OxMIF: a druggable isoform of macrophage migration inhibitory factor in cancer and inflammatory diseases

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ABSTRACT

Macrophage migration inhibitory factor (MIF) is a proinflammatory cytokine with a pleiotropic spectrum of biological functions implicated in the pathogenesis of cancer and inflammatory diseases. MIF is constitutively present in several cell types and non-lymphoid tissues and is secreted after acute stress or inflammation. MIF triggers the release of proinflammatory cytokines, overrides the anti-inflammatory effects of glucocorticoids, and exerts chemokine function, resulting in increased migration and recruitment of leukocytes into inflamed tissue. Despite this, MIF is a challenging target for therapeutic intervention because of its ubiquitous nature and presence in the circulation and tissue of healthy individuals. Oxidized MIF (oxMIF) is an immunologically distinct disease-related structural isoform found in the plasma and tissues of patients with inflammatory diseases and in solid tumor tissues. MIF converts to oxMIF in an oxidizing, inflammatory environment. This review discusses the biology and activity of MIF and the potential for autoimmune disease and cancer modification by targeting oxMIF. Anti-oxMIF antibodies reduce cancer cell invasion/migration, angiogenesis, proinflammatory cytokine production, and ERK and AKT activation. Anti-oxMIF antibodies also elicit apoptosis and alter immune cell function and/or migration. When co-administered with a glucocorticoid, anti-oxMIF antibodies produced a synergistic response in inflammatory models. Anti-oxMIF antibodies therefore counterregulate biological activities attributed to MIF. oxMIF expression has been observed in inflammatory diseases (eg, sepsis, psoriasis, asthma, inflammatory bowel disease, and systemic lupus erythematosus) and oxMIF has been detected in ovarian, colorectal, lung, and pancreatic cancers. In contrast to MIF, oxMIF is specifically detected in plasma and/or tissues of diseased patients, but not in healthy individuals. Therefore, as a druggable isoform of MIF, oxMIF represents a potential new therapeutic target in inflammatory diseases and cancer. Fully human, monoclonal anti-oxMIF antibodies have been shown to selectively bind oxMIF in preclinical and phase I studies; however, additional clinical assessments are necessary to validate their use as either a monotherapy or in combination with standard-of-care regimens (ie, immunomodulatory agents/checkpoint inhibitors, anti-angiogenic drugs, chemotherapeutics, and glucocorticoids).

INTRODUCTION

Oxidized macrophage migration inhibitory factor (oxMIF) is a disease-related conformational isoform of macrophage migration inhibitory factor (MIF) that can be selectively targeted in cancer and inflammatory diseases.[1–3] MIF—first described as a soluble immune cell-derived factor in 1966[4]—is a key mediator in the pathogenesis of cancers and inflammatory diseases.[5–9] MIF is a pro-inflammatory cytokine with a pleiotropic spectrum of associated biological functions.[10] Extracellular MIF binding to CD74 in hetero-complex with CD44, chemokine receptors CXCR2, CXCR4, and/or CXCR7,[10–14] among other activities, initiates activation of the mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) and/or phosphoinositide 3-kinase (PI3K)-protein kinase B (AKT) intracellular signaling pathways,[5 10 12–16] which prompts downstream pro-inflammatory and pro-tumorigenic effects. The extracellular MIF/CD74 interaction increases c-Jun phosphorylation and pro-inflammatory activator protein 1 (AP-1) transcription.[10 17 18] In contrast, intracellular MIF independently binds c-Jun activation domain binding protein-1 (JAB1) and negatively regulates AP-1 transcription and c-Jun amino terminal kinase (JNK) activities, indicating an immunosuppressive role.[10 19 20] The balance between extracellular versus intracellular MIF levels/activities may determine the pro-inflammatory versus anti-inflammatory phenotype, respectively.[10]

Under normal circumstances, preformed MIF is constitutively present in several cell types (eg, monocytes, macrophages, lymphocytes, eosinophils, epithelial cells, and endothelial cells)[19 21–23] and non-lymphoid tissues (eg, pituitary gland, lung, liver, kidney, spleen, adrenal gland, and skin)[19 21 22] (online supplemental figure 1). Preformed MIF is rapidly secreted from intracellular pools after acute stress or inflammatory stimulation (eg, bacterial lipopolysaccharide (LPS), tumor necrosis factor (TNF), or interferon-γ (IFN-γ)).[19 21–24] Once released, MIF triggers the

release of pro-inflammatory cytokines,[19 21 23–26] overrides anti-inflammatory effects of glucocorticoids,[19 24 26–28] and enhances chemokine function, resulting in increased migration and recruitment of leukocytes into inflamed tissue.[11 29] Acting in parallel with endogenous glucocorticoids, MIF controls the threshold and magnitude of immune and inflammatory responses.[19 24 26–28] These distinctive features and functions of MIF distinguish it from other cytokines or hormonal mediators.

