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REVIEWS

D-dopachrome tautomerase in cardiovascular and inflammatory diseases—A new kid on the block or just another MIF?

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Abstract

Macrophage migration inhibitory factor (MIF) as well as its more recently described structural homolog D-dopachrome tautomerase (D-DT), now also termed MIF-2, are atypical cytokines and chemokines with key roles in host immunity. They also have an important pathogenic role in acute and chronic inflammatory conditions, cardiovascular diseases, lung diseases, adipose tissue inflammation, and cancer. Although our mechanistic understanding of MIF-2 is relatively limited compared to the extensive body of evidence available for MIF, emerging

Abbreviations: ACKR3, atypical chemokine receptor 3; AIF, apoptosis-inducing factor; AKT/PKB, protein kinase B; AMPK, AMP-activated protein kinase; AP-1, activator protein-1; ARDS, acute respiratory distress syndrome; ASCVD, atherosclerotic cardiovascular diseases; Asn, asparagine; ATM, adipose tissue macrophage; BNPL1, boehmite nanoparticles ligand 1; CABG, coronary artery bypass grafting; CALC, Cys-Ala-Leu-Cys; CaMKK2, calcium/calmodulin-dependent protein kinase kinase 2; CANTOS, Canakinumab Anti-inflammatory Thrombosis Outcome Study; ccRCCs, clear cell renal cell carcinomas; cDNA, complementary deoxyribonucleic acid; CD74, cluster of differentiation 74; Chr., chromosome; CLIP, class II-associated invariant chain peptide; COPD, chronic obstructive lung disease; COVID-19, coronavirus disease-19; COX2, cyclooxygenase-2; CREB, cAMP-responsive element binding protein; CSN5/JAB1, COP9 signalosome complex subunit 5/c-Jun activation domain-binding protein-1; CVD, cardiovascular disease; CXCR, C-X-C motif chemokine receptor; Cys, cysteine; D-DT, D-dopachrome tautomerase; DHICA, 5,6-dihydroxy-indole-2-carboxylic acid; DKO, Mif/Mif-2 double knockout; DLE, discoid lupus erythematosus; DOPD, D-dopachrome decarboxylase; EAE, experimental autoimmune encephalitis; ERK, extracellular signal-regulated kinase; Gαi, G-protein alpha subunit; GTEx, genotype-tissue expression; HF, heart failure; HIF-1α, hypoxia inducible factor-1α; HPA, human protein atlas; HPAECs, human pulmonary artery endothelial cells; HPP, 3-(4-hydroxyphenyl)pyruvate; hsCRP, high-sensitivity C-reactive protein; HSL, hormone-sensitive lipase; ICD, intracellular domain; IFN-γ, interferon-γ; IL-1β, interleukin-1β; IL-6, interleukin-6; Ile, isoleucine; ISO-1, (S,R)-3-(4-hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid methyl ester; JNK, c-Jun N-terminal kinase; LDL, low density lipoprotein; LPS, lipopolysaccharide; Lys, lysine; MAPK, mitogen-activated protein kinase; MDL, MIF/D-DT-like; MHC, major histocompatibility complex; MIF, macrophage migration inhibitory factor; MIF-2-cKO, cardiomyocyte-specific Mif-2-deficient; MMPs, matrix metalloproteinases; MS, multiple sclerosis; mTOR, mammalian target of rapamycin; mutSOD1, mutant superoxide dismutase 1; NAFLD, non-alcoholic fatty liver disease; NB, neuroblastoma; NF-κB, nuclear factor kappa B; NK, natural killer; NLRP3, NOD-like receptors pyrin domain-containing 3; NSCLC, non-small cell lung cancer; PDAC, pancreatic ductal adenocarcinoma; PGE2, prostaglandin E2; PI3K, phosphoinositide 3-kinase; PKA, protein kinase A; Pro, proline; PRX, peroxiredoxin; p115, Golgi protein p115; p53, tumor protein p53; RA, rheumatoid arthritis; RCT, randomized clinical trial; RIP, regulated intramembrane proteolysis; rMIF-2, recombinant MIF-2; sCD74, soluble CD74; SGBS, Simpson-Golabi-Behmel syndrome; SLE, systemic lupus erythematosus; SREBP, sterol-regulatory element binding protein; SVF, stromal vascular fraction; TAC, transverse aortic constriction; TNF-α, tumor necrosis factor-α; TRP, tyrosine-related protein; TRX, thioredoxin; Tyr, tyrosine; VEGF, vascular endothelial growth factor; WAT, white adipose tissue; ZAP-70, zeta-chain-associated protein kinase 70; 4-IPP, 4-iodo-6-phenylpyrimidine; 3D, three-dimensional; 4-CPPC, 4-(3-Carboxyphenyl)-2,5-pyridinedicarboxylic acid; 6-PP, 6-phenylpyrimidine.

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data suggests that MIF-2 is not only a functional phenocopy of MIF, but may have differential or even oppositional activities, depending on the disease and context. In this review, we summarize and discuss the similarities and differences between MIF and MIF-2, with a focus on their structures, receptors, signaling pathways, and their roles in diseases. While mainly covering the roles of the MIF homologs in cardiovascular, inflammatory, autoimmune, and metabolic diseases, we also discuss their involvement in cancer, sepsis, and chronic obstructive lung disease (COPD). A particular emphasis is laid upon potential mechanistic explanations for synergistic or cooperative activities of the MIF homologs in cancer, myocardial diseases, and COPD as opposed to emerging disparate or antagonistic activities in adipose tissue inflammation, metabolic diseases, and atherosclerosis. Lastly, we discuss potential future opportunities of jointly targeting MIF and MIF-2 in certain diseases, whereas precision targeting of only one homolog might be preferable in other conditions. Together, this article provides an update of the mechanisms and future therapeutic avenues of human MIF proteins with a focus on their emerging, surprisingly disparate activities, suggesting that MIF-2 displays a variety of activities that are distinct from those of MIF.

KEYWORDS
atypical chemokine, chemokine, cytokine, D-dopachrome tautomerase (D-DT), inflammation, macrophage migration inhibitory factor (MIF), receptor, signaling

1 | INTRODUCTION

Cytokines and chemokines orchestrate cellular immune processes and exhibit pivotal roles in host innate and adaptive immunity. Accordingly, deregulated cytokine/chemokine responses are associated with numerous human diseases including inflammatory and autoimmune disorders, cardiovascular, metabolic, and neurodegenerative diseases, as well as cancer.[1] Cytokine and chemokine-directed therapeutic strategies have led to several approved drugs,[2] but in atherosclerotic cardiovascular diseases (now also often abbreviated as ASCVD), cytokine/chemokine-based therapeutics are just emerging.[3] In fact, while preclinical evidence for a causal role of inflammatory cytokines and atherogenic chemokines is overwhelming, no anti-cytokine/chemokine drug has yet reached the clinic for ASCVD. The large-scale randomized clinical trial (RCT) Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS) demonstrated that antibodies against the innate immune cytokine interleukin-1β (IL-1β) reduced recurrent cardiovascular events in atherosclerotic patients with a residual inflammatory risk,[4,5] clinically validating the inflammatory paradigm of atherosclerosis.[6–11] In this context, residual inflammatory risk was defined as on-treatment high-sensitivity C-reactive protein (hsCRP) ≥2 mg/L and low density lipoprotein (LDL)-cholesterol <70 mg/dl after initiating aggressive statin therapy.[12] Similar conclusions were reached by related RCTs, together pointing to the inflammasome-IL-1β-IL-6 innate immune axis as a promising target.[13] However, interfering with this axis came at the expense of a significantly increased mortality risk due to pulmonary infections and no overall effect on all-cause mortality was observed, calling for a follow-up quest to identify more specific targets and tailored therapies. Chemokines could represent such target molecules, as they are locally expressed in the atherogenic vasculature and are the key orchestrators of atherogenic leukocyte recruitment responses. Indeed, several classical chemokines have been pursued as targets in ASCVD, including CC-chemokines such as CCL2 and CCL5, or CXC chemokines such as CXCL1/8, but, while promising, to date none of the inhibitory strategies directed against these chemokines has reached advanced stages of clinical trials in the cardiovascular domain.[9,14–18] In addition to these well-studied classical chemokines, atypical chemokines are an emerging group of inflammatory mediators that could represent interesting target proteins with utility in ASCVD. Atypical chemokines lack the bona fide N-terminal cysteine motif of classical chemokines and the classifying chemokine-fold, but share certain structural properties with chemokines, a phenomenon sometimes termed "chemokine mimicry", and hence are capable of engaging in high-affinity interactions with one or more chemokine receptors. The structural and

functional multitasking properties of atypical chemokines have been summarized in a recent comprehensive review article.[19]

