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REVIEW

Therapeutic potential of midkine in cardiovascular disease

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Ischaemic heart disease, stroke and their pathological consequences are life-threatening conditions that account for about half of deaths in developed countries. Pathology of these diseases includes cell death due to ischaemia/reperfusion injury, vascular stenosis and cardiac remodelling. The growth factor midkine plays a pivotal role in these events. Midkine shows an acute cytoprotective effect in ischaemia/reperfusion injury at least in part via its anti-apoptotic effect. Moreover, while midkine promotes endothelial cell proliferation, it also recruits inflammatory cells to lesions. These activities eventually enhance angiogenesis, thereby preventing cardiac tissue remodelling. However, midkine's activity in recruiting inflammatory cells into the vascular wall also triggers neointima formation, and consequently, vascular stenosis. Moreover, midkine is induced in cancer tissues where it enhances angiogenesis. Therefore, midkine may promote tumour formation through its angiogenic and anti-apoptotic activity. This review focuses on the roles of midkine in ischaemic cardiovascular disease and their pathological consequences, that is angiogenesis, vascular stenosis, and cardiac remodelling, and discusses the possible therapeutic potential of modulation of midkine in these diseases.

LINKED ARTICLES
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Abbreviations
ALK, anaplastic lymphoma kinase; CLL, chronic lymphocytic leukemia; FDA, Food and Drug Administration; FGF, fibroblast growth factor; HIF-1α, hypoxia-inducible factor-1α; HRE, HIF-responsive element; HSCs, hematopoietic stem cells; LDL, low-density lipoprotein; LRP1, LDL receptor-related protein 1; MIF, macrophage migration inhibitory factor; PMNs, polymorphonuclear neutrophils; PTPRZ1, protein tyrosine phosphatase, receptor-type, Z polypeptide 1; SMCs, smooth muscle cells

Midkine in health and disease

The heparin-binding growth factor midkine is a 13kDa secretory protein and comprises a family with another heparin-binding growth factor, pleiotrophin. The major physiological and pathological functions of midkine can be categorized into five areas: (i) tissue protection; (ii) inflammation/immunity; (iii) blood pressure; (iv) development; and (v) cancer (Kadomatsu *et al.*, 2013). This review will focus on the therapeutic potential of midkine in ischaemia/reperfusion injury and cardioprotection, angiogenesis, vascular stenosis, and cardiac remodelling, which are closely related pathologies.

Midkine protects the heart and brain from acute ischaemia/reperfusion injury and infarction at least in part via its anti-apoptotic effect (Qi *et al.*, 2000; Horiba *et al.*, 2006) (Figure 1). Midkine promotes endothelial cell proliferation, leading to angiogenesis (Choudhuri *et al.*, 1997)and it also enhances inflammatory cell infiltration to lesions (Takada *et al.*, 1997; Horiba *et al.*, 2000; Sato *et al.*, 2001) (Figure 1). Inflammatory cells attracted by midkine include macrophages and polymorphonuclear neutrophils (PMNs). Both these cell types are implicated in angiogenesis (Murdoch *et al.*, 2008). The angiogenic activity of midkine is also well documented in cardiac infarction, where midkine is expressed around the

