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MIF family cytokines in cardiovascular diseases and prospects for precision-based therapeutics

Pathricia V. Tilstam¹, Dake Qi^{1,2}, Lin Leng¹, Lawrence Young¹, and Richard Bucala¹

¹Department of Internal Medicine, Yale University School of Medicine, New Haven, CT, USA

²Department of Biomedical Sciences, Memorial University of Newfoundland, St. John's, NL, Canada

Abstract

Introduction: Macrophage migration inhibitory factor (MIF) is a pleiotropic cytokine with chemokine-like functions that is being increasingly studied in different aspects of cardiovascular disease. MIF was first identified as a proinflammatory and pro-survival mediator within the immune system, and a second structurally related MIF family member, D-dopachrome tautomerase (*a.k.a.* MIF-2), was reported recently. Both MIF family members are released by myocardium and modulate the manifestations of cardiovascular disease, specifically in myocardial ischemia.

Areas Covered: A scientific overview is provided for the involvement of MIF family cytokines in the inflammatory pathogenesis of atherosclerosis, myocardial infarction, and ischemia-reperfusion injury. We summarize findings of experimental, human genetic and clinical studies, and suggest therapeutic opportunities for modulating the activity of MIF family proteins that potentially may be applied in a *MIF* allele specific manner.

Expert Opinion: Knowledge of MIF, MIF-2 and their receptor pathways are under active investigation in different types of cardiovascular diseases, and novel therapeutic opportunities are being identified. Clinical translation may be accelerated by accruing experience with MIF-directed therapies currently in clinical testing in cancer and autoimmunity.

Keywords

Atherosclerosis; CXCR2; CXCR4; CD74; D-dopachrome Tautomerase; Ischemia Reperfusion; Macrophage Migration Inhibitory Factor; Myocardial Infarction

Corresponding Author: Pathricia V. Tilstam, Ph.D., Tel.: 203-737-5103, pathricia.tilstam@yale.edu, Dake Qi, Ph.D., Tel.: 709-864-3371, dake.qi@mun.ca, Lin Leng, Ph.D., Tel.: 203-737-5103, linleng@yale.edu, Lawrence Young, M.D., Tel.: 203-785-4102, lawrence.young@yale.edu, Richard Bucala, M.D. Ph.D., Tel.: 203-785-2454, richard.bucala@yale.edu.

Declaration of Interests

L. Young and R. Bucala are co-Inventors on patents describing the potential clinical applications of anti-MIF or MIF agonism. In addition, R. Bucala is a founder of MIFCOR, Inc., which has licensed MIF modulators from Yale University and is endeavoring to develop MIF-2 and MIF agonists for ischemic tissue protection. L. Young has received research funding from MIFCOR. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

1. Introduction

1.1 Cardiovascular Diseases

Cardiovascular disease (CVD) remains the leading cause of mortality in western societies and accounts for an estimated 31% of all deaths worldwide [1]. The most common manifestations of CVD are hypertension, coronary artery disease, stroke, and heart failure [2]. Coronary artery disease is most commonly due to atherosclerosis that presents clinically as angina pectoris, dyspnea on exertion, myocardial infarction and sudden cardiac death [3]. Many of these latter acute cardiovascular events are triggered by the rupture of coronary arterial plaques, made vulnerable by chronic inflammation.

1.2 Atherosclerosis and Restenosis

Atherosclerosis is a progressive disease that is initiated by the adhesion of blood leukocytes to the damaged endothelial monolayer, followed by leukocyte migration into the intimal layers of the blood vessel [4]. Once in the intima, blood monocytes differentiate into macrophages, accumulate lipids, and transform into pathologic foam cells. Continued leukocyte influx, deposition of cholesterol crystals, and apoptosis of macrophage foam cells contributes to the progression of atherosclerosis and the formation of a necrotic core within the vessel wall. Over time, smooth muscle cells migrate into the area, produce collagen fibers, and form a protective fibrous cap over the damaged area [4]. The obstructive thickness of the fibrous cap may decrease with time due to matrix degradation by proteases. However, this remodeling process also may destabilize the plaque, causing it to rupture and produce myocardial infarction or stroke [5]. Percutaneous coronary interventions such as balloon angioplasty and stent implantation aim to re-open and revascularize occluded arteries. Yet, these interventions also may provoke vascular injury, leading to smooth muscle cell proliferation and arterial remodeling with re-narrowing of the blood vessel [6]. Beyond preventative measures, such as statins, anti-thrombotic agents and drugs targeting underlying metabolic dysfunctions, there are no established treatments to combat the inflammatory pathogenesis of atherosclerosis. Given the high incidence of CVD and its attendant morbidity and mortality, finding therapeutic targets for the treatment of progressive atherosclerosis and plaque destabilization remain a priority for medicine.

1.3 Myocardial Ischemia/Reperfusion Injury and Cardiac Hypertrophy

Acute myocardial infarction is a major consequence of atherosclerosis and remains one of the leading causes of death in the developed world [7]. The acute interruption of blood flow to cardiac tissue by the thrombotic occlusion of a coronary artery induces contractile dysfunction and subsequently necrosis of the affected myocardium. An inflammatory response to damaged tissue also ensues, and the resulting interaction between cytokines, cell adhesion molecules and their respective receptors on leukocytes and endothelium triggers the extravasation of neutrophils and mononuclear cells. This response promotes repair and scar formation; however adverse tissue remodeling also impairs contractile function and can contribute to progressive heart failure [8]. Early reperfusion is a key therapeutic strategy to re-establish the oxygen supply to the compromised myocardium and salvage muscle tissue that may not yet be irreversibly damaged. However, reperfusion also may extend myocardial injury by promoting oxidative stress upon reflow [9]. Thus, there has been a long standing

interest in therapeutic interventions that potentially protect the heart against injury ischemia-reperfusion [10].

Cardiac hypertrophy or the abnormal enlargement of the heart muscle is an adaptive response to pressure or volume stress or loss of contractile mass from prior myocardial injury. Hypertrophy can occur as a physiological compensatory response, which is accompanied by normal or enhanced contractile function coupled with normal cardiac structure. However, pathological hypertrophy, such as left ventricular hypertrophy, is associated with significantly increased risk of heart failure and malignant arrhythmia. Causes for left ventricular hypertrophy involve aortic stenosis or insufficiency, hypertension or prior infarction [11]. Despite improved treatments for hypertension, the prevalence of hypertrophic cardiomyopathy is increasing and effective therapeutic approaches remain elusive.

1.4 MIF Family Cytokines

Recent studies implicate the cytokine macrophage migration inhibitory factor (MIF) in various aspects of CVD [12]. MIF is the first cytokine activity to be described and is considered to be a critical mediator in chronic inflammatory and autoimmune diseases [13]. In healthy adults, circulating MIF is present in the plasma in the 2–6 ng/mL range and a circadian rhythm with elevated MIF plasma levels in the morning has been observed [14]. While MIF was first cloned from pituitary cells and stress stimuli promote its release into the circulation [15, 16], the major cellular or tissue source of circulating MIF remains unknown [17]. Immune cells such as monocytes, macrophages, neutrophils, dendritic cells, mast cells, eosinophils, platelets, T- and B-cells express MIF, and the protein is broadly expressed in cells at the outer lining of tissues such as endothelial and epithelial cells, as well as in cardiomyocytes, adipocytes and stromal cells [18]. In contrast to most cytokines, MIF is constitutively synthesized and stored in intracellular pools. The release of intracellular MIF is triggered by proinflammatory and cellular stress, and is followed by subsequent *MIF* gene transcription and RNA translation, which replenishes intracellular stores [19]. Experimental evidence has shown that circulating MIF levels are elevated in different infectious and autoimmune inflammatory diseases, and neutralization of MIF by antibodies or small molecule antagonists improves outcome in numerous animal models of human disease [13, 20].

MIF possesses several intrinsic properties and has been variously described as a cytokine, enzyme, hormone and chemokine. It promotes multiple immunological functions and counter-regulates glucocorticoid immunosuppression to regulate both the set point and the ultimate strength of the inflammatory response [16]. MIF induces rapid and sustained phosphorylation and activation of the ERK1/2-MAPK pathway to directly increase the activity of the cytoplasmic phospholipase A2 (PLA2) [21]. PLA2 is a key target of the anti-inflammatory effects of glucocorticoids, and the ERK1/ERK2-mediated induction of PLA2 is one mechanism whereby MIF overrides the immunosuppressive effects of steroids [22]. MIF additionally downregulates the glucocorticoid-induced expression of the NF- κ B inhibitor I κ B [23] and the mitogen-activated protein kinase phosphatase-1 (MKP-1), which reduces downstream MAPK activation [24]. Importantly, MIF enhances cellular responses

by sustaining cell survival through inhibition of p53 or pAkt dependent activation-induced apoptosis [25, 26]. Intracellular protein-protein interactions of MIF have also been described. One major action involves MIF binding with JUN-activation domain-binding protein 1 (JAB1), also known as COP9 signalosome subunit 5 (CSN5), through which MIF inhibits JAB1-induced c-Jun-N-terminal kinase (JNK) activity and AP-1 transcriptional activation [27]. Additional protein-binding interactions include thioredoxin-interacting protein (TXNIP), a known NF- κ B inhibitor [28], and HSP90, which was shown to stabilize MIF in oncogenically transformed cells [29].

Other intracellular functions include MIF's catalytic properties such as its thiol-protein oxidoreductase (TPOR) activity [30], which may influence cell survival and an intracellular nuclease function, which has been shown to modulate cell death in a pre-clinical model of stroke [31]. MIF's dopachrome tautomerase activity appears vestigial and is dispensable for cytokine activity although the N-terminal proline imparts a necessary structural requirement for CD74 receptor activation [32].