MIF is a critical upstream regulator of innate immunity and inflammation, and excess MIF expression causes exaggerated inflammation and immunopathology, including cancer.[1 2 19 23 24 30–40] For example, MIF is implicated in inflammatory diseases such as sepsis,[19 24 30 41] psoriasis,[11 42] asthma,[1] inflammatory bowel disease,[1] arthritis,[23 31 43–45] and systemic lupus erythematosus (SLE).[23 46] Additionally, high expression of MIF is found in certain solid tumors (ie, colorectal cancer, gastric cancer, non-small cell lung cancer (NSCLC), ovarian cancer, esophageal squamous cell carcinoma, pancreatic cancer, melanoma, neuroblastoma, and osteosarcoma) and is associated with high tumor burden and grade, increased metastasis risk, and poor prognosis.[2 33–40 47–54] Evidence indicates that MIF alleles are associated with incidence and/or severity of these conditions.[23 24 33 44 55–62] Functional MIF gene-promoter polymorphisms (eg, CATT repetition represented 5–8 times and a single nucleotide polymorphism, -173G/C) make individuals susceptible to elevated MIF expression and the consequent exaggerated immune and inflammatory responses, or—in a few instances—result in protection from (or less aggressive) disease.[63 64] For example, individuals with the MIF-173*C allele have increased susceptibility to systemic-onset juvenile idiopathic arthritis, psoriasis, SLE, and prostate cancer.[44 55 57 59 62] In contrast, significantly fewer patients with Crohn's disease who were heterozygous for the MIF-173G→C substitution than patients who were homozygous had upper gastrointestinal tract involvement, and the 5-CATT repeat allele (vs the 6, 7, or 8 repeat alleles) correlated with low disease severity in patients with rheumatoid arthritis.[56 65]

Despite its role in inflammatory disease and cancer pathways, MIF is a challenging target for therapeutic intervention because of its ubiquitous nature and presence in the circulation and tissue of healthy individuals.[2] However, evidence indicates that MIF occurs in two immunologically distinct, conformational isoforms: oxMIF, which is a disease-related structural isoform found predominantly in plasma and tissues of patients with inflammatory diseases and in tumor tissues and corresponding metastases, and reduced MIF (redMIF), which is present in plasma, cells, and tissues of healthy and diseased individuals.[1]

MIF is a 115-amino acid polypeptide, with a homotrimer de facto structure that is toroidal in shape with a central, solvent-filled pore.[66] Unlike other cytokines, MIF has two evolutionarily conserved catalytic activities—a tautomerase activity and a thiol-protein oxidoreductase (TPOR) activity—that are carried out by two distinct catalytic centers.[66 67] The TPOR activity is mediated through a conserved cysteine (Cys)-56-alanine-leucine-Cys-59 (CALC) motif located in the central pore of MIF.[67] The tautomerase activity, which has no known function, is facilitated by the conserved N-terminal proline.[67 68] Some of the pro-inflammatory activities of MIF are inhibited by targeting the N-terminal proline, which is involved in receptor binding.[69–74] MIF is also a poly ADP ribose polymerase-1-dependent, apoptosis-inducing factor-associated nuclease.[75 76] MIF nuclease activity promotes tumor growth in cancer cells[77] and neurodegeneration and neuronal cell death in post-traumatic brain injury.[76]

MIF is modified covalently and structurally, which changes MIF bioactivity. For example, one modification of MIF—mediated by myeloperoxidase (MPO)-derived hypochlorous acid—is oxidation of the N-terminal proline, which eliminates tautomerase activity but spares pro-inflammatory activities.[68] Furthermore, MIF can interconvert between redMIF and oxMIF, and antibodies have been shown to selectively bind oxMIF but not redMIF (figure 1).[1 66 78] One of these antibodies (imalumab) is a recombinant, fully human, immunoglobulin G1 monoclonal antibody. Imalumab binds to an epitope covering the CALC motif and has been tested in clinical studies.[3 66 79] Distal to the CALC motif is a third Cys (Cys-80), which operates as a switch, converting redMIF to oxMIF through post-translational modification.[66 80 81] Redox-sensitive amino acids within MIF (eg, Cys-80, lysine (Lys)-66) were identified as latent sites of functional control.[81] Mutational analyses showed loss of these redox-responsive amino acids attenuated activation of CD74 and altered MIF enzymatic activity.[81]