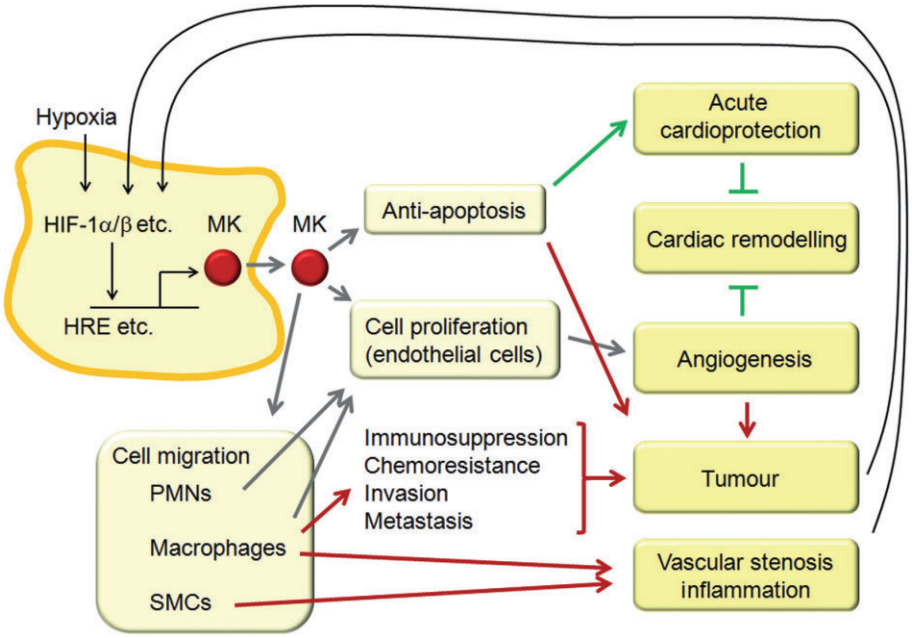

Figure 1

Physiological and pathological effects of midkine. Hypoxia, inflammation and other pathological processes, including tumours, promote midkine (MK) expression. Midkine exerts its functions, including promotion of anti-apoptosis, cell proliferation and cell migration. The anti-apoptotic activity promotes cardiac myocyte protection and may also enhance tumour growth. The cell proliferation activity includes endothelial cell proliferation, which induces angiogenesis. Angiogenesis in turn protects against cardiac remodelling; however, it may also promote tumour growth. The cell migration (chemoattractant) action of midkine targets PMNs, macrophages and vascular SMCs. Infiltration of PMNs and macrophages enhances endothelial cell proliferation, leading to angiogenesis. Macrophages infiltrated in tumour tissues contribute to immunosuppression, resistance to chemotherapy, invasion and metastasis, all of which support tumour growth. Infiltration of macrophages in the vascular wall and migration of vascular SMCs play indispensable roles in vascular stenosis. Green arrows indicate potential therapeutic effects of midkine, while red arrows represent possible harmful effects of chronic midkine treatment.

area of infarction and eventually prevents cardiac remodelling through enhancing angiogenesis (Takenaka *et al.*, 2009) (Figure 1). Therefore, midkine may also improve long-term outcome of myocardial infarction by inhibition of post-infarction cardiac remodelling. On the other hand, the effects on midkine mentioned earlier may also contribute to cancer development (Figure 1). Indeed, in addition to angiogenesis, tumour-associated macrophages attracted by midkine play a role in immunosupression, resistance to chemotherapy, invasion, and metastasis (De Palma and Lewis, 2013).

Vascular stenosis accompanies inflammatory cell infiltration into the vascular wall. Midkine-deficient mice exhibit strikingly less inflammatory cell infiltration, which results in suppression of neointima formation (Horiba *et al.*, 2000) (Figure 1). Exogenous midkine administered to midkine-deficient mice restores neointima formation (Horiba *et al.*, 2000), while knockdown of midkine expression in wild-type animals leads to suppression of neointima formation (Hayashi *et al.*, 2005; Banno *et al.*, 2006).

Ischaemia/reperfusion injury followed by angiogenesis and cardiac remodelling, as well as vascular stenosis share some common mechanisms (e.g. inflammatory cell infiltration and expression of cytokines and growth factors). Therefore, it is reasonable to suggest that the major functions of midkine in cardiovascular disease merge in these events.

Cardioprotective and neuroprotective effect of midkine

Ischaemic heart disease and ischaemic stroke as well as their pathological consequences are life-threatening conditions that account for about half of deaths in developed countries. Therefore, therapeutic strategies to protect the heart and brain against ischaemia/reperfusion injury are much sought after (Ferdinandy *et al.*, 2007; Broussalis *et al.*, 2012; Hausenloy *et al.*, 2013). Midkine has the potential to protect tissues for acute ischaemia/reperfusion injury. We have shown that midkine protected neonatal rat cardiac myocytes subjected to simulated ischaemia and reperfusion. Moreover, acute intravenous bolus treatment with midkine reduced infarct size in anaesthetized rats with coronary occlusion and reperfusion. The cardioprotection of midkine showed a bell-shaped dose–response relationship in both studies (Figure 2). Similarly, in Horiba *et al.*'s (2006) study, infarct size and apoptotic markers in midkine-deficient mice were significantly increased as compared with wild-type mice showing that endogenous midkine expression is cardioprotective. In this study, intramuscular injection of midkine into the peri-infarct area significantly reduced infarct size. Ishiguro *et al.* showed that acute intracoronary bolus injection of midkine

