Macrophage migration inhibitory factor in myocardial ischaemia/reperfusion injury

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Acute myocardial infarction (AMI) remains one of the leading causes of death in the developed world. There is emerging evidence that the cytokine macrophage migration inhibitory factor (MIF) is a crucial player in AMI. Cardioprotection by MIF is likely to be a multifactorial phenomenon mediated by receptor-mediated signalling processes, intracellular protein–protein interactions, and enzymatic redox regulation. Co-ordinating several pathways in the ischaemic heart, MIF contributes to receptor-mediated regulation of cardioprotective AMP-activated protein kinase signalling, inhibition of pro-apoptotic cascades, and the reduction of oxidative stress in the post-ischaemic heart. Moreover, the cardioprotective properties of MIF are modulated by S-nitrosylation. These effects in the pathophysiology of myocardial ischaemia/reperfusion injury qualify MIF as a promising therapeutic target in the future. Here we summarize the findings of experimental and clinical studies and emphasize the therapeutic potential of MIF in AMI.

Keywords

Macrophage migration inhibitory factor • Myocardial ischaemia/reperfusion injury • Cardioprotection

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1. Introduction

Acute myocardial infarction (AMI) remains one of the leading causes of global mortality and illness in the developed world and in developing regions despite recent advances in early myocardial reperfusion therapy.1 The process of restoring blood flow to the ischaemic myocardium, however, can induce injury itself.2 Cell death in terms of apoptosis, necrosis, and autophagy, a hallmark in the development of myocardial damage, is a major determinant of the final infarct size as well as the resulting cardiac function.3 Intensive research to salvage myocardial tissue from ischaemia/reperfusion (I/R) injury by elucidating the pathways involved in tissue damage has been conducted.

In the last decade, a key role for macrophage migration inhibitory factor (MIF) in the development of atherosclerosis, a chronic inflammatory disease of the arterial wall, has been demonstrated.4 Moreover, recent data uncovered MIF as a crucial player in AMI. MIF was characterized in 1966 as one of the first soluble immune mediators secreted from T-cells in delayed-type hypersensitivity reactions exerting inhibitory effects on random macrophage migration.5 Later, it was demonstrated that MIF is stored in and secreted from the pituitary gland upon endotoxaemia and acts as a key regulator of innate immunity by counter-regulating glucocorticoids.6 Today, MIF is known as a pleiotropic inflammatory cytokine with chemokine-like functions and has been recognized as a mediator of a number of acute and chronic inflammatory diseases. MIF is expressed quasi-ubiquitously, but is only secreted from a selected number of endocrine and parenchymal cells as well as from immune cells.5–7 Recently, MIF was implicated more broadly in cardiovascular disease and has been identified as a potent cardioprotective factor in the setting of myocardial I/R injury. Cardioprotection by MIF is likely to be a multifactorial phenomenon mediated by receptor-mediated signalling processes, intracellular protein–protein interactions, and enzymatic redox regulation (Table 1). In fact, MIF contributes to the regulation of cardioprotective AMP-activated protein kinase (AMPK) signalling in a receptor-mediated manner, inhibits pro-apoptotic cascades, and attenuates oxidative stress in the post-ischaemic heart. Remarkably, the cardioprotective properties of MIF are regulated by S-nitrosylation.10 These effects in the pathophysiology of myocardial I/R injury qualify MIF as a promising therapeutic target in the future. We therefore provide a synopsis of MIF’s role in myocardial I/R injury and summarize the findings of experimental and clinical studies, emphasizing the therapeutic potential of MIF in AMI.

2. MIF in cardiovascular disease

A hallmark in the development of atherosclerosis is the recruitment of leucocytes to the arterial wall, where they begin to accumulate lipids, become foam cells, and multiply intravessel inflammation. MIF was identified as a major regulator of atherogenesis by promoting the recruitment of mononuclear cells and the conversion of macrophages into...

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cytes with H2O2, suggesting that MIF could represent an intramyocardial redox-sensitive cytokine. These reports provided a first idea of the role of MIF during I/R. The question whether MIF, which also harbours an evolutionarily conserved catalytic site, acts as a cytokine that is secreted upon redox stress, as an intracellular enzymatic redox regulator, or via regulation of intracellular apoptosis signalling remained unclear.

