

Midkine in Inflammation

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The 13kDa heparin-binding growth factor midkine (MK) was originally identified as a molecule involved in the orchestration of embryonic development. Recent studies provided evidence for a new role of MK in acute and chronic inflammatory processes. Accordingly, several inflammatory diseases including nephritis, arthritis, atherosclerosis, colitis, and autoimmune encephalitis have been shown to be alleviated in the absence of MK in animal models. Reduced leukocyte recruitment to the sites of inflammation was found to be one important mechanism attenuating chronic inflammation when MK was absent. Furthermore, MK was found to modulate expression of proinflammatory cytokines and the expansion of regulatory T-cells. Here, we review the current understanding of the role of MK in different inflammatory disorders and summarize the knowledge of MK biology.

KEYWORDS: Leukocytes, cytokines, immunity.

1. INTRODUCTION

The cytokine midkine (MK) was first discovered in the eighties of the last century as a molecule involved in embryonic development [1]. MK was found to be rarely expressed in the adult organism, but different types of cancer cells showed high MK expression associated with a poor prognosis of the patients [2–6]. Several studies revealed that overexpression of MK promoted tumor growth, survival, invasion, and tumor angiogenesis [7–9]. However, there is growing evidence that MK may also play an important role in chronic inflammatory disorders including, for example, kidney diseases, rheumatoid arthritis, inflammatory bowel disease, and multiple sclerosis as summarized in Table 1 [10–13]. The fact that these diseases exhibit high clinical and epidemiological relevance and affect the patients' quality of life dramatically makes it worthwhile to take a deeper look at a new factor possibly contributing to the induction and/or maintenance of these pathological conditions. In this paper, we summarize the findings on the role of MK in chronic inflammatory diseases and give an overview of MK biology including its gene and protein structure as well as its receptors and signaling.

2. GENE AND PROTEIN

MK was first identified in mouse embryonic carcinoma cells in studies on early stages of embryogenesis. In this model, differentiation of embryonic carcinoma cells by application of retinoic acid, one of the key players coordinating embryogenesis, led to increased MK mRNA expression in these cells [1]. In mouse embryos, MK was found to be induced at day 7 and showed a complex expression pattern at day 11 when organogenesis had started. After the midgestation stage, MK expression rapidly decreased and was thereafter only detectable at restricted sites such as the kidney. In situ hybridization technique in mouse embryos between days 7 and 13 revealed strong MK expression in epithelial tissues interacting with mesenchymal tissues during organ formation, in neuronal tissues, in the mesoderm where remodeling occurred, in the anterior lobe of the pituitary gland, in the retina, and in the kidney. This mode of expression considering gestation in mice lasting about 21 days was one of the reasons to give this molecule the name midkine (midgestation, kidney) [14].

While the human MK gene (MDK) is located on chromosome 11, the mouse MK gene was identified on chromosome 2 [15, 16]. The coding sequence of the human and mouse MK gene consists of 4 exons. In the promoter region of the MK gene, a retinoic acid response element (RARE), a hypoxia-responsive element (HRE), and a binding site for the product of the Wilms tumor suppressor gene WT-1 leading to decreased MK expression upon WT-1 binding were found [17–19].

As shown by NMR technique, the human 13 kDa protein MK consists of two similar domains each containing three antiparallel β -strands which are connected via disulfide bonds (Figures 1(a) and 1(b)) [20, 21]. MK is rich in basic amino acids forming two clusters responsible for heparin binding which are located in the C-terminal domain, namely, cluster 1 (K79, R81, and K102 in human MK) and cluster 2 (K86, K87, R89 in human MK). Although the N-terminal domain also contains several basic residues, the heparin-binding activity is very weak which is probably due to the existence of several acidic amino acids in this domain [20]. The C-terminal domain has been found to be functionally more important than the N-terminal domain [22]. Accordingly, different MK functions, for example, the promotion of neurite outgrowth or plasminogen activator activity are mediated by the C-terminal domain [23–25]. However, only the full length molecule, not the C-terminal domain by itself, was able to promote survival of embryonic brain neurons [23]. For some of its functions as, for example, enhancement of plasminogen activator activity, MK needs to dimerize which is mediated by a head-to-head conformation involving heparin being sandwiched by the cluster 2 heparin binding sites of two MK molecules [20, 26]. Transglutaminase activity and heparin have been found to be capable of promoting MK dimerization [26, 27].

MK represents the founding member of a family of heparin-binding growth factors and is structurally unrelated to any other growth factor family known so far consisting of only one other member named pleiotrophin (PTN). MK and PTN are highly conserved within the different species. While human MK

TABLE 1: Overview of the role of MK in chronic inflammatory diseases.

Organ	Disease	Model/specimen	Publication
Kidney	Diabetic nephropathy	Streptozotocin-induced diabetic nephropathy	Kosugi et al. 2006 [28], Kosugi et al. 2007 [10]
	Tubulointerstitial injury	Ischemia/reperfusion	Sato et al. 2001 [29], Sato et al. 2005 [30]
	Drug side effects	Cisplatin-induced renal damage	Kawai et al. 2004 [31]
Joints	Rheumatoid arthritis	Human synovial fluid, human synovial tissue	Takada et al. 1997 [32]
		Antitype II collagen antibody-induced arthritis	Maruyama et al. 2004 [11]
Vascular System	Atherosclerosis	Intimal hyperplasia in vein grafts	Banno et al. 2006 [33]
		In-stent restenosis	Narita et al. 2008 [34]
		Neointima formation in restenosis	Horiba et al. 2000 [35]
Colon	Crohn's disease	Human blood	Krzystek-Korpacka et al. 2010 [12]
	Ulcerative colitis	Dextran-sulfate-sodium- (DSS-) induced colitis	Yuki et al. 2006 [36]
Central nervous system	Multiple sclerosis	Experimental autoimmune encephalomyelitis (EAE)	Liu et al. 1998 [37], Wang et al. 2008 [13]

shares 87% sequence identity with mouse MK, human MK and PTN have about 50% sequence identity (Figure 2(a)) [38–40]. All cysteine residues in both molecules are conserved in the human and murine system [21]. The basic amino acids that form the clusters for heparin-binding are also highly conserved in MK and PTN (Figure 2(b)). As an exception, the basic amino acid K84 in human MK is changed to R84 in human PTN [22]. Although MK and PTN share distinctive sequence similarities, both proteins show different expression patterns. While MK is highly expressed during midgestation as mentioned above, the expression peak of PTN in mice occurs around birth [41]. In *Drosophila melanogaster*, two MK/PTN homologues have been identified, named *miple1* and *miple2* (midkine, pleiotrophin) [42]. The amino acid sequence of *miple1* and *miple2* is about 60% identical to human MK and human PTN with particularly high homology to the C-terminal domain. The basic amino acids in the heparin-binding clusters are partially conserved in the *miple* proteins [42].

