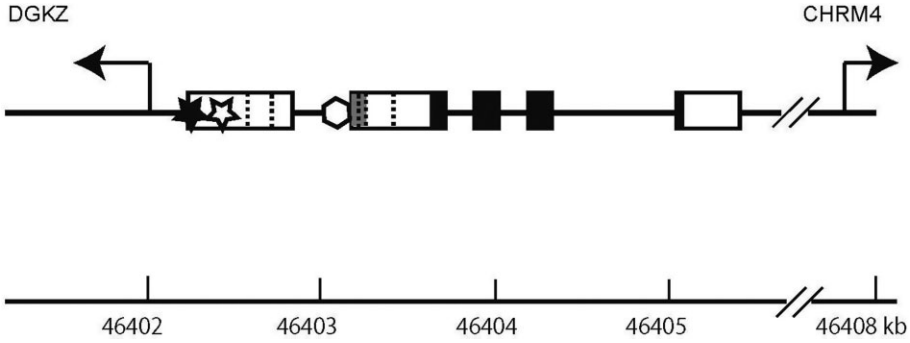

Figure 4

Genomic organization of the human midkine gene (*MDK*). Coding exons are shown by closed boxes, and non-coding exons are shown by open or grey boxes. There are five different structures in the upstream non-coding exons in MK mRNA. When a portion of an exon is shared by different structures, the boundaries are shown by dotted vertical lines. The full information on all MK mRNA isoforms is available (http://www.ncbi.nlm.nih.gov/gene/4192). The first exon shared by three isoforms is shown by a grey box. DGKZ, diacylglycerol kinase ζ gene; CHRM4, muscarinic cholinergic receptor 4 gene; closed star, a retinoic acid responsive element; open star, a binding site for NF-κB; open hexagon, a binding site for the product of Wilms' tumour suppressor gene.

In spite of robust expression of MK in embryos, mice deficient in the MK gene are born without any major morphological defects and reproduce apparently normally (Muramatsu, 2010). The same is true for mice deficient in the PTN gene. However, when mice doubly deficient in both genes are generated by crossing the respective heterozygously deficient mice, they are born only with one-third of the frequency expected by Mendelian segregation (Muramatsu *et al.*, 2006). Furthermore, double-deficient mice are small (Muramatsu *et al.*, 2006) and frequently die before reaching adulthood (H. Muramatsu *et al.*, unpublished observation). MK/PTN double-deficient mice also exhibit severe female infertility. About 80% of double-deficient mice remained infertile even after repeated trials (Muramatsu *et al.*, 2006). The principal reason of the infertility has been concluded to be impaired follicular maturation. MK and PTN expressed in follicular epithelium and granulosa cells in the ovary are considered to be required for follicular maturation. Consistent with this view, MK promotes the maturation of isolated bovine oocytes, and subsequent fertilization and development to blastocysts (Ikeda *et al.*, 2000).

The above results established the importance of the MK/PTN family in development and reproduction, and illustrate that the defect of MK is largely compensated by PTN. This compensation can occur even in tissue with only slight PTN expression, because in some organs, MK suppresses the expression of PTN (Herradon *et al.*, 2005). Thus, in MK-deficient mice, PTN expression is strikingly increased in organs such as the heart, spinal cord, eye and aorta (Herradon *et al.*, 2005).

In addition to defects in development and reproduction, double-deficient mice also exhibit auditory deficit (Zou *et al.*, 2006b; Sone *et al.*, 2011). Although mice deficient in only MK or PTN show an auditory deficit, that in double-deficient mice is severer. The deficit in these mice is considered to be due to a deficit of intermediate cells in the stria vascularis of the cochlear duct (Sone *et al.*, 2011).

The role of MK in development needs a more detailed review. During mouse embryogenesis, MK becomes expressed on embryonic day 5.5 in the embryonic ectoderm (Fan *et al.*, 2000). Consistent with the mode of its expression, MK is expressed in embryonic stem cells, and its role in their survival has been described (Lee *et al.*, 2012).

MK is intensely expressed in the mid-gestation stage, and from the mode of its distribution, MK has been proposed to play roles in neurogenesis, epithelial-mesenchymal interactions and mesoderm remodelling (Kadomatsu *et al.*, 1990).

Concerning neurogenesis, studies using the zebrafish system have revealed that Mdka is involved in the final stage of the fate decision of precursor cells to become either medial floor plate cells constituting the ventral portion of the spinal cord or notochord cells (Schafer *et al.*, 2005). However, Mdkb is required for the development of neural crest cells and sensory neurons (Liedtke and Winkler, 2008). In *X. laevis*, MK enhances neurogenesis and suppresses mesoderm differentiation in the presence of activin (Yokota *et al.*, 1998). Furthermore, MK appears to be involved in dopaminergic development by enhancing the survival of mesencephalic neurons, and Parkinson-like syndrome is observed in MK-deficient mice (Ohgake *et al.*, 2009; Muramatsu, 2011; Prediger *et al.*, 2011).

In the cerebral cortex of the embryonic rat brain, MK is located in both the basal layer where neural stem cells are located and along radial glial processes, which are derived from the stem cells and serve as guides for the migration of differentiated neurons (Matsumoto *et al.*, 1994). This mode of location is consistent with the multiple roles of MK in neurogenesis. As a basis of MK activity in neurogenesis, MK enhances the growth and survival of neural precursor cells, including neural stem cells (Zou *et al.*, 2006a). MK also promotes the survival, migration and neurite outgrowth of embryonic neurons (Michikawa *et al.*, 1993; Muramatsu *et al.*, 1993; Kaneda *et al.*, 1996b; Maeda *et al.*, 1999; Owada *et al.*, 1999; Muramatsu, 2011). In relation to the MK activity to neural precursor cells, MK also enhances growth and survival of primordial germ cells (Shen *et al.*, 2012).

MK is generally more strongly expressed in epithelial tissues than in mesenchymal tissues in embryonic organs where epithelial-mesenchymal interactions are taking place, such as the intestine, pancreas and lung (Kadomatsu *et al.*, 1990; Mitsiadis *et al.*, 1995b). The role of MK in epithelial-mesenchymal interactions has been verified by inhibition of the development of the tooth germ using anti-MK antibodies (Mitsiadis *et al.*, 1995a). The mode of action of MK during epithelial-mesenchymal interactions appears to be complex, as revealed by a study using an artificial blood vessel model, in which collagen gels with smooth muscle cells are covered by endothelial cells (Sumi *et al.*, 2002). In this system, endothelial cells secrete MK, which stimulates smooth muscle cells to secrete IL-8. IL-8 then acts on endothelial cells to promote their growth. Thus, MK is apparently in a central position among the complex interactions of the two cell layers.

Concerning mesoderm remodelling, MK stimulates adipocyte differentiation (Cernkovich *et al.*, 2007), chondrogenesis (Ohta *et al.*, 1999) and osteoclast differentiation (Maruyama *et al.*, 2004), and promotes the differentiation and suppresses the proliferation of osteoblasts (Liedert *et al.*, 2011).

MK in repair and inflammation

MK expression is increased in a number of injured tissues, such as the brain (Yoshida *et al.*, 1995), blood vessels (Horiba *et al.*, 2000), kidney (Sato *et al.*, 2001) and heart (Obama *et al.*, 1998; Horiba *et al.*, 2006). The role of MK in the repair of damaged tissue was demonstrated by the effects of administered MK in reducing light-induced degeneration of the retina (Unoki *et al.*, 1994) and ischaemic brain injury (Yoshida *et al.*, 2001). Then, ischaemic injury of the heart has also been attenuated by MK administration (Horiba *et al.*, 2006). Consistent with the effect of externally administered MK, mice deficient in the MK gene exhibit severer heart damage after ischaemia (Horiba *et al.*, 2006). The basis of this MK activity is anti-apoptotic activity, leading to survival of the affected cells (Horiba *et al.*, 2006).