Functional data then showed that, in the setting of myocardial I/R injury, MIF unexpectedly exerts prominent cytoprotective effects. Receptor-mediated signalling processes, intracellular protein–protein interactions, and enzymatic redox regulation all appear to be involved in mediating cardioprotection by MIF. In fact, MIF contributes to cardioprotective AMPK signalling in a receptor-mediated manner, inhibits pro-apoptotic cascades, and reduces oxidative stress in the post-ischaemic heart. The cardioprotective properties of MIF are (co)-regulated by S-nitrosylation, which leads to a reduction in the infarct size. The different mechanistic aspects of the cardioprotective contributions of MIF and also its emerging more complex role in cardiac disease are reviewed in the following.

4. MIF is a regulator of myocardial glucose metabolism

Disruption of the energy supply during AMI is a hallmark in the development of myocardial damage, and large clinical trials have assessed whether preclinical glucose supplementation might reduce mortality in patients with acute coronary syndrome. These studies have been conducted on the premise that the ischaemic myocardium might benefit from an optimized glucose supply. Of note, glucose administration was not associated with improved survival, but was associated with lower rates of the composite outcome of cardiac arrest or in-hospital mortality.

A potential relationship between MIF and glucose metabolism was first addressed in skeletal myotubes, where MIF mediates tumor necrosis factor effects to promote glycolysis. However, the regulation by MIF of glucose metabolism in cardiomyocytes under I/R stress differs from this effect. With regard to the complex glucose metabolism in the injured heart, Miller et al. were the first to demonstrate a fundamental role of MIF in the setting of myocardial I/R injury in a preclinical study. They identified MIF as an autocrine/paracrine-acting cardiac factor and as an activator of myocardial AMPK, thus regulating the energy-generating and -consuming pathways, and protecting the heart against ischaemic

3. MIF in myocardial infarction and cardioprotective role of MIF in myocardial I/R injury

In contrast to the chronic process of atherosclerosis, myocardial infarction is an acute event that abruptly changes the cellular microenvironment in coronary arteries dramatically within seconds to minutes. The pathophysiological and biochemical events range from rapid changes of the cellular redox environment over acute activation of detrimental and protective signalling pathways to inflammatory cell recruitment and chronic remodelling processes. Experimental studies demonstrated that MIF secretion from cultured rat cardiac myocytes was elevated upon stimulation with hypoxia or H2O2. Concomitantly, MIF mRNA expression was increased after incubation of rat cardiomyocytes with H2O2, suggesting that MIF could represent an intramyocardial