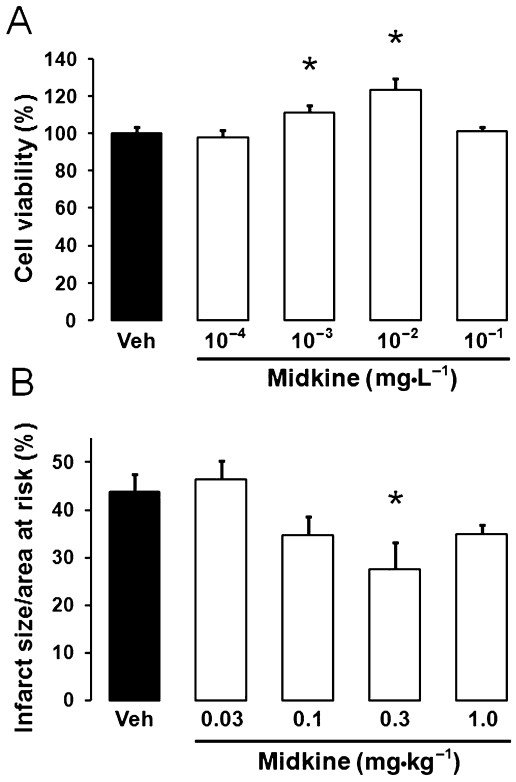

Figure 2

Cardioprotective effects of midkine. (A) Viability of cultured neonatal cardiac myocytes after a 4 h simulated ischaemia followed by 2 h of simulated reperfusion. Midkine and vehicle were applied during simulated ischaemia. (B) Infarct size data of adult male anaesthetized Wistar rats subjected to 30 min coronary occlusion followed by 120 min reperfusion induced by *in vivo* coronary occlusion. Midkine or its vehicle were administered 5 min before the induction of ischaemia. Data are expressed as means ± SEM. *$P < 0.05$, significantly different from vehicle-treated group; one-way ANOVA, followed by Fisher's LSD *post hoc* test.

reduced infarct size and myocardial apoptotic markers and improved myocardial function in pigs with coronary occlusion and reperfusion (Ishiguro *et al.*, 2011). Intraventricular administration of midkine in the brain significantly reduced brain infarction after middle cerebral artery occlusion in rats (Harvey *et al.*, 2004). In an elegant study, Takada *et al.* showed that in male spontaneously hypertensive rats, injection of an adenoviral midkine construct attenuated cerebral ischaemic damage, 2 days after distal middle cerebral artery occlusion (Takada *et al.*, 2005). In this study, midkine reduced the number of TUNEL-positive cells and cleaved caspase-3-positive cells in the peri-ischaemic area of the cerebral cortex and in the caudoputamen. Similarly, infarct size reduction and anti-apoptotic effects were observed by Ishikawa *et al.* in male spontaneously hypertensive rats with photochemical occlusion of the distal MCA, 7 days after the ischaemic insult (Ishikawa *et al.*, 2009). These results show that midkine is able to protect the heart and brain tissue from acute ischaemic injury at least in part via its anti-apoptotic effect. Moreover, by the ability of midkine to stimulate angiogenesis, it inhibits tissue remodelling and may, thereby, further improve long-term outcome of myocardial infarction (see below).

Angiogenic effect of midkine

Vasculogenesis represents *de novo* vessel formation through differentiation of angioblasts, while angiogenesis requires pre-existing vessels, from which new vessels are formed through proliferation of endothelial cells (Poole and Coffin, 1989; Risau, 1997). Vasculogenesis occurs in developing embryos, but can also occur during vascular repair in adults; the latter is accomplished through differentiation of endothelial progenitor cells (Asahara *et al.*, 2011). Stepwise development of vessels is undertaken during embryogenesis. The yolk sac and the embryo proper undergo distinct vasculogenesis. These steps of vasculogenesis are precisely regulated by transcriptional programmes and cell–cell and cell–tissue interactions (Marcelo *et al.*, 2013; Park *et al.*, 2013). The role of midkine in vasculogenesis has not yet been studied extensively; however, there are no reports in the literature showing that midkine-deficient mice would exhibit any gross abnormality of vascular formation. In contrast, midkine is involved in angiogenesis.