Using MIF-specific and oxMIF-specific antibodies, total MIF—but not oxMIF—was detected in plasma (by quantitative ELISA), and in the cytosol (by immunoprecipitation and western blot analysis), and on the surface of immune cells (by flow cytometry), from healthy controls.[1] In contrast, oxMIF was detected in plasma and on the surface of granulocytes, macrophages, and NK cells (but not T or B cells) only under inflammatory disease conditions.[1] Furthermore, using immunohistochemistry methods which prevented an artificial conversion of MIF to oxMIF (eg, by fixatives or oxidative agents), oxMIF-specific antibodies detected oxMIF in primary tumors (eg, pancreatic, colorectal, ovarian, and lung) but not in adjacent non-tumoural tissue; and in corresponding metastatic tissue.[2] oxMIF was also detected via immunohistochemistry in metastases from biopsied patients in the imalumab phase I trial; results were interpreted using digital pathology algorithms.[3] Therefore, oxMIF represents a potential new therapeutic target in solid tumors and inflammatory diseases. Here, we discuss the biology and activity of MIF, as well as the potential for autoimmune disease and cancer modification by targeting oxMIF.
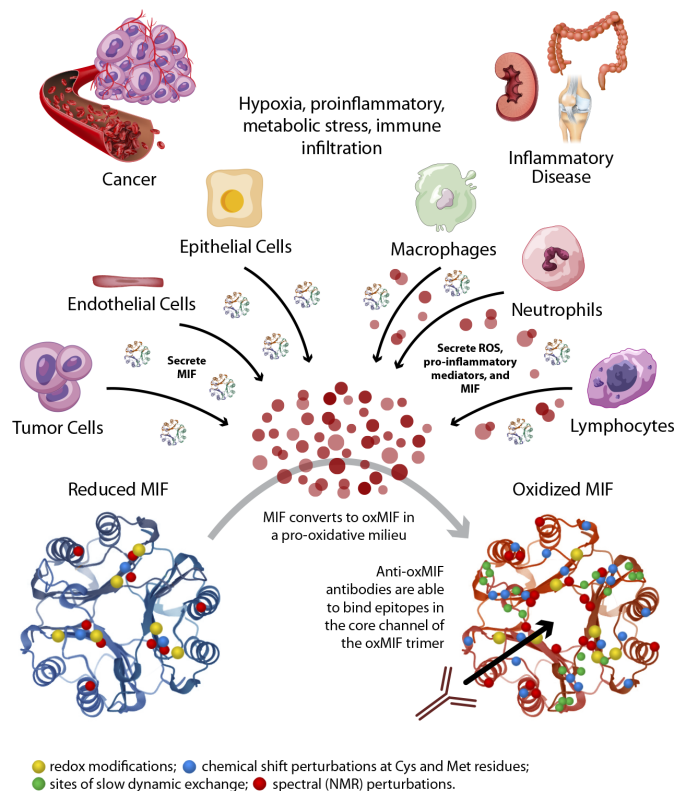
● redox modifications; ● chemical shift perturbations at Cys and Met residues;
● sites of slow dynamic exchange; ● spectral (NMR) perturbations.

Figure 1 Redox-dependent macrophage migration inhibitory factor (MIF) structure. Hypoxic pro-inflammatory conditions are common in cancer and inflammatory disease microenvironments. Macrophages, neutrophils, and other immune cells contribute to the generation of ROS and pro-inflammatory mediators. Under these oxidizing conditions, MIF, which is secreted by tumor cells, as well as endothelial cells, epithelial cells, and other immune cells, can convert to oxMIF. oxMIF has increased redox modifications (yellow dots) and chemical shift perturbations at Cys and Met residues (blue dots), more sites of slow dynamic exchange (green dots), and more spectral (NMR) perturbations (red dots) compared with redMIF. Once in the oxMIF isoform, anti-oxMIF antibodies can access and bind to epitopes in the core channel of the trimer. Cys, cysteine; Met, methionine; MIF, macrophage migration inhibitory factor; NMR, nuclear magnetic resonance; oxMIF, oxidized macrophage migration inhibitory factor; ROS, reactive oxygen species.

MIF biology and disease modification by targeting oxMIF

Owing to its pleiotropic action, MIF promotes inflammation, cell proliferation, and inhibition of cell death by apoptosis (figure 2).[19 82] During the early phases of tumor development, MIF/oxMIF elicits pro-inflammatory immune responses in the tumor microenvironment.[2 10] In contrast, during later-stage disease the effects of MIF more closely mimic wound-resolution activity, increasing neovascularization and immune evasion.[10] In addition, MIF regulates migration, activation, differentiation, and reprogramming of immune and non-immune cells (figures 2 and 3).[10 19 82] For example, inhibition of MIF in solid tumors efficiently repolarizes tumor-associated macrophages (TAMs) within tumors from an immunosuppressive and pro-angiogenic/pro-tumorigenic phenotype

to an immunostimulatory and non-angiogenic/anti-tumor phenotype.[10]

The role of MIF in tumor invasion and metastasis, tumor cell proliferation, apoptosis, angiogenesis, pro-inflammatory mediator production, counterregulation of glucocorticoids, and chemokine function, and the effects of anti-oxMIF antibodies on these activities are summarized in figure 4 and below.