### Table 1 Review of literature summarizing the effects of MIF in myocardial I/R injury in vivo

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Effect of MIF</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Takahashi et al. (2001)</td>
<td>MIF is expressed in rat cardiomyocytes in response to hypoxia and hydrogen peroxide.</td>
<td>11</td>
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<tr>
<td>Hattori et al. (2004)</td>
<td>MIF mRNA is up-regulated during LV remodelling after chronic LAD ligation.</td>
<td>12</td>
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<tr>
<td>Miller et al. (2008)</td>
<td>Macrophage MIF stimulates AMPK in the ischaemic heart.</td>
<td>13</td>
</tr>
<tr>
<td>Qi et al. (2009)</td>
<td>MIF inhibits activation of the JNK pathway during myocardial I/R.</td>
<td>14</td>
</tr>
<tr>
<td>Ma et al. (2010)</td>
<td>MIF–AMPK activation during myocardial I/R is impaired in the senescent heart.</td>
<td>15</td>
</tr>
<tr>
<td>Koga et al. (2011)</td>
<td>MIF reduces oxidative stress in the post-ischaemic heart.</td>
<td>16</td>
</tr>
<tr>
<td>Gao et al. (2011)</td>
<td>MIF promotes inflammatory cell recruitment and leads to exacerbation of myocardial damage after myocardial I/R.</td>
<td>17</td>
</tr>
<tr>
<td>Luedike et al. (2012)</td>
<td>S-nitrosylation of MIF provides enhanced activity resulting in increased cytoprotection in myocardial I/R.</td>
<td>10</td>
</tr>
<tr>
<td>Wang et al. (2013)</td>
<td>Small-molecule MIF agonists enhance AMPK activation and reduce myocardial I/R.</td>
<td>18</td>
</tr>
<tr>
<td>Liehn et al. (2013)</td>
<td>CXCR2 increases MIF-dependent monocyte infiltration and impairs myocardial function after I/R.</td>
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LV, left ventricular; LAD, left anterior descending artery.
injury and apoptosis. The AMPK pathway is known to be a key regulator of metabolism controlling glucose and lipid uptake, storage, and use. Mice deficient in MIF (Mif<sup>-/-</sup>) as well as Cd74-deficient mice displayed a blunted AMPK activation and reduced glucose uptake during ex vivo global I/R paralleled by an impaired post-ischaemic ventricular function and increased infarct size, suggesting that the cardioprotective effect of MIF is mediated through the CD74/AMPK axis. These findings and the fact that huge amounts of MIF were found in the coronary effluent after I/R suggested that I/R triggers MIF secretion from the myocardium leading to a subsequent activation of AMPK in an autocrine/paracrine manner. In line with these results, it was recently demonstrated that pharmacological administration of small-molecule MIF agonists enhances AMPK activation, showing that this pathway is therapeutically addressable through MIF. The pharmacological agonist used in that study, a small-molecule called MIF-20, was pulled out from an in silico screen of MIF activity modulating small molecular weight compounds and found to alter the receptor-active conformation of MIF. Studies on cell cultures lacking both the MIF ligand-binding component CD74 and the signal-transducing component CD44 showed no AMPK response to either hypoxia or exogenously added MIF. In addition, human fibroblasts with a low-activity MIF promoter polymorphism showed diminished MIF release and AMPK activation during hypoxia that could be overcome by exogenous MIF. This indicates that genetic variation in MIF expression may impact on the response of the human heart to ischaemia, and that diagnostic MIF genotyping might predict risk in patients with coronary artery disease. Recent data from patients with symptomatic coronary artery disease show that MIF is associated with established inflammatory markers and correlates with the extent of cardiac necrosis marker release after percutaneous coronary intervention. Remarkably, the senescent murine heart exhibits a reduced MIF expression accompanied by a decreased AMPK activation. Total myocardial MIF content was also decreased in the aged heart which appears counter-intuitive at first sight since one would expect an increase of MIF with aging considering that aging is a well-recognized mediator of atherosclerosis and that MIF is an established component in the development of atherosclerosis. However, these findings reflect the broad range and in part dichotomous manner of physiological effects of MIF and emphasize that it is of great importance to differentiate the cellular origin and the mode of action of this protein. Nevertheless, it could be clearly demonstrated that extracellular MIF-mediated AMPK activation constitutes an established cardioprotective pathway that is impaired in the senescent heart (Figure 1).

In addition, recent evidence suggested that the cardioprotective effects of MIF are at least in part also mediated by cardiac CXCR2, whereas MIF signalling through leucocyte-expressed CXCR2 exacerbates myocardial I/R injury. This implies compartmentalized protective and detrimental effects of endogenous MIF mediated by CXCR2 in the mouse model of myocardial I/R. Cardioprotection mediated via MIF has also been shown in a mouse model of myocardial hypertrophy induced by transverse aortic coarctation. In this model, MIF antagonizes hypertrophy and fibrosis in response to haemodynamic stress by maintaining a redox homeostatic phenotype and by attenuating the stress-induced activation of hypertrophic signalling cascades.

**Figure 1** Effects of macrophage MIF during myocardial I/R injury. The role of MIF in the setting of cardiac ischaemia and infarction is complex, time-dependent and involves different cellular components, e.g. cardiomyocytes, myocytes, fibroblasts, the microvasculature, as well as the extracellular matrix. All these components act as an integrated structural and functional unit. (A) Cardioprotective effects of MIF after ischaemia. Cardioprotective effects of MIF are mediated via endogenous and extracellular MIF in a paracrine/autocrine fashion. Extracellular MIF interacts with the CD74/CD44<sup>13,14</sup> complex—CXCR2 is further supposed to be a mandatory compound of the receptor complex on cardiomyocytes, but could also act independently. MIF receptor interaction mediates activation of the AMPK, leading to membrane translocation of glucose transporters (GLUT 4) and subsequent increased glucose uptake during reperfusion.<sup>13,15,18</sup> Receptor-mediated inhibition of the JNK leads to suppression of apoptotic downstream signalling (BAD) and decreased induction of apoptosis after myocardial I/R injury.<sup>19</sup> Intracellular MIF functions as a potent oxidoreductase, is able to reduce the ROS level, and thus inhibits apoptosis induction.<sup>16</sup> Formation of S-nitrosylated MIF (SNO-MIF) increases the oxidoreductase activity of MIF, decreases interaction of MIF with c-Jun N-terminal activation domain-binding protein-1 (JAB1/CSN5) and increases apoptosis inhibition.<sup>19</sup> (B) Cardiodepressive effects of MIF after prolonged ischaemia. MIF up-regulates Toll-like receptor signalling (TLR4), promotes nuclear translocation of NF-κB p65, and subsequently increases apoptosis induction after 60 min of ischaemia.<sup>17</sup> Interaction of extracellular MIF with CXCR2 receptor-bearing monocytes, increases inflammatory cell recruiting, and leads to impaired cardiac function.<sup>19</sup>
5. MIF regulates apoptotic signalling in myocardial I/R injury