Macrophage migration inhibitory protein (MIF) is an evolutionarily conserved inflammatory cytokine and prototypical atypical chemokine. MIF was discovered as one of the first cytokines over half a century ago,[20] and re-discovered as T-cell factor, pituitary-derived inflammatory cytokine, and endogenous glucocorticoid antagonist. Following the molecular identification of the *Mif* gene[21–23] a quarter of a century later, today, MIF as well as its more recently described structural homolog D-dopachrome tautomerase (D-DT; now also termed MIF-2), are known as multifunctional cytokines and chemokines with key roles in host immunity and homeostasis.[19,24–26] They also have an important pathogenic role in acute and chronic inflammatory conditions, cardiovascular diseases, lung diseases, neurodegenerative diseases, adipose tissue inflammation, and cancer, as summarized in previous review articles.[27–40] Curiously, both MIF and MIF-2 share a striking structural similarity and conserved N-terminal tautomerase pocket with a family of bacterial tautomerases, and display catalytic tautomerase activity in vitro[41–43] (Figure 1). Although the physiological relevance of this activity has yet to be elucidated, the existence of a catalytic pocket in the structure of these cytokines offers intriguing possibilities for the development of small molecule inhibitors.[44,45]

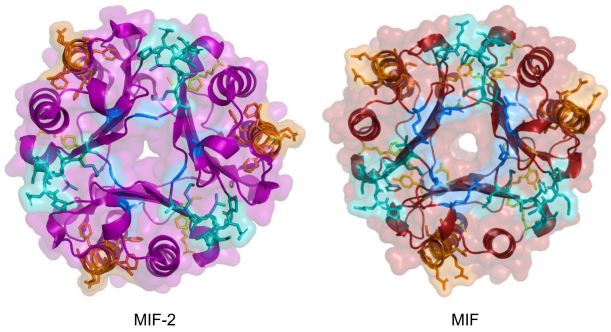

FIGURE 1 Structures of MIF-2 and MIF. Depicted are the three-dimensional structures of human MIF-2 (left, magenta) and human MIF (right, red). The trimeric structures are depicted as cartoon models and their surfaces are shown. Residues predicted to be relevant for receptor interactions are rendered as stick models and colored as follows: the N-terminal tautomerase pocket is highlighted in yellow. Proline-2 is indicated and contributes to CD74 binding for both MIF-2 and MIF together with residues 80–87 (orange). Of note, MIF-2 does not contain a pseudo-*E*LR motif which MIF features, leading to a major predicted functional difference between MIF-2 and MIF. The pseudo-*E*LR motif (blue) and N-like loop (cyan) are important for MIF's interaction with the CXC-type chemokine receptor CXCR2, while the N-like loop (cyan) also is involved in CXCR4 binding. On MIF-2, the corresponding residues are highlighted according to the same color scheme. Both proteins are visualized based on PDB-entry 1MIF (MIF) and 7MSE (MIF-2), using the PyMOL molecular graphics system, Version 2.0 (Schrödinger, LLC.).

Although our mechanistic understanding of MIF-2 is relatively limited compared to the extensive body of evidence available for MIF, emerging data suggests that MIF-2 is not only a functional phenocopy of MIF, but may have differential or even oppositional activities, depending on the disease and context. For example, while MIF and MIF-2 appear to exhibit unidirectional or cooperative activity in endotoxemia, some cancers, chronic obstructive pulmonary disease (COPD), and cardiac ischemia/reperfusion injury and heart failure,[27,46–54] distinct or opposite effects of the homologs are emerging in adipose tissue inflammation, fatty liver disease and hepatic fibrosis, or advanced atherosclerosis.[55–60]

Considering the remarkable 3D structural homology but remote sequence identity of MIF and MIF-2 (Figure 1), we here summarize and discuss the emerging evidence that these sister molecules may have context-dependent distinct activities. While primarily focusing on their roles in cardiovascular, metabolic, autoimmune, and inflammatory conditions, we will cover characteristics of MIF and MIF-2 in other disease entities as well. Most importantly, we will make an attempt to scrutinize the potential molecular underpinnings, such as structural distinctions, receptor specificities and downstream signaling pathways, as well as cell- and tissue-specific expression characteristics. Lastly, we discuss how an improved understanding of such differential structure–activity features might enable for future precision medicine approaches to selectively -or jointly- target MIF and MIF-2, depending on the disease and patient context.

2 | DISCOVERY, EVOLUTIONARY ORIGIN, TAUTOMERASE ACTIVITY, AND STRUCTURAL CHARACTERISTICS OF MIF-2 IN COMPARISON TO MIF

MIF was identified as a soluble, T cell-derived factor inhibiting random macrophage migration in 1966 with additional indirect evidence dating back to the year 1932, making it one of the first cytokines to be discovered.[20,61] Its molecular cloning and re-discovery as pituitary-derived mediator of endotoxemia, macrophage cytokine, and endogenous glucocorticoid antagonist led to a re-definition of MIF as more widely expressed pleiotropic inflammatory cytokine.[21–23,62] Further discoveries and the establishment of molecular tools in the ensuing years encompassed the elucidation of the three-dimensional structure of MIF,[63,64] protocols to produce endotoxin-free recombinant MIF,[65] the generation of a *Mif*-deficient knockout mouse,[66] or the

production of neutralizing monoclonal antibodies,[67] and provided invaluable assets for structure–function studies and the exploration of MIF's in vivo significance in a variety of disease models.

Although recent analyses of the evolutionary tree of MIF proteins relating it to a highly conserved family of MIF/D-DT-like (MDL) proteins in plants and other kingdoms suggest that D-DT is the older family member, D-DT/MIF-2 research in humans and mammalians has been lagging behind. MIF is established as a pivotal pathogenic player in numerous acute and chronic inflammation conditions, autoimmunity, atherosclerotic diseases, and cancer. On the other hand, identified tissue-protective activities of MIF in hepatic fibrosis and non-alcoholic fatty liver disease (NAFLD), COPD, and in the early phase of cardiac ischemia/reperfusion injury, also established that MIF may display context-dependent dichotomous activities. Very recent findings indicate that oxidative redox alterations as they might occur in inflammation-related microenvironments could lead to molecular signatures of the MIF molecule that could be a structural basis for its pathogenic activities.[68,69] As mentioned above, little is known about MIF-2 and studies exploring its structure–activity relationships and role in diseases have been conducted only relatively recently.

First reports on the purification of a dopachrome tautomerase activity from B16 mouse melanoma tumor tissue date back to the year 1990. It was named according to its property to catalyze the discoloration of dopachrome through catalyzing a tautomeric shift on the dopachrome ring structure.[70] D-dopachrome tautomerase (D-DT, MIF-2), also termed D-dopachrome decarboxylase (DOPD), was unexpectedly discovered when tautomerization reactions of L-dopachrome were investigated in cultured melanoma cells, using D-dopachrome as a supposed control substrate.[71] The cytoplasmic enzymatic tautomerase activity that was linked to the conversion of D-dopachrome into 5,6-dihydroxy-indole-2-carboxylic acid (DHICA) was then named D-DT. As alluded to above, we here mostly refer to this MIF homolog as MIF-2. MIF-2 is widely expressed in almost all tissues and cells, but its expression abundance differs appreciably from that of MIF, with the most prominent expression of MIF-2 noted in the liver[25,46,71] (Figure 2, Table 1). It is well known that transcriptional regulation of *MIF* gene expression upon hypoxic or inflammatory/infectious stimulation is controlled by hypoxia-inducible factor-1 (HIF-1)α and cAMP-responsive element binding protein (CREB) or SP1 and CREB, respectively.[72–74] Moreover, glucocorticoids (involving the GRE and ATF/CRE transcription factor binding sites), peptide hormones, cancerogenic stress, and glucose have also been implicated in controlling MIF gene expression.[75,76] Importantly, analysis of upstream regulatory regions has revealed a key role for the -794 $CATT_{5-8}$ microsatellite and the transcription factor ICBP90 as well as the -173-G/C SNP in controlling MIF gene expression.[29,77–79] In comparison, little is known about the regulation of *MIF-2* gene expression and the regulated secretion of this MIF homolog. For example, upstream regulatory elements or microsatellites such as -794 $CATT_{5-8}$ have not been identified for the MIF-2 gene. However, Iwata and colleagues obtained evidence for MIF-2 transcriptional upregulation by adenosine-monophosphate (AMP) kinase activation in adipocytes[80] and Pasupuleti et al. showed that MIF-2 is a hypoxia-inducible gene in renal cancer cells indicating HIF-1-controlled gene expression similar to the MIF gene.[81] Similarly, while the release pathway of MIF has been amply characterized to