However, ischaemic injury in the kidney is severer in wild-type mice than in MK-deficient mice (Sato *et al.*, 2001). This is because MK induced by ischaemia recruits inflammatory leukocytes to the injured renal epithelium. In the case of partial hepatectomy, induced MK enhances tissue repair as

well as inflammation, and the balance is slightly in favour of repair: the reparative process is slightly more enhanced in wild-type mice than in MK-deficient mice (Ochiai *et al.*, 2004).

In the brain, MK does not appear to play important roles in neuroinflammation because MK does not activate astrocytes or microglias (Muramoto *et al.*, 2013). Generally, MK exerts protective roles in the brain (Muramatsu, 2011). As an example, MK-deficient mice exhibit increased amphetamine-induced astrocytosis (Gramage *et al.*, 2011). Furthermore, MK enhances migration of microglias and is likely to contribute to the clearance of amyloid β-peptide (Muramatsu, 2011).

MK participates in inflammatory processes by exerting two different activities. Firstly, MK enhances the migration of neutrophils and macrophages both by direct action of MK and by inducing chemokine expression (Takada *et al.*, 1997; Horiba *et al.*, 2000; Sato *et al.*, 2001). Secondly, MK suppresses differentiation of regulatory T-cells partly by inhibiting the development of tolerogenic dendric cells, which are essential for the differentiation (Wang *et al.*, 2008; Sonobe *et al.*, 2012). Inflammation, which is enhanced by MK, is part of the immune system. MK also has anti-microbial activities and is regarded as a component of the innate immune system (Svensson *et al.*, 2010; Nordin *et al.*, 2013). Furthermore, MK has been found to enhance the survival of B cells (Cohen *et al.*, 2012). Thus, MK plays diverse roles in counteracting infection upon injury.

MK is also known to have angiogenic activity (Choudhuri *et al.*, 1997; Weckbach *et al.*, 2012). We can regard these different activities of MK mentioned in this section are all aimed at promoting the repair of injured tissue, while excessive action of some activities leads to tissue damage.

MK in diseases

The diverse activities of MK described in the previous sections form the basis of its involvement in the pathogenesis of various diseases. As this subject will be fully explained in subsequent articles, only a brief summary is described here.

Consistent with the important role of MK in inflammation, MK deficiency attenuates various inflammation-related diseases, namely neointima formation upon ischaemia (a model of restenosis) (Horiba *et al.*, 2000), ischaemic renal injury (Sato *et al.*, 2001), drug-induced renal injury (Muramatsu, 2010; Kadomatsu *et al.*, 2013), antibody-induced arthritis (a model of rheumatoid arthritis) (Maruyama *et al.*, 2004), adhesion after surgery (Inoh *et al.*, 2004) and experimental autoimmune encephalitis (a model of multiple sclerosis) (Wang *et al.*, 2008), raising the possibility of drug development targeting MK for treatment of the above-mentioned diseases. Drugs targeting MK include antibodies, aptamers, low molecular weight compounds and nucleic acid reagents suppressing the formation and/or action of MK mRNA (Muramatsu, 2011; Kadomatsu *et al.*, 2013).

MK is also up-regulated in the majority of human malignant tumours and contributes to tumour development and progression by enhancing the growth, survival, migration, epithelial-mesenchymal transition and angiogenic activity of these cells (Tsutsui *et al.*, 1993; Aridome *et al.*, 1995; Kadomatsu and Muramatsu, 2004; Muramatsu, 2010, 2011; Erguven *et al.*, 2012). Strong MK expression in the tumour is correlated with the poor prognosis of patients and resistance to chemotherapy (Muramatsu, 2010; 2011; Kadomatsu *et al.*, 2013). Thus, anti-MK reagents are expected to be effective in the treatment of malignant diseases (Takei *et al.*, 2001; Muramatsu, 2010; 2011; Erguven *et al.*, 2012; Kadomatsu *et al.*, 2013). In addition, the promoter region of MK can be used to express toxic genes in tumour cells (Tagawa *et al.*, 2012). Furthermore, circulating MK is a promising tumour marker (Krzystek-Korpacka and Matusiewicz, 2012).

MK also plays important roles in the regulation of blood pressure via the renin-angiotensin system (Ezquerra *et al.*, 2005; Hobo *et al.*, 2009). Partial nephrectomy induces MK expression in the lung, and the induced MK acts on endothelial cells to promote the expression of angiotensin-converting enzyme, leading to hypertension (Hobo *et al.*, 2009). Therefore, MK appears to be a key molecule in the aetiology of hypertension upon chronic nephritis.

MK signalling

Consistent with the various *in vivo* activities of MK mentioned above, MK exerts many *in vitro* activities. Prominent activities fall into the following three categories: (i) enhancement of the survival of target cells such as embryonic neurons (Michikawa *et al.*, 1993; Owada *et al.*, 1999), neural precursor cells (Zou *et al.*, 2006a) and B cells (Cohen *et al.*, 2012); (ii) promotion of the expression of other cytokines by MK in renal epithelial cells (Sato *et al.*, 2001), endothelial cells (Sumi *et al.*, 2002) and bone marrow cells (Sonobe *et al.*, 2012); and (iii) promotion of the migration of embryonic neurons (Maeda *et al.*, 1999), macrophages (Horiba *et al.*, 2000), neutrophils (Takada *et al.*, 1997; Horiba *et al.*, 2000) and osteoblast-like cells (Qi *et al.*, 2001). Promotion of migration is mainly performed by substratum-bound MK, while other activities are mainly performed by soluble MK. In addition to the promotion of migration, MK has the following activities with potential involvement of the cytoskeleton, namely promotion of neurite outgrowth, clustering of ACh receptor in the neuromuscular junction and induction of collagen gel contraction by fibroblasts (Muramatsu *et al.*, 1993; Kaneda *et al.*, 1996b; Muramatsu, 2010; 2011). Besides cytokines, MK induces the expression of molecules in extracellular matrices (Muramatsu, 2010) and angiotensin-converting enzyme (Hobo *et al.*, 2009). MK activities related to cell differentiation include enhanced differentiation of regulatory T-cells via tolerogenic dendritic cells (Wang *et al.*, 2008; Sonobe *et al.*, 2012), induction of adipocyte differentiation (Cernkovich *et al.*, 2007) and induction of epithelial-mesenchymal transition (Huang *et al.*, 2008a).

Other activities are enhancement of growth (Muramatsu *et al.*, 1993; Zou *et al.*, 2006a; Muramatsu, 2010; Reiff *et al.*, 2011; Shen *et al.*, 2012), fibrinolytic activity (Kojima *et al.*, 1997), angiogenic activity (Weckbach *et al.* 2012) and antimicrobial activity (Svensson *et al.*, 2010). Some of these activities have been used to analyse the signalling system of MK.

MK signalling is largely mediated by cell surface receptors, and several membrane proteins have been identified as MK receptors. Among them, the most established is PTPζ.

PTPζ is a transmembrane protein with intracellular tyrosine phosphatase domain and extracellular chondroitin sulfate chain and also serves as a PTN receptor (Maeda *et al.*, 1999). Chondroitin sulfate portion of PTPζ is important for binding to MK as after enzymatic removal of chondroitin sulfate, the high-affinity binding site to MK (K_d, 0.58 nM) disappears, and only the low-affinity binding site (K_d, 8.8 nM) remains (Maeda *et al.*, 1999).