3. RECEPTORS AND SIGNALING

MK binds a variety of different receptors and an overview of these receptors is shown in Table 2. MK has been found to promote, for example, growth, survival, migration, and gene expression of different cell types probably via a multiprotein receptor complex consisting of several molecules [43]. Within this complex, the best characterized receptor represents the receptor-like protein tyrosine phosphatase β /protein tyrosine phosphatase ζ (PTP ζ), which is abundantly expressed in the central nervous system mediating cell adhesion and signaling during embryonic development [44]. PTP ζ is a transmembrane protein with intracellular tyrosine phosphatase activity linked to an extracellular chondroitin sulfate chain [45]. The fact that the binding affinity of MK to PTP ζ decreased from a Kd of 0.56 nM to 8.8 nM after removal of the chondroitin sulfate chain indicated its importance for ligand-receptor recognition [45]. Binding of MK to PTP ζ induced migration of embryonic neurons as well as UMR106 osteoblast-like cells and enhanced survival of neurons during embryonic development [45–47]. Inhibition of different kinases such as PI3 kinase, MAP kinases, Src family kinases, and protein kinase C impaired MK-dependent migration of UMR-106 cells implying the involvement of multiple kinases in downstream signaling upon MK binding to PTP ζ [46].

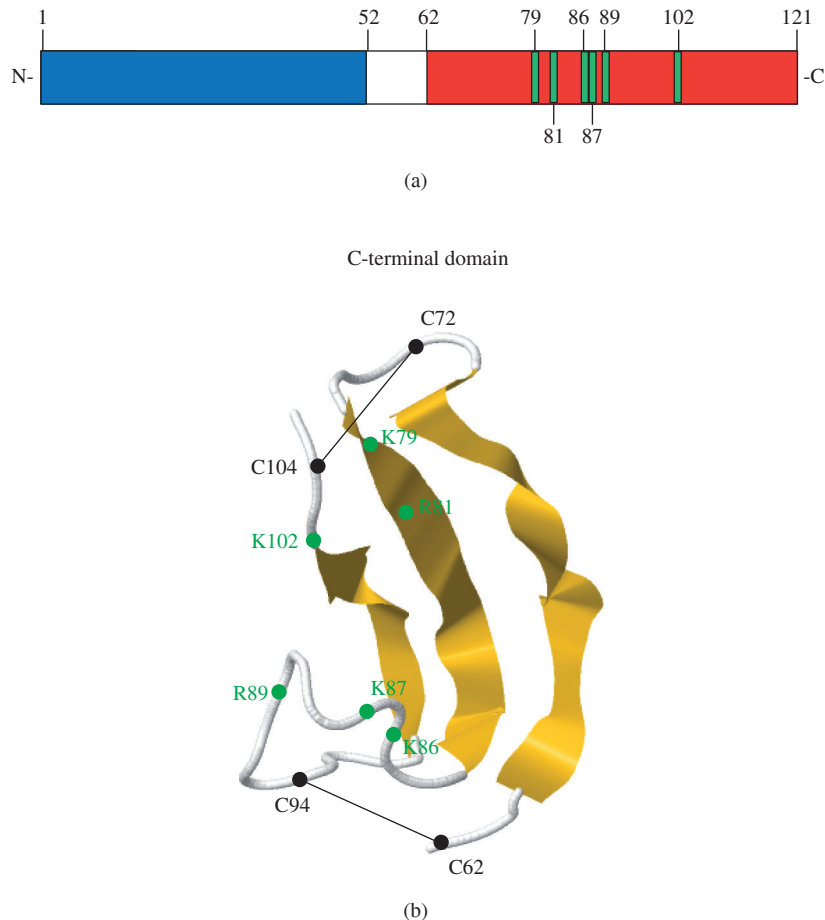


FIGURE 1: Protein structure of MK. (a) MK protein structure is shown. MK consists of two domains with similar size connected by an interdomain (white box). Numbers indicate amino acid position within the protein. The heparin-binding sites consisting of basic amino acids are located in the C-terminal domain (green boxes) [20]. (b) Tertiary structure of the C-terminal domain of MK protein “taken from the protein data bank PDB via <http://www.rcsb.org/pdb/explore.do?structureId=1MKC>.” The C-terminal domain contains three β -strands (yellow structure). Heparin binding clusters consisting of basic amino acids (cluster 1: K79, R81, K102; cluster 2: K86, K87, R89 in human MK) are displayed as green dots. C62, C72, C94, and C104 represent highly conserved cysteine residues forming disulfide bonds (black lines) [20].

In addition to PTP ζ , members of the low-density-lipoprotein receptor-related protein (LRP) family have been identified as components of the MK receptor complex including LRP-1, megalin/brushin, LRP-6, and apoE receptor-2 [47, 48]. LRP-1 induced survival of embryonic neurons and prevented hypoxic injury in mouse embryonic stem cells upon MK binding with a K_d of 3.5 nM, whereas binding affinity of MK to the other LRP family members mentioned above was significantly lower [47–49].

In 13-day-old mouse embryos, β_1 integrins were identified as functional MK receptors. MK binding to $\alpha_4\beta_1$ -integrin mediated migration of UMR-106 osteoblast-like cells [51]. In these cells, MK treatment led to increased tyrosine phosphorylation of paxillin which interacts with the cytoplasmic tail of β integrins [52, 61]. Via $\alpha_6\beta_1$ -integrin binding, MK induced neurite outgrowth of embryonic neurons [51]. Furthermore, MK promoted the association of the $\alpha_6\beta_1$ -integrin and the tetraspanin CD9, which belongs to a family of highly conserved receptor proteins forming multimeric complexes with other membrane proteins and thereby modulating cell adhesion, proliferation, and metastasis [52, 62]. The fact that $\alpha_4\beta_1$ and $\alpha_6\beta_1$ integrins coimmunoprecipitated with the LRP-6 ectodomain and PTP ζ upon MK treatment suggested a functional interaction of these molecules in an MK receptor complex [51].

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Human MK 1 KKKDKVKKGGPGSECAEWAWGPCTPSSKDCGVGFREGTCGAQTQRI RCRVPCNWKKEFGA
Murine MK 1 KKKKEKVKK---GSECSEWTWGPCTPSSKDCGMGFREGTCGAQTQRVHCKVPCNWKKEFGA
          ***  *****   *****  **  *****  *****  *****  *****  *****
          *  *****