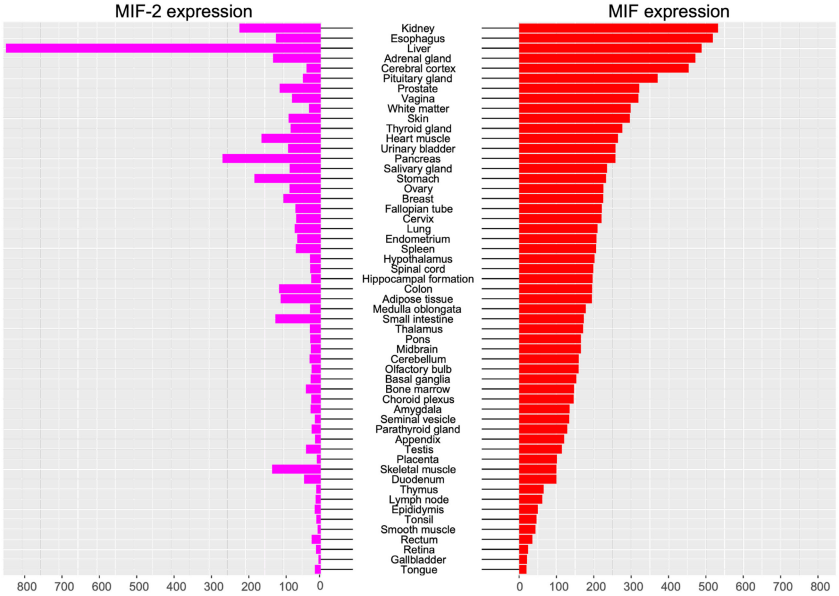

FIGURE 2 Expression pattern of MIF-2 and MIF across different human tissues. Relative mRNA levels of MIF-2 (left, magenta bars) and MIF (right, red bars) in 56 human tissues are mapped according to RNA consensus tissue gene data from https://www.proteinatlas.org/. Transcriptomic data for the samples from Genotype-Tissue Expression (GTEx) and human protein atlas (HPA) are summarized and normalized as nTPM values via a tailored pipeline to enable comparisons among different tissues.

TABLE 1 Genes, structures, expression, tautomerase activity, structure–activity features, receptors/interactors: commonalities and differences between D-DT/MIF-2 and MIF

Commonalities between MIF-2 and MIF			
Features	**Human**	**Rat**	**Mouse**
Genomic location	Chr. 22	Chr. 20	Chr. 10
cDNA	50% identity	25% identity	40% identity
Protein	34% sequence identity	27% sequence identity	27% sequence identity
	49% homology	53% homology	48% homology
Monomer	Each monomer possesses two βαβ motifs and an additional two β-strands to form the interface between monomers		
Trimer	Each trimer has similar trimeric packing and a highly similar 3D topology		

Differences between MIF-2 and MIF			
Features	**Sub-features**	**MIF-2/D-DT**	**MIF**
Expression	Tissue distribution	Abundant MIF-2 expression is largely limited to liver and kidney	Abundant MIF expression is relatively ubiquitous
Structural differences	Cysteine residues (human)	Cys-24, Cys-57	Cys-57, Cys-60, Cys-81
	Potential glycosylation sites (human)	Asn-39, Asn-74	Asn-73, Asn-110
	Active site[a] and surrounding area	MIF-2 is positively charged in the active site, whereas negatively charged in the surrounding area	MIF protein is positively charged in the active site as well as the surrounding area
Tautomerase properties	Reaction type	Tautomerization followed by decarboxylation	Pure tautomerization
	Tautomerized product	5,6-dihydroxyindole	5,6-dihydroxyindole-2-carboxylic acid
	Residues implicated in substrate binding	Pro-2, Lys-33, Ile-65	Pro-2, Lys-33, Ile-65, Tyr-96, Asn-98
	Enzymatic parameters	K_M: 1.13 mM (HPP as substrate)	K_M: 1.20 mM (HPP as substrate)
		k_{cat}: 62.4 s^{-1}	k_{cat}: 38 s^{-1}
Receptors and other interactors	Surface receptors	CD74, ACKR3, (CXCR4)	CD74, CXCR2, CXCR4, ACKR3
	Other binding proteins	Unknown	CSN5/JAB1, mutSOD1, p53, BNPL1, PRX, TRX, AIF, p115

Abbreviations: ACKR3, atypical chemokine receptor 3; AIF, apoptosis-inducing factor; Asn, asparagine; BNPL1, boehmite nanoparticles ligand 1; cDNA, complementary deoxyribonucleic acid; CD74, cluster of differentiation 74; Chr, chromosome; CSN5/JAB1, COP9 signalosome complex subunit 5/c-Jun activation domain-binding protein-1; CXCR, C-X-C motif chemokine receptor; Cys, cysteine; D-DT/MIF-2, d-dopachrome tautomerase; HPP, 3-(4-hydroxyphenyl)pyruvate; Ile, isoleucine; Lys, lysine; MIF, macrophage migration inhibitory factor; mutSOD1, mutant superoxide dismutase 1; Pro, proline; PRX, peroxiredoxin; p115, Golgi protein p115; p53, tumor protein p53; TRX, thioredoxin; Tyr, tyrosine; 3D, three-dimensional.

[a]Related to the tautomerase activity.

follow an unconventional secretion mechanism involving the NLRP3 inflammasome, ATP-binding cassette transporters and/or gasdermin-like pores as well as the Golgi-associated protein p115,[82–87] virtually nothing is known about the secretion mechanism of MIF-2, except that it is promoted by inflammatory and hypoxic conditions and cancerogenic cell stress.[46,53,88]

The studies on MIF-2 from rat liver and later on from red blood cells also led to its initial biochemical characterization and determination of a molecular weight of about 12 kDa for its monomeric form.[89] MIF-2 has no similarities to any other enzymes that catalyze the conversion of L-dopachrome into DHICA during melanin biosynthesis, nor to D-amino acid oxidase[90] or the L-dopachrome tautomerases tyrosine-related proteins TRP-1 and TRP-2.[91] A first link to MIF was noticed in the EST databank by Zhang et al. after they cloned a cDNA encoding rat D-DT/MIF-2.[92] Rat MIF-2 shares 27% identity and 53% homology with rat MIF,[92] while human MIF-2 and MIF are 34% identical.[46,93] The open reading frame of human MIF-2 encodes for 118 amino acids compared to 115 for MIF, with the N-terminal methionine of both homologs being cleaved immediately upon

biosynthesis in most tissues.[46] Esumi and co-workers also found that both proteins locate to the same overall genomic locus; chromosome 10 in the mouse and chromosome 22 in the human genome (Table 1). Importantly, the X-ray crystallographic analysis revealed that the three-dimensional (3D) structures of MIF and MIF-2 are strikingly similar[63,94] (Figure 1). Both proteins crystallize as trimers with essentially identical folds and topology and with a total molecular weight of about 37 kDa (Table 1, Figure 1). Trimerization is essential for the catalytic tautomerase activity, as residues from adjacent subunits contribute to the catalytic cavity. Although trimerization also appears to be necessary for the interaction of both homologs with the cognate receptor cCD74,[95–98] the role of oligomerization is less clear for MIF- versus MIF-2-mediated effects through other receptors/interactors (see also chapter on MIF-2 receptors below). Of note, it is unlikely that MIF-2 is extensively prone to redox modification and redox regulation similar to MIF, as the MIF-2 sequence lacks the redox-sensitive cysteines within the Cys-Ala-Leu-Cys (CALC) motif and at sequence position 81 (Cys-81)[68,99–101] (Table 1). Whether Cys-24 and Cys-57 of MIF-2 may fulfill this property has not been studied. In this context, we refer to the comprehensive review article of Schindler et al.,[102] in which the hitherto detected post-translational modifications of MIF have been summarized. In addition to the aforementioned processing of the N-terminal methionine residue and the oxidation of the MIF cysteine residues, these are an S-nitrosylation of Cys-81, a cysteinylation of Cys-60, a non-canonical O-glycosylation of Ser-122/Thr-113, and a phosphorylation of Ser-91.[102] In addition, due to its unusual nucleophilic property, the reactive N-terminal Pro-2 residue has been found to be prone to numerous modifications, such as an oxidation under formation of a prolinimine, a carbamylation, or the covalent binding of isothiocyanates and tautomerase pocket-targeting small molecule inhibitors such as 4-IPP.[102] Not surprisingly given the structural conservation of the tautomerase pocket between MIF and MIF-2, modifications such as prolinimine formation and 4-IPP modification have also been identified for MIF-2.[103–105] Functional consequences of the various post-translational modifications of MIF and MIF-2 and effects on pathogenic activities are not yet understood well. The determined enhancing effect of the S-nitrosylation of Cys-81 on the cardioprotective activity of MIF in the early phase of ischemia–reperfusion injury in the heart is an exception in this regard[106] (see also the chapter on myocardial diseases). Systematically studying the post-translational modifications of MIF and MIF-2 in a disease context e.g., by proteomic approaches will be helpful to broaden our context-related understanding of these cytokines.