Tumor cell migration, invasion, and metastasis

Several biological activities of MIF favor cancer development, growth, and metastasis, including sustained ERK activation, activation of the AKT pathway, cyclooxygenase-2/prostaglandin E-2 (PGE$_2$) induction, p53 inhibition, and endothelial cell proliferation and differentiation.[5 10 14 19 82 83] MIF plays a critical role in tumor cell migration, invasion, and metastasis[10 19 82 83] as demonstrated in in vitro and in vivo studies, including studies of pancreatic,[36 47 84–86] prostate,[33] lung,[34 52] colorectal,[35 87 88] ovarian,[48 89] esophageal squamous cell carcinoma,[49 53] osteosarcoma,[50] melanoma,[51] and nasopharyngeal cancers.[90 91] MIF overexpression was associated with cancer cell invasion and particularly aggressive disease.[33 35 36 47 53 86 91] Potential mechanisms for the role of MIF in tumor cell migration, invasion, and metastasis include: the dependence of liver premetastatic niche formation on tumor exosome-derived MIF during pancreatic ductal adenocarcinoma (PDAC) metastasis[84]; activation of AKT serine/threonine kinase and ERK[36]; activation of cyclin D1 expression[36]; activation of matrix metalloproteinase expression[36 88]; regulation of the tumor microenvironment (eg, cytokines, angiogenic factors, and chemokines)[48]; induced epithelial-to-mesenchymal transition (evidenced by decreased E-cadherin and increased mesenchymal markers)[47 53]; loss of cell adhesion and anchorage-dependent growth[34]; promotion of RAC1 activity and subsequent tumor cell motility through lipid raft stabilization[34]; increased vascular endothelial growth factor (VEGF) expression and formation of new tumor vasculature[87]; decreased CD74 and Tiam1 expression[87]; JNKII-dependent and nuclear factor-κB (NF-κB)-dependent induction of release by macrophages[89]; and activation of the Rho pathway.[88]

Blocking MIF activity using antibodies, small interfering ribonucleic acids (siRNAs), or microRNAs inhibited invasion and reduced metastasis in multiple studies.[33 34 36 78 83 86 87 90 92] Anti-oxMIF antibodies, which have binding regions that include the MIF oxidoreductase motif, were effective at inhibiting the capacity of prostate cancer cells to invade and migrate through extracellular matrix components.[78 93] In the same study, anti-oxMIF antibodies reduced active ERK1/2 and AKT levels. Finally, in a study of bevacizumab-resistant glioblastomas, xenografts expressed high levels of MIF, and exposure to imalumab reduced tumor cell invasion in Matrigel and bioengineered three-dimensional hyaluronic acid-based assays.[94]