Human MK 61 DCKYKFENWGACDGGTGTKVVRQGLTKKARYNAQCQETIRVTKPCTPKTKAKAKAKKKGK
Murine MK 58 DCKYKFESWGACDGSSTGTKARQGLTKKARYNAQCQETIRVTKPCTSKTKSKTKAKKKGK
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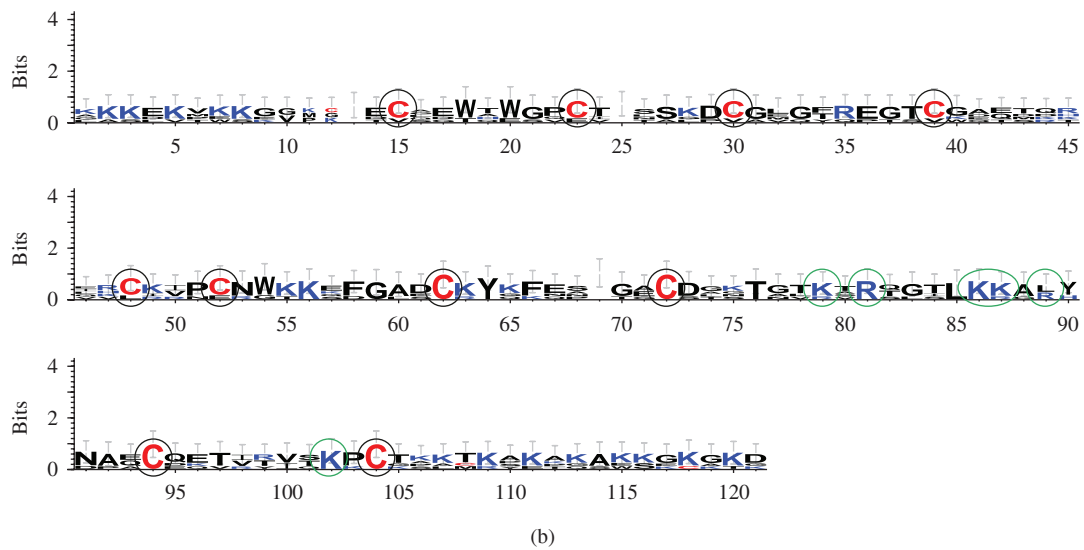
Human MK 121 D
Murine MK 118 D
          *

Human MK 2 KKKDKVKKGGPGSSECAEWAWGPCTPSSKDCGVGFREGT-----CGAQTQRI RCRVPCNWK
Human PTN 2 KKEKPEKVKKSDCGEWQWSVCVPTSGDCGLGTREGT RTGAECKQTMKTQRCKI PCNWK
          ** *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *
          *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *

Human MK 57 EFGADCKYKFENWGACDGGTGTKVVRQGLTKKARYNAQCQETIRVTKPCTPKTKAKAKK
Human PTN 62 QFGAECKYQFQAWGECDLNTALKTRTGS LKRALHNAECQKTVTISKPCGKLTQPKPOAES
          ***  ***  *  **  **  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *
          *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *

Human MK 117 GKKG
Human PTN122 KKKK
    
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(a)



(b)

FIGURE 2: Sequence homologies of MK and PTN. (a) Amino acid sequence of human MK and murine MK as well as human MK and human PTN. Human and murine MK show 87% amino acid sequence identity; human MK and PTN share 50% sequence homology. * = residues identical in both sequences [38–40]. (b) Web logos displaying sequence homology of human MK, murine MK, chicken MK, miple1, and human PTN in information bits [50]. A large overall height indicates strong conservation of the corresponding amino acid in that position; the height within the stack shows the relative frequency of the amino acid at that position. Cysteine residues (black circle) and basic amino acids (green circle) are highly conserved within different species of the MK family.

TABLE 2: Overview of MK receptors.

Receptor family	MK receptor	Function	Publication
PTP		Migration of embryonic neurons	Maeda et al. 1999 [45]
	PTP ζ	Migration of UMR106 osteoblast-like cells	Qi et al. 2001 [46]
		Survival of embryonic neurons	Sakaguchi et al. 2003 [47]
LRP	LRP-1	Survival of embryonic neurons	Muramatsu et al. 2000 [48]
		Prevention of hypoxic injury in mouse embryonic stem cells	Lee et al. 2011 [49]
LRP	megalim/brushin		Muramatsu et al. 2000 [48]
	LRP-6		Muramatsu et al. 2000 [48], Muramatsu et al. 2004 [51]
	apoE receptor-2		Muramatsu et al. 2000 [48]
	$\alpha_4\beta_1$	Migration of UMR106 osteoblast-like cells	Muramatsu et al. 2004 [51]
	$\alpha_6\beta_1$	Neurite outgrowth of embryonic neurons	Muramatsu et al. 2004 [51], Huang et al. 2008 [52]
Integrins			
Notch		Epithelial mesenchymal transition of immortalized HaCaT keratinocytes	Huang et al. 2008 [52]
	notch2		
		Epithelial mesenchymal transition and chemoresistance in pancreatic ductal adenocarcinoma cells	Güngör et al. 2011 [53]
Receptor tyrosine kinase	ALK	Growth of SW-13 cells in soft agar	Stoica et al. 2007 [7]
	Neuroglycan C		Ichihara-Tanaka et al. 2006 [54]
Carbohydrates			Kaneda et al. 1996 [55], Ueoka et al. 2000 [56], Zou et al. 2003 [57]
	Heparan sulfate trisulfated units	Process elongation of oligodendrocyte precursor-like cells	Ueoka et al. 2000 [56], Zou et al. 2003 [57]
	Chondroitin sulfate E units		Ueoka et al. 2000 [56], Zou et al. 2003 [57]
	Syndecan-1		Mitsiadis et al. 1995 [58]
	Syndecan-3		Mitsiadis et al. 1995 [58], Nakanishi et al. 1997 [59]
	Glypican-2		Kurosawa et al. 2001 [60]

Recently, neuroglycan C was found to serve as an MK receptor in an oligodendrocyte precursor-like cell line [54]. Neuroglycan C is a chondroitin sulfate proteoglycan exclusively expressed in the central nervous system contributing to brain development [63]. Similar to PTP ζ , the binding affinity of neuroglycan C to MK was impaired when chondroitin sulfate chains were absent [54]. In addition, MK binds to Notch2, a transmembrane protein belonging to the Notch family. Here, MK was found to be involved in induction of epithelial mesenchymal transition (EMT) of immortalized HaCaT keratinocytes indicated by a decrease of epithelial and an increase of fibroblast markers [64]. EMT is a process that occurs during embryonic development as well as in tumor metastasis and is characterized by loss of cell contacts in order to allow cell migration [64]. An interaction between MK and Notch2 promoting EMT was also shown in pancreatic ductal adenocarcinoma cells (PDACs) [53]. Furthermore, Notch2 signaling upon MK binding upregulated NF κ B implying an involvement of MK in inflammatory pathways [53]. Moreover, anaplastic lymphoma kinase (ALK), a transmembrane tyrosine kinase mediating survival, proliferation, and differentiation of normal and tumor cells was identified as MK receptor [7, 65]. MK promoted colony formation of the adrenal gland SW-13 tumor cell line in soft agar and induced proliferation of human endothelial cells via ALK signaling [7]. In WI-38 human fibroblasts, MK stimulation led to phosphorylation of ALK and subsequent activation of PI3 kinase and MAP kinase [7].

The fact that most MK effects were diminished upon administration of heparin or treatment with heparitinase or chondroitinase indicated the importance of carbohydrate recognition in MK signaling. Two specific carbohydrate structures, namely, heparan sulfate trisulfated units or chondroitin sulfate E units—when present as oligomers—have been shown to bind to MK with high affinity [55–57]. As mentioned above, chondroitin sulfate chains linked to PTP ζ enhanced MK binding affinity. Syndecans and glypican-2, carbohydrates involved in the neuronal development, also showed MK binding activity contributing to development of the central nervous system, neuronal cell migration, and neurite outgrowth [58–60].