Interestingly, recent cross-kingdom studies of D-DT/MIF family proteins, including plant MDLs, not only revealed that the family is evolutionarily highly conserved, with its orthologs in other phylogenetic branches dating back by over 900 000 000 years, but also provided evidence that MIF-2 is likely more closely related to these ancestral *MIF*-related genes than MIF, and suggest that MIF proteins have similar 3D structures across kingdoms.[107,108] This is consistent with the high degree of conservation of the catalytic site with other non-mammalian tautomerases.[41]

In view of an as yet elusive physiological substrate for the tautomerase activity of mammalian MIF and MIF-2, it is currently believed that the tautomerase pocket is an evolutionary remainder without intrinsic catalytic utility in mammalian cells.[109,110] Intriguingly though, structure–activity studies demonstrated that mutations of residues constituting the catalytic pocket of human MIF, such as substitutions of the proline-2 (Pro-2) residue or adjacent insertions, lead to an impairment of MIF binding to its cognate receptor CD74 and its non-cognate receptor CXCR4. A conformational involvement of residues forming the pocket was confirmed by studies employing small molecule tautomerase inhibitors, together suggesting that the pocket is an interesting target structure in MIF-directed drug development.[95,105,111–113] Although these studies have so far mostly been performed for MIF, and although the cavity-forming residues slightly differ between MIF and MIF-2, it is likely that residues of the pocket also contribute to the receptor binding affinity of MIF-2. This suggests that small molecule tautomerase inhibitors such as ISO-1, 4-IPP, and MIF98 for MIF, or 4-CPPC and thieno[2,3-d]pyrimidine-2,4(1H,3H)-dione for MIF-2, respectively, could be valuable tools and drug leads, when it comes to develop receptor-specific MIF or MIF-2 inhibitors.[45,114] To this end, it is of note that 4-CPPC, which binds to MIF-2 by a unique induced fit mechanism involving C-terminal residues and exhibits a 15-fold selectivity for MIF-2 versus MIF,[113,115] effectively inhibits MIF-2/CD74 binding without affecting the binding of MIF to CD74.[113] Consistently, 4-CPPC inhibited MIF-2-mediated activation of CD74 and reduced CD74-dependent signal transduction.[113] Furthermore, it cannot be excluded that multi-omics screenings might eventually lead to an identification of a physiologically relevant tautomerase substrate, with implications for functional insights and additional translational opportunities.

3 | RECEPTORS AND SIGNALING PATHWAYS INSTIGATED BY MIF-2 AND COMPARISON TO MIF

The cell surface receptor spectrum engaged by MIF-2 is similar but not identical to that of MIF. Both homologs bind to cluster-of-differentiation 74 (CD74), also known as the invariant chain (Ii) of major histocompatibility (MHC)

class II. While endolysosomal CD74/Ii serves as critical MHC class II chaperone involved in the loading process of antigenic peptides, cell surface-expressed CD74 functions as the cognate receptor for both MIF and MIF-2, a property that appears to be fully independent of its class II accessory function.[116–118] Structure–activity and mutational studies have partially elucidated the binding regions on MIF and CD74 that form the interface, with a prominent role for MIF residue Pro-2, the amino acid sequence containing residues 79 to 87 and the C-terminus, as well as the class II-associated invariant chain peptide (CLIP)-containing ectodomain of CD74.[95,119] Depending on the cellular or disease context, MIF-mediated CD74 activation can trigger different downstream signaling pathways. Most notably, these are the ERK–MAPK/prostaglandin and PI3K/AKT pathways (Table 2). Activation of these pathways requires the recruitment of CD44 as signaling-competent accessory protein.[136,137] Alternatively, MIF ligation of CD74 can lead to regulated intramembrane proteolysis (RIP) and the release of an intracellular domain (ICD) of CD74 that has a nuclear trafficking capacity, acts as a transcription factor, and can activate NF-κB.[138–141] In turn, the liberated soluble CD74 ectodomain has been found to serve as a circulating scavenger to neutralize MIF-elicited signaling responses and its plasma levels have been (inversely) associated with disease progression.[118,119,142–145] How soluble CD74 regulates MIF-2 activity is poorly understood, but initial studies have observed correlations between soluble CD74 plasma levels and disease activity in burn and cardiac surgery patients.[145,146] The cardio- and

TABLE 2 Receptor activities triggered by MIF-2 and comparison to MIF

Receptors	Proteins	Context	Cells	Signaling	References
CD74	MIF-2	LPS-induced inflammation	Macrophages	MAPK/ERK	(46)
		Spinal cord injury	Astrocytes, neurons	COX2/PGE2	(120)
		Atherosclerosis, hepatic steatosis	Macrophages, hepatocytes	AMPK, AKT	(58)
		Adipogenesis	SGBS cells	MAPK/ERK	(121)
		Acute myocardial ischemia/reperfusion injury	Cardiomyocytes	AMPK	(48)
	MIF	LPS-induced inflammation	Macrophages	MAPK/ERK	(46)
		Acute myocardial ischemia/reperfusion injury	Macrophages, cardiomyocytes, myofibroblasts	JNK, AMPK, AKT	(49,50,122,123)
		Fatty liver disease	Hepatocytes	AMPK	(124)
		COPD	Epithelial cells	PI3K/AKT, MAPK/ERK	(125,126)
		Acute lung injury	Neutrophils, macrophages	MAPK/ERK	(127)
CXCR2	MIF-2	Not applicable	Not applicable	Not applicable	Not applicable (88)
	MIF	Atherosclerosis/cardiac ischemia	Monocytes, neutrophils, cardiomyocytes	Gαi-dependent signaling	(57,128–130)
		Sepsis	Macrophages	–	(88)
CXCR4	MIF-2	Atherosclerosis, hepatic steatosis	Macrophages, hepatocytes	AMPK, AKT	(58)
	MIF	Atherosclerosis	Monocytes, T cells, B cells	Gai-dependent signaling	(57,131)
		Infection	Neutrophils	Neutrophil extracellular traps	(132)
ACKR3	MIF-2	COPD	Epithelial cells, lung fibroblasts	PI3K/AKT	(56,133)
	MIF	Atherosclerosis	B lymphocytes	ERK1/2, ZAP-70	(134)
		Thrombosis, inflammation	Platelets	AKT	(135)

Abbreviations: ACKR3, atypical chemokine receptor 3; AKT/PKB, protein kinase B; AMPK, AMP-activated protein kinase; CD74, cluster of differentiation 74; COPD, chronic obstructive pulmonary disease; COX2, cyclooxygenase-2; CXCR, C-X-C motif chemokine receptor; ERK, extracellular signal-regulated kinase; Gαi, G-protein alpha subunit; JNK, c-Jun N-terminal kinase; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MIF, macrophage migration inhibitory factor; PGE2, prostaglandin E2; PI3K, phosphoinositide 3-kinase; SGBS, Simpson-Golabi-Behmel syndrome; ZAP-70, zeta-chain-associated protein kinase 70.

hepatoprotective activities of the MIF/CD74 axis in the ischemic heart and during hepatic fibrosis, respectively, involve signaling through the metabolic stress enzyme AMP-activated protein kinase (AMPK) as well as Jun kinase (JNK) pathways.[49,50] MIF-2 also activates protective CD74/AMPK signaling in cardiac ischemia, but unlike MIF, activation of this pathway by MIF-2, also involves an upstream activation of calcium/calmodulin-dependent protein kinase kinase 2 (CaMKK2). On the other hand, PI3K/AKT has been implicated as an upstream signaling component in the MIF/CD74/AMPK pathway.[48] It is currently unknown whether CD44 functions as a CD74 accessory protein in the MIF-2 pathway as well and whether MIF-2 also triggers RIP and the formation of a CD74-ICD.