Tumor cell proliferation

The role of MIF in tumor cell proliferation has been demonstrated in various in vitro and in vivo studies
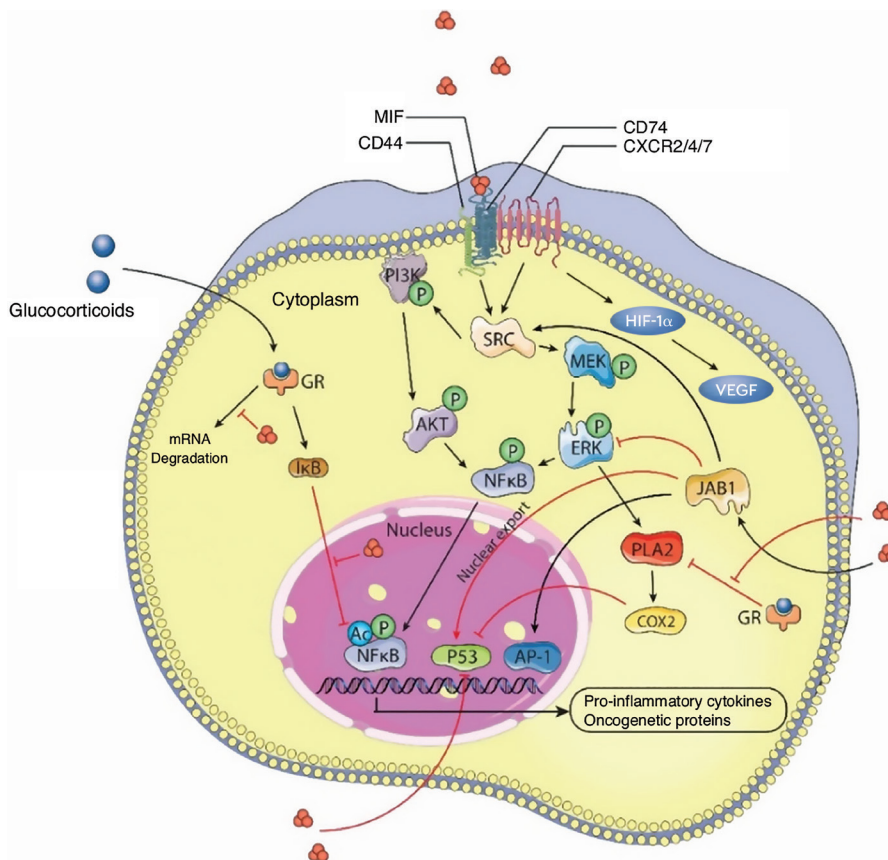
Figure 2 Role of MIF in activation of anti-apoptotic, pro-angiogenic, and pro-proliferative pathways. MIF binds the ligand-binding protein CD74 and the signal transducer CD44. The MIF-CD74-CD44 complex activates transcription factors that regulate the Src proto-oncogene, non-receptor tyrosine kinase (SRC) and ERK-MAPK pathways, which control gene expression and cellular proliferation. The MIF-CD74 interaction activates the AKT pathway via the mediation of kinases SRC and PI3K. MIF also interacts with JAB1 to activate the JNK and works as a co-activator of the transcription factor AP-1. MIF also limits the immunosuppressive effects of glucocorticoid and revokes the glucocorticoid-mediated inhibition of PLA2 and arachidonic acid production. MIF can inactivate p53-mediated apoptosis and growth arrest, and many pieces of evidence connect MIF with inflammation and tumorigenesis. AP-1, activator protein 1; ERK, extracellular signal-regulated kinase; GR, glucocorticoid receptor; IκB, inhibitor of NF-κB; JNK, JUN N-terminal kinase; MAPK, mitogen-activated protein kinase; MIF, macrophage migration inhibitory factor; NF-κB, nuclear factor kappa B. Figure has been modified from Osipyan A, Chen D, Dekker FJ. Epigenetic regulation in macrophage migration inhibitory factor (MIF)-mediated signaling in cancer and inflammation. *Drug Discov Today* 2021;26(7):1728–1734.

involving multiple tumor types (eg, pancreatic, colorectal, prostate, ovarian, nasopharyngeal, gastric, and melanoma).[15 33 36 47 48 51 86 87 90 95 96] MIF triggers cell proliferation, at least in part, by activating MAPK/ERK and AKT intracellular signaling.[15 16 95] Both endogenous and exogenous MIF stimulate cellular proliferation in association with MAPK/ERK activation; resulting sustained cytoplasmic phospholipase A2 activation leads to the production of arachidonic acid, which is a precursor for the synthesis of prostaglandins and leukotrienes.[15] MIF knockdown suppressed proliferation of DU-145 (human prostate cancer) cells, and MIF-specific inhibition suppressed proliferation and tumor growth (in part, by inhibiting angiogenesis) in a prostate cancer xenograft model.[33] Furthermore, MIF knockdown, which suppressed proliferation of PDAC cells, also significantly inhibited activation of ERK and AKT signaling.[36]

In an in vitro study in PC3 (human prostate cancer) cells, anti-oxMIF antibodies inhibited MIF-induced phosphorylation of ERK1/2 and AKT activation.[78] Additionally, satisfactory tissue penetration of imalumab was observed in pretherapy and on-therapy advanced solid tumor biopsies, with regulation of PI3K/AKT/mammalian target of rapamycin (mTOR) downstream signaling, in all biopsy-evaluable patients.[3 79]