As an MK receptor, PTPζ has been shown to be involved in migration of embryonic neurons (Maeda *et al.*, 1999) and UMR106 osteoblast-like cells (Qi *et al.*, 2001), survival of embryonic neurons (Sakaguchi *et al.*, 2003) and B cells (Cohen *et al.*, 2012), and suppression of the proliferation of osteoblasts (Liedert *et al.*, 2011). Consistent with the important role of PTPζ in MK signalling, genetic analysis suggests that *Drosophila* miple functions also through PTPζ (Munoz-Soriano *et al.*, 2013).

After ligand receptor interactions of PTPζ with MK or PTN, tyrosine phosphorylation is increased in cytoplasmic signalling molecules such as β-catenin, (Meng *et al.*, 2000; Liedert *et al.*, 2011), β-adducin (Pariser *et al.*, 2005a), Fyn (Pariser *et al.*, 2005b), Syk (Cohen *et al.*, 2012) and Akt (Qi *et al.*, 2001; Cohen *et al.*, 2012). It is likely that after ligand stimulation, PTPζ is dimerized, leading to inactivation of the intracellular phosphatase domain, and results in increased tyrosine phosphorylation. The finding that oligomerization of PTPζ by antibodies or an artificial dimerizer leads to its inactivation (Fukada *et al.*, 2006) is consistent with this view.

There are two isoforms of PTPζ, the full-length form and the short form. They are functionally different, and the full-length form appears to mediate the action of PTN in hippocampal neurons. PTN increases the density of hippocampal dendritic synapses, and overexpression of the full-length form decreases the density (Asai *et al.*, 2009). This observation is consistent with the action mechanism of PTPζ described above. Finally, it is proper to mention that another mechanism has been proposed for the action of PTPζ, namely PTPζ dephosphorylates Src, leading to its activation (Polykratis *et al.*, 2005).

Multiple kinases, that is, PI3K, MAPK, a Src family kinase and PKC, appear to be involved in signalling downstream from PTPζ, as revealed by their inhibitors (Qi *et al.*, 2001). MK-induced phosphorylation of Akt in these cells has verified the role of PI3K in the signalling system (Qi *et al.*, 2001). In the promotion of survival of B lymphocytes by MK, increased phosphorylation of Syk and Akt via PTPζ leads to increased Bcl-2 expression (Cohen *et al.*, 2012).

Dephosphorylation of β-catenin is a critical step in canonical Wnt signalling. In osteoblasts, MK has been shown to inhibit osteoblast proliferation by interfering in Wnt signalling by inhibiting PTPζ-mediated dephosphorylation of β-catenin (Liedert *et al.*, 2011).

DNER, a Notch-related receptor, forms a complex with PTPζ (Fukazawa *et al.*, 2008). After PTN stimulation, tyrosine phosphorylation of DNER is increased, probably by the inhibition of PTPζ activity, leading to the inhibition of DNER endocytosis, and results in the promotion of neurite outgrowth in neuroblastoma cells. A similar mechanism can be considered upon MK-induced neurite outgrowth in embryonic neurons.

New classes of MK receptors, namely low-density lipoprotein receptor-related protein-1 (LRP-1) (Muramatsu *et al.*, 2000), $\alpha_4\beta_1$-integrin and $\alpha_6\beta_1$-integrin (Muramatsu *et al.*, 2004) have been identified by analysing MK-binding proteins from day 13 mouse embryos. LRP-1 is a member of the low-density lipoprotein receptor family and binds to MK with a K_d of 3.5 nM (Muramatsu *et al.*, 2000). Among the family members, megalin (Muramatsu *et al.*, 2000) and ectodomains of LRP-6 and apo-E receptor-2 (Sakaguchi *et al.*, 2003) also bind to MK, but with less affinity than that of LRP-1. LRP-1 serves as an MK receptor upon survival of embryonic neurons (Muramatsu *et al.*, 2000) and prevention of hypoxic injury in mouse embryonic stem cells (Lee *et al.*, 2012). In the latter system, MK signal is transmitted through Akt and hypoxia-induced factor (HIF)1α.

$\alpha_4\beta_1$-integrin serves as an MK receptor upon migration of UMR-106 osteoblastic cells, and $\alpha_6\beta_1$-integrin for neurite outgrowth of embryonic neurons (Muramatsu *et al.*, 2004). Furthermore, these integrins, LRP-6 ectodomain and PTPζ form a molecular complex, and MK promotes complex formation (Muramatsu *et al.*, 2004). As the result of MK action, tyrosine phosphorylation of paxillin, which is downstream in the integrin signalling system, is increased in UMR-106 cells (Muramatsu *et al.*, 2004). In human head and neck carcinoma cells, MK binds to tetraspanin and $\alpha_6\beta_1$-integrin and enhances their association, leading to activation of focal adhesion kinase, paxillin and STAT1α pathway, resulting in enhanced migration and invasiveness of these cells (Huang *et al.*, 2008b). $\alpha_V\beta_3$-integrin and PTPζ are receptors for PTN upon migration of human umbilical vein endothelial cells (Mikelis *et al.*, 2009). PTN binds directly to both receptors, which interact mutually. Interestingly, MK does not bind to $\alpha_V\beta_3$-integrin and does not enhance the migration of these cells (Mikelis *et al.*, 2009). All these results indicate that integrins are components of MK and PTN receptors to enhance cell migration and function in a molecular complex.

Neuroglycan C is a brain-specific chondroitin sulfate proteoglycan and serves as an MK receptor upon process elongation of CG-4 oligodendroglial precursor-like cells (Ichihara-Tanaka *et al.*, 2006). MK can bind to neuroglycan C devoid of chondroitin sulfate, but the presence of chondroitin sulfate increases binding affinity (Ichihara-Tanaka *et al.*, 2006). The K_d value of MK to recombinant neuroglycan C fusion protein without chondroitin sulfate is 51.8 nM (Ichihara-Tanaka *et al.*, 2006). Further studies revealed that neuroglycan C also functions in a molecular complex (K. Ichihara-Tanaka *et al.*, unpublished results).

Anaplastic lymphoma kinase (ALK) is a transmembrane tyrosine kinase involved in the tumourigenesis of at least several malignant tumours, such as anaplastic large cell lymphoma (Kadomatsu *et al.*, 2013). ALK has been shown to be a receptor of MK (Stoica *et al.*, 2002) and PTN. MK-dependent growth of SW-13 cells in soft agar requires ALK (Stoica *et al.*, 2002). Furthermore, ALK plays the central role in MK-dependent proliferation of immature sympathetic neurons (Reiff *et al.*, 2011) and MK-dependent resistance to cannabinoid of glioblastoma cells (Lorente *et al.*, 2011).

After stimulation by MK, ALK phosphorylates insulin receptor substrate-1, leading to activation of MAPK, PI3K and NF-κB (Kuo *et al.*, 2007). The apparent K_d value between MK and ALK was estimated to be 170 pM, based on the binding to

ALK-expressing cells (Stoica *et al.*, 2002). However, it is not established whether ALK directly binds to MK with high affinity. Furthermore, ALK formed a complex with LRP-6 ectodomain and integrins (H. Muramatsu *et al.* unpublished results). Therefore, ALK may work in the receptor complex for MK. Indeed, PTN-induced activation of ALK is mediated by PTPζ in MCF-7 cells (Perez-Pinera *et al.*, 2007).