While vasculogenesis is the principal mechanism of vessel formation, angiogenesis is the predominant means of vascularization for all organs. In addition, angiogenesis occurs in a variety of pathological settings, such as cancer and inflammation, and often plays critical roles in their pathogenesis. The relationship between angiogenesis and midkine was first demonstrated by the promotion of proliferation of HUVECs by midkine purified through a heparin-affinity column from midkine-transfected MCF-7 breast cancer cells (Choudhuri *et al.*, 1997). Midkine-expressing MCF-7 cells promoted angiogenesis in the rabbit corneal assay, compared with non-expressing MCF-7 cells. Midkine overexpression in MCF-7 cells also enhanced not only tumour growth, but also angiogenesis in a subcutaneous xenograft model (Choudhuri *et al.*, 1997). Furthermore, Weckbach *et al.* have recently reported that hypoxia induced midkine production by HUVECs (Weckbach *et al.*, 2012)and that exogenous midkine induced neovascularization in a chorioallantoic membrane assay (Weckbach *et al.*, 2012). Although it is well known that endothelial cells and resident tissue cells are essential for angiogenesis, inflammatory cells, such as PMNs and monocytes/macrophages, also play important roles (Murdoch *et al.*, 2008). For example, M2-polarized macrophages are essential for angiogenesis (Mantovani *et al.*, 2013) and PMNs secrete molecules important for angiogenesis, such as VEGF, oncostatin M and MMP9 (Tazzyman *et al.*, 2009). In this context, it should be noted that midkine acts as an chemoattractant for inflammatory cells in a variety of conditions involving inflammation (Horiba *et al.*, 2000; Sato *et al.*, 2001; Banno *et al.*, 2006).

Midkine inhibits cardiac remodelling

Midkine expression was progressively increased after myocardial infarction in a mouse model of ligation of the left coronary artery (Takenaka *et al.*, 2009). Midkine-deficient mice showed a higher mortality compared with wild-type mice.

Exogenous administration of midkine improved survival and left ventricular function in both wild-type and midkine-deficient mice. Notably, this treatment enhanced angiogenesis in the peri-infarct zone. Similar results were obtained in a rat model of chronic cardiac infarction (Fukui *et al.*, 2008). In this study, recombinant midkine was injected into hearts, 2 weeks after induction of myocardial infarction. Six weeks later, cardiac remodelling was significantly and dose-dependently attenuated by midkine treatment. Midkine treatment facilitated angiogenesis in the infarcted area, and the viable muscle area after myocardial infarction dose-dependently increased. Despite this increase of viable muscle area, midkine-treated hearts showed significantly less cardiomyocyte hypertrophy than vehicle-treated hearts. These results show that midkine prevents cardiac remodelling, at least in part, through its angiogenic activity.

Tumourigenic effect of midkine

Anti-angiogenic therapy for cancer was proposed by Judah Folkman in 1971 (Folkman, 1971), more than 10 years before the vascular permeability factor, now known as VEGF, was isolated in 1983 (Senger *et al.*, 1983). The N-terminal amino acid sequence of VEGF was determined in 1989 (Ferrara and Henzel, 1989). VEGF and its receptor VEGFR2 are major regulators of angiogenesis. Besides VEGF, many other growth factors, such as fibroblast growth factor (FGF) and PDGF, are involved in tumour angiogenesis (Claesson-Welsh, 2012). Most types of cells in the tumour microenvironment produce VEGF which promotes vascular growth and sprouting by accelerating endothelial cell proliferation. PDGF is produced by endothelial cells, and attracts pericytes to support the vasculature, while FGF is expressed by tumour cells and enhances endothelial cell growth in tumours. Tumour blood vessels, however, are incomplete and leaky compared with normal blood vessels. This is in part due to incomplete perivascular support and high expression of VEGF, which stimulates endothelial cell proliferation, rounds up cells, breaks cell–cell junctions and consequently increases vascular permeability (Baluk *et al.*, 2005; Fukumura and Jain, 2007). The leaky tumour vasculature increases interstitial pressure, which then becomes a barrier against anti-tumour drug delivery to tumour tissues. Nevertheless, tumour blood vessels do transport nutrients and oxygen to tumour tissue, and thus, support growth of tumour cells.