Work exploring the chemokine-like activity of MIF and its pro-atherogenic role in atherosclerosis demonstrated that MIF engages in non-cognate binding of three CXC-type chemokine receptors, i.e., CXCR2, CXCR4, and CXCR7/ACKR3.[128,134,147] Structure–activity studies performed for the MIF/CXCR2 and MIF/CXCR4 interaction suggest that this is enabled by (partially) mimicking the classical chemokine ligand pseudo-*E*LR motif and N-loop for CXCR2, and the CXCL12 N-loop for CXCR4, respectively, representing features of chemokine mimicry.[112,128,148,149] Thus, MIF is a member of the emerging group of atypical chemokines that "highjack" classical chemokine receptors as a basis for primary or secondary extracellular "moonlighting" activities that enable these mediators to expand their functional spectrum. Such properties of MIF and other atypical chemokines have been previously summarized,[19] and will not be further elaborated on here. Regarding MIF, it could, however, be speculated that this evolutionarily conserved molecule with a supposed intracellular function in other kingdoms, may have acquired two moonlighting functions as an extracellular acting cytokine/chemokine: (i) one by highjacking the MHC class II invariant chain CD74, and (ii) a second one via highjacking certain CXC-type chemokine receptors. The structure–activity studies suggest this type of mimicry is specific, as MIF features certain structural similarities with the MHC protein fold and the receptor binding motifs of CXC chemokines such as CXCL1/8, respectively, whereas it does not bind to other chemokine receptors such as CXCR1, CXCR3, or CCR5, overall guaranteeing signaling specificity.[57,128,148,150] In contrast, MIF-2 only engages ACKR3[56]; plus, there is preliminary evidence that MIF-2 binds to CXCR4 owing to partially mimicking the N-loop of CXCL12 from work that is published on a preprint server but has not yet been peer-reviewed.[58] MIF-2 cannot interact with CXCR2 due to its missing pseudo-*E*LR motif[88] (Tables 1 and 2).

Moreover, CD74 has been suggested to form complexes with the MIF chemokine receptors CXCR2, CXCR4, and ACKR3 to fine-tune MIF-triggered atherogenic leukocyte migration responses.[57,134] Complex formation has so far mostly been studied by cell biology methods using overexpression of fusion proteins and a role for MIF-2 in heterologous CD74/CXCR complex formation has not yet been explored. Intriguingly, MIF but not MIF-2 was recently identified to elicit cooperative-sequential inflammatory macrophage activation involving CD74 followed by CXCR2 signaling in the context of polymicrobial sepsis.[88] Thus, different signaling bias paradigms can be realized by receptor complex formation or receptor cooperation in a longitudinal manner.[151]

The differential expression pattern of CD74 and the non-cognate receptor(s) of MIF-2 and MIF further informs about their potential involvement in different disease settings. CD74 is not only expressed by MHC class II-positive cells, i.e., mononuclear phagocytes, dendritic cells, and B cells, but also by thymic epithelial cells, cardiomyocytes, type II alveolar epithelial cells activated endothelial cells, as well as various kinds of tumor cells.[46,116,118,152] CXCR4 is essentially ubiquitously expressed, but may be profoundly upregulated upon inflammatory or redox stimulation.[153,154] Atypical chemokine receptor 3 is often co-expressed with CXCR4 in the same cell types such as fibroblasts, B cells, platelets, endothelial cells, and tumor cells, but overall, its expression is more restricted than that of CXCR4; thereby, at least partly, sharing similar signaling pathways with CXCR4 through interacting with CXCL12 or MIF.[56,134,135,147,155] CXCR2 has traditionally been viewed to be expressed by neutrophils, natural killer (NK) cells, dendritic cells, monocytes, some epithelial cells, and tumor cells,[156–158] but an upregulated expression upon inflammatory or ischemic stimulation has been observed in cardiomyocytes and additional tumor cells as well.[129] The tissue and cell-specific expression characteristics of the MIF and MIF-2 receptors may contribute to certain MIF- or MIF-2-specific pathogenic activities in a corresponding disease context.

4 | ROLE OF MIF-2 IN INFLAMMATORY DISEASES, INFECTION, AND CANCER

Although the number of studies on MIF-2 is still fairly limited compared to MIF, it has become clear that MIF-2 plays a pivotal role in a number of diseases. Here, we focus on its role in cardiovascular and metabolic/adipose tissue diseases, and summarize its contribution to acute inflammation, sepsis, COVID-19, cancer, and COPD/chronic pulmonary inflammation. We discuss the cooperative

versus disparate properties that MIF-2 may have compared to MIF (Figure 3).

4.1 | MIF-2 in acute inflammation, sepsis, and COVID-19, and comparison to MIF

Macrophages were found to produce MIF-2 as well as MIF in response to lipopolysaccharide (LPS). MIF-2 concentrations peaked 16 h after LPS stimulation. However, twenty-fold more MIF than MIF-2 was found to be released by cultured macrophages stimulated with LPS.[46] In addition, Merk and coworkers generated an anti-MIF-2-specific antibody, which showed a protective effect on mice upon lethal endotoxic shock by lowering the levels of pro-inflammatory cytokines such as interferon (IFN)-γ, interleukin (IL)-1β, tumor necrosis factor (TNF)-α, and IL-12p70. They also demonstrated that MIF-2 could activate inflammatory signaling through high affinity-binding to CD74 similar to MIF, for instance regulating macrophage migration and counteracting immunosuppressive effects of glucocorticoids.[46] However, a recent study by Tilstam et al. found that MIF-2, unlike MIF, did not support the recruitment of small inflammatory macrophages in a mouse model of polymicrobial sepsis. Mechanistically, this was attributed to the failure of MIF-2 to activate CXCR2-mediated