Notch-2, a transmembrane protein belonging to the Notch family, is an MK receptor inducing epithelial-mesenchymal transition of immortalized HaCaT keratinocytes (Huang *et al.*, 2008a). Direct binding of MK and Notch-2 has been shown by yeast 2-hybrid analysis and co-immunoprecipitation. After MK stimulation, JAK2 and STAT3 are recruited to hairy and enhancer of split-1 (Hes1), a transcription factor of the Notch signalling system, resulting in increased phosphorylation and activation of STAT3 (Huang *et al.*, 2008a). Notch-2 also serves as an MK receptor in pancreatic ductal adenocarcinoma cells to drive epithelial-mesenchymal transition and chemoresistance (Gungor *et al.*, 2011). In these cells, MK induces cleavage of the Notch-2 cytoplasmic domain and stimulates the expression of Hes1 and NF-κB. Furthermore, Notch-2 participates in MK signalling upon development of neuroblastoma (Kishida *et al.*, 2012). The Notch signalling system might also be involved in MK-induced production of chemokines because *IL8* gene is activated after MK stimulation in keratinocytes (Huang *et al.*, 2008a). The possibility that Notch-2 acts in the receptor complex is not excluded because DNER, a Notch-related transmembrane protein, forms a complex with PTPζ (Fukazawa *et al.*, 2008).

Most MK activities are inhibited by heparin or digestion with heparitinase or chondroitinase, indicating the importance of carbohydrate recognition in MK signalling (Muramatsu, 2010). Two oligomeric carbohydrate structures, namely heparan sulfate trisulfated units and chondroitin sulfate E units, have been shown to bind to MK strongly (Kaneda *et al.*, 1996a; Ueoka *et al.*, 2000; Muramatsu *et al.*, 2003; Zou *et al.*, 2003; Muramatsu, 2010). Chondroitin sulfate chains are present in PTPζ and neuroglycan C, as mentioned above. Furthermore, versican, another chondroitin sulfate proteoglycan, also binds to MK (Zou *et al.*, 2000). Although versican is not a transmembrane protein, but a pericellular protein, it can function in the delivery of MK to the receptor.

Syndecans and glypican-2 are heparan sulfate proteoglycans with MK binding activity (Muramatsu, 2010). K_d of syndecan-4 to MK is 0.30 nM (Kojima *et al.*, 1996). Transfection of syndecan-3 or glypican-2 cDNA to neurobalstoma cells results in extension of neurites on the MK-coated substratum (Kurosawa *et al.*, 2001).

Although several transmembrane proteins have been identified as MK receptors, as mentioned above, none has very high affinity to MK or is specific only to the MK/PTN family. Therefore, it might be reasonable to assume that MK frequently binds to more than one component in the receptor complex to exert its function. That MK can form a dimer (Kojima *et al.*, 1997) is important as dimeric MK is suitable for enhancing the association of different components in the receptor complex.

The signalling systems downstream from the MK receptors are diverse, as mentioned above, while the PI3K/Akt system and MAPK appear to play central roles in many cases. Notably, the PI3K /Akt system is involved in the survival of embryonic neurons (Owada *et al.*, 1999) and B cells (Cohen *et al.*, 2012), prevention of hypoxic injury in embryonic stem cells (Lee *et al.*, 2012), and migration of osteoblast-like cells (Qi *et al.*, 2001). Upon promotion of survival by MK, suppression of caspase and activation of Bcl-2 are observed after signalling (Owada *et al.*, 1999; Qi *et al.*, 2000; Muramatsu, 2010; Cohen *et al.*, 2012).

Transcriptional factors, whose activities are regulated by signalling cascades triggered by MK, include NF-κB (Kuo *et al.*, 2007; Gungor *et al.*, 2011), Hes-1 (Gungor *et al.*, 2011), HIF-1α (Lee *et al.*, 2012) and STATs (Cernkovich *et al.*, 2007; Huang *et al.*, 2008a; b; Sonobe *et al.*, 2012).

Among them, STATs require further description. Activation of STAT1α is involved in the migration of head and neck carcinoma cells (Huang *et al.*, 2008b), while activation of STAT3 is important in epithelial-mesenchymal transition of keratinocytes (Huang *et al.*, 2008a) and the proliferation and adipogenesis of 3T3-L1 cells (Cernkovich *et al.*, 2007). However, in bone marrow cells containing tolerogenic dendritic cells, MK activates SHP-2 (src homology region 2 domain-containing phosphatase-2) (Sonobe *et al.*, 2012). This effect leads to decreased phosphorylation of STAT3α upon IL-10 stimulation and to increased production of IL-12.

In addition to receptor-mediated signalling, a portion of MK has been shown to exert its effect directly in the nucleus. MK is internalized after binding to LRP-1 and is then transported to the nucleus by binding to nucleolin (Shibata *et al.*, 2002) or to laminin-binding protein precursor (Salama *et al.*, 2001), and is involved in the promotion of cell survival (Shibata *et al.*, 2002). Furthermore, MK transferred to the nucleolus enhances the synthesis of ribosomal RNA (Dai *et al.*, 2008).

In conclusion, various aspects of MK signalling have been clarified in detail, while further efforts are required to reveal the complete image of MK signalling. Thus, there remain possibilities that new targets will emerge for suppression of the MK signalling system.

Conflict of interest

None declared.

References

Adachi Y, Matsubara S, Pedraza C, Ozawa M, Tsutsui J, Takamatsu H *et al.* (1996). Midkine as a novel target gene for the Wilms' tumor suppressor gene (WT1). Oncogene 13: 2197–2203.

Ahmed KM, Shitara Y, Takenoshita S, Kuwano H, Saruhashi S, Shinozawa T (2002). Association of an intronic polymorphism in the midkine gene with human sporadic colorectal cancer. Cancer Lett 180: 159–163.

Akhter S, Ichihara-Tanaka K, Kojima S, Muramatsu H, Inui T, Kimura T *et al.* (1998). Clusters of basic amino acids in midkine: roles in neurite-promoting activity and plasminogen activator-enhancing activity. J Biochem 123: 1127–1136.

Aridome K, Tsutsui J, Takao S, Kadomatsu K, Ozawa M, Aikou T *et al.* (1995). Increased midkine gene expression in human gastrointestinal cancers. Jpn J Cancer Res 86: 655–661.

Asai H, Yokoyama S, Morita S, Maeda N, Miyata S (2009). Functional difference of receptor-type protein tyrosine phosphatase ζ/β isoforms in neurogenesis of hippocampal neurons. Neuroscience 164: 1020–1030.

Asai T, Watanabe K, Ichihara-Tanaka K, Kaneda N, Kojima S, Iguchi A *et al.* (1997). Identification of heparin-binding sites in midkine and their role in neurite-promotion. Biochem Biophys Res Commun 236: 66–70.

Cernkovich ER, Deng J, Hua K, Harp JB (2007). Midkine is an autocrine activator of signal transducer and activator of transcription 3 in 3T3-L1 cells. Endocrinology 148: 1598–1604.

Choudhuri R, Zhang HT, Donnini S, Ziche M, Bicknell R (1997). An angiogenic role for the neurokines midkine and pleiotrophin in tumorigenesis. Cancer Res 57: 1814–1819.

Cohen S, Shoshana OY, Zelman-Toister E, Maharshak N, Binsky-Ehrenreich I, Gordin M *et al.* (2012). The cytokine midkine and its receptor RPTPζ regulate B cell survival in a pathway induced by CD74. J Immunol 188: 259–269.