Recently, a second member of the MIF protein superfamily was identified. The gene for the protein D-dopachrome tautomerase (D-DT/ MIF-2) (Figure 1) is closely adjacent to the *MIF* gene on human chromosome 22q11.23 and studies to date suggest that it exhibits a similar spectrum of activity as MIF [13]. Like MIF, MIF-2 is expressed in most tissues and by a variety of immune cells, circulates in serum at similar concentrations as MIF, and binds with high affinity to the cognate MIF cell surface receptor CD74 [33, 34]. MIF-2 also induces similar signaling cascades and counter-regulates the immunosuppressive effects of glucocorticoids [33]. However, differences in dose-response profiles are observed when comparing MIF and MIF-2, and MIF-2's action may be reduced at low concentrations when compared to MIF. Despite structural and signaling similarities, there are a few distinct differences between MIF and MIF-2 that may distinguish these proteins in the context of myocardial injury, as discussed further below.

1.5 MIF Family Receptors

CD74 is the surface expressed form of the Class II invariant chain, which functions intracellularly to facilitate Class II peptide loading. However, CD74 also is expressed on multiple cell types independently of Class II, and indeed was initially cloned from endothelial cells [34]. A circulating soluble form of CD74 (sCD74) also has been recently identified that appears to form by proteolytic truncation of the MIF binding ectodomain [35]. sCD74 binds MIF and transduces signal activity *in vivo*, and recent studies have implicated it in the clinical expression of autoimmune liver disease, acute lung injury, burn injury, and postoperative inflammatory responses [35, 36, 37, 38].

MIF signaling through CD74 requires the recruitment and intracytoplasmic phosphorylation of CD44, which is a broadly expressed transmembrane protein that recruits members of the nonreceptor Src tyrosine kinase family (e.g. Lck). CD74 is sufficient for MIF/MIF-2 high affinity cell surface binding, but CD44 is essential for downstream MAP kinase (e.g. ERK1/2, JNK), Akt kinase and AMPK signaling [33, 39, 40]. MIF engagement of CD74 leads to the horizontal membrane recruitment of CD44; both proteins then undergo intracellular phosphorylation to initiate downstream signal transduction [39, 41]. MIF

additionally activates the chemokine receptors CXCR2 and CXCR4, alone or in a complex with CD74 [42]. MIF-2 induces similar cell signaling as MIF, and the two ligands may work cooperatively [13]. There exist structural differences between MIF and MIF-2 that may impart unique functional properties for the two proteins. First, MIF-2 lacks the MIF CXXC motif that has been hypothesized to contribute to redox protection or modulation of nitric oxide activity [43, 44]. MIF-2 also lacks the *pseudo-(E)LR* motif by which MIF interacts with the chemokine receptor CXCR2 [45]. This motif consists of the MIF residues Arg-11 and Asp-44 that are closely apposed in the mature folded protein, while MIF-2 contains the residues Ala-11 and Gly-44 in these respective positions. Signaling through CD74/CD44 induces cell proliferation and inhibits apoptosis, and MIF interaction with the non-cognate receptors CXCR2 and CXCR4 modulates the migration of immune cells (Figure 2). The MIF/CXCR axis was reported to be critical for MIF-dependent monocyte recruitment processes in atherosclerotic arteries [42] but it remains to be demonstrated experimentally if MIF-2 fails to activate CXCR2 due to the absent *pseudo-(E)LR* motif.

2. Targeting MIF Family in Atherosclerosis

2.1 *MIF* Gene Polymorphisms

Several studies implicate MIF in the initiation and progression of atherosclerosis, both in experimental animal and human clinical studies [42, 46, 47, 48]. Of importance, the transcriptional regulation of MIF is influenced by a commonly occurring promoter polymorphism. There is a CATT microsatellite repeat at position -794 in the 5' promoter of *MIF* that exists in five to eight copy variants (CATT₅₋₈) (Figure 3) [49]. Functional studies indicate that CATT repeat number is associated with both constitutive and inducible *MIF* expression such that the CATT₅ repeat is a low expression allele and the CATT₆, CATT₇, and CATT₈ repeats are progressively higher expression alleles. Increasing repeat length also is associated with increased MIF plasma levels [50]. The transcription factor ICBP90 is essential for *MIF* expression and Toll like receptor-induced MIF transcription in both immune cells (*e.g.* monocytes, T and B lymphocytes) and stromal cells (*e.g.* fibroblasts), and *MIF* expression is regulated in both an ICBP90- and -794 CATT₅₋₈ length-dependent manner [51]. *MIF* CATT repeat length has been associated with autoimmune diseases and with the inflammatory sequelae of different infections [35, 49, 50, 52]. Notably, this structural variation is not represented in the DNA chips employed in Genome-Wide Association Studies (GWAS) and must be investigated by a candidate gene approach using specialized methodologies. There additionally is a G/C single nucleotide polymorphisms (SNP) at position -173 (-173°C) in the *MIF* promoter that has been found to be associated with a more inflammatory clinical phenotype - presumably due to linkage disequilibrium with the high expression -794 CATT₇ allele [53].

In 2008, data from 363 patients in the MONICA/KORA Augsburg case-cohort study showed that female carriers of the -173°C *MIF* allele had a higher risk for coronary heart disease (HR=2.44) [47]. These findings were recently affirmed in two studies of Chinese populations [54, 55]. In a Mexican-Mestizo population, the frequency of the -794 CATT_{6/7} genotype also was significantly higher in patients with acute coronary artery syndromes when compared to healthy subjects (OR=1.77) [56]. A relationship between both promoter

polymorphisms (–173*G/C and –794 CATT₅₋₈) and the severity of carotid artery disease has been reported in subjects carrying the high expression –794 CATT₇ allele, the –173*C allele, or the –794 CATT₇/ –173*C haplotype [57]. These reported associations between high expression *MIF* gene polymorphisms, which promote an inflammatory phenotype [35, 49, 50, 52], and coronary or carotid artery disease suggest that *MIF* genotype may be a useful tool for risk stratification or potentially for selecting treatments targeting atherosclerosis

2.2 MIF in Atherosclerotic Plaque Vulnerability

MIF is produced by different cell types in human atherosclerotic lesions and may contribute both to early plaque development and to pathologic progression to more advanced and complicated lesions [58]. As plaque rupture with subsequent thrombus formation is a major pathogenic mechanism for acute coronary syndromes, several studies have investigated MIF in this process, both as an inciting mediator and as a biomarker. MIF induces the expression of matrix metalloproteinases (MMP) in model systems [59] as well as in relevant vascular smooth muscle cells and macrophages [60]. High MIF plasma levels correlate with plaque rupture in patients with acute coronary artery syndromes and elevated MIF levels are associated with elevations in C-reactive protein, IL-6, and the myocardial necrosis marker troponin-I [61]. A subsequent study of patients with acute coronary artery syndrome that evaluated circulating levels of Gremlin1, which binds and inhibits MIF activity, reported elevated plasma levels of MIF in patients with unstable plaques. Patients with elevated Gremlin1 levels were judged to have stable coronary artery disease and there is potential for a Gremlin1/MIF ratio measurement to quantify risk of coronary artery disease and the grade of plaque stability [62]. Elevated serum MIF also has been described to reflect the presence and severity of angiographically complex coronary lesions [63]. MIF additionally has been reported to be overexpressed in aortas, with correlation with coronary artery disease severity [64]. Similarly, increased *MIF* expression was observed in aneurysmal abdominal aorta, where elevated *MIF* expression was linked to enhanced production of matrix damaging MMPs [65].

Promoting plaque regression and stability are desirable therapeutic goals for the treatment of atherosclerosis. A number of reports support a role for MIF in the initiation, progression, and ultimate destabilization of atherosclerotic plaques [12]. MIF induces atherogenesis by enhancing monocyte arrest on endothelium and by increasing the expression of adhesion molecules such as E-selectin, ICAM-1, and VCAM-1 that mediate leukocyte transmigration [66, 67]. Bernhagen *et al.* have established MIF's ability to influence monocyte and T cell migration via the receptors CXCR2 and CXCR4 in monocytes and T-cells respectively [42]. Advanced atheromatous plaques have been shown to express high amounts of MIF *in situ* and given MIF's ability to induce MMP expression [46], there is a compelling argument for its therapeutic targeting. In a recent pre-clinical study of diabetes-associated atherosclerosis, adenovirus-mediated RN interference of the *Mif* gene led to plaque stabilization and decreased macrophage and lipid deposition in large vessels [68]. A neutralizing anti-MIF monoclonal antibody, which is in human clinical investigation, has been shown to attenuate intimal inflammation and promote plaque regression in mice with advanced atherosclerosis [42, 69]. MIF blockade also reduces vascular inflammation, cellular proliferation, and

neointimal thickening after experimental angioplasty in atherosclerotic-prone mice [46]. Furthermore, in a mouse model of restenosis, MIF inhibition stabilized neointimal lesions, reduced macrophage/foam cell content, and increased smooth muscle cell content [70]. Wirtz and colleagues also have proposed a role for MIF in atherothrombosis as they found MIF to be a major platelet-derived chemotactic recruitment factor with significant clot-modulating properties [71]. These findings make it attractive to consider the therapeutic potential of emerging anti-MIF therapies in the treatment of progressive atherosclerosis or restenosis.