In conclusion, MK represents a promiscuous ligand that binds different receptors thereby activating several intracellular signaling events. However, the intracellular signal transduction pathways that mediate the effects of MK in inflammation are widely unknown.

4. MK AS A MODULATOR OF THE INFLAMMATORY RESPONSE

4.1. Kidney

There is a growing body of evidence that MK plays a critical role during inflammation. In the adult organism, MK shows constitutive expression in proximal renal tubular epithelial cells, and different pathological kidney conditions including diabetic nephropathy have been linked to enhanced MK expression [10, 29–31]. Diabetic nephropathy caused by diabetes mellitus represents the main cause of end-stage renal failure and dialysis requirement accompanied by high mortality and exceeding cost of care [66]. During diabetic nephropathy which is associated with tubulointerstitial inflammation, glomeruli, interstitium, and tubules of patients showed highly increased MK expression in contrast to healthy patients [10]. In a diabetes mouse model where hyperglycemia was induced by application of streptozotocin which impairs insulin production by selective destruction of β -cells of the pancreas and results in elevated glucose levels, tubulointerstitial damage was less severe in mice lacking MK expression compared to WT control animals within 2 to 6 months after the onset of the experiment. This suggests that MK may aggravate the inflammatory response in diabetic nephropathy [10, 28].

Inflammation induced by ischemia/reperfusion (I/R) in the kidney, which is one of the main causes of acute renal failure in humans, led to increased MK expression in the renal tubules two days after ligation of renal arteries for 90 minutes in a mouse model. Seven days after I/R, MK expression returned to baseline values [29, 30]. In MK-deficient mice or mice treated with MK antisense oligodeoxyribonucleotides, tubulointerstitial damage was reduced compared to control conditions [29, 30]. Furthermore, renal damage caused by cisplatin—a chemotherapeutic drug used for the treatment of different kinds of cancer—was found to be reduced in MK-deficient mice implying that MK may exacerbate side effects of drugs in the

kidney [31]. However, in all pathological conditions mentioned above, reduced renal damage in the absence of MK was associated with impaired infiltration of inflammatory cells into the renal tissue [10, 29–31]. This is in line with the finding that monocyte chemoattractant protein 1 (MCP-1, CCL2) and macrophage inflammatory protein (MIP-2, CXCL2) were upregulated in WT mice upon I/R of the kidney. Similarly, MCP-1 expression was found to be increased in streptozotocin-induced diabetic nephropathy [10, 29, 30]. Thus, MK may be able to contribute to the recruitment of leukocytes by induction of chemokine expression in the kidney. Both chemokines are known to mediate leukocyte infiltration and upregulation of these proteins was diminished in mice lacking MK expression [10, 29]. Studies with cultured tubular epithelial cells (TEC) revealed that high glucose levels mimicking the diabetes model and H₂O₂ mimicking conditions during ischemia induced MK expression in TEC and increased MCP-1 and MIP-1 expression in these cells. This effect was not observed in cells of MK-deficient mice or in cells treated with an MK blocking antibody [10, 29]. In addition, recombinant MK enhanced MCP-1 and MIP-1 expression in TEC [29]. Thus, induction of chemokines by MK may at least partially explain the reduced recruitment of inflammatory cells into the kidney under inflammatory conditions in the absence of MK. In addition, MK by itself has been reported to act as a haptotactic and chemotactic agent for neutrophils in a Boyden chamber [32]. However, leukocyte infiltration was diminished during Cisplatin-induced renal inflammation in the genetic absence of MK or upon downregulation of MK by antisense technique, whereas no difference in expression pattern of MCP-1 was observed [31]. Thus, the induction of chemokines may represent not the only mechanism mediating leukocyte recruitment in the kidney. Taken together, MK may aggravate different pathologies of the kidney by enhanced recruitment of leukocytes. The question whether MK is able to induce additional inflammatory chemokines besides MCP-1 and MIP-2 and how MK exactly contributes to leukocyte trafficking still needs to be investigated.

4.2. Joints

Rheumatoid arthritis (RA), an autoimmune disease leading to chronic joint inflammation, subsequent joint destruction, and deformities, is of high clinical and epidemiological relevance affecting approximately from 0.5% to 1% of the adults worldwide with a higher prevalence in women [67]. High levels of RF—autoantibodies against the Fc fragment of IgG—can be detected in 80% of RA patients and correlate with the susceptibility to developing RA and severe joint damage. However, RF is also elevated in other autoimmune diseases and does not correlate with disease activity [68]. Of note, synovial fluid and sera of 90% of patients suffering from RA showed enhanced MK levels. MK levels correlated positively with RF [11]. Inflamed tissues of patients with active inflammatory synovitis of RA expressed high concentrations of MK, whereas noninflamed tissue showed no MK expression at all [32]. Elevated MK expression in a large number of RA patients indicated that MK may represent a new additional biomarker for the diagnosis of RA. Furthermore, the functional relevance of MK during arthritis was investigated in a murine arthritis model where joint inflammation was induced by injection of an antitype II collagen Ab followed by intra-articular LPS administration [69]. While control mice developed severe arthritis until day 7 measured by joint cavity size, synovial membrane thickening, and accumulation of synovial fluid, the inflammatory response was almost completely absent in MK-deficient mice. However, intraperitoneal administration of recombinant MK restored this effect and led to severe arthritis in this model. As seen in the kidney, the number of extravasated neutrophils and macrophages into the synovial tissue in MK-deficient mice was decreased after 2 to 7 days when compared to WT control animals at the same time point indicating that MK may represent a critical player for pathogenesis of RA [11].

4.3. Vascular System

Although risk factors for development and maintenance of atherosclerosis including hypercholesterolemia, free radicals from cigarette smoking, diabetes mellitus, and hypertension have been clearly identified, cardiovascular diseases still represent the main cause of death in Europe and in the United States.

Atherosclerosis is a chronic inflammatory disease of large- and medium-sized arteries characterized by endothelial dysfunction and subsequent plaque formation of the vascular wall. Under healthy conditions, arteries and veins express MK at a very low level [34]. However, injury of the endothelium caused by experimental interventions led to increased MK expression in different rabbit and mouse models [33, 35]. Macrophages infiltrating the injured vascular wall after stenting have been found to be a major source of MK, whereas freshly isolated monocytes did not express MK [34]. Clinically, mechanic vessel injury is followed by an inflammatory response causing neointima formation which may lead to restenosis after therapeutic intervention. Leukocytes play an important role in this context by secreting factors which promote migration and proliferation of smooth muscle cells (SMCs) and maintain an inflammatory environment [70]. Compared to control mice, neointima formation was almost completely absent in MK-deficient mice [35]. This was in line with the finding that leukocyte recruitment to the injured vessel wall was reduced in MK-deficient mice compared to control animals. However, intra-arterial administration of recombinant MK restored neointima formation [35]. Endothelial cells of implanted vein grafts interpositioned into an artery in rabbits also strongly expressed MK. When grafts were treated with MK siRNA, intima thickness and leukocyte infiltration into the vessel wall was significantly reduced suggesting that MK promoted leukocyte infiltration into the vascular wall after intervention-associated vessel injury [33].