Dai LC, Shao JZ, Min LS, Xiao YT, Xiang LX, Ma ZH (2008). Midkine accumulated in nucleolus of HepG2 cells involved in rRNA transcription. World J Gastroenterol 14: 6249–6253.

Englund C, Birve A, Falileeva L, Grabbe C, Palmer RH (2006). Miple1 and miple2 encode a family of MK/PTN homologues in *Drosophila melanogaster*. Dev Genes Evol 216: 10–18.

Erguven M, Muramatsu T, Bilir A (eds) (2012). Midkine: From Embryogenesis to Pathogenesis and Therapy. Springer: Dordrecht, the Netherlands.

Ezquerra L, Herradon G, Nguyen T, Silos-Santiago I, Deuel TF (2005). Midkine, a newly discovered regulator of the renin-angiotensin pathway in mouse aorta: significance of the pleiotrophin/midkine developmental gene family in angiotensin II signaling. Biochem Biophys Res Commun 333: 636–643.

Fabri L, Maruta H, Muramatsu H, Muramatsu T, Simpson RT, Burges AW *et al.* (1993). Structural characterisation of native and recombinant forms of the neurotrophic cytokine MK. J Chromatogr 646: 213–225.

Fan QW, Muramatsu T, Kadomatsu K (2000). Distinct expression of midkine and pleiotrophin in the spinal cord and placental tissues during early mouse development. Dev Growth Differ 42: 113–119.

Fukada M, Fujikawa A, Chow JP, Ikematsu S, Sakuma S, Noda M (2006). Protein tyrosine phosphatase receptor type ζ is inactivated by ligand-induced oligomerization. FEBS Lett 580: 4051–4056.

Fukazawa N, Yokoyama S, Eiraku M, Kengaku M, Maeda N (2008). Receptor type protein tyrosine phosphatase ζ-pleiotrophin signaling controls endocytic trafficking of DNER that regulates neuritogenesis. Mol Cell Biol 28: 4494–4506.

Gramage E, Martin YB, Ramanah P, Perez-Garcia C, Herradon G (2011). Midkine regulates amphetamine-induced astrocytosis in striatum but has no effects on amphetamine-induced striatal dopaminergic denervation and addictive effects: functional differences between pleiotrophin and midkine. Neuroscience 190: 307–317.

Gungor C, Zander H, Effenberger KE, Vashist YK, Kalinina T, Izbicki JR *et al.* (2011). Notch signaling activated by replication stress-induced expression of midkine drives epithelial-mesenchymal transition and chemoresistance in pancreatic cancer. Cancer Res 71: 5009–5019.

Herradon G, Ezquerra L, Nguyen T, Silos-Santiago I, Deuel TF (2005). Midkine regulates pleiotrophin organ-specific gene expression: evidence for transcriptional regulation and functional redundancy within the pleiotrophin/midkine developmental gene family. Biochem Biophys Res Commun 333: 714–721.

Hobo A, Yuzawa Y, Kosugi T, Kato N, Asai N, Sato W *et al.* (2009). The growth factor midkine regulates the renin-angiotensin system in mice. J Clin Invest 119: 1616–1625.

Horiba M, Kadomatsu K, Nakamura E, Muramatsu H, Ikematsu S, Sakuma S *et al.* (2000). Neointima formation in a restenosis model is suppressed in midkine-deficient mice. J Clin Invest 105: 489–495.

Horiba M, Kadomatsu K, Yasui K, Lee JK, Takenaka H, Sumida A *et al.* (2006). Midkine plays a protective role against cardiac ischemia/reperfusion injury through a reduction of apoptotic reaction. Circulation 114: 1713–1720.

Huang Y, Hoque MO, Wu F, Trink B, Sidransky D, Ratovitski EA (2008a). Midkine induces epithelial-mesenchymal transition through Notch2/Jak2-Stat3 signaling in human keratinocytes. Cell Cycle 7: 1613–1622.

Huang Y, Sook-Kim M, Ratovitski E (2008b). Midkine promotes tetraspanin-integrin interaction and induces FAK-Stat1α pathway contributing to migration/invasiveness of human head and neck squamous cell carcinoma cells. Biochem Biophys Res Commun 377: 474–478.

Ichihara-Tanaka K, Oohira A, Rumsby M, Muramatsu T (2006). Neuroglycan C is a novel midkine receptor involved in process elongation of oligodendroglial precursor-like cells. J Biol Chem 281: 30857–30864.

Ikeda S, Ichihara-Tanaka K, Azuma T, Muramatsu T, Yamada M (2000). Effects of midkine during *in vitro* maturation of bovine oocytes on subsequent developmental competence. Biol Reprod 63: 1067–1074.

Inazumi T, Tajima S, Nishikawa T, Kadomatsu K, Muramatsu H, Muramatsu T (1997). Expression of the retinoid-inducible polypeptide, midkine, in human epidermal keratinocytes. Arch Dermatol Res 289: 471–475.

Inoh K, Muramatsu H, Ochiai K, Torii S, Muramatsu T (2004). Midkine, a heparin-binding cytokine, plays key roles in intraperitoneal adhesions. Biochem Biophys Res Commun 317: 108–113.

Iwasaki W, Nagata K, Hatanaka H, Inui T, Kimura T, Muramatsu T *et al.* (1997). Solution structure of midkine, a new heparin-binding growth factor. EMBO J 16: 6936–6946.

Kadomatsu K, Muramatsu T (2004). Midkine and pleiotrophin in neural development and cancer. Cancer Lett 204: 127–143.

Kadomatsu K, Tomomura M, Muramatsu T (1988). cDNA cloning and sequencing of a new gene intensely expressed in early differentiation stages of embryonal carcinoma cells and in mid-gestation period of mouse embryogenesis. Biochem Biophys Res Commun 151: 1312–1318.

Kadomatsu K, Huang RP, Suganuma T, Murata F, Muramatsu T (1990). A retinoic acid responsive gene MK found in the teratocarcinoma system is expressed in spatially and temporally controlled manner during mouse embryogenesis. J Cell Biol 110: 607–616.

Kadomatsu K, Kishida S, Tsubota S (2013). The heparin-binding growth factor midkine: the biological activities and candidate receptors. J Biochem 153: 511–521.

Kaname T, Kuwano A, Murano I, Uehara K, Muramatsu T, Kajii T (1993). Midkine gene (MDK), a gene for prenatal differentiation

and neuroregulation, maps to band 11p11.2 by fluorescence *in situ* hybridization. Genomics 17: 514–515.

Kaname T, Kadomatsu K, Aridome K, Yamashita S, Sakamoto K, Ogawa M *et al.* (1996). The expression of truncated MK in human tumors. Biochem Biophys Res Commun 219: 256–260.

Kaneda N, Talukder AH, Ishihara M, Hara S, Yoshida K, Muramatsu T (1996a). Structural characteristics of heparin-like domain required for interaction of midkine with embryonic neurons. Biochem Biophys Res Commun 220: 108–112.

Kaneda N, Talukder AH, Nishiyama H, Koizumi S, Muramatsu T (1996b). Midkine, a heparin-binding growth/differentiation factor, exhibits nerve cell adhesion and guidance activity for neurite outgrowth *in vitro*. J Biochem 119: 1150–1156.

Kaplan F, Comber J, Sladek R, Hudson TJ, Muglia LJ, Macrae T *et al.* (2003). The growth factor midkine is modulated by both glucocorticoid and retinoid in fetal lung development. Am J Respir Cell Mol Biol 28: 33–41.