2.3 MIF-2: A Potential Effector in Atherogenesis?

While MIF's role in atherosclerosis has been studied extensively, the function of MIF-2 remains relatively unknown. The *MIF-2* gene was described originally as encoding an enzyme that converts D-dopachrome into 5,6-dihydroxyindole and was detected in the cytoplasm of human melanoma, liver and different parenchymal cells [72]. As the D-stereoisomer of dopachrome does not occur naturally (all dopachrome pathways utilize L-dopachrome derived from L-tyrosine), this enzymatic activity, while similar to that of MIF [32], appears vestigial and not a physiologic property of the protein. *MIF* and *MIF-2* most likely arose by an ancestral gene duplication, and the MIF-2 protein product binds to the CD74/CD44 receptor complex to activate an identical signaling cascade as MIF [33]. These properties together with the close structural similarity between MIF-2 and MIF ascribe potential clinical significance to MIF-2. Like MIF, MIF-2 also promotes inflammatory IL-6 secretion by preadipocytes via CD74-dependent ERK1/2 MAPK signaling, and it upregulates the mRNA levels of MMP-1 and MMP-3 [73]. As expression of the common MIF receptor CD74 is increased in vascular plaques and in the peripheral blood mononuclear cells of patients with atherosclerosis [74], MIF-2 likely contributes to disease progression via signaling pathways similar to MIF. Nevertheless, while MIF has been positively correlated with obesity as well as insulin resistance, and contributes to adipose tissue inflammation by activating adipose tissue macrophages, there are reports suggesting distinct, if not opposite effects for MIF-2. MIF-2 was observed to be negatively correlated with obesity and to reverse glucose intolerance [75]. MIF-2 is produced by human adipocytes and MIF-2 knockdown enhanced the expression of lipolysis and lipogenesis genes [76]. MIF-2 expression was recently examined in an *in vivo* model of adipose tissue inflammation and distinct temporal differences were noted between its expression and that of MIF [75]. A protective effect of MIF-2 also was reported in wound healing by improving fibroblast survival and proliferation [77].

3. MIF Family Proteins in Myocardial Infarction and Ischemia Reperfusion Injury

3.1 Spectrum of MIF Proinflammatory Action

Initial studies reported an increase in circulating MIF levels following myocardial ischemia [78], and experimental evidence for both proinflammatory and cardioprotective effects for MIF have since been postulated. Hypoxia and oxidative stress stimulate the secretion of MIF from cardiomyocytes, both *in vitro* and *in vivo* in acute myocardial ischemia [78, 79, 80],

Whether MIF renders protective or detrimental effects appears to depend on the experimental model of myocardial damage and the duration of ischemic injury. Local MIF release by ischemic myocardium initiates signaling pathways mediating cardioprotection (Figure 4) [81]. MIF release activates the cardioprotective AMP-activated protein kinase (AMPK) signaling pathway via the CD74/CD44 MIF receptor complex to upregulate glucose transport and utilization that provides for metabolic adaptation to ischemia (Figure 4) [82]. Miller *et al.* also established that high expression *MIF* alleles (CATT_{non-5}) are associated with an augmented human cellular response to hypoxic stress and increased AMPK phosphorylation [82]. The MIF/AMPK signaling pathway is blunted in aged hearts, which supports the observation that elderly patients are more susceptible to myocardial ischemia and suffer more extensive post-infarction myonecrosis compared to younger patients. This age-related defect may be caused by reduced expression in elderly hearts of the transcription factor hypoxia-inducible factor (HIF-1 α), which upregulates *MIF* transcription [83]. Additional cardioprotective actions of MIF in the ischemic heart include inhibition of JNK signaling and a reduction in cardiomyocyte apoptosis during reperfusion injury [84]. Some intracellular functions of MIF also may be postulated to be cardioprotective. MIF's TPOR activity reduces cellular oxidative stress [85] and the formation of S-nitros(yl)ated MIF inhibits MIF's interaction with JAB1/CSN5 and increases apoptosis inhibition [44].

Early human genetic studies show that high *MIF* expressers, when compared to low *MIF* expressers, are more likely to survive from septic shock (a state of systemic hypoperfusion) with less end-organ failure [86]. Low-expression *MIF* alleles also correlate with adverse surgical outcomes. In a prospective study of 100 patients who underwent elective cardiac surgery, those subjects with the lowest *MIF* expression genotype (−794 CATT_{5/5}) exhibited an increased incidence of post-operative organ dysfunction when compared to patients with a high expression *MIF* genotype (CATT_{non-5/5}). Clinical outcomes were especially notable for acute kidney injury with a 5.5-fold increased incidence in low-genotypic *MIF* (−794 CATT_{5/5}) expressing patients. These human genetic data support the importance of MIF in tissue protection and suggest that therapeutic augmentation of MIF levels may be especially beneficial in those subjects who are low genotypic *MIF* expressers and more susceptible to ischemic complications [38].

Although the majority of studies suggest a cardioprotective role for MIF, recent findings also suggest a detrimental effect of MIF in myocardial ischemia under certain circumstances. As MIF is released from damaged tissue and as a consequence of the systemic stress response [87], elevations in circulating MIF in the peri-infarct period are within expectation. White *et al.* explored the role of MIF in mediating inflammatory responses following acute myocardial ischemia and found MIF to be rapidly released from the damaged myocardium into the circulation [88]. Plasma MIF levels also have been reported to be associated with infarct size and the extent of post-infarct cardiac remodeling [89]. Mice with genetic *Mif* deficiency were found to be protected from prolonged and severe ischemia reperfusion injury, and this effect was associated with reduced expression of inflammatory cytokines, a decrease in the myocardial infiltration of neutrophils and macrophages, and increased macrophage apoptosis [90]. Anti-MIF intervention in mice with myocardial ischemia reduced inflammatory cell infiltration and decreased the incidence of post-myocardial

ischemia cardiac rupture [88]. Notably, patients carrying the -173^*C *MIF* promoter SNP (associated with increased *MIF* expression) who underwent surgical revascularization exhibited increased levels of circulating MIF and an elevated inflammatory profile [91].

Additionally, differential and cell-specific roles for MIF during myocardial ischemia have been postulated, with leukocyte-derived MIF promoting inflammatory responses after infarction and cardiac-derived MIF mediating an adaptive metabolic response that promotes cardiac healing [92]. Likewise, a cell-specific role for CXCR2 was demonstrated experimentally with MIF conferring protective effects through CXCR2 in resident cells and detrimental effects in CXCR2- expressing circulating leukocytes, leading in the later case to increased monocyte infiltration and impaired heart function [93]. Temporal or cell-specific differences in *MIF* expression may explain conflicting reports of the impact of exogenous MIF administration in myocardial reperfusion injury. One recent study showed no evident protective effect of exogenous MIF before ischemia or after reperfusion [94], while a second study reported that intracellular accumulation of MIF, via a post-translational S-nitrosylation, accounted for MIF's ability to reduce reperfusion injury [95].

In summary, the literature on MIF's precise role in myocardial ischemia is in evolution. Methodologic differences in the ischemia models with some studies employing models of chronic ischemia and others employing myocardial ischemia-reperfusion injury may account for the variation in experimental outcomes. The duration of artery occlusion and thus the phase of the ischemia injury also varies between studies. These findings reflect the multifaceted effects of MIF and emphasize the need for more focused approaches that examine cellular sources, intracellular versus extracellular MIF functions, as well as the temporal expression of MIF in different stages of cardiac ischemia and repair. The sum of published literature appears to support two distinct functions of MIF during myocardial ischemia and subsequent reperfusion injury. In the initial phase of ischemia, MIF promotes metabolic adaptation and recovery. With prolonged ischemia and myonecrosis, MIF's proinflammatory actions supervene, resulting in detrimental effects on contractility, functional recovery, and ventricular remodeling [96].

3.2 Augmenting MIF's Cardioprotective Action

Recent insights into MIF structure-function that enabled the structure-based molecular design of small molecule MIF antagonists also led to the serendipitous discovery of small molecule agonists of MIF signaling [97]. MIF binding ligands were designed by computational modeling, tested for their ability to block MIF interaction with CD74 *in vitro*, and refined by medicinal chemistry until high potency molecules were obtained [98]. MIF agonists, such as MIF20, thus were discovered as small molecules that bind to MIF but increase the MIF interaction with CD74 to enhance signal transduction [97]. These first-in-class compounds induce a concentration-dependent increase in the amount of MIF bound to CD74 (Figure 5A, B). Measurement of the equilibrium constants between MIF and the recombinant CD74 ectodomain in the presence of MIF20 revealed a 2.7-fold increase in the high-affinity binding interaction between MIF and its receptor versus MIF alone (MIF: $KD=6.06 \times 10^{-9}$ mol/L vs. MIF/MIF20: $KD=2.25 \times 10^{-9}$ mol/L) (Figure 5C). The main effect of MIF20 was to increase the association rate between MIF and sCD74 by 5.3-fold

(MIF: $k_a=1.85 \times 10^5$ mol/L vs. MIF/MIF20: $k_a=9.89 \times 10^5$ mol/L). These data suggest that MIF20 alters the conformation of MIF in a manner that enhances its initial association with the CD74 ectodomain (Figure 5C, D). Importantly, small molecule MIF agonists exhibit no activity in the genetic absence of MIF or CD74, verifying specificity of action for the MIF signaling pathway [99]. Pre-clinical validation for these first-in-class molecules has been obtained in mouse models of hyperoxic lung injury and cardiac ischemic injury [99, 100]. These experiments also verified the ability of MIF20 to enhance MIF signaling both in human as well as mouse target cells, which was predicted given the 90% sequence identity between human and mouse MIF. MIF20 was shown to increase AMPK phosphorylation and significantly reduce infarct size when studied in *in vivo* and *in situ* murine models of myocardial ischemia reperfusion injury [99]. These data additionally validate the potential of targeting the MIF pathway in treating cardiac ischemia and suggest that this pathway can be pharmacologically augmented.