4.4. Colon

Approximately 1.4 million people in Europe are diagnosed—generally in late adolescence or early adulthood—with inflammatory bowel disease (IBD) which consists of two major forms, namely, ulcerative colitis (UC) and Crohn’s disease (CD) [71]. Both pathologic entities are characterized by autoimmune-mediated inflammation of the gastrointestinal tract. In CD, MK serum levels showed positive correlation with Crohn’s Disease Activity Index (CDAI) which includes vital parameters, clinical findings, and medical history [12, 72]. Accordingly, MK has been found to be a sensitive biomarker of diagnostic value comparable with the gold standard C-reactive protein (CRP) in this disease [12]. In an experimental colitis model, where bowel inflammation was induced by dextran sulfate sodium (DSS) and which represents a well-established model for UC, MK was abundantly expressed in fibroblasts of the mucosal and submucosal layers of the rat distal colon [36]. Here, $\text{TNF-}\alpha$ and $\text{IL-1}\beta$, which show high local expression during active IBD in humans leading to infiltration of innate immune cells, were expressed in the distal rat colon [36, 73]. In addition, both cytokines induced MK expression in the rat fibroblast cell line 3Y1. Moreover, MK was found to accelerate intestinal wound repair in vitro using cultured epithelial monolayer sheets suggesting that MK may contribute to mucosal repair [36]. Thus, the inflammatory cytokines $\text{TNF-}\alpha$ and $\text{IL-1}\beta$ may facilitate wound repair by upregulating MK. However, the question whether MK is beneficial for mucosa repair or rather promotes inflammation requires further investigation.

4.5. Central Nervous System

MK has also been investigated in a chronic inflammatory disease affecting the central nervous system, namely, multiple sclerosis (MS). In Europe around 500,000 people suffer from MS [74]. MS is a chronic autoimmune disease that is characterized by demyelination of axons of the central nervous system caused by autoreactive T-cells and demyelinating antibodies. To study MS, experimental autoimmune encephalomyelitis (EAE) has been used as a widely accepted animal model where demyelination is induced by injection of myelin-oligodendrocyte-glycoprotein leading to $\text{T}_\text{H}1$ and $\text{T}_\text{H}17$ cell-mediated inflammation [75, 76]. After induction of EAE in rats, increased MK mRNA expression in the spinal cord correlated with the severity of clinical symptoms such as limb and tail weakness. Recovery of clinical symptoms led to decreased MK expression in the spinal cord again [37]. EAE induction of WT mice caused severe clinical symptoms 18 days after onset of the experiment. MK-deficient mice also developed clinical signs of encephalomyelitis. However, clinical scores were significantly lower than in WT

control animals. Administration of recombinant MK with a subcutaneously implanted microosmotic pump deteriorated clinical scores [13]. Histological analysis of the spinal cord revealed decreased infiltration of inflammatory cells in the spinal cord in MK-deficient mice compared to WT control animals, whereas MK application restored this effect showing positive correlation of clinical findings and histological analysis [13]. This indicates that MK was important for induction and development of EAE. CD4+ and CD8+ T cell populations were similar in peripheral lymph nodes of WT and MK-deficient mice and, therefore, did not explain reduced clinical and histopathological severity in MK-deficient mice. After onset of EAE, expansion of CD4+/CD25+ regulatory T-cells (T_{reg}), which have the ability to suppress autoreactive T_H1 and T_H17 cells, was significantly increased in peripheral lymph nodes in the absence of MK [13, 77]. Accordingly, administration of MK critically suppressed T_{reg} expansion in a dose-dependent manner in vitro and in vivo. When T_{reg} were targeted with a CD25 blocking antibody in MK-deficient mice, suppression of EAE was abolished [13]. These findings indicate that an increased number of T_{reg} were recruited to peripheral lymph nodes when MK was absent after induction of EAE leading to attenuated clinical and histopathological symptoms in this model [13]. Furthermore, it was shown that CD4+ T-cells from MK-deficient mice after induction of EAE expressed lower amounts of IFN- γ and IL-17, cytokines which are critically involved in the pathogenesis of several autoimmune diseases [78]. It could also be shown that targeting MK by RNA aptamers significantly increased T_{reg} expansion and improved clinical signs after induction of EAE in WT mice [13]. Taken together, MK expression has been shown to be enhanced during induction and progression of EAE which correlated positively with clinical scores and histopathological signs of inflammation of the myelin sheath. MK suppressed expansion of T_{reg} and enhanced autoreactive injury of the myelin sheath. Targeting MK led to increased T_{reg} expansion and subsequently suppressed autoreactive T_H1 and T_H17 cell populations in this model which are critically involved in the pathogenesis of autoimmune diseases.

5. OUTLOOK AND PERSPECTIVES

Being diagnosed with autoimmune diseases such as multiple sclerosis, ulcerative colitis, Crohn's disease, or rheumatoid arthritis critically affects the patients' quality of life [79, 80]. Especially the early onset and gradual progression of disease over a long period of time makes therapy and treatment for clinicians and patients very challenging [81]. Immunosuppressive drugs, corticosteroids, cytostatics, and biological drugs specifically targeting proteins involved in the inflammatory response can effectively suppress autoimmune responses. However, severe side effects, for example, increased risk of infection, cancer, and other autoimmune diseases, make treatment options unsatisfying [81]. Similar to autoimmune disorders, a number of additional widespread diseases including atherosclerosis are characterized by chronic inflammatory processes [70]. Different studies have shown that MK plays a role in inflammation by induction of leukocyte infiltration and chemokine expression as well as by suppression of T_{reg} expansion. As MK shows highly restrictive expression patterns in healthy tissues of the adult organism, targeting MK may represent a promising new approach for treatment of chronic inflammation including autoimmune diseases.

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REFERENCES

- [1] K. Kadomatsu, M. Tomomura, and T. Muramatsu, "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," *Biochemical and Biophysical Research Communications*, vol. 3, pp. 1312–1318, 1988.