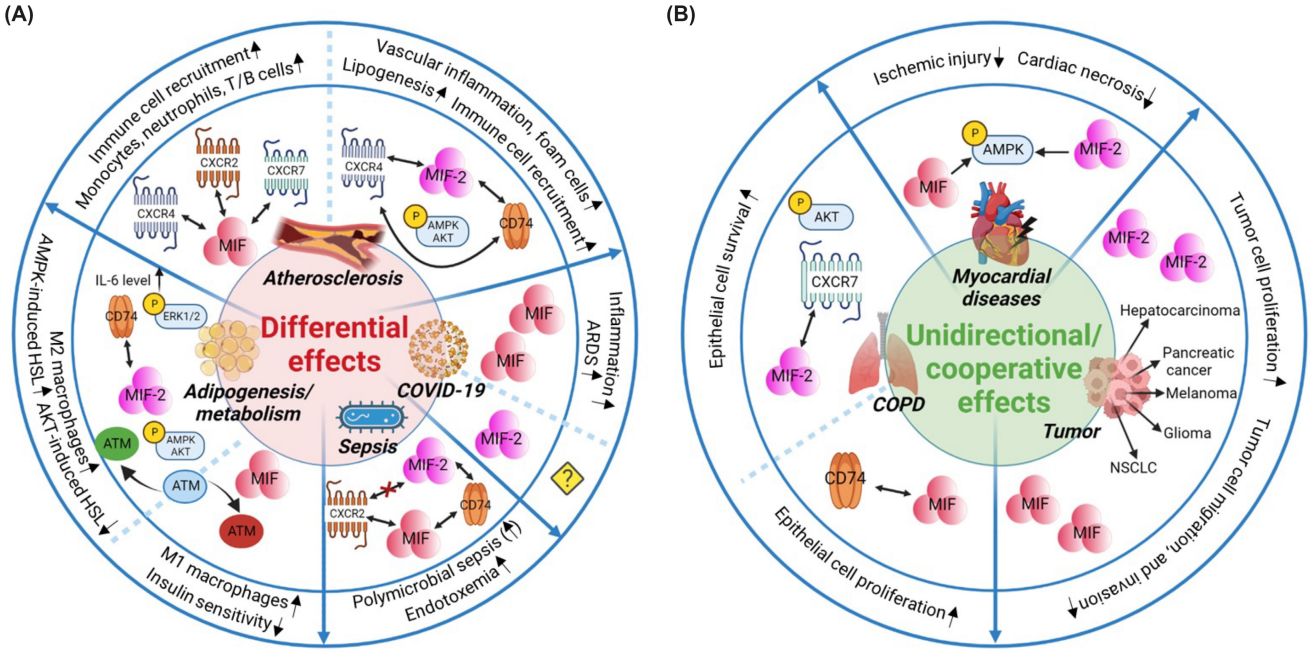

FIGURE 3 Cartoons summarizing current knowledge on the role of MIF-2 in various diseases, and comparison with MIF. (A) Focus on differential effects between MIF-2 and MIF as suggested in adipose tissue inflammation/metabolic disease, infectious diseases, and atherosclerosis. *Top central*, role of MIF-2 and MIF in atherosclerosis. Both cytokines have exacerbating effects in atherosclerosis. While the mechanisms are overlapping but not identical, both homologs promote leukocyte recruitment and vascular inflammation (for details see main text). *Bottom left*, role of MIF-2 and MIF in adipose tissue inflammation and metabolism. Opposite effects of MIF-2 and MIF have been suggested in adipose tissue inflammation and adipose tissue macrophage differentiation. *Bottom right*, role of MIF-2 and MIF in COVID-19, endotoxemia and sepsis. MIF levels correlate with inflammation and ARDS severity in COVID-19 patients, but MIF-2 has not been studied. Both MIF-2 and MIF promote endotoxemia pathogenesis, but MIF-2 does not promote polymicrobial sepsis due to a lack of effect on small inflammatory macrophages in the peritoneum. (B) focus on unidirectional/cooperative effects of MIF-2 and MIF as suggested for myocardial diseases, COPD/chronic pulmonary inflammation, and cancer. *Top central*, role of MIF-2 and MIF in myocardial diseases. MIF-2 and MIF have beneficial effects in acute myocardial ischemia and heart failure. While the CD74/AMPK pathway is involved for both homologs, the downstream pathways are overlapping but not identical (not shown in detail in the cartoon). *Left/bottom left*, role of MIF-2 and MIF in COPD/chronic pulmonary inflammation. Unidirectional effects on COPD, although through different receptors are observed for MIF-2 and MIF as indicated. *Bottom right*, role of MIF-2 and MIF in cancer. Both proteins promote tumorigenesis in several organs, affecting similar mechanisms. MIF, macrophage migration inhibitory factor; MIF-2/D-DT, D-dopachrome tautomerase; COPD, chronic obstructive pulmonary disease; ARDS: acute respiratory distress syndrome; COVID-19: coronavirus disease-19; CD74, cluster of differentiation 74; CXCR2, C-X-C motif chemokine receptor 2; CXCR4, C-X-C motif chemokine receptor 4; CXCR7, C-X-C motif chemokine receptor 7; AMPK, AMP-activated protein kinase; AKT/PKB, protein kinase B; ERK, extracellular signal-regulated kinase; IL-6: interleukin-6; ATM: adipose tissue macrophage; HSL, hormone-sensitive lipase; NSCLC, non-small cell lung cancer.

responses associated with an observed absence of a CXCR2-interrogating pseudo-*E*LR motif in MIF-2.[88] In contrast, Rajasekaran and colleagues found that MIF-2 promotes the recruitment of neutrophils into inflamed lungs.[105] While the role of CXCR2, an established neutrophil recruitment chemokine receptor, was not specifically explored in that study, the observed lack of involvement of CXCR2 in the macrophage recruitment experiments of Tilstam et al.[88] may suggest that MIF-2-mediated neutrophil recruitment may be supported by a CD74 and/or CXCR4 mechanism, or via an indirect mechanism, as implicated by Schindler et al., who obtained evidence for monocyte/macrophage—neutrophil crosstalk.[103]

Winner et al. revealed a covalent modification at the N-terminal proline of both MIF-2 and MIF by 4-IPP, which leads to the production of 6-phenylpyrimidine (6-PP) adduct.[159] These findings were confirmed by Rajasekaran and colleagues.[105] Despite their different tautomerase sites (Table 1), 4-IPP is likely to modify MIF-2 and MIF in a similar fashion. When Rajasekaran et al. individually administered modified MIF-2-6-PP or MIF-6-PP adducts in their lung neutrophil recruitment model, a partial reduction in recruitment by 50% in comparison with the effect induced by the apo proteins was noted. However, the joint administration of MIF-2-6-PP and MIF-6-PP did not lead to a synergistic effect.[105]

From a clinical perspective, Kim and coworkers investigated the significance of MIF-2 and soluble CD74 (sCD74) in twenty burn patients.[146] The study confirmed earlier data showing that MIF could be an independent predictive biomarker for patients with burn injury[160] and revealed that MIF-2 and sCD74 levels were elevated in burn patients compared to healthy controls. MIF-2 also displayed a positive correlation with other indices of burn in early stages such as procalcitonin levels. This indicates that circulating MIF-2 levels have a predictive value for burn patients.[146] Pohl et al. evaluated several laboratory parameters and mortality for 72 critically ill patients and showed that mortality was higher for patients with elevated plasma MIF-2, supporting the notion that MIF-2 could be a marker to assess the prognosis of critically ill patients.[161] In the context of acute inflammation and infections, MIF has been suggested to be a predictive biomarker in sepsis,[162–165] a good predictor of septic acute kidney injury,[166] and was shown to be an independent predictor of disease outcome in acute pancreatitis.[167] Baron-Stefaniak et al. reported that MIF-2 predicts outcome of acute kidney injury after orthotopic liver transplantation.[168] At last, MIF proteins have been assessed as early-stage predictive biomarkers for the severity of Coronavirus Disease 2019 (COVID-19), which is causing the worldwide Corona pandemic, and have been under active investigation so far. Even though there are no published data on MIF-2 yet, elevated MIF levels have been detected in COVID-19 patients, which may be related to the severity of ARDS in those patients.[169,170] In conclusion, MIF-2 appears to aggravate acute inflammatory conditions, including sepsis and burn injury. Overall, the observed effects are similar to those previously reported for MIF,[24] but distinct mechanistic differences have also been noted. While MIF is an established biomarker for acute disease conditions, in part with added value as "independent" predictor, much less is known for MIF-2, but data for this homolog are emerging.

4.2 | MIF-2 in COPD and chronic pulmonary inflammation, and comparison to MIF

In addition to acute inflammation, COPD is a good example for an involvement of MIF-2 in chronic (pulmonary) inflammation, with functional similarities but also differences observed between MIF-2 and MIF. MIF-2 was found to promote the proliferation and survival of lung epithelial cells. Moreover, a recent study reported that MIF-2 enhanced lung epithelial repair in COPD patients through interaction with ACKR3/CXCR7.[56] They observed that MIF-2 promoted A549 epithelial cell proliferation and conferred protection against apoptosis. Mechanistically, the ERK–MAPK and PI3K-AKT signaling pathways were identified to participate in this process. Overall, this study suggests MIF-2 as a novel regulator in COPD,[56] and also highlights ACKR3 as the second known receptor for MIF-2.[56,133] Similar to MIF-2, MIF has been identified as a lung-protective factor against COPD. However, unlike MIF-2, MIF maintains alveolar structures in the lung in a CD74-dependent manner.[125] MIF also acts as a repressor of p53 and a mediator of apoptosis, leading to an anti-apoptotic effect on human pulmonary artery endothelial cells (HPAECs).[171] Thus, even though MIF-2 and MIF exhibit mechanistic differences in their effects on different types of lung cells, they also share some common mechanisms to protect against COPD.[133]

4.3 | MIF-2 in cancer and comparison to MIF

Not surprisingly and in line with the paradigm of causality between chronic inflammation and cancer,[172,173] MIF-2 was found to be associated with tumorigenesis derived from organs such as lung,[53] colon,[174] kidney,[175] or pancreas.[176] Accordingly, MIF-2 is highly expressed in several tumor cell types. Coleman et al. found that MIF-2 and/or MIF enhance CXCL8 and vascular endothelial growth

factor (VEGF) expression in A549 lung cancer cells along with the activation of c-Jun N-terminal kinase (JNK), c-Jun phosphorylation, and activator protein-1 (AP-1) activation.[53] In addition, Xin et al. reported that MIF-2-activated cyclooxygenase-2 (COX-2) transcription was partially reliant on the stabilization and transcriptional regulation of β-catenin, as observed in colorectal adenocarcinoma cell lines.[174] In another study, they showed that CD74 is expressed at moderately high levels in HT-29 and HCT-16 cells, which endorsed previous results showing upregulated CD74 in a large portion of samples from patients with colorectal adenomas.[177] Thus, CD74 appears to be a key receptor for MIF-2 in colorectal cancerogenesis.