3.3 MIF-2: Protection against Ischemic Injury

Recent evidence suggests that MIF-2 can activate similar pathways as MIF but with a more selective tissue protective action, possibly due to the absence in MIF-2 of the MIF *pseudo-(E)LR* domain that promotes inflammatory leukocyte infiltration. Qi *et al.* recently reported cardioprotective activities for recombinant MIF-2 in the ischemic heart in murine experimental models. Both antibody-based MIF-2 neutralization and cardiomyocyte-specific conditional deletion of the *Mif-2* gene resulted in cardiac dysfunction and increased infarct size in a mouse model of myocardial ischemia. The impairment in post-ischemic cardiac function was reversed by administration of recombinant MIF-2, which acts by a CD74/CD44/CaMKK2/AMPK pathway (Figure 4). Notably, when recombinant MIF-2 was administered prior to the induction of ischemia in the isolated Langendorff perfused heart and *in vivo* coronary artery occlusion models, it improved cardiac function and reduced infarct size after ischemia-reperfusion by >70% [101]. As pretreatment is difficult to translate into clinical utility, the efficacy of MIF-2 at the time of reperfusion was additionally evaluated, since this strategy could be applied during interventional procedures at the time of threatened myocardial infarction. In the perfused heart model, reperfusion with MIF-2 significantly improved the recovery of contractile function of the heart and decreased infarct size by ~80%. In contrast to studies with MIF, MIF-2 administration did not result in a negative inotropic action in the ischemic heart, supporting the potential superiority of MIF-2 over MIF as a therapeutic agent. The deleterious role of MIF's chemotactic *pseudo-(E)LR* domain in cardiac ischemia-reperfusion injury also has been affirmed in a study where myocardial Cxcr2-deficient chimeric mice showed less inflammatory injury after ischemia-reperfusion injury than wild type mice [93].

3.4 MIF-Mediated Cardioprotection in Hypertrophy

Recent reports have described a role for MIF in pre-clinical mouse models of myocardial hypertrophy. Koga and coworkers reported that genetic absence of *Mif* augments the pathologic hypertrophic response of the heart induced by experimental constriction of the aorta that leads to left ventricular pressure overload [102]. The role of MIF in this response appears to be by regulating autophagy via AMPK-mTOR signaling during pressure-overload stress. In tissue from failing hypertrophic human hearts, elevations in MIF, AMPK

activation, and autophagy were detected [103]. Xu *et al.* also found *Mif*-deficient mice to show an attenuation in aging-induced structural and functional changes in the heart that was associated with a reduction in autophagy and increased ATP availability [104]. These initial results suggest that MIF may play a previously unsuspected role in modulating the cardiac hypertrophic response to pressure overload, with potential regulatory points that may be amenable to therapeutic intervention.

4. Therapeutic Implications and Future Directions

The wealth of experimental and clinical data that support a regulatory role of MIF in the inflammatory response has prompted the development of pharmacologic strategies to inhibit MIF directed pathways for therapeutic applications. The clinical targeting of MIF has been further validated by human genetic studies that have linked severe inflammatory complications with a commonly occurring high expression *MIF* promoter polymorphism [50, 86, 105]. These genetic data also support important mechanistic features of MIF action [106, 107, 108, 109]. The strategies that have advanced the furthest in clinical development are the anti-CD74 antibody (*Milatuzumab*), which was approved for applications in oncology. The fully human anti-MIF monoclonal antibody (*Imalumab*) also has completed phase II clinical testing (ClinicalTrials.gov, NCT02448810) with acceptable toxicity [110, 111, 112]. An early stage competitive peptide *RTL1000*, which antagonizes MIF interaction with CD74, also has completed phase I clinical testing in multiple sclerosis (ClinicalTrials.gov, NCT00411723) [113, 114]. Several small molecule approaches for interfering with MIF:CD74 remain in pre-clinical development; these have exploited the facility of docking small molecules into the MIF tautomerase site, which interfaces with CD74 to initiate signal transduction [98, 114, 115, 116, 117, 118, 119, 120, 121, 122]. Notably, the asthma drug *Ibudilast*, initially developed for its ability to block phosphodiesterase activity, inhibits MIF allosterically [123]. MIF-2 has been crystallized and found to have a structurally distinct dopachrome binding pocket when compared to MIF [124, 125]. Accordingly, there is a firm molecular basis for developing small molecule inhibitors that may be selective for MIF or MIF-2. Notably, the small molecule 4-IPP (4-iodo-6-phenylpyrimidine), which is a suicide substrate for MIF that covalently modifies Pro1, also reacts with the MIF-2 Pro1 to inhibit its tautomerase activity [125].

There are currently no clinical development programs aimed at augmenting MIF action, however the recent studies that indicate a tissue protective role for CD74 signaling in myocardial ischemia suggest that the regional application of MIF could be especially beneficial in this common clinical scenario. The administration of MIF, or preferentially MIF-2, which may act more selectively through the MIF receptor CD74, would serve to augment the endogenous expression of MIF-2 to levels that act therapeutically to upregulate protective metabolic responses and salvage ischemic heart tissue at risk for necrosis. Conceivably, recombinant MIF-2 could be delivered in conjunction with primary angioplasty treatment of acute ST-segment myocardial infarction to protect at risk myocardium downstream of the occluded coronary artery. Such proof-of-concept has been obtained in mouse models of cardiac ischemia, with benefit observed by administering MIF-2 after reperfusion, which mimics the desired clinical scenario [101]. The prototypic small molecule MIF agonist MIF20 and its triazole-based congeners additionally introduce a

novel approach for augmenting signal transduction through CD74. Small molecules offer the possibility of oral administration and greater target tissue penetration than recombinant protein (as well as at lower cost).

5. Expert Opinion

Significant experimental, clinical and human genetic evidence implicates MIF family proteins in the pathogenesis of atherosclerosis, myocardial infarction, and ischemic myocardial injury. While MIF exerts upstream inflammatory and survival functions, structural features in the recently described MIF homolog MIF-2 suggest it to have a more selective survival and tissue protective function via CD74 signaling. Experimental knowledge of MIF proteins in different aspects of cardiovascular disease continues to accrue and proof-of-concept has been obtained for therapeutic opportunities in ameliorating plaque progression and reducing injury from myocardial ischemia. Both biologic and small molecule approaches for neutralizing MIF have been developed, with one antibody receiving FDA approval. Novel opportunities for small molecule development also exist based on the targeting of a small molecule binding site at the MIF:CD74 interface, and both MIF antagonistic and MIF agonistic molecules are under study in pre-clinical models. Nevertheless, small molecule approaches remain challenging due to the difficulty in effectively interfering with the large surface contacts necessary for high affinity protein ligand:receptor interaction and it remains to be seen if the targeting of the MIF tautomerase pocket is sufficient for therapeutic CD74 antagonism. An additional impediment to small molecule development has been the long-standing failure to obtain a MIF:CD74 co-crystal structure to define precisely the molecular interaction between the two proteins. To date only an NMR structure of a truncated CD74 ectodomain has been reported [126] and there is evidence that the CD74 intramembrane domain provides crucial three-dimensional stabilization for the protein. Computational modeling studies support the possibility of a dodecamer involving either MIF:CD74 or MIF-2:CD74 (Figure 5E) [114, 127] and mutational data support aspects of this working model [127, 128]. Strong interest in MIF-pathway based interventions is further prompted by the possibility that such agents could be applied in a pharmacogenomic approach to modulate MIF signaling.

The human studies that have investigated *MIF* genotype suggest distinct clinical relationships. While high *MIF* expressers appear more susceptible to metabolic syndrome, atherosclerosis, enhanced vascular inflammation, and acute coronary artery syndromes, high *MIF* expressers may be protected against ischemic injury and post-operative organ dysfunction. The precise role of MIF in the pathogenesis and progression of CVD may be multifaceted, and determination of *MIF* genotype may be useful in predicting disease course within a particular clinical context or targeting of a particular treatment modality.

Evidence for MIF's role in the inflammatory pathogenesis of atherosclerosis suggest that MIF inhibition may be a potential strategy for primary or secondary prevention in CVD. Such an approach would require the careful selection of patients likely to benefit from long-term MIF inhibition and close supervision of potential immunosuppressive side effects. As functional *MIF* promoter variants occur commonly in the population, it may be argued that there already exists a genetically-defined therapeutic window to guide the pharmacologic

targeting of MIF. Conceivably, anti-MIF interventions would need only to decrease MIF expression to the level observed in low-genotypic *MIF* expressers to reduce risk and effect a long-term therapeutic action. The recent discovery of the -794 CATT₅₋₈ specific transcription factor ICBP90 lends further impetus for a precision medicine approach to MIF inhibition by the opportunity to design small molecules that act furthest upstream on the transcriptional activation of the *MIF* gene.

In the case of augmenting MIF action for ischemic tissue protection, initial clinical testing may be simplified and reduced in cost by designing efficacy studies to focus on those subjects who are low-genotypic *MIF* expressers. Toward this end, rapid and inexpensive biochip methodologies have been developed that allow for *MIF* genotype determination at point of care. One technology is based on a solid-phase optical biosensor chip that requires no detector instrumentation and can be read by simple visual inspection [53]. Initially developed for genetic epidemiological studies in rural Africa, the platform is robust, inexpensive, and can be applied in acute settings for patient enrollment and genetic risk stratification.