The anti-VEGF antibody bevacizumab was the first anti-angiogenic agent drug to be approved for cancer therapy by the Food and Drug Administration (FDA) in 2004 (Grothey and Galanis, 2009; Van Meter and Kim, 2010; Kieran *et al.*, 2012). Although most VEGF blocking therapies require adjuvant chemotherapy, as demonstrated in the first trial of bevacizumab for metastatic colon cancer (Hurwitz *et al.*, 2004), anti-angiogenic therapy is effective. However, the majority of patients eventually succumb to their disease. Resistance to anti-angiogenic therapy relies on tumour plasticity. Thus, sensitivity to anti-angiogenic therapy decreases with progression of disease, probably because of changes in the characteristics of tumour cells with accumulated mutations or intratumour heterogeneity (Bergers *et al.*, 1999; Gerlinger *et al.*, 2012). Tumour cells may also acquire vasculogenic mimicry, with tumour cells differentiating to endothelial cell-like cells and/or pericyte-like cells (Ricci-Vitiani *et al.*, 2010; Cheng *et al.*, 2013). Furthermore, tumour cells are switched to respond more to compensatory growth factors such as FGF and PDGF rather than to VEGF if the VEGF axis is blocked (Casanovas *et al.*, 2005); (Orimo *et al.*, 2005; Crawford *et al.*, 2009). Therefore, identification of these angiogenic factors and verification of the mechanisms of their actions are important for further development of cancer therapy.

The degree of midkine expression correlates with microvessel density in salivary gland tumours (Ota *et al.*, 2010). Midkine expression is high in human neurofibromas, schwannomas and various nervous system tumours associated with neurofibromatosis type 1 or 2 suppressor gene, where midkine expression can be detected in endothelial cells of tumour blood vessels, but not in normal blood vessels (Mashour *et al.*, 2001). Finally, midkine stimulates proliferation of human systemic and brain endothelial cells *in vitro* (Mashour *et al.*, 2001). These observations suggest that midkine is involved in cancer progression through its angiogenic activity. Consistent with this idea, a high expression of midkine correlates with a poor prognosis in patients with invasive bladder cancers (O'Brien *et al.*, 1996). Levels of midkine expression are significantly correlated with microvessel density, tumour size, clinical stage and prognosis in oral squamous cell carcinoma (Ruan *et al.*, 2007). Midkine overexpression also enhances tumour growth and microvessel density of human UM-UC-3 bladder cancer cells in both subcutaneous and orthotropic xenograft models. It also increases their sensitivity to anti-angiogenic therapy (Muramaki *et al.*, 2003). Moreover, midkine antisense oligonucleotides inhibit not only proliferation of HUVECs, but also angiogenesis induced by HEPG2 human hepatocellular cancer cells in chorioallantoic membranes (Dai *et al.*, 2007).

Mice deficient in midkine are useful tools in the investigation of the role of host midkine in tumour progression. Salama *et al.* reported that lung metastasis of Lewis lung carcinoma cells was less in midkine-deficient mice (Salama *et al.*, 2006). Because the Lewis lung carcinoma cells do not significantly express midkine, this result suggests that midkine is also a host factor regulating metastasis. Furthermore, Kishida *et al.*, have recently reported that midkine-deficient mice show significantly less tumourigenesis of neuroblastoma in *MYCN* transgenic mice, which spontaneously develop neuroblastoma (Kishida *et al.*, 2013). Although angiogenesis has not been examined in these models, it would be an intriguing subject of future studies.

Midkine induces vascular stenosis

Atherosclerosis is the primary cause of life-threatening events such as stroke and heart attack. Low-density lipoprotein (LDL) diffuses passively through the endothelial cell junction and enters the space between the endothelium and internal elastic lamina, the so-called intima. High serum concentrations of LDL in blood therefore increase the chance of LDL entering the intima, where it is trapped by proteoglycans in the extracellular matrix and undergoes oxidation through interaction with reactive oxygen species, including hydroperoxyeicosatetraenoic acids, the products of 12/15 lipoxygenase (Lusis, 2000).

Minimally oxidized LDL stimulates endothelium to produce chemoattractants such as CCL2 (MCP-1) and M-CSF, and as a result, recruits monocytes to the intima. Monocytes

recruited to the intima become activated macrophages that produce M-CSF, other cytokines and proteoglycans, laying the groundwork for further inflammation. Highly oxidized LDL forms aggregates that are engulfed by macrophages via scavenger receptors such as scavenger receptor A and CD36 (Lusis, 2000; Moore and Tabas, 2011). Engulfment of oxidized LDL turns macrophages into foam cells. Foam cells undergo apoptosis and secondary necrosis, leading to formation of a necrotic core rich in extracellular lipids. On the other hand, activated macrophages and infiltrated T-cells produce cytokines and growth factors, which stimulate proliferation of smooth muscle cells (SMCs). The SMCs migrate from their original space underneath the internal elastic lamina to the intima. As a result, the intimal region significantly expands and becomes so-called neointima.