After re-opening an occluded coronary artery, reperfusion of the infarcted myocardium with the ensuing oxygen burst leads to dysfunctional mitochondrial function with subsequent DNA and cell fragmentation. c-Jun N-terminal kinase (JNK) is a member of the mitogen-activated protein kinase family and controls essential processes such as inflammation, proliferation, cell differentiation, and apoptosis. JNK acts in a pro-apoptotic manner by promoting the dephosphorylation of the Bcl-2 family protein Bad. Dephosphorylated Bad forms a heterodimer with Bcl-2 and Bcl-xL, inactivating them and thus allowing Bax/Bak-triggered apoptosis. Inversely, BAD phosphorylation leaves Bcl-2 free to inhibit Bax-triggered apoptosis. BAD phosphorylation is thus anti-apoptotic. MIF has been shown to inhibit JNK-mediated apoptotic signalling during myocardial I/R injury and thus favours BAD phosphorylation and decreases infarct size. Utilizing Mif1/2 mice, it could be demonstrated that MIF deficiency leads to increased JNK activity after global myocardial I/R and that this enhanced activation can be reversed by application of extracellular recombinant MIF. Even after pharmacological activation—/phosphorylation of the JNK pathway via its well-known activator anisomycin, JNK activation was depressed by application of recombinant MIF in rat cardiomyocytes. Cardiomyocytes challenged by I/R injury showed up-regulated JNK-dependent, pro-apoptotic Bad dephosphorylation, which was accentuated in Mif1/2 mice compared with wild-type mice in vivo—revealing the apoptotic downstream signalling of the MIF—JNK interaction. Reconstitution of wild-type conditions by application of recombinant MIF in Mif1/2 mice furthermore led to a decreased infarct size and improvement of left ventricular function. Furthermore, Mif1/2 animals exhibit increased levels of apoptotic cells in the reperfused myocardium in vivo. Since activation of the JNK pathway was markedly elevated in both CD74−/− and Mif1/2 mouse hearts compared with wild-type conditions, a CD74 receptor—MIF interaction was suggested to underlie the inhibition of apoptotic JNK signalling. Further investigations revealed that activation of JNK signalling in fibroblasts and T-cell lines by MIF involved the upstream kinases phosphatidylinositol 3-kinase (PI3K)/S6 and was found to be not only dependent on CD74 but also on the alternate MIF receptor CXCR4, which engages in crosstalk with CD74. However, it remains unclear whether these results from cell culture experiments are transferable to in vivo situations. This limitation with regard to myocardial I/R injury is of great importance when discussing the potential effects of MIF on apoptotic signalling during I/R injury since recent evidence suggested a role for CD74 and CXCR4 in clathrin-dependent endocytosis of MIF in vitro. It could be demonstrated that MIF endocytosis in HEK293 cells can be markedly increased by ectopic overexpression of CD74, whereas pharmacological blockade of CXCR4 led to reduced endocytosis. Endocytosis of a receptor-bound ligand can also trigger endosomal signalling. MIF affects intracellular protein—protein interactions after endocytosis, and the results of MIF on I/R injury may be discussed against the background of these new insights into MIF—CD74—CXCR4 signalling.