Current anti-MIF interventions target MIF's extracellular function, which raises the question if intracellular MIF properties e.g. its nuclease function or the JAB1 regulation also have a role in CVD. Nevertheless, the clinical translation of MIF-directed therapies into CVD will be facilitated by information accruing from the ongoing clinical testing of anti-MIF and anti-CD74. Selective and pharmacologic modulation of MIF action in prevalent cardiovascular conditions such as advanced atherosclerosis or acute ischemia appears possible, and the testing and ultimate application of novel agents may be accelerated by a precision medicine approach in patients with risk *MIF* alleles.

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Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

References

1. McAloon CJ, Boylan LM, Hamborg T, et al. The changing face of cardiovascular disease 2000–2012: An analysis of the world health organisation global health estimates data. *Int J Cardiol.* 2016;224:256–64. [PubMed: 27664572]
2. A Nationwide Framework for Surveillance of Cardiovascular and Chronic Lung Diseases. The National Academies Collection: Reports funded by National Institutes of Health. Washington (DC)2011.
3. Cassar A, Holmes DR, Jr., Rihal CS, et al. Chronic coronary artery disease: diagnosis and management. *Mayo Clin Proc.* 2009;84:1130–46. [PubMed: 19955250]
4. Libby P, Ridker PM, Hansson GK. Progress and challenges in translating the biology of atherosclerosis. *Nature.* 2011;473:317–25. [PubMed: 21593864]

5. Hansson GK, Libby P, Tabas I. Inflammation and plaque vulnerability. *J Intern Med*. 2015;278:483–93. [PubMed: 26260307]
6. Simsekylmaz S, Liehn EA, Militaru C, et al. Progress in interventional cardiology: challenges for the future. *Thromb Haemost*. 2015;113:464–72. [PubMed: 25608683]
7. Lopez AD, Mathers CD, Ezzati M, et al. Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. *Lancet*. 2006;367:1747–57. [PubMed: 16731270]
8. Fang L, Moore XL, Dart AM, et al. Systemic inflammatory response following acute myocardial infarction. *J Geriatr Cardiol*. 2015;12:305–12. [PubMed: 26089856]
9. Yellon DM, Hausenloy DJ. Myocardial reperfusion injury. *N Engl J Med*. 2007;357:1121–35. [PubMed: 17855673]
10. Hausenloy DJ, Yellon DM. Myocardial ischemia-reperfusion injury: a neglected therapeutic target. *J Clin Invest*. 2013;123:92–100 [PubMed: 23281415]
11. Kehat I, Molkentin JD. Molecular pathways underlying cardiac remodeling during pathophysiological stimulation. *Circulation*. 2010;122:2727–35. [PubMed: 21173361]
12. Zernecke A, Bernhagen J, Weber C. Macrophage migration inhibitory factor in cardiovascular disease. *Circulation*. 2008;117:1594–602. [PubMed: 18362243]
13. Merk M, Mitchell RA, Endres S, et al. D-dopachrome tautomerase (D-DT or MIF-2): doubling the MIF cytokine family. *Cytokine*. 2012;59:10–7. Epub 2012/04/18. [PubMed: 22507380]
14. Petrovsky N, Socha L, Silva D, et al. Macrophage migration inhibitory factor exhibits a pronounced circadian rhythm relevant to its role as a glucocorticoid counter-regulator. *Immunol Cell Biol*. 2003;81:137–43. [PubMed: 12631237]
15. Bernhagen J, Calandra T, Mitchell RA, et al. MIF is a pituitary-derived cytokine that potentiates lethal endotoxaemia. *Nature*. 1993;365:756–9. [PubMed: 8413654]
16. Flaster H, Bernhagen J, Calandra T, et al. The macrophage migration inhibitory factor-glucocorticoid dyad: regulation of inflammation and immunity. *Mol Endocrinol*. 2007;21:1267–80. [PubMed: 17389748]
17. Roger T, Schlapbach LJ, Schneider A, et al. Plasma Levels of Macrophage Migration Inhibitory Factor and d-Dopachrome Tautomerase Show a Highly Specific Profile in Early Life. *Front Immunol*. 2017;8:26. [PubMed: 28179905]
18. Calandra T, Roger T. Macrophage migration inhibitory factor: a regulator of innate immunity. *Nat Rev Immunol*. 2003;3:791–800. [PubMed: 14502271]
19. Merk M, Baugh J, Zierow S, et al. The Golgi-associated protein p115 mediates the secretion of macrophage migration inhibitory factor. *J Immunol*. 2009;182:6896–906. [PubMed: 19454686]
20. Calandra T, Echtenacher B, Roy DL, et al. Protection from septic shock by neutralization of macrophage migration inhibitory factor. *Nat Med*. 2000;6:164–70. [PubMed: 10655104]
21. Lue H, Kapurniotu A, Fingerle-Rowson G, et al. Rapid and transient activation of the ERK MAPK signalling pathway by macrophage migration inhibitory factor (MIF) and dependence on JAB1/CSN5 and Src kinase activity. *Cell Signal*. 2006;18:688–703. [PubMed: 16122907]
22. Mitchell RA, Metz CN, Peng T, et al. Sustained mitogen-activated protein kinase (MAPK) and cytoplasmic phospholipase A2 activation by macrophage migration inhibitory factor (MIF). Regulatory role in cell proliferation and glucocorticoid action. *J Biol Chem*. 1999;274:18100–6. [PubMed: 10364264]
23. Daun JM, Cannon JG. Macrophage migration inhibitory factor antagonizes hydrocortisone-induced increases in cytosolic IkappaBalpha. *Am J Physiol Regul Integr Comp Physiol*. 2000;279:R1043–9. [PubMed: 10956264]
24. Roger T, Chanson AL, Knaup-Reymond M, et al. Macrophage migration inhibitory factor promotes innate immune responses by suppressing glucocorticoid-induced expression of mitogen-activated protein kinase phosphatase-1. *Eur J Immunol*. 2005;35:3405–13. [PubMed: 16224818]
25. Mitchell RA, Liao H, Chesney J, et al. Macrophage migration inhibitory factor (MIF) sustains macrophage proinflammatory function by inhibiting p53: regulatory role in the innate immune response. *Proc Natl Acad Sci U S A*. 2002;99:345–50. [PubMed: 11756671]

26. Lue H, Thiele M, Franz J, et al. Macrophage migration inhibitory factor (MIF) promotes cell survival by activation of the Akt pathway and role for CSN5/JAB1 in the control of autocrine MIF activity. *Oncogene*. 2007;26:5046–59. [PubMed: 17310986]
27. Kleemann R, Hausser A, Geiger G, et al. Intracellular action of the cytokine MIF to modulate AP-1 activity and the cell cycle through Jab1. *Nature*. 2000;408:211–6. [PubMed: 11089976]
28. Kim MJ, Kim WS, Kim DO, et al. Macrophage migration inhibitory factor interacts with thioredoxin-interacting protein and induces NF-kappaB activity. *Cell Signal*. 2017;34:110–20. [PubMed: 28323005]
29. Schulz R, Marchenko ND, Holembowski L, et al. Inhibiting the FISP90 chaperone destabilizes macrophage migration inhibitory factor and thereby inhibits breast tumor progression. *J Exp Med*. 2012;209:275–89. [PubMed: 22271573]
30. Kleemann R, Kapurniotu A, Frank RW, et al. Disulfide analysis reveals a role for macrophage migration inhibitory factor (MIF) as thiol-protein oxidoreductase. *J Mol Biol*. 1998;280:85–102. [PubMed: 9653033]
31. Wang Y, An R, Umanah GK, et al. A nuclease that mediates cell death induced by DNA damage and poly(ADP-ribose) polymerase-1. *Science*. 2016;354.
32. Fingerle-Rowson G, Kaleswarapu DR, Schlender C, et al. A tautomerase-null macrophage migration-inhibitory factor (MIF) gene knock-in mouse model reveals that protein interactions and not enzymatic activity mediate MIF-dependent growth regulation. *Mol Cell Biol*. 2009;29:1922–32. [PubMed: 19188446]
- 33 ••. Merk M, Zierow S, Leng L, et al. The D-dopachrome tautomerase (DDT) gene product is a cytokine and functional homolog of macrophage migration inhibitory factor (MIF). *Proc Natl Acad Sci U S A*. 2011;108:E577–85. Epub 2011/08/06.
The first paper to study D-DT/MIF-2 and its similarities/differences to MIF.
[PubMed: 21817065]
- 34 •. Leng L, Metz CN, Fang Y, et al. MIF signal transduction initiated by binding to CD74. *J Exp Med*. 2003;197:1467–76.
The cloning of the MIF receptor.
[PubMed: 12782713]
35. Assis DN, Leng L, Du X, et al. The role of macrophage migration inhibitory factor in autoimmune liver disease. *Hepatology*. 2014;59:580–91. [PubMed: 23913513]
36. Wu G, Sun Y, Wang K, et al. Relationship between elevated soluble CD74 and severity of experimental and clinical ALI/ARDS. *Sci Rep*. 2016;6:30067. [PubMed: 27444250]
37. Kim BS, Stoppe C, Grieb G, et al. The clinical significance of the MIF homolog d-dopachrome tautomerase (MIF-2) and its circulating receptor (sCD74) in burn. *Burns*. 2016;42:1265–76. [PubMed: 27209369]
- 38 •. Stoppe C, Rex S, Goetzenich A, et al. Interaction of MIF Family Proteins in Myocardial Ischemia/Reperfusion Damage and Their Influence on Clinical Outcome of Cardiac Surgery Patients. *Antioxid Redox Signal*. 2015;23:865–79.
Study establishing elevated circulating MIF and MIF-2 levels in cardiac surgery patients and increased risk of post-surgical complications in MIF low-genotypic expressers.
[PubMed: 26234719]
39. Yoo SA, Leng L, Kim BJ, et al. MIF allele-dependent regulation of the MIF coreceptor CD44 and role in rheumatoid arthritis. *Proc Natl Acad Sci USA*. 2016;113:E7917–E26. [PubMed: 27872288]
40. Gore Y, Starlets D, Maharshak N, et al. Macrophage migration inhibitory factor induces B cell survival by activation of a CD74-CD44 receptor complex. *J Biol Chem*. 2008;283:2784–92. [PubMed: 18056708]
41. Shi X, Leng L, Wang T, et al. CD44 is the signaling component of the macrophage migration inhibitory factor-CD74 receptor complex. *Immunity*. 2006;25:595–606. [PubMed: 17045821]
- 42 ••. Bernhagen J, Krohn R, Lue H, et al. MIF is a noncognate ligand of CXC chemokine receptors in inflammatory and atherogenic cell recruitment. *Nat Med*. 2007;13:587–96. Epub 2007/04/17.