- [2] T. O'Brien, D. Cranston, S. Fuggle, R. Bicknell, and A. L. Harris, "The angiogenic factor midkine is expressed in bladder cancer, and overexpression correlates with a poor outcome in patients with invasive cancers," *Cancer Research*, vol. 56, no. 11, pp. 2515–2518, 1996.
- [3] M. Ruan, T. Ji, Z. Wu, J. Zhou, and C. Zhang, "Evaluation of expression of midkine in oral squamous cell carcinoma and its correlation with tumour angiogenesis," *International Journal of Oral and Maxillofacial Surgery*, vol. 36, no. 2, pp. 159–164, 2007.
- [4] S. Maeda, H. Shinchi, H. Kurahara et al., "Clinical significance of midkine expression in pancreatic head carcinoma," *British Journal of Cancer*, vol. 97, no. 3, pp. 405–411, 2007.
- [5] G. A. Mashour, N. Ratner, G. A. Khan, H. L. Wang, R. L. Martuza, and A. Kurtz, "The angiogenic factor midkine is aberrantly expressed in NF1-deficient Schwann cells and is a mitogen for neurofibroma-derived cells," *Oncogene*, vol. 20, no. 1, pp. 97–105, 2001.
- [6] K. Aridome, J. Tsutsui, S. Takao et al., "Increased Midkine gene expression in human gastrointestinal cancers," *Japanese Journal of Cancer Research*, vol. 86, no. 7, pp. 655–661, 1995.
- [7] G. E. Stoica, A. Kuo, C. Powers et al., "Midkine binds to anaplastic lymphoma kinase (ALK) and acts as a growth factor for different cell types," *Journal of Biological Chemistry*, vol. 277, no. 39, pp. 35990–35998, 2002.
- [8] R. Choudhuri, H. T. Zhang, S. Donnini, M. Ziche, and R. Bicknell, "An angiogenic role for the neurokinins midkine and pleiotrophin in tumorigenesis," *Cancer Research*, vol. 57, no. 9, pp. 1814–1819, 1997.
- [9] M. Muramaki, H. Miyake, I. Hara, and S. Kamidono, "Introduction of midkine gene into human bladder cancer cells enhances their malignant phenotype but increases their sensitivity to antiangiogenic therapy," *Clinical Cancer Research*, vol. 9, no. 14, pp. 5152–5160, 2003.
- [10] T. Kosugi, Y. Yuzawa, W. Sato et al., "Midkine is involved in tubulointerstitial inflammation associated with diabetic nephropathy," *Laboratory Investigation*, vol. 87, no. 9, pp. 903–913, 2007.
- [11] K. Maruyama, H. Muramatsu, N. Ishiguro, and T. Muramatsu, "Midkine, a heparin-binding growth factor, is fundamentally involved in the pathogenesis of rheumatoid arthritis," *Arthritis and Rheumatism*, vol. 50, no. 5, pp. 1420–1429, 2004.
- [12] M. Krzystek-Korpacka, K. Neubauer, and M. Matusiewicz, "Circulating midkine in Crohn's disease: clinical implications," *Inflammatory Bowel Diseases*, vol. 16, no. 2, pp. 208–215, 2010.
- [13] J. Wang, H. Takeuchi, Y. Sonobe et al., "Inhibition of midkine alleviates experimental autoimmune encephalomyelitis through the expansion of regulatory T cell population," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 105, no. 10, pp. 3915–3920, 2008.
- [14] K. Kadomatsu, R. P. Hung, T. Suganuma, F. Murata, and T. Muramatsu, "A retinoic acid responsive gene MK found in the teratocarcinoma system is expressed in spatially and temporally controlled manner during mouse embryogenesis," *Journal of Cell Biology*, vol. 110, no. 3, pp. 607–616, 1990.
- [15] T. Kaname, A. Kuwano, I. Murano, K. Uehara, T. Muramatsu, and T. Kajii, "Midkine gene (MDK), a gene for prenatal differentiation and neuroregulation, maps to band 11p11.2 by fluorescence in situ hybridization," *Genomics*, vol. 17, no. 2, pp. 514–515, 1993.
- [16] D. Simon-Chazottes, S. Matsubara, T. Miyauchi, T. Muramatsu, and J. L. Guenet, "Chromosomal localization of two cell surface-associated molecules of potential importance in development: Midkine (Mdk) and basigin (Bsg)," *Mammalian Genome*, vol. 2, no. 4, pp. 269–271, 1992.
- [17] P. R. Reynolds, M. L. Mucenski, T. D. Le Cras, W. C. Nichols, and J. A. Whitsett, "Midkine is regulated by hypoxia and causes pulmonary vascular remodeling," *Journal of Biological Chemistry*, vol. 279, no. 35, pp. 37124–37132, 2004.
- [18] Y. Adachi, S. Matsubara, C. Pedraza et al., "Midkine as a novel target gene for the Wilms' tumor suppressor gene (WT1)," *Oncogene*, vol. 13, no. 10, pp. 2197–2203, 1996.
- [19] S. Matsubara, M. Take, C. Pedraza, and T. Muramatsu, "Mapping and characterization of a retinoic acid-responsive enhancer of midkine, a novel heparin-binding growth/differentiation factor with neurotrophic activity," *Journal of Biochemistry*, vol. 115, no. 6, pp. 1088–1096, 1994.
- [20] W. Iwasaki, K. Nagata, H. Hatanaka et al., "Solution structure of midkine, a new heparin-binding growth factor," *EMBO Journal*, vol. 16, no. 23, pp. 6936–6946, 1997.