Finally, fibrous plaques consisting of extracellular lipids, SMCs and SMC-derived extracellular matrix are formed. Thrombosis associated with fibrous plaques is the major cause of acute coronary events, as well as of stroke. It is triggered by the rupture of a plaque. In addition, new vessel formation occurs in the plaque. This angiogenesis is induced by local ischaemia and inflammation, and is promoted by monocytes recruited to the arterial wall (Jaipersad *et al.*, 2013).

Midkine expression is increased in the rat common carotid artery after intraluminal balloon injury (Horiba *et al.*, 2000). In addition, ligation of the common carotid artery induces neointima at the site of ligation in wild-type mice, but this neointimal formation is diminished in midkine-deficient mice. Exogenous midkine restores neointima formation in midkine-deficient mice. Midkine promotes macrophage migration *in vitro* and, consistent with this, leukocytes are less recruited to the vascular wall in midkine-deficient mice. Midkine also promotes migration of SMCs *in vitro*. These data suggest that midkine plays a pivotal role in neointima formation (Horiba *et al.*, 2000).

Midkine antisense oligodeoxynucleotides transfected by means of lipofection to the vascular wall suppressed neointima formation after the rabbit carotid artery balloon injury (Hayashi *et al.*, 2005). Increased midkine expression is also found in jugular vein-to-carotid artery interposition vein grafts in rabbits (Figure 3) (Banno *et al.*, 2006). Controlled release of siRNA to rabbit midkine, which is accomplished by wrapping the grafted vein with atelocollagen containing the siRNA, markedly suppressed inflammatory cell infiltration and SMC proliferation, and consequently suppressed neointima formation. Indeed, this method of perivascular application of siRNA using atelocollagen efficiently delivers siRNA to the vascular wall (Figure 3) (Banno *et al.*, 2006). The same animal model was used to evaluate the effect of statin in vascular stenosis, with pitavastatin suppressing midkine expression, and consequently, neointima formation (Fujita *et al.*, 2008).

Compared with balloon injury, stenting induces more prolonged inflammation and more macrophage infiltration in the vascular wall. Midkine expression is also increased in the neointima when induced by a bare metal stent, which is implanted in the atheromatous lesion of hypercholesterolemic rabbits. The main source of midkine expression is macrophages in this model (Narita *et al.*, 2008). These data suggest that midkine is important for pathogenesis of vascular restenosis not only after ballooning and vein grafting, but also after stenting, and can be a target of therapy for these conditions.

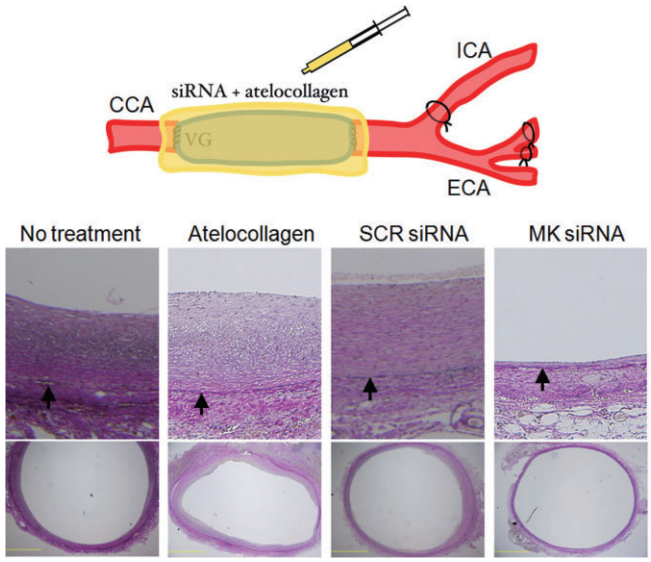

Figure 3

Suppression of neointima formation by knockdown of midkine. Increased midkine expression was found in jugular vein-to-carotid artery interposition vein grafts in rabbits (Banno *et al.*, 2006). To accomplish a controlled release of siRNA to the grafted vein, the drug delivery system of atelocollagen mixed with siRNA was put around the vein. Because atelocollagen is solidified at around 37°C, the vein was wrapped with this mixture. Midkine siRNA (MKsiRNA), but not control siRNA (SCRsiRNA), markedly suppressed neointima formation. This figure was modified from Banno *et al.*, 2006. with permission. CCA, common carotid artery; ECA, external carotid artery; ICA, internal carotid artery; SCR, scramble; VG, vein graft. Arrows indicate internal elastic lamina.