Apoptosis

Initially, MIF was shown to inhibit apoptosis by overcoming p53 activity in mouse embryonic fibroblasts, macrophages from LPS-stimulated mice, and MIF-transfected murine macrophages.[97 98] Further research revealed several MIF-involved mechanisms associated with apoptosis. Tumors arising from MIF knockdown in PDAC cells and ID8 (murine ovarian cancer) syngeneic tumors had significantly increased apoptosis, which was associated with reduced AKT phosphorylation and increased p53 phosphorylation.[48] MIF, through interaction with
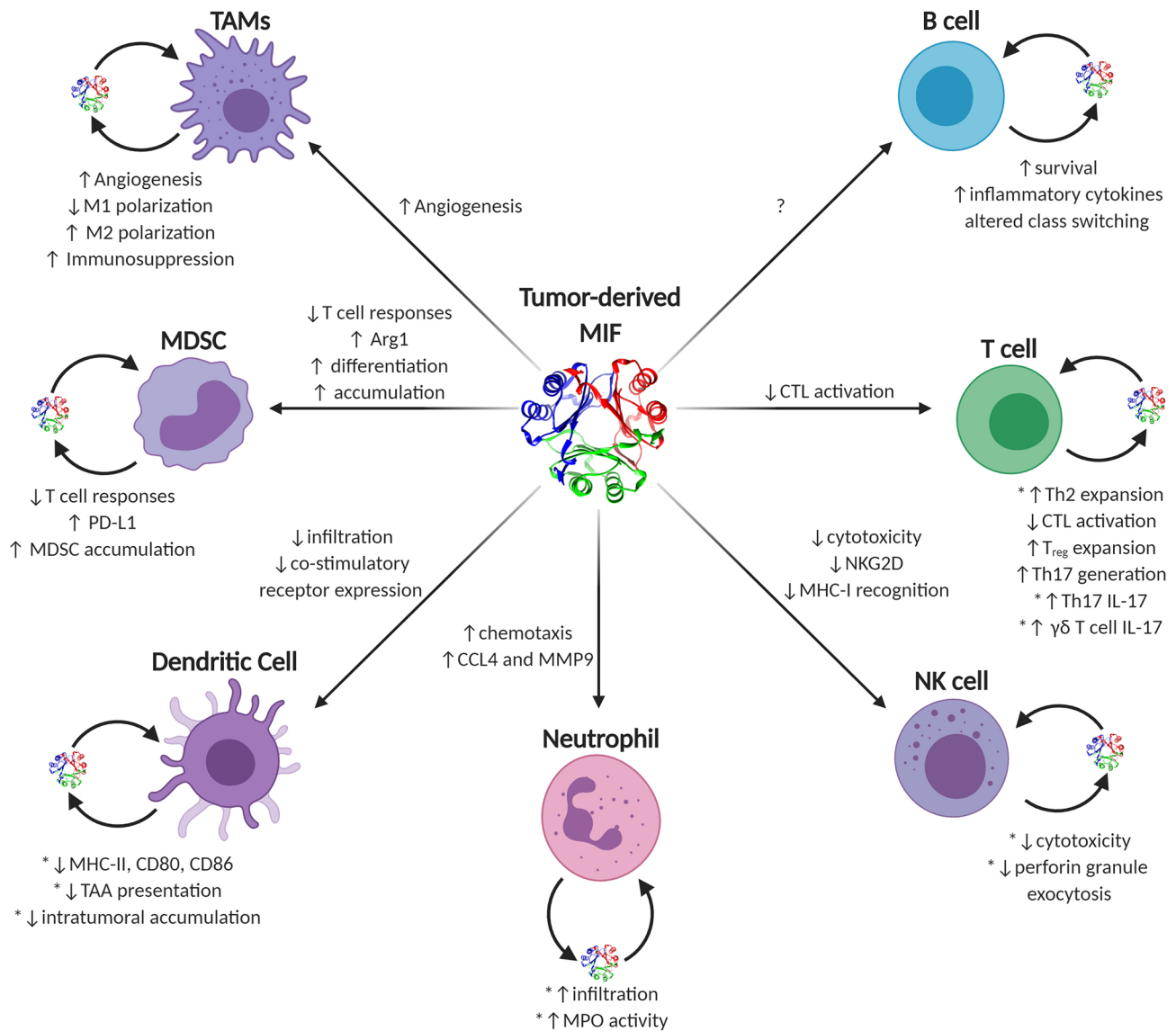
Figure 3 Known and putative effects of MIF on tumor-infiltrating immune cells. Graphical depiction of immune effector cells known to be influenced by MIF. Sources of intratumoral MIF include both paracrine acting tumor-secreted and autocrine acting immune cell-secreted MIF. Phenotypes ascribed to tumor-derived MIF on individual cell types are listed next to each corresponding arrow to each cell type, while autocrine-associated activities are noted next to each cell with the caveat that those activities validated using recombinant MIF sources are noted with an asterisk. CCL4, inflammatory chemokine (C-C motif) ligand 4; CTL, cytotoxic T lymphocyte; MDSC, myeloid-derived suppressor cells; MHC, major histocompatibility complex; MIF, macrophage migration inhibitory factor; MMP9, matrix metalloproteinase 9; MPO, myeloperoxidase; NK, natural killer; PD-L1, programmed death-ligand 1; TAA, tumor-associated antigens; TAMs, tumor-associated macrophages; Th, T helper. Figure has been reprinted from Noe JT, Mitchell RA. MIF-dependent control of tumor immunity. *Front Immunol* 2020;11:609948.[10]