Establishment of MIF's chemokine-like functions by its ability to signal through CXCR2/4 and its role in atherosclerosis.

[PubMed: 17435771]

43. Nguyen MT, Lue H, Kleemann R, et al. The cytokine macrophage migration inhibitory factor induces pro-oxidative stress-induced apoptosis. *J Immunol.* 2003;170:3337–47. [PubMed: 12626594]
 44. Luedike P, Hendgen-Cotta UB, Sobierajski J, et al. Cardioprotection through S-nitros(yl)ation of macrophage migration inhibitory factor. *Circulation.* 2012;125:1880–9. [PubMed: 22415145]
 45. Weber C, Kraemer S, Drechsler M, et al. Structural determinants of MIF functions in CXCR2-mediated inflammatory and atherogenic leukocyte recruitment. *Proc Natl Acad Sci USA.* 2008;105:16278–83. [PubMed: 18852457]
 46. Chen Z, Sakuma M, Zago AC, et al. Evidence for a role of macrophage migration inhibitory factor in vascular disease. *Arterioscler Thromb Vase Biol.* 2004;24:709–14.
 - 47 •. Herder C, Illig T, Baumert J, et al. Macrophage migration inhibitory factor (MIF) and risk for coronary heart disease: results from the MONICA/KORA Augsburg case-cohort study, 1984–2002. *Atherosclerosis.* 2008;200:380–8.
- An early human clinical cohort study describing a detrimental role for MIF in coronary heart disease.

[PubMed: 18242614]

48. Verschuren L, Kooistra T, Bernhagen J, et al. MIF deficiency reduces chronic inflammation in white adipose tissue and impairs the development of insulin resistance, glucose intolerance, and associated atherosclerotic disease. *Circ Res.* 2009;105:99–107. Epub 2009/05/30. [PubMed: 19478200]
 49. Baugh JA, Chitnis S, Donnelly SC, et al. A functional promoter polymorphism in the macrophage migration inhibitory factor (MIF) gene associated with disease severity in rheumatoid arthritis. *Genes Immun.* 2002;3:170–6. [PubMed: 12070782]
 50. Sreih A, Ezzeddine R, Leng L, et al. Dual effect of the macrophage migration inhibitory factor gene on the development and severity of human systemic lupus erythematosus. *Arthritis Rheum.* 2011;63:3942–51. [PubMed: 22127710]
 - 51 •. Yao J, Leng L, Sauler M, et al. Transcription factor ICBP90 regulates the MIF promoter and immune susceptibility locus. *J Clin Invest.* 2016;126:732–44.
- Study establishing ICBP90 as a transcription factor regulating CATT-dependent MIF expression.

[PubMed: 26752645]

52. Renner P, Roger T, Calandra T. Macrophage migration inhibitory factor: gene polymorphisms and susceptibility to inflammatory diseases. *Clin Infect Dis.* 2005;41 Suppl 7:S513–9. [PubMed: 16237655]
53. Zhong XB, Leng L, Beitin A, et al. Simultaneous detection of microsatellite repeats and SNPs in the macrophage migration inhibitory factor (MIF) gene by thin-film biosensor chips and application to rural field studies. *Nucleic Acids Res.* 2005;33:e121. [PubMed: 16077028]
54. Luo JY, Xu R, Li XM, et al. MIF Gene Polymorphism rs755622 Is Associated With Coronary Artery Disease and Severity of Coronary Lesions in a Chinese Kazakh Population: A Case-Control Study. *Medicine (Baltimore).* 2016;95:e2617. [PubMed: 26825917]
55. Ji K, Wang X, Li J, et al. Macrophage migration inhibitory factor polymorphism is associated with susceptibility to inflammatory coronary heart disease. *Biomed Res Int.* 2015;2015:315174. [PubMed: 25821795]
56. Valdes-Alvarado E, Munoz-Valle JF, Valle Y, et al. Association between the –794 (CATT)₅–8 MIF gene polymorphism and susceptibility to acute coronary syndrome in a western Mexican population. *J Immunol Res.* 2014;2014:704854. [PubMed: 25105152]
57. Lan MY, Chang YY, Chen WH, et al. Association between MIF gene polymorphisms and carotid artery atherosclerosis. *Biochem Biophys Res Commun.* 2013;435:319–22. [PubMed: 23537651]

58. Burger-Kentischer A, Goebel H, Seiler R, et al. Expression of macrophage migration inhibitory factor in different stages of human atherosclerosis. *Circulation*. 2002;105:1561–6. [PubMed: 11927523]
59. Onodera S, Kaneda K, Mizue Y, et al. Macrophage migration inhibitory factor up-regulates expression of matrix metalloproteinases in synovial fibroblasts of rheumatoid arthritis. *J Biol Chem*. 2000;275:444–50. [PubMed: 10617637]
60. Kong YZ, Huang XR, Ouyang X, et al. Evidence for vascular macrophage migration inhibitory factor in destabilization of human atherosclerotic plaques. *Cardiovasc Res*. 2005;65:272–82. [PubMed: 15621056]
61. Muller II, Muller KA, Schonleber H, et al. Macrophage migration inhibitory factor is enhanced in acute coronary syndromes and is associated with the inflammatory response. *PLoS One*. 2012;7:e38376. [PubMed: 22693633]
62. Muller II, Muller KA, Karathanos A, et al. Impact of counterbalance between macrophage migration inhibitory factor and its inhibitor Gremlin-1 in patients with coronary artery disease. *Atherosclerosis*. 2014;237:426–32. [PubMed: 25463068]
63. Hao Y, Yi SL, Zhong JQ. Serum macrophage migration inhibitory factor levels are associated with angiographically complex coronary lesions in patients with coronary artery disease. *Genet Test Mol Biomarkers*. 2015;19:556–60. [PubMed: 26263432]
64. Gong Z, Xing S, Zheng F, et al. Increased expression of macrophage migration inhibitory factor in aorta of patients with coronary atherosclerosis. *J Cardiovasc Surg (Torino)*. 2015;56:631–7.
65. Verschuren L, Lindeman JH, van Bockel JH, et al. Up-regulation and coexpression of MIF and matrix metalloproteinases in human abdominal aortic aneurysms. *Antioxid Redox Signal*. 2005;7:1195–202. [PubMed: 16115023]
66. Cheng Q, McKeown SJ, Santos L, et al. Macrophage migration inhibitory factor increases leukocyte-endothelial interactions in human endothelial cells via promotion of expression of adhesion molecules. *Journal of immunology*. 2010;185:1238–47.
67. Schober A, Bernhagen J, Weber C. Chemokine-like functions of MIF in atherosclerosis. *J Mol Med (Berl)*. 2008;86:761–70. [PubMed: 18385967]
68. Sun H, Zhang X, Zhao L, et al. Attenuation of atherosclerotic lesions in diabetic apolipoprotein E-deficient mice using gene silencing of macrophage migration inhibitory factor. *J Cell Mol Med*. 2015;19:836–49. [PubMed: 25661015]
- 69 •. Burger-Kentischer A, Gobel H, Kleemann R, et al. Reduction of the aortic inflammatory response in spontaneous atherosclerosis by blockade of macrophage migration inhibitory factor (MIF). *Atherosclerosis*. 2006;184:28–38.
An early study showing benefit of MIF neutralization in experimental atherosclerosis.
[PubMed: 15921687]
70. Schober A, Bernhagen J, Thiele M, et al. Stabilization of atherosclerotic plaques by blockade of macrophage migration inhibitory factor after vascular injury in apolipoprotein E-deficient mice. *Circulation*. 2004;109:380–5. [PubMed: 14691040]
71. Wirtz TH, Tillmann S, Strussmann T, et al. Platelet-derived MIF: a novel platelet chemokine with distinct recruitment properties. *Atherosclerosis*. 2015;239:1–10. [PubMed: 25561410]
72. Odh G, Hindemith A, Rosengren AM, et al. Isolation of a new tautomerase monitored by the conversion of D-dopachrome to 5,6-dihydroxyindole. *Biochem Biophys Res Commun*. 1993;197:619–24. Epub 1993/12/15. [PubMed: 8267597]
73. Ishimoto K, Iwata T, Taniguchi H, et al. D-dopachrome tautomerase promotes IL-6 expression and inhibits adipogenesis in preadipocytes. *Cytokine*. 2012;60:772–7. Epub 2012/09/07. [PubMed: 22951300]
74. Martin-Ventura JL, Madrigal-Matute J, Munoz-Garcia B, et al. Increased CD74 expression in human atherosclerotic plaques: contribution to inflammatory responses in vascular cells. *Cardiovasc Res*. 2009;83:586–94. [PubMed: 19423618]
75. Kim BS, Pallua N, Bernhagen J, et al. The macrophage migration inhibitory factor protein superfamily in obesity and wound repair. *Exp Mol Med*. 2015;47:e161 Epub 2015/05/02. [PubMed: 25930990]