- [21] L. Fabri, H. Maruta, H. Muramatsu et al., “Structural characterisation of native and recombinant forms of the neurotrophic cytokine MK,” *Journal of Chromatography*, vol. 646, no. 1, pp. 213–225, 1993.
- [22] T. Asai, K. Watanabe, K. Ichihara-Tanaka et al., “Identification of heparin-binding sites in midkine and their role in neurite-promotion,” *Biochemical and Biophysical Research Communications*, vol. 236, no. 1, pp. 66–70, 1997.
- [23] H. Muramatsu, T. Inui, T. Kimura et al., “Localization of heparin-binding, neurite outgrowth and antigenic regions in midkine molecule,” *Biochemical and Biophysical Research Communications*, vol. 203, no. 2, pp. 1131–1139, 1994.
- [24] S. Kojima, T. Inui, T. Kimura et al., “Synthetic peptides derived from midkine enhance plasminogen activator activity in bovine aortic endothelial cells,” *Biochemical and Biophysical Research Communications*, vol. 206, no. 2, pp. 468–473, 1995.
- [25] H. Muramatsu and T. Muramatsu, “Purification of recombinant midkine and examination of its biological activities: Functional comparison of new heparin binding factors,” *Biochemical and Biophysical Research Communications*, vol. 177, no. 2, pp. 652–658, 1991.
- [26] S. Kojima, T. Inui, H. Muramatsu et al., “Dimerization of midkine by tissue transglutaminase and its functional implication,” *Journal of Biological Chemistry*, vol. 272, no. 14, pp. 9410–9416, 1997.
- [27] S. Kojima, H. Muramatsu, H. Amanuma, and T. Muramatsu, “Midkine enhances fibrinolytic activity of bovine endothelial cells,” *Journal of Biological Chemistry*, vol. 270, no. 16, pp. 9590–9596, 1995.
- [28] T. Kosugi, Y. Yuzawa, W. Sato et al., “Growth factor midkine is involved in the pathogenesis of diabetic nephropathy,” *American Journal of Pathology*, vol. 168, no. 1, pp. 9–19, 2006.
- [29] W. Sato, K. Kadomatsu, Y. Yuzawa et al., “Midkine is involved in neutrophil infiltration into the tubulointerstitium in ischemic renal injury,” *Journal of Immunology*, vol. 167, no. 6, pp. 3463–3469, 2001.
- [30] W. Sato, Y. Takei, Y. Yuzawa, S. Matsuo, K. Kadomatsu, and T. Muramatsu, “Midkine antisense oligodeoxyribonucleotide inhibits renal damage induced by ischemic reperfusion,” *Kidney International*, vol. 67, no. 4, pp. 1330–1339, 2005.
- [31] H. Kawai, W. Sato, Y. Yuzawa et al., “Lack of the growth factor midkine enhances survival against cisplatin-induced renal damage,” *American Journal of Pathology*, vol. 165, no. 5, pp. 1603–1612, 2004.
- [32] T. Takada, K. Toriyama, H. Muramatsu, X. J. Song, S. Torii, and T. Muramatsu, “Midkine, a retinoic acid-inducible heparin-binding cytokine in inflammatory responses: chemotactic activity to neutrophils and association with inflammatory synovitis,” *Journal of Biochemistry*, vol. 122, no. 2, pp. 453–458, 1997.
- [33] H. Banno, Y. Takei, T. Muramatsu, K. Komori, and K. Kadomatsu, “Controlled release of small interfering RNA targeting midkine attenuates intimal hyperplasia in vein grafts,” *Journal of Vascular Surgery*, vol. 44, no. 3, pp. 633–641, 2006.
- [34] H. Narita, S. Chen, K. Komori, and K. Kadomatsu, “Midkine is expressed by infiltrating macrophages in in-stent restenosis in hypercholesterolemic rabbits,” *Journal of Vascular Surgery*, vol. 47, no. 6, pp. 1322–1329, 2008.
- [35] M. Horiba, K. Kadomatsu, E. Nakamura et al., “Neointima formation in a restenosis model is suppressed in midkine-deficient mice,” *Journal of Clinical Investigation*, vol. 105, no. 4, pp. 489–495, 2000.
- [36] T. Yuki, S. Ishihara, M. A. K. Rumi et al., “Increased expression of midkine in the rat colon during healing of experimental colitis,” *American Journal of Physiology, Gastrointestinal and Liver Physiology*, vol. 291, no. 4, pp. G735–G743, 2006.
- [37] X. Liu, G. A. Mashour, H. D. Webster, and A. Kurtz, “Basic FGF and FGF receptor 1 are expressed in microglia during experimental autoimmune encephalomyelitis: temporally distinct expression of midkine and pleiotrophin,” *GLIA*, vol. 24, no. 4, pp. 390–397, 1998.
- [38] K. Uehara, S. Matsubara, K. Kadomatsu, J. Tsutsui, and T. Muramatsu, “Genomic structure of human midkine (MK), a retinoic acid-responsive growth/differentiation factor,” *Journal of Biochemistry*, vol. 111, no. 5, pp. 563–567, 1992.
- [39] Y. S. Li, P. G. Milner, A. K. Chauhan et al., “Cloning and expression of a developmentally regulated protein that induces mitogenic and neurite outgrowth activity,” *Science*, vol. 250, no. 4988, pp. 1690–1694, 1990.
- [40] J. I. Tsutsui, K. Uehara, K. Kadomatsu, S. Matsubara, and T. Muramatsu, “A new family of heparin-binding factors: Strong conservation of midkine (MK) sequences between the human and the mouse,” *Biochemical and Biophysical Research Communications*, vol. 176, no. 2, pp. 792–797, 1991.

- [41] H. Rauvala, “An 18-kd heparin-binding protein of developing brain that is distinct from fibroblast growth factors,” *EMBO Journal*, vol. 8, no. 10, pp. 2933–2941, 1989.
- [42] C. Englund, A. Birve, L. Falileeva, C. Grabbe, and R. H. Palmer, “Miple1 and miple2 encode a family of MK/PTN homologues in *Drosophila melanogaster*,” *Development Genes and Evolution*, vol. 216, no. 1, pp. 10–18, 2006.
- [43] T. Muramatsu, “Midkine, a heparin-binding cytokine with multiple roles in development, repair and diseases,” *Proceedings of the Japan Academy Series B: Physical and Biological Sciences*, vol. 86, no. 4, pp. 410–425, 2010.
- [44] S. Bouyain and D. J. Watkins, “The protein tyrosine phosphatases PTPRZ and PTPRG bind to distinct members of the contactin family of neural recognition molecules,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 107, no. 6, pp. 2443–2448, 2010.
- [45] N. Maeda, K. Ichihara-Tanaka, T. Kimura, K. Kadomatsu, T. Muramatsu, and M. Noda, “A receptor-like protein-tyrosine phosphatase PTP ζ /RPTP β binds a heparin-binding growth factor midkine: involvement of arginine 78 of midkine in the high affinity binding to PTP ζ ,” *Journal of Biological Chemistry*, vol. 274, no. 18, pp. 12474–12479, 1999.
- [46] M. Qi, S. Ikematsu, N. Maeda et al., “Haptotactic migration induced by midkine: Involvement of protein-tyrosine phosphatase ζ , mitogen-activated protein kinase, and phosphatidylinositol 3-kinase,” *Journal of Biological Chemistry*, vol. 276, no. 19, pp. 15868–15875, 2001.
- [47] N. Sakaguchi, H. Muramatsu, K. Ichihara-Tanaka et al., “Receptor-type protein tyrosine phosphatase ζ as a component of the signaling receptor complex for midkine-dependent survival of embryonic neurons,” *Neuroscience Research*, vol. 45, no. 2, pp. 219–224, 2003.
- [48] H. Muramatsu, K. Zou, N. Sakaguchi, S. Ikematsu, S. Sakuma, and T. Muramatsu, “LDL receptor-related protein as a component of the midkine receptor,” *Biochemical and Biophysical Research Communications*, vol. 270, no. 3, pp. 936–941, 2000.
- [49] S. H. Lee, H. N. Suh, Y. J. Lee, B. N. Seo, J. W. Ha, and H. J. Han, “Midkine prevented hypoxic injury of mouse embryonic stem cells through activation of Akt and HIF-1 α via low-density lipoprotein receptor-related protein-1,” *Journal of Cellular Physiology*. In press.
- [50] G. E. Crooks, G. Hon, J. M. Chandonia, and S. E. Brenner, “WebLogo: a sequence logo generator,” *Genome Research*, vol. 14, no. 6, pp. 1188–1190, 2004.
- [51] H. Muramatsu, P. Zou, H. Suzuki et al., “ $\alpha 4\beta 1$ - and $\alpha 6\beta 1$ -integrins are functional receptors for midkine, a heparin-binding growth factor,” *Journal of Cell Science*, vol. 117, no. 22, pp. 5405–5415, 2004.
- [52] Y. Huang, M. Sook-Kim, and E. Ratovitski, “Midkine promotes tetraspanin-integrin interaction and induces FAK-Stat1 α pathway contributing to migration/invasiveness of human head and neck squamous cell carcinoma cells,” *Biochemical and Biophysical Research Communications*, vol. 377, no. 2, pp. 474–478, 2008.
- [53] C. Güngör, H. Zander, K. E. Effenberger et al., “Notch signaling activated by replication stress-induced expression of midkine drives epithelial-mesenchymal transition and chemoresistance in pancreatic cancer,” *Cancer Research*, vol. 71, no. 14, pp. 5009–5019, 2011.
- [54] K. Ichihara-Tanaka, A. Oohira, M. Rumsby, and T. Muramatsu, “Neuroglycan C is a novel midkine receptor involved in process elongation of oligodendroglial precursor-like cells,” *Journal of Biological Chemistry*, vol. 281, no. 41, pp. 30857–30864, 2006.
- [55] N. Kaneda, A. H. Talukder, M. Ishihara, S. Hara, K. Yoshida, and T. Muramatsu, “Structural characteristics of heparin-like domain required for interaction of midkine with embryonic neurons,” *Biochemical and Biophysical Research Communications*, vol. 220, no. 1, pp. 108–112, 1996.
- [56] C. Ueoka, N. Kaneda, I. Okazaki, S. Nadanaka, T. Muramatsu, and K. Sugahara, “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,” *Journal of Biological Chemistry*, vol. 275, no. 48, pp. 37407–37413, 2000.
- [57] P. Zou, K. Zou, H. Muramatsu et al., “Glycosaminoglycan structures required for strong binding to midkine, a heparin-binding growth factor,” *Glycobiology*, vol. 13, no. 1, pp. 35–42, 2003.
- [58] T. A. Mitsiadis, M. Salmivirta, T. Muramatsu et al., “Expression of the heparin-binding cytokines, midkine (MK) and HB-GAM (pleiotrophin) is associated with epithelial-mesenchymal interactions during fetal development and organogenesis,” *Development*, vol. 121, no. 1, pp. 37–51, 1995.