Kilpelainen I, Kaksonen M, Kinnunen T, Avikainen H, Fath M, Linhardt RJ *et al.* (2000). Heparin-binding growth-associated molecule contains two heparin-binding β-sheet domains that are homologous to the thrombospondin type I repeat. J Biol Chem 275: 13564–13570.

Kishida S, Mu P, Miyakawa S, Fujiwara M, Abe T, Sakamoto K *et al.* (2012). Midkine promotes neuroblastoma through Notch2 signaling. Cancer Res 73: 1318–1327.

Kojima S, Inui T, Muramatsu H, Suzuki Y, Kadomatsu K, Yoshizawa M *et al.* (1997). Dimerization of midkine by tissue transglutaminase and its functional implication. J Biol Chem 272: 9410–9416.

Kojima T, Katsumi A, Yamazaki T, Muramatsu T, Nagasaka T, Ohsumi K *et al.* (1996). Human ryudocan from endothelium-like cells binds basic fibroblast growth factor, midkine, and tissue factor pathway inhibitor. J Biol Chem 271: 5914–5920.

Krzystek-Korpacka M, Matusiewicz M (2012). Circulating midkine in malignant, inflammatory and infectious diseases: a systematic review. In: Erguven M, Muramatsu T, Bilir A (eds). Midkine: From Embryogenesis to Pathogenesis and Therapy. Springer: Dordrecht, the Netherlands, pp. 69–85.

Kuo AH, Stoica GE, Riegel AT, Wellstein A (2007). Recruitment of insulin receptor substrate-1 and activation of NF-κB essential for midkine growth signaling through anaplastic lymphoma kinase. Oncogene 26: 859–869.

Kurosawa N, Chen GY, Kadomatsu K, Ikematsu S, Sakuma S, Muramatsu T (2001). Glypican-2 binds to midkine: the role of glypican-2 in neuronal cell adhesion and neurite outgrowth. Glycoconj J 18: 499–507.

Lee SH, Suh HN, Lee YJ, Seo BN, Ha JW, Han HJ (2012). Midkine prevented hypoxic injury of mouse embryonic stem cells through activation of Akt and HIF-1α via low-density lipoprotein receptor-related protein-1. J Cell Physiol 227: 1731–1739.

Liedert A, Mattausch L, Rontgen V, Blakytny R, Vogele D, Pahl M *et al.* (2011). Midkine-deficiency increases the anabolic response of cortical bone to mechanical loading. Bone 48: 945–951.

Liedtke D, Winkler C (2008). Midkine-b regulates cell specification at the neural plate border in zebrafish. Dev Dyn 237: 62–74.

Lim J, Yao S, Graf M, Winkler C, Yang D (2013). Structure-function analysis of full-length midkine reveals novel residues important for heparin-binding and zebrafish embryogenesis. Biochem J 451: 407–415.

Lorente M, Torres S, Salazar M, Carracedo A, Hernández-Tiedra S, Rodríguez-Fornés F *et al.* (2011). Stimulation of the midkine/ALK axis renders glioblastoma cells resistant to cannabinoid antitumoral action. Cell Death Differ 18: 959–973.

Maeda N, Ichihara-Tanaka K, Kimura T, Kadomatsu K, Muramatsu T, Noda M (1999). A receptor-like protein-tyrosine phosphatase PTPζ/RPTPβ binds a heparin-binding growth factor midkine. Involvement of arginine 78 of midkine in the high affinity binding to PTPζ. J Biol Chem 274: 12474–12479.

Maruyama K, Muramatsu H, Ishiguro N, Muramatsu T (2004). Midkine, a heparin-binding growth factor, is fundamentally involved in the pathogenesis of rheumatoid arthritis. Arthritis Rheum 50: 1420–1429.

Matsuda Y, Talukder AH, Ishihara M, Hara S, Yoshida K, Muramatsu T *et al.* (1996). Limited proteolysis by chymotrypsin of midkine and inhibition by heparin binding. Biochem Biophys Res Commun 228: 176–181.

Matsumoto K, Wanaka A, Takatsuji K, Muramatsu H, Muramatsu T, Tohyama M (1994). A novel family of heparin-binding growth factors, pleiotrophin and midkine, is expressed in the developing rat cerebral cortex. Brain Res Dev Brain Res 79: 229–241.

Meng K, Rodriguez-Pena A, Dimitrov T, Chen W, Yamin M, Noda M *et al.* (2000). Pleiotrophin signals increased tyrosine phosphorylation of β-catenin through inactivation of the intrinsic catalytic activity of the receptor-type protein tyrosine phosphatase β/ζ. Proc Natl Acad Sci U S A 97: 2603–2608.

Michikawa M, Kikuchi S, Muramatsu H, Muramatsu T, Kim SU (1993). Retinoic acid responsive gene product, midkine, has neurotrophic functions for mice spinal cord and dorsal root ganglion neurons in culture. J Neurosci Res 35: 530–539.

Mikelis C, Sfaelou E, Koutsioumpa M, Kieffer N, Papadimitriou E (2009). Integrin αvβ3 is a pleiotrophin receptor required for pleiotrophin-induced endothelial cell migration through receptor protein tyrosine phosphatase β/ζ. FASEB J 23: 1459–1469.

Milner PG, Li YS, Hoffman RM, Kodner CM, Siegel NR, Deuel TF (1989). A novel 17 kD heparin-binding growth factor (HBGF-8) in bovine uterus: purification and N-terminal amino acid sequence. Biochem Biophys Res Commun 165: 1096–1103.

Mitsiadis TA, Muramatsu T, Muramatsu H, Thesleff I (1995a). Midkine (MK), a heparin-binding growth/differentiation factor, is regulated by retinoic acid and epithelial-mesenchymal interactions in the developing mouse tooth, and affects cell proliferation and morphogenesis. J Cell Biol 129: 267–281.

Mitsiadis TA, Salmivirta M, Muramatsu T, Muramatsu H, Rauvala H, Lehtonen E *et al.* (1995b). Expression of the heparin-binding cytokines, midkine (MK) and HB-GAM (pleiotrophin) is associated with epithelial-mesenchymal interactions during fetal development and organogenesis. Development 121: 37–51.

Munoz-Soriano V, Ruiz C, Perez-Alonso M, Mlodzik M, Paricio N (2013). Nemo regulates cell dynamics and represses the expression of *miple*, a midkine/pleiotrophin cytokine, during ommatidial rotation. Dev Biol 377: 113–125.

Muramatsu H, Shirahama H, Yonezawa S, Maruta H, Muramatsu T (1993). Midkine, a retinoic acid-inducible growth/differentiation factor: immunochemical evidence for the function and distribution. Dev Biol 159: 392–402.

Muramatsu H, Inui T, Kimura T, Sakakibara S, Song XJ, Maruta H *et al.* (1994). Localization of heparin-binding, neurite outgrowth and antigenic regions in midkine molecule. Biochem Biophys Res Commun 203: 1131–1139.

Muramatsu H, Zou K, Sakaguchi N, Ikematsu S, Sakuma S, Muramatsu T (2000). LDL receptor-related protein as a component of the midkine receptor. Biochem Biophys Res Commun 270: 936–941.

Muramatsu H, Zou P, Suzuki H, Oda Y, Chen GY, Sakaguchi N *et al.* (2004). 4β1- and α6β1-integrins are functional receptors for midkine, a heparin-binding growth factor. J Cell Sci 117: 5405–5415.