In earlier studies, Kleemann et al. demonstrated that MIF endocytosis is coupled with MIF’s intracellular interaction with c-Jun N-terminal activation domain-binding protein-1 (JAB1/CSN5). JAB1/CSN5 is a component of the COP9 signalosome (CSN), a multiprotein complex with important roles in cell cycle control and ubiquitin/proteasome-dependent protein degradation processes in the cell. Monomeric JAB1 is also an activator of JNK signalling and AP-1 transcriptional activity. and MIF was found to be able to inhibit JNK activation via JAB1/CSN5 interaction. Moreover, later studies showed that JAB1 controls autocrine MIF-mediated Akt signalling by inhibition of MIF secretion in HeLa cervix carcinoma cells. We demonstrated that this interaction critically depends on Cys81 of the amino acid structure of MIF, and that pin-pointed S-nitrosylation at this cysteine residue led to a decreased interaction of MIF with JAB1/CSN5 after myocardial I/R in vivo. Thus, it appears that the MIF-JAB1/CSN5 interaction is a key regulatory component that controls intracellular JNK signalling and also MIF secretion. The second established mechanism is mediated via MIF—CD74—CXCR4 interaction and subsequent JNK activation and MIF endocytosis. Since most of the experimental work was not only performed in cardiomyocytes but also in different tumour cell lines or HEK293 cells, it will be of importance to further investigate the detailed mechanism of MIF-mediated apoptosis regulation during myocardial I/R (Figure 1).

6. MIF is a redox regulator in myocardial I/R injury

Beside disruption of the energy supply and the rapid activation of detrimental signalling pathways, generation of reactive oxygen species (ROS) has been early identified to be a major mediator of myocardial damage. Targeting the reduction of oxidative stress might be an attractive strategy to reduce myocardial injury, but its translation into the clinical setting failed to date. Considering the broad impact of ROS during myocardial I/R, it is of note that MIF exhibits a thiol-protein oxidoreductase (TPOR) activity that contributes to cellular redox regulation in the post-ischaemic heart by reducing oxidative stress. MIF’s role in redox regulation is due to its structural properties, which are unique within the protein family of cytokines. The TPOR activity of MIF is based on a conserved Cys57-Ala-Leu-Cys60 motif resembling the CXXC motif of classical TPORs like thioredoxin. Mouse hearts exhibit larger infarct size. Moreover, left ventricular contractile function of MIF-deficient hearts subjected to ischaemia and subsequent reperfusion compared with wild-type mouse hearts. Basic haemodynamic parameters between Mif1/2 and wild-type mice display no difference considering cardiac function between the two groups before I/R injury. After experimental occlusion of the left coronary artery and subsequent reperfusion, Mif1/2 mouse hearts exhibited markedly elevated levels of oxidative stress compared with wild-type mouse hearts. A decreased GSH/GSSG ratio, increased protein oxidation, reduced aconitase activity, and increased mitochondrial injury as indicated by increased cytochrome c release were observed. Cardiac fibroblasts isolated from wild-type and Mif1/2 mouse hearts were expanded in culture and underwent treatment with H2O2 to imitate oxidative stress. As a result of H2O2 treatment, generation of oxidative species was significantly greater in MIF-deficient cells than in wild-type fibroblasts. Re-expression of MIF protein in MIF-deficient fibroblasts with an adenoviral delivery approach reduced the oxidative response to the level observed in wild-type cells. Although the outlined experiments were not performed in cultured cardiomyocytes, the results were congruent with the in vivo findings from heart homogenates and one can speculate whether these effects might be limited to cardiac fibroblasts. Interestingly, our own recent studies could demonstrate that S-nitrosylation of MIF at Cys81 increased the inherent catalytic oxidoreductase activity of this protein in vitro, and that this post-translational protein modification also increased the
cardioprotective potential of MIF in vivo.\textsuperscript{10} S-nitros(y)lated MIF catalysed the formation of 2-hydroxyethyldisulfide as a marker of the oxidoreductase activity of MIF at a markedly faster rate than wild-type recombinant MIF. Since the site of S-nitros(y)lation at Cys-81 is located remote from the redox active CXXC motif, one could speculate that modification of Cys-81 resulted in a conformational change that promoted the redox activity of MIF.\textsuperscript{37} Future structural studies will be needed to characterize the detailed effects of these conformational changes on MIF function. Finally, one could state that the redox-regulating effects of intracellular MIF have the potential to preserve cardiac function after ischaemic injury (Figure 1).