76. Iwata T, Taniguchi H, Kuwajima M, et al. The action of D-dopachrome tautomerase as an adipokine in adipocyte lipid metabolism. *PLoS One*. 2012;7:e33402 Epub 2012/03/20. [PubMed: 22428043]
77. Kim BS, Tilstam PV, Hwang SS, et al. D-dopachrome tautomerase in adipose tissue inflammation and wound repair. *J Cell Mol Med*. 2017;21:35–45. [PubMed: 27605340]
78. Yu CM, Lau CP, Lai KW, et al. Elevation of plasma level of macrophage migration inhibitory factor in patients with acute myocardial infarction. *Am J Cardiol*. 2001;88:774–7. [PubMed: 11589847]
79. Takahashi M, Nishihira J, Shimpō M, et al. Macrophage migration inhibitory factor as a redox-sensitive cytokine in cardiac myocytes. *Cardiovasc Res*. 2001;52:438–45. [PubMed: 11738060]
80. Yu CM, Lai KW, Chen YX, et al. Expression of macrophage migration inhibitory factor in acute ischemic myocardial injury. *J Histochem Cytochem*. 2003;51:625–31. [PubMed: 12704210]
81. Rassaf T, Weber C, Bernhagen J. Macrophage migration inhibitory factor in myocardial ischaemia/reperfusion injury. *Cardiovasc Res*. 2014;102:321–8. [PubMed: 24675723]
- 82 •. Miller EJ, Li J, Leng L, et al. Macrophage migration inhibitory factor stimulates AMP-activated protein kinase in the ischaemic heart. *Nature*. 2008;451:578–82. Epub 2008/02/01.
First study to observe the cardioprotective MIF/AMPK signaling pathway in cardiac ischemic injury.
[PubMed: 18235500]
- 83 •. Ma H, Wang J, Thomas DP, et al. Impaired macrophage migration inhibitory factor-AMP-activated protein kinase activation and ischemic recovery in the senescent heart. *Circulation*. 2010;122:282–92.
Article explaining the age-related susceptibility to ischemic injury caused by deficient HIF-1 α expression.
[PubMed: 20606117]
84. Qi D, Hu X, Wu X, et al. Cardiac macrophage migration inhibitory factor inhibits JNK pathway activation and injury during ischemia/reperfusion. *J Clin Invest*. 2009;119:3807–16. [PubMed: 19920350]
85. Koga K, Kenessey A, Powell SR, et al. Macrophage migration inhibitory factor provides cardioprotection during ischemia/reperfusion by reducing oxidative stress. *Antioxid Redox Signal*. 2011;14:1191–202. [PubMed: 20831446]
86. Yende S, Angus DC, Kong L, et al. The influence of macrophage migration inhibitory factor gene polymorphisms on outcome from community-acquired pneumonia. *The FASEB Journal*. 2009;23:2403–11 [PubMed: 19346297]
87. Fingerle-Rowson G, Koch P, Bikoff R, et al. Regulation of macrophage migration inhibitory factor expression by glucocorticoids in vivo. *Am J Pathol*. 2003;162:47–56. [PubMed: 12507889]
88. White DA, Fang L, Chan W, et al. Pro-inflammatory action of MIF in acute myocardial infarction via activation of peripheral blood mononuclear cells. *PLoS One*. 2013;8:e76206. [PubMed: 24098445]
89. Fan F, Fang L, Moore XL, et al. Plasma Macrophage Migration Inhibitor Factor Is Elevated in Response to Myocardial Ischemia. *J Am Heart Assoc*. 2016;5.
90. Gao XM, Liu Y, White D, et al. Deletion of macrophage migration inhibitory factor protects the heart from severe ischemia-reperfusion injury: a predominant role of anti-inflammation. *J Mol Cell Cardiol*. 2011;50:991–9. [PubMed: 21215754]
91. Lehmann LE, Schroeder S, Hartmann W, et al. A single nucleotide polymorphism of macrophage migration inhibitory factor is related to inflammatory response in coronary bypass surgery using cardiopulmonary bypass. *Eur J Cardiothorac Surg*. 2006;30:59–63. [PubMed: 16527487]
92. White DA, Su Y, Kanellakis P, et al. Differential roles of cardiac and leukocyte derived macrophage migration inhibitory factor in inflammatory responses and cardiac remodelling post myocardial infarction. *J Mol Cell Cardiol*. 2014;69:32–42. [PubMed: 24508700]
- 93 •. Liehn EA, Kanzler I, Konschalla S, et al. Compartmentalized protective and detrimental effects of endogenous macrophage migration-inhibitory factor mediated by CXCR2 in a mouse model of myocardial ischemia/reperfusion. *Arterioscler Thromb Vasc Biol*. 2013;33:2180–6.

Article describing a cell source specific effect of MIF/CXCR2 signaling in cardiac ischemic injury.

[PubMed: 23868943]

94. Rossello X, Burke N, Stoppe C, et al. Exogenous Administration of Recombinant MIF at Physiological Concentrations Failed to Attenuate Infarct Size in a Langendorff Perfused Isolated Mouse Heart Model. *Cardiovasc Drugs Ther.* 2016;30:445–53. [PubMed: 27335054]
95. Pohl J, Hendgen-Cotta UB, Rammos C, et al. Targeted intracellular accumulation of macrophage migration inhibitory factor in the reperfused heart mediates cardioprotection. *Thromb Haemost.* 2016;115:200–12. [PubMed: 26310191]
96. Dayawansa NH, Gao XM, White DA, et al. Role of MIF in myocardial ischaemia and infarction: insight from recent clinical and experimental findings. *Clin Sci (Lond).* 2014;127:149–61. [PubMed: 24697297]
97. Jorgensen WL, Gandavadi S, Du X, et al. Receptor agonists of macrophage migration inhibitory factor. *Bioorg & Med Chem Letts.* 2010;20:7033–6. [PubMed: 20971005]
98. Cournia Z, Leng L, Gandavadi S, et al. Discovery of human MIF-CD74 antagonists via virtual screening. *J Med Chem.* 2009;52:416–24. [PubMed: 19090668]
- 99 ••. Wang J, Tong C, Yan X, et al. Limiting cardiac ischemic injury by pharmacologic augmentation of MIF-AMPK signal transduction. *Circulation.* 2013;128:225–36.

Article observing the cardioprotective effect of small molecule MIF agonist in augmenting CD74 signaling in cardiac ischemic injury.

[PubMed: 23753877]

100. Sun H, Choo-Wing R, Fan J, et al. Small molecular modulation of macrophage migration inhibitory factor in the hyperoxia-induced mouse model of bronchopulmonary dysplasia. *Respir Res.* 2013;14:27–38. [PubMed: 23448134]
- 101 ••. Qi D, Atsina K, Qu L, et al. The vestigial enzyme D-dopachrome tautomerase protects the heart against ischemic injury. *J Clin Invest.* 2014;124:3540–50.

First study to investigate the role of MIF-2 in myocardial ischemic injury and describe its protective role.

[PubMed: 24983315]

102. Koga K, Kenessey A, Ojamaa K. Macrophage migration inhibitory factor antagonizes pressure overload-induced cardiac hypertrophy. *Am J Physiol Heart Circ Physiol.* 2013;304:H282–93. [PubMed: 23144312]
103. Xu X, Hua Y, Nair S, et al. Macrophage migration inhibitory factor deletion exacerbates pressure overload-induced cardiac hypertrophy through mitigating autophagy. *Hypertension.* 2014;63:490–9. [PubMed: 24366076]
104. Xu X, Pang J, Chen Y, et al. Macrophage Migration Inhibitory Factor (MIF) Deficiency Exacerbates Aging-Induced Cardiac Remodeling and Dysfunction Despite Improved Inflammation: Role of Autophagy Regulation. *Sci Rep.* 2016;6:22488. [PubMed: 26940544]
105. Wu SP, Leng L, Feng Z, et al. Macrophage migration inhibitory factor promoter polymorphisms and the clinical expression of scleroderma. *Arthritis Rheum.* 2006;54:3661–9. [PubMed: 17075815]
106. Vivarelli M, D'Urbano LE, Insalaco A, et al. Macrophage migration inhibitory factor (MIF) and oligoarticular juvenile idiopathic arthritis (o-JIA): association of MIF promoter polymorphisms with response to intra-articular glucocorticoids. *Clin Exp Rheumatol.* 2007;25:775–81. [PubMed: 18078632]
107. Berdeli A, Mir S, Ozkayin N, et al. Association of macrophage migration inhibitory factor –173C allele polymorphism with steroid resistance in children with nephrotic syndrome. *Pediatr Nephrol.* 2005;20:1566–71. [PubMed: 16133063]
108. Griga T, Wilkens C, Wirkus N, et al. A polymorphism in the macrophage migration inhibitory factor gene is involved in the genetic predisposition of Crohn's disease and associated with cumulative steroid doses. *Hepatogastroenterology.* 2007;54:784–6. [PubMed: 17591062]