Jun activation domain-binding protein-1/constitutive photomorphogenic-9 signalosome, modulated activator protein-1 activity and cell proliferation, and inactivated p53.[19 82] Murine CT26 and LoVo (colon carcinoma) cells incubated with a MIF-specific inhibitor reduced cell proliferation, suggesting that increased MIF expression in cancer may inhibit p53-mediated apoptosis.[35] Blocking the MIF-CD74 interaction decreased ERK1/2 activation and increased apoptosis in DU-145 (prostate cancer) cells.[33] MIF overexpression in Capan 2 and Panc 1 (pancreatic cancer) cell lines decreased apoptosis, as measured by lower caspase-3 and caspase-7 activity relative to controls.[47] Similarly, MIF activation of the PI3K/AKT pathway led to inactivation of pro-apoptotic proteins in mouse embryonic fibroblasts and NIH/3T3 (immortalized fibroblasts), HeLa (cervical carcinoma), and various breast cancer cell lines[14] and expression of anti-apoptotic proteins in chronic lymphocytic leukemia B cells.[99] MIF knockdown in EC9706 and NE6-T (esophageal squamous cell carcinoma) cells increased apoptosis with elevated levels of cleaved caspase-3.[53]

Reduced levels of active ERK1/2 and AKT favor caspase activation, thereby promoting apoptosis.[78] When PC3 cells were incubated with anti-oxMIF antibodies, which
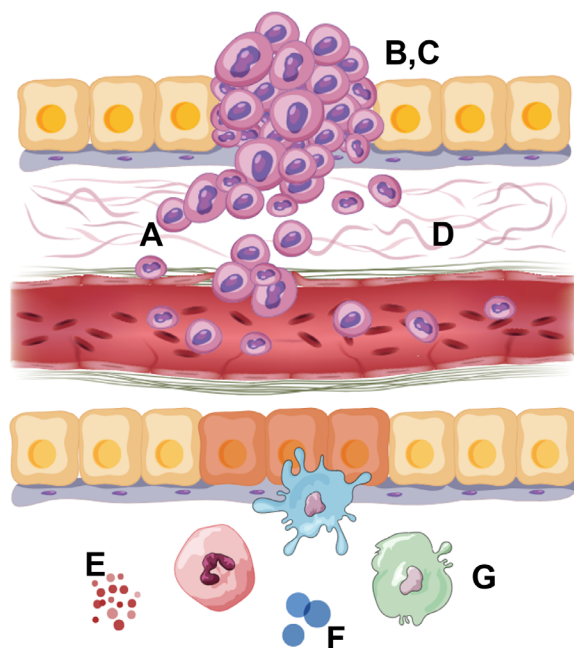
MIF activity in cancer	Effects of anti-oxMIF antibodies on MIF activity
A. Tumor cell migration, invasion, and metastasis[33–36,47,49–51,84–87,89–91]	Inhibition of invasion and migration of tumor cells, and reduction in metastasis (mechanisms/MIF activities affected undefined)[78,94]
B. Tumor cell proliferation via ERK, AKT, and Rho activation[14,15,33,36,48,86,87,90,95]	Inhibited MIF-induced phosphorylation of ERK1/2 and AKT activation and regulation of PI3K-AKT-mTOR downstream signaling[78,79]
C. Apoptosis inhibition via reduced p53, ERK1/2, and AKT activity and inhibition of caspases[33,35,47,48,96–98]	Reduction in active ERK1/2 and AKT levels, and increased caspase activity[78]
D. Angiogenesis and (neo)vascularization via upregulation of VEGF and IL-8[15,21,25,27,35,48–50,100,108]	Reduced tumor vessel density and tumor cell intravasation[105]

MIF activity in inflammation	Effects of anti-oxMIF antibodies on MIF activity
E. Pro-inflammatory cytokines and growth factor mediation[15,21,25,27,48]	Reduction of pro-inflammatory mediators (TNF-α, IL-6, IL-1ß, and MCP-1) and increase in anti-inflammatory cytokines (IL-1, IL-1RA, and IL-10)[1,93,109]
F. Counter-regulation of glucocorticoids[19,24,26–28]	Synergistic effect with glucocorticoids[1]
G. Chemokine function[11,29,100]	Reduction of infiltration of extravascular leucocytes/macrophages to sites of inflammation[1,93,109,111]

Figure 4 In vitro and in vivo studies demonstrate that MIF activity can be regulated by targeting oxMIF. AKT, protein kinase B; ERK, extracellular signal-regulated kinases; IL, interleukin; IL-1RA, IL-1 receptor agonist; MIF, macrophage migration inhibitory factor; MCP-1, monocyte chemoattractant protein-1; mTOR, mammalian target of rapamycin; TNF-α, tumor necrosis factor-alpha; VEGF, vascular endothelial growth factor. Tumor image has been modified from Novikov NM, Zolotaryova SY, Gautreau AM et al. Mutational drivers of cancer cell migration and invasion. *Br J Cancer* 2021;124:102–114 (http://creativecommons.org/licenses/by/4.0/).