7. Detrimental effects of MIF on the myocardium

The majority of experimental reports on the effect of MIF during myocardial I/R injury demonstrate an overall cardioprotective effect. However, a couple of recent observations draw a more complex picture.\textsuperscript{37} For example, it has been suggested that MIF deficiency protects the heart from prolonged and severe I/R injury by suppressing inflammatory responses in vivo. One fundamental finding was a decreased infarct size and a reduction in cardiomyocyte apoptosis, associated with preserved contractile force after prolonged I/R injury in MIF\textsuperscript{-/-} compared with wild-type mice. These seemingly contradictory results are likely due to the multifarious effects of MIF and the different experimental settings using varying ischaemia durations ranging from 15 to 60 min and studying short- vs. mid-term heart parameters including mid-term inflammatory reactions. Prolonged ischaemia might have resulted in the activation of different MIF-associated pathways. The post-ischaemic inflammatory response was suppressed in MIF\textsuperscript{-/-} mice as shown by decreased expression and production of inflammatory mediators and cytokines as well as reduced neutrophil and macrophage infiltration to the site of myocardial damage. Furthermore, Toll-like receptor 4 (TLR-4) expression was significantly reduced in MIF\textsuperscript{-/-} mouse myocardium, when compared with wild-type mice.\textsuperscript{17} TLRs are known as key recognition components of the innate immune system in mammals, including endogenous danger signals, and to be also involved in the cardiac inflammatory response in heart failure. TLR-4 modulates survival by induction of left ventricular remodelling after myocardial infarction in mice.\textsuperscript{41} Earlier studies could demonstrate that MIF regulates innate immune responses through modulation of TLR-4. This points to a possible role of MIF that may promote inflammation in response to severe myocardial I/R injury through TLR-4 signalling. These observations were in line with studies on cardiac function during experimental lipopolysaccharides (LPS)-induced sepsis in mice.\textsuperscript{42,43} Elevated MIF plasma levels were associated with depressed cardiac function after endotoxin challenge, and this effect could be reversed by application of MIF-neutralizing antibodies.\textsuperscript{42,43} Inactivation of MIF significantly reversed the imbalance of pro-apoptotic to pro-survival pathways after LPS-induced sepsis in vivo.\textsuperscript{43} Furthermore, one study has observed that MIF seems to induce cardiomyocyte apoptosis by activating stress kinases and mitochondria-associated apoptotic mechanisms, whereas inactivation of MIF improved cardiomyocyte survival in vitro.\textsuperscript{44} This finding may be explainable by the different effects that MIF has on JNK. MIF regulates JNK activation variably, inhibiting or activating JNK, depending on the cell type and conditions in isolated cells.\textsuperscript{14,21,38}

Taken together, there is obvious evidence demonstrating certain detrimental effects of MIF on the myocardium that should be taken into consideration when discussing the emerging role of MIF in the setting of myocardial I/R injury (Figure 1). Further studies will be needed to differentiate between systemic inflammatory response effects of circulating MIF on the myocardium, local effects of cardiac-derived MIF, and cardiac MIF. As indicated above, the first study addressing this contradiction emphasizes the compartmentalization of MIF effects demonstrating that on the one hand, the MIF–CXCR2 interaction increased monocyte infiltration to the site of myocardial injury, impairing cardiac function.\textsuperscript{19} In contrast, MIF conferred protective effects by improving myocardial function in CXCR2-bearing cardiomyocytes, whereas these effects were abolished in CXCR2-deficient hearts. These findings emphasize the importance of MIF effects on circulating CXCR2-bearing inflammatory cells like monocytes and the subsequent consequences on myocardial wound healing after ischaemia and differentiate them from autocrine/paracrine cardioprotective effects of MIF through CXCR2-expressing cardiomyocytes, the latter possibility through complex formation or crosstalk with cardiac CD74. To assess the physiological and pathophysiological impact of these investigations, it is also inevitable to consider the rapid changes during the early phase of lethal reperfusion injury. These include the generation of ROS, excessive intracellular Ca\textsuperscript{2+} overload, and the rapid restoration of the physiologic pH, all of which interact with each other to mediate cardiomyocyte death through the damage of the integrity of mitochondrial membranes and count up for the final infarct size. Due to its multifarious functions, MIF has the potential to be involved in the different stages of myocardial I/R injury, but the final judging whether the detrimental or cardioprotective properties prevail necessitates further investigation. MIF measurements in cardiac surgery patients undergoing open-heart surgery, representing a defined ‘model’ of myocardial I/R injury in humans, correlate intraoperative MIF levels with a better outcome, suggesting that the cardioprotective properties of MIF prevail.\textsuperscript{45,46}

8. Comparison between MIF- and AMPK-driven phenotypes in I/R injury

It is of interest to compare the phenotypes of rodent models of I/R injury following Mif gene knockout, Mif receptor knockout and/or MIF blockade, and agonistic activation vs. Ampk gene deficiency or pharmacologic AMPk agonism in more detail.