Muramatsu H, Zou P, Kurosawa N, Ichihara-Tanaka K, Maruyama K, Inoh K *et al.* (2006). Female infertility in mice deficient in midkine and pleiotrophin, which form a distinct family of growth factors. Genes Cells 11: 1405–1417.

Muramatsu T (2002). Midkine and pleiotrophin: two related proteins involved in development, survival, inflammation and tumorigenesis. J Biochem 132: 359–371.

Muramatsu T (2010). Midkine, a heparin-binding cytokine with multiple roles in development, repair and diseases. Proc Jpn Acad Ser B Phys Biol Sci 86: 410–425.

Muramatsu T (2011). Midkine: a promising molecule for drug development to treat diseases of the central nervous system. Curr Pharm Des 17: 410–423.

Muramatsu T, Muramatsu H, Kaneda N, Sugahara K (2003). Recognition of glycosaminoglycans by midkine. Methods Enzymol 363: 365–376.

Muramoto A, Imagama S, Natori T, Wakao N, Ando K, Tauchi R *et al.* (2013). Midkine overcomes neurite outgrowth inhibition of chondroitin sulfate proteoglycan without glial activation and promotes functional recovery after spinal cord injury. Neurosci Lett 550: 150–155.

Nordin SL, Andersson C, Bjermer L, Bjartell A, Morgelin M, Egesten A (2013). Midkine is part of the antibacterial activity released at the surface of differentiated bronchial epithelial cells. J Innate Immun 5: 519–530.

Novotny WF, Maffi T, Mehta RL, Milner PG (1993). Identification of novel heparin-releasable proteins, as well as the cytokine midkine and pleiotrophin, in human postheparin plasma. Arterioscler Thromb 13: 1798–1805.

Obama H, Biro S, Tashiro T, Tsutsui J, Ozawa M, Yoshida H *et al.* (1998). Myocardial infarction induces expression of midkine, a heparin-binding growth factor with reparative activity. Anticancer Res 18: 145–152.

Ochiai K, Muramatsu H, Yamamoto S, Ando H, Muramatsu T (2004). The role of midkine and pleiotrophin in liver regeneration. Liver Int 24: 484–491.

Ohgake S, Shimizu E, Hashimoto K, Okamura N, Koike K, Koizumi H *et al.* (2009). Dopaminergic hypofunctions and prepulse inhibition deficits in mice lacking midkine. Prog Neuropsychopharmacol Biol Psychiatry 33: 541–546.

Ohta S, Muramatsu H, Senda T, Zou K, Iwata H, Muramatsu T (1999). Midkine is expressed during repair of bone fracture and promotes chondrogenesis. J Bone Miner Res 14: 1132–1144.

Ohyama Y, Miyamoto K, Minamino N, Matsuo H (1994). Isolation and identification of midkine and pleiotrophin in bovine follicular fluid. Mol Cell Endocrinol 105: 203–208.

Owada K, Sanjo N, Kobayashi T, Mizusawa H, Muramatsu H, Muramatsu T *et al.* (1999). Midkine inhibits caspase-dependent apoptosis via the activation of mitogen-activated protein kinase and phosphatidylinositol 3-kinase in cultured neurons. J Neurochem 73: 2084–2092.

Pariser H, Herradon G, Ezquerra L, Perez-Pinera P, Deuel TF (2005a). Pleiotrophin regulates serine phosphorylation and the cellular distribution of β-adducin through activation of protein kinase C. Proc Natl Acad Sci U S A 102: 12407–12412.

Pariser H, Ezquerra L, Herradon G, Perez-Pinera P, Deuel TF (2005b). Fyn is a downstream target of the pleiotrophin/receptor protein tyrosine phosphatase β/ζ signaling pathway: regulation of tyrosine phosphorylation of Fyn by pleiotrophin. Biochem Biophys Res Commun 332: 664–669.

Paul S, Mitsumoto T, Asano Y, Kato S, Kato M, Shinozawa T (2001). Detection of truncated midkine in Wilms' tumor by a monoclonal antibody against human recombinant truncated midkine. Cancer Lett 163: 245–251.

Pedraza C, Matsubara S, Muramatsu T (1995). A retinoic acid-responsive element in human midkine gene. J Biochem 117: 845–849.

Perez-Pinera P, Zhang W, Chang Y, Vega JA, Deuel TF (2007). Anaplastic lymphoma kinase is activated through the pleiotrophin/receptor protein-tyrosine phosphatase β/ζ signaling pathway: an alternative mechanism of receptor tyrosine kinase activation. J Biol Chem 282: 28683–28690.

Polykratis A, Katsoris P, Courty J, Papadimitriou E (2005). Characterization of heparin affin regulatory peptide signaling in human endothelial cells. J Biol Chem 280: 22454–22461.

Prediger RD, Rojas-Mayorquin AE, Aguiar AS, Jr, Chevarin C, Mongeau R, Hamon M *et al.* (2011). Mice with genetic deletion of the heparin-binding growth factor midkine exhibit early preclinical features of Parkinson's disease. J Neural Transm 118: 1215–1225.

Qi M, Ikematsu S, Ichihara-Tanaka K, Sakuma S, Muramatsu T, Kadomatsu K (2000). Midkine rescues Wilms' tumor cells from cisplatin-induced apoptosis: regulation of Bcl-2 expression by midkine. J Biochem 127: 269–277.

Qi M, Ikematsu S, Maeda N, Ichihara-Tanaka K, Sakuma S, Noda M *et al.* (2001). Haptotactic migration induced by midkine. Involvement of protein-tyrosine phosphatase ζ. Mitogen-activated protein kinase, and phosphatidylinositol 3-kinase. J Biol Chem 276: 15868–15875.

Raulais D, Lagente-Chevallier O, Guettet C, Duprez D, Courtois Y, Vigny M (1991). A new heparin binding protein regulated by retinoic acid from chick embryo. Biochem Biophys Res Commun 174: 708–715.

Rauvala H (1989). An 18-kd heparin-binding protein of developing brain that is distinct from fibroblast growth factors. EMBO J 8: 2933–2941.

Reiff T, Huber L, Kramer M, Delattre O, Janoueix-Lerosey I, Rohrer H (2011). Midkine and Alk signaling in sympathetic neuron proliferation and neuroblastoma predisposition. Development 138: 4699–4708.

Reynolds PR, Mucenski ML, Le Cras TD, Nichols WC, Whitsett JA (2004). Midkine is regulated by hypoxia and causes pulmonary vascular remodeling. J Biol Chem 279: 37124–37132.

Sakaguchi N, Muramatsu H, Ichihara-Tanaka K, Maeda N, Noda M, Yamamoto T *et al.* (2003). Receptor-type protein tyrosine phosphatase ζ as a component of the signaling receptor complex for midkine-dependent survival of embryonic neurons. Neurosci Res 45: 219–224.

Salama RH, Muramatsu H, Zou K, Inui T, Kimura T, Muramatsu T (2001). Midkine binds to 37-kDa laminin binding protein precursor, leading to nuclear transport of the complex. Exp Cell Res 270: 13–20.

Sato W, Kadomatsu K, Yuzawa Y, Muramatsu H, Hotta N, Matsuo S *et al.* (2001). Midkine is involved in neutrophil infiltration into the tubulointerstitium in ischemic renal injury. J Immunol 167: 3463–3469.

Schafer M, Rembold M, Wittbrodt J, Schartl M, Winkler C (2005). Medial floor plate formation in zebrafish consists of two phases and requires trunk-derived Midkine-a. Genes Dev 19: 897–902.