Midkine signalling

Hypoxia-inducible factor-1α (HIF-1α) and midkine

Hypoxia induces midkine protein expression in human PMNs, monocytes and HUVECs (Weckbach *et al.*, 2012). In a hind limb ischaemia model, a striking angiogenesis was observed in wild-type mice, but not in midkine-deficient mice (Weckbach *et al.*, 2012). CAST/eiJ mice, which are susceptible to hypoxia and show increased muscularization of small pulmonary arteries after chronic hypoxia, exhibited an increase in midkine expression in the hypoxic lung. Double transgenic mice, in which midkine expression is specifically induced upon doxycycline administration in the lung epithelium, demonstrated that midkine increases muscularization of small pulmonary arteries (Reynolds *et al.*, 2004).

Hypoxia induces HIF-1α. While HIF-1α is susceptible to proteasomal degradation via the E3 ligase von Hippel–Lindau (VHL) protein in a normoxic condition, HIF-1β, which is a stable protein and constitutively expressed, protects HIF-1α from proteasomal degradation by forming a complex with it. Therefore, the chance of complex formation is increased in

hypoxic conditions and the complex is transported to the nucleus, where it binds to the so-called HIF-responsive element (HRE) and drives gene expression. Reynolds *et al*. found a HRE in the promoter of the midkine gene, and demonstrated that if the HRE is mutated, midkine expression is no longer induced by hypoxia (Reynolds *et al*., 2004).

HIF-1α plays an indispensable role in the maintenance of stem cells. For example, haematopoietic stem cells (HSCs) are kept quiescent in the stem cell niche, which is a hypoxic zone of the bone marrow. While HIF–1α-deficient mice exhibit loss of quiescence of HSCs, as well as a decrease in HSCs during stress such as aging and bone marrow transplantation, VHL-deficient mice show overstabilization of HIF-1α and transplantation impairment, suggesting an appropriate HIF-1α level is required for HSCs to maintain cell-cycle quiescence (Takubo *et al*., 2010). HIF-1α is also an important factor in chronic lymphocytic leukemia (CLL). CLL is the most common adult leukaemia in developed countries and decreased apoptosis of malignant cells is its main feature. CLL cells accumulate in hypoxic zones of bone marrow where these cells overexpress HIF-1α. HIF-1α then induces expression of three important factors, VEGF, macrophage migration inhibitory factor (MIF) and midkine (Shachar *et al*., 2012). VEGF stimulates proliferation of CLL cells and inhibits their apoptosis, whereas MIF promotes HIF-1α stabilization and VEGF expression. MIF also enhances IL-8 expression, which in turn induces Bcl-2 expression, which consequently inhibits apoptosis and VLA-4 integrin, which facilitates CLL cells' homing, retention and survival in the bone marrow (Shachar *et al*., 2012). Furthermore, MIF induces midkine expression in CLL, in which serum midkine levels are high, regardless of the disease stage (Cohen *et al*., 2012). Midkine thus plays a major role against apoptosis in CLL.

On the other hand, it is possible that midkine might regulate HIF-1α expression. Under hypoxic conditions, midkine binds to its receptor, the LDL receptor-related protein 1 (LRP1). Akt is activated downstream of LRP1, and expression of HIF-1α and haem oxygenase-1 is increased. These consequently protect mouse embryonic stem cells from hypoxia-induced apoptosis (Lee *et al*., 2012).

Midkine signalling to support inflammation, angiogenesis and anti-apoptosis

Midkine receptors so far identified include anaplastic lymphoma kinase (ALK), protein tyrosine phosphatase, receptor-type, Z polypeptide 1 (PTPRZ1), LRP1, integrins, nucleolin and proteoglycans. Some of them might make a complex, depending on cellular context (Muramatsu, 2010; Kadomatsu *et al*., 2013).

LRP1 was identified through the midkine-affinity column, and mediates the actions of midkine on neuronal cell survival (Muramatsu *et al*., 2000). It also endocytoses midkine, leading to intracellular binding to nucleolin and then translocation to the nucleus, which plays an indispensable role in cell survival (Shibata *et al*., 2002). Intracellular trapping of midkine with a specific peptide named midkine-trap results in midkine-LRP1 aggregation in the endoplasmic reticulum, and suppression of LRP1 translocation to the cell surface (Sakamoto *et al*., 2011). It also enhances PDGF-mediated cell migration and PDGF receptor β phosphorylation in response to exogenous PDGF-BB. This phenomenon supports the report that LRP1 binds to and inhibits PDGF receptors (Boucher *et al*., 2003). LRP1-deficient mice show enhanced migration of SMCs to the neointima region and enhanced atherosclerosis (Boucher *et al*., 2003). Midkine promotes migration of not only macrophages but also of SMCs in the milieu of neointima formation (Horiba *et al*., 2000). Because both midkine and PDGF are important for neointima formation, further investigation of the midkine-LRP1-PDGF axis would provide new insights into the pathogenesis of vascular stenosis.