As outlined above, modulating MIF activity or that of its receptors in I/R injury draws a complex picture. While the studies that have explored an interference with MIF-driven pathways unanimously suggest that cardioprotection by MIF is associated with decreased glucose uptake,\textsuperscript{13} which in turn correlates with impaired post-ischaemic ventricular function and increased infarct size, the contribution of MIF pathways to the post-I/R inflammatory response is complex. While the MIF–CXCR2 axis is responsible for compartmentalized cardiac vs. peripheral effects,\textsuperscript{19} Mif deficiency protects the heart from prolonged I/R injury by suppressing inflammatory responses, suggesting a role for MIF in mid- to long-term inflammatory cell recruitment into the injured heart tissue.\textsuperscript{17} How MIF-mediated redox effects and the regulation of MIF activity by oxidation and/or nitrosylation affect the balance between these cardioprotective and cardio-exacerbating effects of MIF is currently unclear and intensively investigated in several laboratories.
In contrast, Ampk gene deficiency or Ampk-depending preconditioning has unanimously led to a reduction in inflammation and leucocyte recruitment. This is suggested by studies employing the AMPK agonist S-aminomimidazole-4-carboxamide 1-B-o-ribosafuransode (AICAR) and Ampk α(1)- or α(2)-knockout mice. These studies also revealed interesting mechanistic aspects. The anti-inflammatory effect of AMPK-mediated preconditioning 24 h before I/R injury (i.e. ‘late’ preconditioning) was dependent on nitric oxide (NO) production and endothelial NO-synthase (eNOS) activity. Of note, AMPK phosphorylates eNOS, leading to its activation. In contrast, the anti-inflammatory activity exerted by ‘early’ AMPK-mediated preconditioning was NO-independent. It is currently unknown whether S-nitrosylation of MIF at cysteine residue 81 might be linked to the ‘late’ preconditioning effect of the AMPK pathway.

With respect to cardiac function, various animal studies that also included diabetic models and the examination of the AMPK activator adiponectin also uniformly demonstrated that AMPK activation substantially protects the heart from I/R injury. To this end, COX-2 dependency, inhibition of mitochondrial permeability transition pore (mPTP) opening, and AMPK subunit isoform-specific effect could be delineated. It is unclear at this point whether the alpha(2) isoform, which appears to predominantly be responsible for the cardioprotective effects of AMPK in cardiac I/R injury, is modulated by the MIF/CD74 pathway.

Interestingly, overexpression of a dominant-negative Ampk mutant in mice, while leading to increased myocardial I/R injury, did not interfere with the cardioprotective effects of adiponectin, in particular pertaining to the cardiac anti-oxidative and nitrative stress-reducing effects of this adipocytokine. While somewhat speculative at this time, this could suggest that the cardioprotective anti-oxidative and/or pro-nitrosative molecules might not be directly associated with MIF-mediated AMPK activation. In fact, as briefly discussed above, it has been proposed that redox-related cardioprotection by MIF primarily is based on intracellular MIF activities that could be linked to the MIF–JAB1 interaction and quenching effect on pro-oxidative and/or pro-nitrosative molecules.

9. Links to ischaemic brain injury

A similar complex picture for MIF in neuronal injury exists. MIF has been demonstrated to be involved in the pathogenesis of reperfusion injury of the brain. MIF gene expression is up-regulated after stroke, and it seems that hypoxia signalling plays an important role. MIF promotes neuronal death and aggravates neurological deficits during the first week after experimental stroke in a murine model. However, it seems that MIF does not affect major components of the inflammatory response during the first week after experimental stroke, but that the deleterious effects of MIF depend on an intra- and interneuronal action.