Shen W, Park BW, Toms D, Li J (2012). Midkine promotes proliferation of primordial germ cells by inhibiting the expression of the deleted in azoospermia-like gene. Endocrinology 153: 3482–3492.

Shibata Y, Muramatsu T, Hirai M, Inui T, Kimura T, Saito H *et al.* (2002). Nuclear targeting by the growth factor midkine. Mol Cell Biol 22: 6788–6796.

Sone M, Muramatsu H, Muramatsu T, Nakashima T (2011). Morphological observation of the stria vascularis in midkine and pleiotrophin knockout mice. Auris Nasus Larynx 38: 41–45.

Sonobe Y, Li H, Jin S, Kishida S, Kadomatsu K, Takeuchi H *et al.* (2012). Midkine inhibits inducible regulatory T cell differentiation by suppressing the development of tolerogenic dendritic cells. J Immunol 188: 2602–2611.

Stoica GE, Kuo A, Powers C, Bowden ET, Sale EB, Riegel AT *et al.* (2002). Midkine binds to anaplastic lymphoma kinase (ALK) and acts as a growth factor for different cell types. J Biol Chem 277: 35990–35998.

Sumi Y, Muramatsu H, Takei Y, Hata K, Ueda M, Muramatsu T (2002). Midkine, a heparin-binding growth factor, promotes growth and glycosaminoglycan synthesis of endothelial cells through its action on smooth muscle cells in an artificial blood vessel model. J Cell Sci 115: 2659–2667.

Svensson SL, Pasupuleti M, Walse B, Malmsten M, Mörgelin M, Sjögren C *et al.* (2010). Midkine and pleiotrophin have bactericidal properties: preserved antibacterial activity in a family of heparin-binding growth factors during evolution. J Biol Chem 285: 16105–16115.

Tagawa M, Kawamura K, Yu L, Tada Y, Hiroshima K, Shimada H (2012). A gene medicine with the midkine-mediated transcriptional regulation as new cancer therapeutics. In: Erguven M, Muramatsu T, Bilir A (eds). Midkine: From Embryogenesis to Pathogenesis and Therapy. Springer: Dordrecht, the Netherlands, pp. 237–246.

Takada T, Toriyama K, Muramatsu H, Song XJ, Torii S, Muramatsu T (1997). Midkine, a retinoic acid-inducible heparin-binding cytokine in inflammatory responses: chemotactic activity to neutrophils and association with inflammatory synovitis. J Biochem 122: 453–458.

Takei Y, Kadomatsu K, Matsuo S, Itoh H, Nakazawa K, Kubota S *et al.* (2001). Antisense oligodeoxynucleotide targeted to Midkine, a heparin-binding growth factor, suppresses tumorigenicity of mouse rectal carcinoma cells. Cancer Res 61: 8486–8491.

Tan K, Duquette M, Liu JH, Dong Y, Zhang R, Joachimiak A *et al.* (2002). Crystal structure of the TSP-1 type 1 repeats: a novel layered fold and its biological implication. J Cell Biol 159: 373–382.

Tomomura M, Kadomatsu K, Matsubara S, Muramatsu T (1990). A retinoic acid-responsive gene, MK, found in the teratocarcinoma system. Heterogeneity of the transcript and the nature of the translation product. J Biol Chem 265: 10765–10770.

Tsutsui J, Uehara K, Kadomatsu K, Matsubara S, Muramatsu T (1991). A new family of heparin-binding factors: strong conservation of midkine (MK) sequences between the human and the mouse. Biochem Biophys Res Commun 176: 792–797.

Tsutsui J, Kadomatsu K, Matsubara S, Nakagawara A, Hamanoue M, Takao S *et al.* (1993). A new family of heparin-binding growth/differentiation factors: increased midkine expression in Wilms' tumor and other human carcinomas. Cancer Res 53: 1281–1285.

Uehara K, Matsubara S, Kadomatsu K, Tsutsui J, Muramatsu T (1992). Genomic structure of human midkine (MK), a retinoic acid-responsive growth/differentiation factor. J Biochem 111: 563–567.

Ueoka C, Kaneda N, Okazaki I, Nadanaka S, Muramatsu T, Sugahara K (2000). Neuronal cell adhesion, mediated by the heparin-binding neuroregulatory factor midkine, is specifically inhibited by chondroitin sulfate E. Structural and functional implications of the over-sulfated chondroitin sulfate. J Biol Chem 275: 37407–37413.

Unoki K, Ohba N, Arimura H, Muramatsu H, Muramatsu T (1994). Rescue of photoreceptors from the damaging effects of constant light by midkine, a retinoic acid-responsive gene product. Invest Ophthalmol Vis Sci 35: 4063–4068.

Vanucci S, Minerdi D, Kadomatsu K, Mengoni A, Bazzicalupo M (2005). Putative midkine family protein up-regulated in *Patella caerulea* (Mollusca, Gastropoda) exposed to sublethal concentration of cadmium. Aquat Toxicol 75: 374–379.

Wang J, Takeuchi H, Sonobe Y, Jin S, Mizuno T, Miyakawa S *et al.* (2008). Inhibition of midkine alleviates experimental autoimmune encephalomyelitis through the expansion of regulatory T cell population. Proc Natl Acad Sci U S A 105: 3915–39120.

Weckbach LT, Muramatsu T, Walzog B (2011). Midkine in inflammation. ScientificWorldJournal 11: 2491–2505.

Weckbach LT, Groesser L, Borgolte J, Pagel JI, Pogoda F, Schymeinsky J *et al.* (2012). Midkine acts as proangiogenic cytokine in hypoxia-induced angiogenesis. Am J Physiol Heart Circ Physiol 303: H429–H438.

Winkler C, Schafer M, Duschl J, Schartl M, Volff JN (2003). Functional divergence of two zebrafish midkine growth factors following fish-specific gene duplication. Genome Res 13: 1067–1081.

Yokota C, Takahashi S, Eisaki A, Asashima M, Akhter S, Muramatsu T *et al.* (1998). Midkine counteracts the activin signal in mesoderm induction and promotes neural formation. J Biochem 123: 339–346.

Yoshida Y, Goto M, Tsutsui J, Ozawa M, Sato E, Osame M *et al.* (1995). Midkine is present in the early stage of cerebral infarct. Brain Res Dev Brain Res 85: 25–30.

Yoshida Y, Ikematsu S, Moritoyo T, Goto M, Tsutsui J, Sakuma S *et al.* (2001). Intraventricular administration of the neurotrophic factor midkine ameliorates hippocampal delayed neuronal death following transient forebrain ischemia in gerbils. Brain Res 894: 46–55.

Zou K, Muramatsu H, Ikematsu S, Sakuma S, Salama RH, Shinomura T *et al.* (2000). A heparin-binding growth factor, midkine, binds to a chondroitin sulfate proteoglycan, PG-M/versican. Eur J Biochem 267: 4046–4053.

Zou P, Zou K, Muramatsu H, Ichihara-Tanaka K, Habuchi O, Ohtake S *et al.* (2003). Glycosaminoglycan structures required for strong binding to midkine, a heparin-binding growth factor. Glycobiology 13: 35–42.

Zou P, Muramatsu H, Miyata T, Muramatsu T (2006a). Midkine, a heparin-binding growth factor, is expressed in neural precursor cells and promotes their growth. J Neurochem 99: 1470–1479.

Zou P, Muramatsu H, Sone M, Hayashi H, Nakashima T, Muramatsu T (2006b). Mice doubly deficient in the midkine and pleiotrophin genes exhibit deficits in the expression of β-tectorin gene and in auditory response. Lab Invest 86: 645–653.