- [59] T. Nakanishi, K. Kadomatsu, T. Okamoto et al., “Expression of syndecan-1 and -3 during embryogenesis of the central nervous system in relation to binding with midkine,” *Journal of Biochemistry*, vol. 121, no. 2, pp. 197–205, 1997.
- [60] N. Kurosawa, G. Y. Chen, K. Kadomatsu, S. Ikematsu, S. Sakuma, and T. Muramatsu, “Glypican-2 binds to midkine: the role of glypican-2 in neuronal cell adhesion and neurite outgrowth,” *Glycoconjugate Journal*, vol. 18, no. 6, pp. 499–507, 2001.
- [61] D. M. Rose, “The role of the $\alpha 4$ integrin-paxillin interaction in regulating leukocyte trafficking,” *Experimental and Molecular Medicine*, vol. 38, no. 3, pp. 191–195, 2006.
- [62] D. Powner, P. M. Kopp, S. J. Monkley, D. R. Critchley, and F. Berditchevski, “Tetraspanin CD9 in cell migration,” *Biochemical Society Transactions*, vol. 39, no. 2, pp. 563–567, 2011.
- [63] A. Oohira, T. Shuo, Y. Tokita, K. Nakanishi, and S. Aono, “Neuroglycan C, a brain-specific part-time proteoglycan, with a particular multidomain structure,” *Glycoconjugate Journal*, vol. 21, no. 1-2, pp. 53–57, 2004.
- [64] Y. Huang, M. O. Hoque, F. Wu, B. Trink, D. Sidransky, and E. A. Ratovitski, “Midkine induces epithelial-mesenchymal transition through Notch2/Jak2-Stat3 signaling in human keratinocytes,” *Cell Cycle*, vol. 7, no. 11, pp. 1613–1622, 2008.
- [65] A. Barreca, E. Lasorsa, L. Riera et al., “Anaplastic lymphoma kinase in human cancer,” *Journal of Molecular Endocrinology*, vol. 47, no. 1, pp. R11–R23, 2011.
- [66] G. Remuzzi, A. Schieppati, and P. Ruggenti, “Nephropathy in patients with type 2 diabetes,” *New England Journal of Medicine*, vol. 346, no. 15, pp. 1145–1151, 2002.
- [67] S. E. Gabriel and K. Michaud, “Epidemiological studies in incidence, prevalence, mortality, and comorbidity of the rheumatic diseases,” *Arthritis Research and Therapy*, vol. 11, no. 3, article 229, 2009.
- [68] D. L. Scott, F. Wolfe, and T. W. J. Huizinga, “Rheumatoid arthritis,” *The Lancet*, vol. 376, no. 9746, pp. 1094–1108, 2010.
- [69] K. Terato, K. A. Hasty, R. A. Reife, M. A. Cremer, A. H. Kang, and J. M. Stuart, “Induction of arthritis with monoclonal antibodies to collagen,” *Journal of Immunology*, vol. 148, no. 7, pp. 2103–2108, 1992.
- [70] R. Ross, “Atherosclerosis—an inflammatory disease,” *New England Journal of Medicine*, vol. 340, no. 2, pp. 115–126, 1999.
- [71] M. A. Engel and M. F. Neurath, “New pathophysiological insights and modern treatment of IBD,” *Journal of Gastroenterology*, vol. 45, no. 6, pp. 571–583, 2010.
- [72] M. Rentsch, A. Beham, H. J. Schlitt, and K. W. Jauch, “Crohn’s disease activity index and Vienna classification—is it worthwhile to calculate before surgery?” *Digestive Surgery*, vol. 23, no. 4, pp. 241–249, 2006.
- [73] C. Abraham and J. H. Cho, “Inflammatory bowel disease,” *New England Journal of Medicine*, vol. 361, no. 21, pp. 2066–2078, 2009.
- [74] P. Flachenecker, L. Khil, S. Bergmann et al., “Development and pilot phase of a European MS register,” *Journal of Neurology*, vol. 257, no. 10, pp. 1620–1627, 2010.
- [75] X. Liu, D. L. Yao, C. A. Bondy et al., “Astrocytes express insulin-like growth factor-I (IGF-I) and its binding protein, IGFBP-2, during demyelination induced by experimental autoimmune encephalomyelitis,” *Molecular and Cellular Neurosciences*, vol. 5, no. 5, pp. 418–430, 1994.
- [76] D. L. Yao, X. Liu, L. D. Hudson, and H. D. Webster, “Insulin-like growth factor I treatment reduces demyelination and up-regulates gene expression of myelin-related proteins in experimental autoimmune encephalomyelitis,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 92, no. 13, pp. 6190–6194, 1995.
- [77] K. H. G. Mills, “Regulatory T cells: friend or foe in immunity to infection?” *Nature Reviews Immunology*, vol. 4, no. 11, pp. 841–855, 2004.
- [78] A. Brüstle, S. Heink, M. Huber et al., “The development of inflammatory TH-17 cells requires interferon-regulatory factor 4,” *Nature Immunology*, vol. 8, no. 9, pp. 958–966, 2007.
- [79] J. H. Noseworthy, C. Lucchinetti, M. Rodriguez, and B. G. Weinshenker, “Multiple sclerosis,” *New England Journal of Medicine*, vol. 343, no. 13, pp. 938–952, 2000.

- [80] W. N. Gray, L. A. Denson, R. N. Baldassano, and K. A. Hommel, “Disease activity, behavioral dysfunction, and health-related quality of life in adolescents with inflammatory bowel disease,” *Inflammatory Bowel Diseases*, vol. 17, no. 7, pp. 1581–1586, 2011.
- [81] A. Davidson and B. Diamond, “Autoimmune diseases,” *New England Journal of Medicine*, vol. 345, no. 5, pp. 340–350, 2001.

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