10. MIF in clinical studies

MIF exhibits several properties affecting all stages of myocardial I/R injury. As a consequence, a growing number of clinical studies (Table 2) were conducted in order to assess the clinical meaning of the data of the preclinical experimental models. To date, there is few information of how MIF influences the progression or recovery of AMI and there is a need of more comprehensive studies. Considering the available data on elevated circulating MIF levels in patients suffering from AMI, cardiac arrest, or cardiac surgery, it is of great importance to note that the reported values are in the same range like comparable data from investigations in patients with metabolic syndrome, diabetes, or chronic kidney disease—which are common co-morbidities in patients presenting with AMI. Despite an ongoing overall consistent trend that severe cardiac illness is accompanied by an increase of circulating MIF, a common limitation of most reports has to be considered. The most important restriction concerns the broad range of reported circulating MIF levels in different studies. These range from ~1.5 to ~30 ng/mL in patients with AMI constituting a 20-fold difference (Table 2). The control group levels even vary from 0.6 to 96 ng/mL, corresponding to a 170-fold discrepancy. To compare between the different reports and to assess the scientific and pathophysiological value of the measurements, a detailed patient characteristic is needed. We recently demonstrated that proper sample preparation and processing is essential to obtain reproducible results. Notwithstanding, recent studies showed a correlation between MIF and established inflammatory markers as well as the extent of cardiac necrosis markers after AMI. By now, MIF plasma levels are far from being solid markers of myocardial damage or inflammation compared with established markers like troponin or C-reactive protein, and the cellular origin of the measured MIF remains elusive. Clinical assessment of MIF in AMI must consider the multitude of potential confounders when we discuss the value of elevated plasma MIF levels in patients. Nevertheless,

### Table 2: Review of literature summarizing relevant clinical studies

<table>
<thead>
<tr>
<th>Author (year)</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Yu et al. (2001)</td>
<td>MI levels remain high in patients with AMI (~4.3 ng/mL) compared with those without chest pain (~1.9 ng/mL).</td>
</tr>
<tr>
<td>Takahashi et al. (2002)</td>
<td>Elevated MI levels in patients with AMI (~25 ng/mL) compared with controls (~4 ng/mL).</td>
</tr>
<tr>
<td>Boekholdt et al. (2004)</td>
<td>Patients with history of myocardial infarction show increasing MI serum levels (~107 ng/mL) compared with controls (~91 ng/L).</td>
</tr>
<tr>
<td>Stoppe et al. (2012)</td>
<td>Elevated postoperative MI levels are inversely correlated with organ dysfunction in patients after cardiac surgery (~107 ng/mL) compared with controls (~13 ng/mL).</td>
</tr>
<tr>
<td>Stoppe et al. (2012)</td>
<td>MI shows a rapid and pronounced increase following CPR (~475 ng/mL) compared with healthy volunteers (~13 ng/mL).</td>
</tr>
<tr>
<td>Muller et al. (2012)</td>
<td>MI is associated with established inflammatory markers and correlates with the extent of cardiac necrosis marker release after PCI in ACS patients (~2.9 ng/mL) compared with controls (~1.2 ng/mL).</td>
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</table>

CPR, cardiopulmonary resuscitation; PCI, percutaneous coronary intervention.
against the background of the unequivocal trend of elevated MIF in cardiovascular disease, further clinical studies are needed to define the precise role of this protein and to potentially develop new therapeutic strategies.

11. Future directions
Translation of infarct size-reducing strategies from animal models into the clinical practice has not been successful so far. Further investigations are needed to identify the key regulatory events in the pathophysiology of AMI. In the recent decade, an unequivocal impact of MIF in myocardial I/R injury has been uncovered. The multifarious functions of MIF like control of glucose metabolism, apoptosis reduction, and redox regulation take place throughout the different temporal stages of myocardial I/R injury. Those protective properties can be enhanced by post-translational modification of MIF via S-nitrosylation or modulation of the receptor-active conformation of endogenous MIF, which opens therapeutic options in the treatment of myocardial I/R injury. Survey of reliable MIF plasma quantifications and the established MIF promoter polymorphism may have a diagnostic value. Further experimental studies need to be conducted in order to identify the precise mode of action of MIF, the cells of its origin in heart disease, and interaction partners during the different stages of disease. The involvement of MIF during the whole process of myocardial infarction and regeneration and also a segregation of its effects both locally to the heart by autocrine/paracrine cardiac effects and also on inflammatory cells make this protein an outstanding novel candidate for therapeutic interventions.

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