In a similar vein, MIF-2 plays a critical role in clear cell renal cell carcinomas (ccRCCs) by cooperation with MIF through survival signaling pathways.[175] MIF-2 displayed a similar expression pattern as MIF in ccRCC sections, accompanied by a high correlation between MIF-2 and MIF levels. More importantly, dual inhibition of MIF-2 and MIF resulted in a more pronounced phenotype than inhibition of each cytokine alone, indicating cooperativity and suggesting that a dual targeting strategy may have utility in cancer therapy. Guo and coworkers studied the significance of MIF-2 in pancreatic ductal adenocarcinoma (PDAC) and found that dual knockdown of MIF-2 and MIF attenuated the phosphorylation of AKT and ERK1/2, and upregulated the expression of p53. This was paralleled by inhibition of cell proliferation and invasion and led to inhibition of tumor growth.[176] Furthermore, 4-IPP, a non-selective inhibitor of both MIF and MIF-2, was capable of inhibiting cell proliferation and tumor formation.[176] Additionally, using HeLa and SiHa cell lines, Wang et al. demonstrated that the knockdown of MIF-2 and MIF not only inhibited proliferation, migration, and invasion of tumor cells, but also constrained the growth of xenograft tumors.[178] Other data from Gavalli and coworkers pointed out that MIF-2 and MIF might have overlapping effects on neuroblastoma (NB) tumorigenesis.[179] Of note, Brock et al. showed that in non-small cell lung cancer, MIF and MIF-2 act cooperatively to inhibit activation of AMPK in an LKB1-independent but CD74-dependent manner.[180] In summary, several studies have consistently shown an upregulated expression of MIF-2 and MIF in different kinds of human cancers, although much less data are available for MIF-2. Moreover, in NSCLC, pancreatic and gastric cancer, MIF-2 was shown to "cooperate" with MIF in regulating tumorigenesis.

5 | MIF-2 IN CARDIOVASCULAR DISEASES

Cytokines and chemokines including atypical chemokines are major drivers of CVDs. That also includes MIF proteins, which first became evident about two decades ago, when MIF was found to be upregulated in different atherosclerosis-relevant cell types such as immune cells, vascular smooth muscle cells, endothelial cells, and platelets, and was detected in different stages of atherosclerotic plaques in humans, mice, and rabbits.[181,182] These initial studies on the role of MIF in atherosclerosis kicked off extensive research of MIF family proteins in various cardiovascular disease conditions. The role of MIF in CVDs has been summarized before[34] and also discussed in two recent review articles.[27,28] Here, we summarize and discuss the emerging evidence on a key role of MIF-2 in three types of CVDs, i.e., myocardial ischemia/reperfusion injury, heart failure (HF), and atherosclerosis.

5.1 | Role of MIF-2 in myocardial infarction and ischemia/reperfusion injury, and comparison to MIF

An involvement of MIF-2 in CVDs was first studied in the context of myocardial ischemia and ischemia/reperfusion injury. Qi et al. noticed that murine cardiomyocytes express increased levels of MIF-2 and that MIF-2 is secreted by the heart after ischemic stress.[48] They generated conditional cardiomyocyte-specific *Mif-2*-deficient (MIF-2-cKO) mice and studied cardiac ischemia/reperfusion in an experimental model. To this end, the left descending coronary artery (LAD) was ligated for 20 min, followed by 3 h of reperfusion. Using this genetic model in combination with ischemia/reperfusion stress, cardiomyocyte *Mif-2*-deficient mice displayed an exacerbated response to ischemia/reperfusion under normal baseline.[48] In addition, they applied an isolated heart perfusion procedure (the "Langendorff heart"), featuring no-flow global ischemia for 15 min followed by reperfusion for 30 min. More necrosis and left ventricle contractile dysfunction were observed in hearts from MIF-2-cKO mice in comparison with control hearts. Moreover, and in line with the genetic data, administration of recombinant MIF-2 protected isolated Langendorff hearts from contractile dysfunction as well as ischemia–reperfusion injury. Mechanistically and similar but not identical to previous findings for MIF, this was found to be mediated by the AMPK signaling pathway. Together, these studies suggested that MIF-2, similar to MIF, has protective effects during ischemic heart injury.[48] Interestingly, a correlative clinical study undertaken in one hundred cardiovascular patients undergoing elective cardiac surgery by coronary artery bypass grafting (CABG),[145] indicated a differential relationship between MIF-2 and MIF plasma levels with the risk of developing post-surgery atrial fibrillation. Although this apparent discrepancy with the data obtained in the mouse model

could be explained by species differences and various clinical variables, it should be emphasized that associations observed in clinical studies do not necessarily indicate causality. Compensatory effects between MIF, MIF-2, and disease severity may also play a role. A clarification or specification of this point is needed, as it would guide future MIF-2-based therapeutic approaches.[27]

Beyond the cardiomyocyte CD74/AMPK axis,[48] MIF-2 receptor pathways have not specifically been studied in cardiac ischemia/reperfusion injury. MIF exerts cardioprotective activity in ischemia/reperfusion injury via CD74 and its cardioprotective effect is further enhanced by S-nitrosylation at Cys-81, in particular during the ischemic phase and in the early reperfusion phase. MIF also likely contributes to inflammatory leukocyte recruitment in the medium-late reperfusion phase through CXCR2 and CXCR4,[27,28,50,129] whereas MIF-2 is not a ligand for CXCR2. This may support the hypothesis that MIF-2-triggered inflammatory effects in the late ischemic or reperfusion phase are not as pronounced as for MIF. More mechanistic studies on MIF-2 in the context of cardiac ischemia/reperfusion injury are needed in the future to deepen our understanding of the mechanisms and to scrutinize therapeutic options.

5.2 | Role of MIF-2 in heart failure and comparison to MIF

Emerging studies also suggest a role for MIF-2 in heart failure (HF) and may indicate that MIF-2 behaves somewhat differently from MIF.[47] Luedike et al. detected circulating MIF in patients with HF, and further revealed a potential association between MIF levels and clinical endpoints of these patients,[183] offering clues for further studies. However, there are no clinical data available on MIF-2 yet.

Experimental evidence is available from an extensive study in a mouse model of HF. Ma and coworkers investigated the involvement of MIF-2 in HF development based on an observed high expression of MIF-2 in cardiomyocytes. To induce cardiac pressure overload, they performed surgical transverse aortic constriction (TCA) on MIF-2-cKO mice and compared these animals to corresponding control mice. Following this HF-mimicking procedure, significantly more pronounced cardiac contractile dysfunctions, pulmonary edema, and cardiac dilatation were observed in MIF-2-cKO mice.[47] Mechanistically, cardiomyocytes isolated from MIF-2-cKO mice showed impaired contractility, calcium transients, and downregulated sarcoplasmic reticulum calcium ATPase after TCA, when compared with control cardiomyocytes.[47] Additionally, recombinant MIF-2 (rMIF-2) showed an anti-fibrotic function through diminishing TGF-β-induced SMAD-2 activation in cardiac fibroblasts, confirming a protective function for MIF-2 in HF.

To some extent, there are distinct pharmacological activities of MIF-2 and MIF in the heart. For example, in contrast to MIF, MIF-2 was incapable of inhibiting cardiac contractility, which could be due to a lack of a negative inotropic effect in the presence of MIF-2.[47,48]

5.3 | Role of MIF-2 in atherosclerosis and comparison to MIF

MIF has been amply studied in atherosclerosis. A unanimous body of evidence now suggests that MIF has proatherogenic properties through promoting lesional leukocyte recruitment, upregulating adhesion molecules, and enhancing vascular inflammation via several other pathways including cytokine secretion and activation of matrix metalloproteinases (MMPs).[9,27,28,34,35]

In contrast, there is no peer-reviewed published study available yet that has interrogated, whether MIF-2 also contributes to atherosclerosis. However, emerging data now provides preliminary evidence for a causative role of MIF-2 in atherosclerosis, while a distinct activity profile different from that of MIF also likely.[58] In this study, we generated *Mif-2* knockout mice in an *Apoe*$^{-/-}$ background, studied these mice in models of early and advanced atherosclerosis, and explored the atherogenic mechanisms of MIF-2. The data suggest that MIF-2 is an atherogenic chemokine that promotes monocyte and lymphocyte migration and supports arterial monocyte arrest via CXCR4.[58] While this is an activity that is similar to MIF's pro-atherogenic chemokine activities, MIF-2 appears to be the even stronger chemokine, as indicated by MIF-2/MIF competition 3D migration experiments.[58] At the same time, chemotactic effects elicited by MIF-2 are unlikely to involve CXCR2 engagement.[88] Morphometric plaque analysis suggests that *Mif-2* knockout mice exhibit reduced lesions and plaque macrophage counts in both early and advanced stages of atherosclerosis. The latter also implies that MIF-2-elicited lesional leukocyte recruitment promotes atherogenesis. Unexpectedly, the attenuating effect on plaque formation and vascular inflammation was accompanied by a reduction in hepatic lipid accumulation and steatosis. The latter is a surprising observation, because *Mif*$^{-/-}$*Apoe*$^{-/-}$ mice do not show a hepatic phenotype.[184] In line with this observation, *Mif-2*$^{-/-}$*Apoe*$^{-/-}$ mice exhibited reduced plasma triglyceride and cholesterol levels, which points towards a hepatic lipid metabolism phenotype of *Mif-2* knockout mice. Initial mechanistic studies implicate MIF-2 receptors CD74 and CXCR4 and the sterol-regulatory element binding proteins-1 and 2 (SREBP-1, SREBP-2) and their lipogenic downstream targets in this phenotype. While

preliminary,[58] these findings let us hypothesize that MIF-2 could be a driver of atherosclerosis that, unlike its homolog MIF, not only promotes atherogenic leukocyte recruitment and vascular inflammation, but also acts as a contributor to hepatic lipid accumulation (Figure 3).