In terms of cell migration that may be relevant to angiogenesis, vascular stenosis and cardiac remodelling, other receptors may also be involved. PTPRZ1 was identified as a midkine receptor for the migration of neuronal cells (Maeda *et al*., 1999). PTPRZ1 is a proteoglycan carrying chondroitin sulfate, and its affinity to midkine strikingly decreases if this long sugar chain is removed (Maeda *et al*., 1999; Qi *et al*., 2001). PTPRZ1, PI3-kinase and MAPK pathways are coordinately involved in midkine-mediated cell migration (Qi *et al*., 2001). With regard to neointima formation, it is noteworthy that one of the characteristics of the intima is that it is rich proteoglycans, which show high affinity for LDL (Lusis, 2000; Moore and Tabas, 2011). Midkine might be retained in the intima through binding to proteoglycans in the extracellular matrix, leading to enhanced inflammation.

Midkine-mediated inhibition of myocardial remodelling through enhanced angiogenesis has been demonstrated in a coronary artery occlusion model (Takenaka *et al*., 2009). On the other hand, midkine also protects myocytes from reperfusion injury (Horiba *et al*., 2006). In this case, midkine protects cells from apoptosis. This mechanism of action might be relevant to the involvement of midkine in cancer development. PTPRZ1-deficient mice exhibit a reduced proportion and number of mature B cells. Midkine enhances survival of B cells of wild-type mice compared with those of PTPRZ1-deficient mice; it also enhances survival of CLL cells, while the antibody-blocking PTPRZ1 induces apoptosis of these cells (Cohen *et al*., 2012). In addition, as described earlier, LRP1 plays a critical role in cell survival (Shibata *et al*., 2002). LRP1 might also trigger HIF-1α expression, which in turn promotes expression of midkine and other important genes for cancer development (Lee *et al*., 2012).

Midkine-mediated migration of macrophages and PMNs might enhance angiogenesis, but midkine may also directly promote proliferation of HUVECs (Choudhuri *et al*., 1997). In this context, ALK may mediate the midkine signal. Midkine binds to ALK and promotes its phosphorylation, consequently activating PI3-kinase and MAP kinase signal transduction in various cell lines, including HUVECs (Stoica *et al*., 2002). Alternatively, midkine may act on the crosstalk between endothelial cells and SMCs (Sumi *et al*., 2002). Midkine promotes proliferation and stratification of HUVECs when cultured with human aortic SMCs in collagen gel. Midkine acts on SMCs and enhances secretion of IL-8, which in turn causes stratification of endothelial cells (Sumi *et al*., 2002).

Conclusions

Midkine exerts acute cardioprotection and neuroprotection against ischaemia/reperfusion injury and inhibits cardiac

remodelling at least in part via its anti-apoptotic and angiogenic effect. These events require inflammatory cell infiltration and expression of cytokines and growth factors. Midkine is a common and indispensable factor in these contexts.

Evidence shows that acute treatment with midkine induces tissue protection in ischaemia/reperfusion injury thereby reducing infarct size and provides long-term benefit in the prevention of cardiac remodelling. Therefore, after clinical translation of these results, midkine therapy may be useful in the treatment of acute myocardial infarction for cardioprotection and prevention of long-term consequences of myocardial infarction, such as heart failure. Moreover, because the beneficial effects of midkine on cardiac function and mortality in cardiac failure/remodelling models have been shown when midkine was applied 2 weeks after the coronary occlusion, potential midkine therapy may be also useful not only in the prevention, but also in the treatment of cardiac remodelling. Nevertheless, long-term midkine treatment might lead to some adverse effects such as vascular stenosis, inflammation, and possibly tumour formation.

Taken together, midkine is a promising biological drug candidate for different cardiovascular diseases. Further elucidation of the mechanisms of midkine action, its chronic toxicity and safety profile, optimal therapeutic dosing regimen and duration, as well as optimal targeted tissue delivery is necessary for rational development of midkine therapy.

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Conflict of interest

None.

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