109. Wang FF, Zhu LA, Zou YQ, et al. New insights into the role and mechanism of macrophage migration inhibitory factor in steroid-resistant patients with systemic lupus erythematosus. *Arthritis Res Ther*. 2012;14:R103. [PubMed: 22551315]
110. Liu X, Rafaat-Adib D, Barak H, et al. Development of a phase Ib/IIa proof-of-concept study of imalumab (BAX69), a first-in-class anti-macrophage migration inhibitory factor (MIF) antibody, as the 3rd or 4th line treatment in metastatic colorectal cancer (mCRC). 2015 ASCO Annual Meeting; 2015.
111. Kaufman JL, Niesvizky R, Stadtmauer EA, et al. Dose-escalation trial of milatuzumab (humanized anti-CD74 monoclonal antibody) in multiple myeloma. 2009 ASCO Annual Meeting; 2009.
112. Bucala R, Donnelly SC. Macrophage migration inhibitory factor: a probable link between inflammation and cancer. *Immunity*. 2007;26:281–5. [PubMed: 17376392]
113. Offner H, Sinha S, Burrows GG, et al. RTL therapy for multiple sclerosis: a Phase I clinical study. *J Neuroimmunol*. 2011;231:7–14. [PubMed: 20965577]
114. Meza-Romero R, Benedek G, Leng L, et al. Predicted structure of MIF/CD74 and RTL1000/CD74 complexes. *Metab Brain Dis*. 2016;31:249–55. [PubMed: 26851955]
115. Senter PD, Al-Abed Y, Metz CN, et al. Inhibition of macrophage migration inhibitory factor (MIF) tautomerase and biological activities by acetaminophen metabolites. *Proc Natl Acad Sci USA*. 2002;99:144–9. [PubMed: 11773615]
116. Orita M, Yamamoto S, Katayama N, et al. Coumarin and chromen-4-one analogues as tautomerase inhibitors of macrophage migration inhibitory factor: discovery and X-ray crystallography. *J Med Chem*. 2001;44:540–7. [PubMed: 11170644]
117. Lubetsky JB, Dios A, Han J, et al. The tautomerase active site of macrophage migration inhibitory factor is a potential target for discovery of novel anti-inflammatory agents. *J Biol Chem*. 2002;277:24976–82. [PubMed: 11997397]
118. Al-Abed Y, Dabideen D, Aljabari B, et al. ISO-1 binding to the tautomerase active site of MIF inhibits its pro-inflammatory activity and increases survival in severe sepsis. *J Biol Chem*. 2005;280:36541–4. [PubMed: 16115897]
119. Arjona A, Foellmer HG, Town T, et al. Abrogation of macrophage migration inhibitory factor decreases West Nile virus lethality by limiting viral neuroinvasion. *J Clin Invest*. 2007;117:3059–66. [PubMed: 17909632]
120. Ouertani-Sakouhi H, El-Turk F, Fauvet B, et al. Identification and characterization of novel classes of macrophage migration inhibitory factor (MIF) inhibitors with distinct mechanisms of action. *J Biol Chem*. 2010;285:26581–98. [PubMed: 20516071]
121. Leng L, Chen L, Fan J, et al. A small-molecule macrophage migration inhibitory factor antagonist protects against glomerulonephritis in lupus-prone NZB/NZW F1 and MRL/lpr mice. *Journal of immunology*. 2011;186:527–38.
122. Hare AA, Leng L, Gandavadi S, et al. Optimization of N-benzyl-benzoxazol-2-ones as receptor antagonists of macrophage migration inhibitory factor (MIF). *Bioorg Med Chem Lett*. 2010;20:5811–4. [PubMed: 20728358]
123. Cho Y, Crichlow GV, Vermeire JJ, et al. Allosteric inhibition of macrophage migration inhibitory factor revealed by ibudilast. *Proc Natl Acad Sci U S A*. 2010;107:11313–8. [PubMed: 20534506]
124. Sugimoto H, Taniguchi M, Nakagawa A, et al. Crystal structure of human D-dopachrome tautomerase, a homologue of macrophage migration inhibitory factor, at 1.54 Å resolution. *Biochemistry*. 1999;38:3268–79. Epub 1999/03/17. [PubMed: 10079069]
125. Rajasekaran D, Zierow S, Syed M, et al. Targeting distinct tautomerase sites of D-DT and MIF with a single molecule for inhibition of neutrophil lung recruitment. *FASEB J*. 2014;28:4961–71. [PubMed: 25016026]
126. Jasanoff A, Wagner G, Wiley DC. Structure of a trimeric domain of the MHC class II-associated chaperonin and targeting protein Ii. *EMBO J*. 1998;17:6812–8. [PubMed: 9843486]
127. Meza-Romero R, Benedek G, Jordan K, et al. Modeling of both shared and distinct interactions between MIF and its homologue D-DT with their common receptor CD74. *Cytokine*. 2016;88:62–70. [PubMed: 27573366]

128. Pantouris G, Syed MA, Fan C, et al. An Analysis of MIF Structural Features that Control Functional Activation of CD74. *Chem Biol.* 2015;22:1197–205. [PubMed: 26364929]
129. Qi D, Young LH. AMPK: energy sensor and survival mechanism in the ischemic heart. *Trends Endocrinol Metab.* 2015;26:422–9. [PubMed: 26160707]

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Article Highlight Box

- Experimental animal studies of *MIF* gene deletion or anti-MIF intervention establish an upstream role for MIF in the endothelial response to injury and atherosclerosis disease progression.
- Commonly occurring high expression *MIF* alleles defined by >5 CATT repeats at the -794 *MIF*(CATT)₅₋₈ promoter site or the closely associated, which in linkage disequilibrium with CATT₇, are associated with more advanced atherosclerosis and CVD events in different population-based cohort studies.
- MIF and its homolog MIF-2 exhibit cardioprotective function in murine models of acute myocardial ischemia by signaling through the common CD74/CD44 MIF receptor complex to activate AMPK and promote energy conserving pathways.
- MIF-2 lacks the MIF *pseudo-(E)LR* domain necessary for CXCR2 activation and is likely to be a preferential agent for therapeutic cardioprotection during acute myocardial ischemia.
- The small molecule MIF agonist MIF20 enhances MIF signaling through CD74 and shows cardioprotective action in mouse models of cardiac ischemia.

Fig. 1: MIF protein family homology.

The significant similarity between the three-dimensional X-ray crystal structures of human MIF and human MIF-2. (A) Left panel: Human MIF monomer, Right panel: Human MIF trimer. (B) Left panel: Human MIF-2 monomer, Right panel: Human MIF-2 trimer.

Structures reused from “Merk M, Mitchell RA, Endres S, Bucala R. D-dopachrome tautomerase (D-DT or MIF-2): doubling the MIF cytokine family. Cytokine. 2012;59:10–7. Epub 2012/04/18. <http://www.sciencedirect.com/science/article/pii/S104346661200097X>”

Fig. 2: MIF family receptor signaling pathways in myocardial ischemia reperfusion and atherosclerosis.

Both MIF and MIF-2 bind to CD74, which results in its phosphorylation and the recruitment of CD44 into a signaling complex and MIF additionally signals through the chemokine receptors CXCR2 and CXCR4. Signaling through CD74/CD44 leads to apoptosis inhibition and metabolic adaptation resulting in cardioprotection. MIF interaction with the non-cognate receptors CXCR2 and CXCR4 modulates the migration of leukocytes and their migration arrest to chemokine stimulus at sites of inflammation. Figure compiled from references [41, 42].

Fig. 3: The human *MIF* gene.

Shown is the human *MIF* locus with its three exons, the microsatellite –794 CATT_{5–8}, and the ICBP90 transcription factor binding site. Also shown is the –173*G/C SNP that is in linkage with –794 CATT₇.

Fig. 4: MIF and MIF-2 cardioprotective CD74/AMPK signaling.

Hypoxia resulting from ischemic stress releases intracellular MIF. MIF and MIF-2 activate the cardioprotective AMPK signaling pathway in aCD74:CD44 dependent manner. Activation of AMPK promotes cellular glucose uptake by inducing the trafficking of GLUT4-containing membrane vesicles to the membrane, inhibits pro-apoptotic cascades and enhances pro-survival signaling. Figure compiled from references [82, 101, 129].

Fig. 5: MIF agonists enhance MIF binding to its receptor, CD74.

(A) Chemical structures of three different MIF agonists MIF20, MIF21 and MIF33. (B) MIF binding to immobilized soluble CD74 (sCD74=CD74⁷³⁻²³²) in the presence of increasing concentrations of MIF agonists. (C) Biacore analysis and MIF/sCD74 equilibrium constants. MIF introduced into the flow phase with vehicle (upper panel) or MIF20 (lower panel). Figures A–C reused from “Wang J, Tong C, Yan X, Yeung E, Gandavadi S, Hare AA, Du X, Chen Y, Xiong H, Ma C, Leng L, Young LH, Jorgensen WL, Li J, Bucala R. Limiting cardiac ischemic injury by pharmacological augmentation of macrophage migration inhibitory factor-AMP-activated protein kinase signal transduction. *Circulation*. 2013;128:225–36. <http://circ.ahajournals.org/content/128/3/225.long>” (D) Computational model showing three MIF20 molecules bound within the MIF tautomerase site, which forms at the interface of two adjacent monomers. (E) Computational model showing MIF interaction with three MIF receptor (CD74) proteins forming a dodecamer. Model taken from “Meza-Romero R, Benedek G, Leng L, Bucala R, Vandenbark AA. Predicted structure of MIF/CD74 and RTL1000/CD74 complexes. *Metab Brain Dis*. 2016;31:249–55. <http://link.springer.com/article/10.1007/s11011-016-9798-x>”.