6 | MIF-2 IN AUTOIMMUNE DISEASES

MIF is an established key player in the pathogenesis of rheumatoid arthritis (RA)[79,85,86,137,185–188] and systemic lupus erythematosus (SLE).[86,189–193] The available data on the mechanisms and clinical significance have been summarized in several excellent review articles[29,35,194–197] and will not be further discussed here. In contrast, very little is known about the role of MIF-2 in RA and SLE, but Vincent et al. recently compared the serum levels of MIF and MIF-2 from patients with systemic sclerosis and SLE. Their study encompassing 105 and 184 patients, respectively, suggests that serum MIF, but not MIF-2, is significantly higher in systemic sclerosis patients than in SLE patients and healthy controls,[193] indicating differential roles of MIF and MIF-2.

There is also initial evidence for a role of MIF-2 in other autoimmune conditions. Benedek et al. demonstrated that both MIF-2 and MIF levels went up in male patients with progressive multiple sclerosis (MS) compared with female MS patients, and inversely, *Mif-* or *Mif-2* deficient male mice developed less severe MS-like signs. This suggested that MIF-2 and MIF contributors to MS progression in a sex-specific manner, although this has not been systematically studied further, neither for MIF nor for MIF-2.[198] Moreover, Vandenbark et al. for the first time generated *Mif/Mif-2* double knockout (DKO) mice ($Mif^{-/-}$ $Mif\text{-}2^{-/-}$) mice and revealed a reduction by ~25% in moderate experimental autoimmune encephalitis (EAE) in either *Mif* or *Mif-2* single knockout mice in comparison with controls.[199] Interestingly, there were no further reductions in EAE severity detected in the $Mif^{-/-}Mif\text{-}2^{-/-}$ DKO mice, suggesting an absence of a synergistic mechanism, at least in the specific EAE model studied.[199] The notion that disease- or model-specific aspects may govern the mode of cooperativity or non-cooperativity between MIF-2 and MIF is further supported by a study on discoid lupus erythematosus (DLE). In this context, MIF and MIF-2 were suggested to display opposite properties.[200]

7 | MIF-2 IN ADIPOSE TISSUE INFLAMMATION AND LIPID METABOLISM

Several studies have addressed the role of MIF-2 in adipose tissue inflammation and lipid metabolism. Ishimoto et al. identified MIF-2 as a unique adipokine with the function of regulating lipid metabolism.[121] They used rMIF-2 protein to stimulate the human preadipocyte cell line SGBS in vitro and found that rMIF-2 could upregulate IL-6 expression and secretion as well as ERK1/2 phosphorylation. Moreover, pretreatment with U0126, an ERK inhibitor, reduced IL-6 expression. Knockdown of CD74 in SGBS cells suppressed rMIF-2-elicited IL-6 upregulation on mRNA level, which indicates that the MIF-2-CD74-ERK signaling pathway participates in the upregulation of IL-6 expression, while at the same time inhibiting adipogenesis.[121] In summary, the evidence linking MIF, IL-6, and adipose inflammation is complicated due to the multiplicity and distinct effects of IL-6 in various organs. The same group reported that glucose intolerance was improved whilst serum-free fatty acids were reduced in *db/db* mice after administration with rMIF-2.[201] Furthermore, Iwata et al. investigated signaling pathways involved in this process, and observed that rMIF-2 administration in vivo increased AMPK phosphorylation-induced hormone-sensitive lipase (HSL) expression, whereas it decreased protein kinase A (PKA) activity-mediated HSL levels.[201] This suggests that both AMPK and PKA pathways are involved in MIF-2-regulated lipid metabolism.

Of interest, MIF-2 has been reported to show functional properties distinct from MIF in adipose tissue inflammation.[80,202,203] One report suggests that MIF-2 has protective effects on adipogenesis and pointed out that AMPK phosphorylation could promote MIF-2 transcription in SGBS adipocytes in vitro via modulating mammalian target of rapamycin (mTOR) signaling.[80] In contrast, the insulin sensitivity was improved in $Mif^{-/-}$ mice through inducing glucose uptake[202] and inhibiting adipose tissue macrophage infiltration,[203] implying that MIF plays an unfavorable role in the inflamed adipose tissue. Kim and colleagues investigated the potential effects of MIF family proteins, namely comparing MIF-2 and MIF, on white adipose tissue (WAT) in a murine endotoxemia model and observed an opposite effects.[55] Of note, *Mif-2* gene deletion induced the transition of adipose tissue macrophages (ATM) towards a pro-inflammatory type, whereas ATMs were found to display an anti-inflammatory type upon *Mif* gene deficiency. This suggests that MIF-2 skews ATMs towards an anti-inflammatory, M2-like, phenotype, whereas MIF promotes macrophage skewing towards an inflammatory subtype. Additionally, they observed that LPS stimulation could reduce MIF-2 levels in adipocytes, but did not affect its expression in the stromal vascular fraction (SVF), suggesting that adipocytes may be the main cellular source of MIF-2 in the inflamed WAT.[55]

Collectively, due to high expression of MIF-2 in liver and adipose tissue,[46] the roles of MIF-2 in adipose tissue inflammation and metabolic diseases are worth exploring. However, it is currently unclear, whether MIF-2 affects

physiological functions of adipocytes or hepatocytes and even pathological developments. With more studies of *Mif-2*$^{-/-}$ mouse models and the *Mif*$^{-/-}$*Mif-2*$^{-/-}$ DKO mouse model emerging, the role of MIF-2 in adipose tissue and metabolic diseases and inter-relations to CVDs will become clearer in the near future.

8 | CONCLUDING REMARKS

The importance of MIF family proteins in cardiovascular, autoimmune, and inflammatory diseases is well established, but most of the evidence comes from studies on MIF. However, as outlined in this review article, findings on MIF-2 are emerging, complementing the picture on the role of these cytokines in these diseases. These studies also indicate that MIF-2, despite being a close structural homolog of MIF, behaves in an, at least partially, distinct or even oppositional manner to MIF. Accordingly, while overlapping and partially identical, the utilized receptors and signaling pathways are distinct as well. Thus, future mechanistic studies are likely to lead to the identification of specific MIF-2- versus MIF-biased signaling paradigms with cell type-specific characteristics. This may eventually explain, on molecular level, the cooperative, synergistic, or antagonistic activities that are emerging for these two cytokines. While, overall, direct comparative evidence in the cardiovascular, autoimmune, and inflammatory disease area is still scarce, initial studies in tumor, HF, and COPD models suggest cooperative/synergistic MIF-2/MIF activities. This notion would argue for the development of joint targeting strategies for such conditions. On the other hand, MIF-2 and MIF appear to display at least partly oppositional functions in adipose tissue inflammation, metabolic disease, and atherosclerosis. While an extensive body of in-depth basic, pre-clinical, and eventually clinical studies in these areas is clearly mandated, the recently emerging evidence might already be suggestive of a need for ligand-specific precision strategies for these disorders. In any case, future mechanistic studies on MIF-2 including those directly comparing the effects of both agonists as well as studies capitalizing on single versus double-knockout approaches or using targeted pharmacological tools will greatly help us to understand the mechanistic basis of how these cytokines signal in a given cell, tissue, or disease context.

AUTHOR CONTRIBUTIONS

Chunfang Zan and Jürgen Bernhagen conceived and designed the contents, structure and layout of the review article with help from Bishan Yang, Omar El Bounkari, and Markus Brandhofer. The first draft of the manuscript was written by Chunfang Zan and edited by Jürgen Bernhagen. All authors revised and commented on the manuscript drafts. Jürgen Bernhagen, Chunfang Zan, Bishan Yang, and Markus Brandhofer provided the funding.

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DISCLOSURES

J.B. and O.E.B. are inventors on patent applications related to anti-MIF strategies in inflammatory and cardiovascular diseases. The other authors declare no competing interests.

DATA AVAILABILITY STATEMENT

All materials are available in the manuscript.

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