reduced active ERK1/2 and AKT levels, increased caspase activity was detected in lysates.[78] In addition, satisfactory tissue penetration of imalumab was observed along with apoptosis in biopsy-evaluable patients in a phase I study.[79]

Angiogenesis and neovascularization

MIF is an angiogenesis-promoting factor during neoplastic transformation and neovascularization, the latter of which is controlled by angiogenic factors (eg, VEGF) that are secreted mainly by tumor cells and is essential for tumor growth.[100–102] In addition, MIF activates hypoxia-induced factor-1a (HIF-1α), directly and indirectly, which leads to interleukin (IL)-8 and VEGF expression.[54] The role of MIF in angiogenesis was evaluated in various in vitro and in vivo studies of multiple tumor types, including colon/colorectal cancer, prostate cancer, lymphoma, osteosarcoma, melanoma, and esophageal squamous cell carcinoma.[33,35,49–51,88,92,100,103,104] In these studies, MIF stimulated angiogenesis in a MAPK/ERK-dependent and PI3K/AKT-dependent manner[92,100] and induced angiogenic factors (eg, VEGF, IL-8).[35,49,50,100] Additionally, MIF serum levels correlated with serum VEGF levels and microvessel density correlated with MIF expression.[49,50] Furthermore, angiogenesis and tumor (out)growth were suppressed when MIF activity was blocked (eg, MIF siRNA, MIF-specific inhibitor, anti-MIF monoclonal antibodies, and MIF-deficient tumors).[33,35,50,88,100,103,104] Together, these findings suggest MIF participates in tumor angiogenesis in an autocrine fashion.[49,100] Reduced vessel density and tumor cell intravasation were observed in tumors from

human prostate cancer cell-line xenografted mice that were treated with a novel anti-oxMIF antibody[105]; however, additional studies are needed to evaluate whether the HIF-1α/VEGF axis contributed to these effects and whether MIF-dependent effects on stromal macrophages contribute to these phenotypes.[49,106,107]

Pro-inflammatory cytokine and growth factor mediation

As a pro-inflammatory cytokine, MIF promotes production of pro-inflammatory and pro-tumorigenic mediators (eg, TNF-α, nitric oxide, and PGE₂, and the cytokines IL-1β, IL-6, and IFN-γ).[26,35,49,50,100,108] oxMIF-specific antibodies reduced TNF-α and IL-6 levels in an endotoxic shock model.[93] Reductions in TNF-α, IL-6, and monocyte chemoattractant protein-1 were observed after treatment with oxMIF antibodies in in vivo animal models of chronic inflammation.[1,109] Additionally, imalumab downregulated TNF-α and IL-6 in murine models of inflammatory disease.[1,93,109] In a phase I study of multiple solid tumors, satisfactory tissue penetration of imalumab was observed, along with regulation of TNF-α signaling and anti-inflammatory cytokines (IL-1 and IL-10), in biopsy-evaluable patients.[79]

Counterregulation of glucocorticoids

On stimulation by glucocorticoids, MIF is secreted from monocytes and macrophages.[26–28] A hallmark of MIF is its ability to override the immunosuppressive effects of glucocorticoids on activation of immune cells and production of pro-inflammatory cytokines, which, in

turn, promotes and aggravates local and systemic inflammatory responses mediated by macrophages and monocytes.[19 24 26–28] Proposed mechanisms for the physiological antagonistic effect of MIF on glucocorticoids include: interference with glucocorticoids at a transcriptional and post-transcriptional level; counteracting the steroid-mediated induction of cytosolic inhibitor of NF-κB (IκB), thus antagonizing the effect of glucocorticoids on the NF-κB signal-transduction pathway; inhibiting the regulation of cytokine production at the transcriptional and post-transcriptional level; and activating the ERK1/ERK2 pathway, which, in turn, induces cytoplasmic phospholipase A2—a key target of the anti-inflammatory effects of glucocorticoids.[19]

Combination treatment with a MIF inhibitor and glucocorticoid (dexamethasone) acted synergistically in an in vitro inflammatory model to attenuate LPS-induced TNF-α release, as well as in vivo in an experimental autoimmune encephalitis model, decreasing disease scores.[110] In mouse models of acute and chronic enterocolitis, anti-oxMIF antibodies reduced disease severity and, when administered with a glucocorticosteroid (dexamethasone) in a rat model of crescentic glomerulonephritis, had a synergistic effect, resulting in greater renal improvement than with dexamethasone monotherapy.[1]

Chemokine function

Chemokines coordinate the activation and recruitment of leukocytes during inflammation.[11] As mentioned, MIF is a cognate ligand for CD74 and a non-cognate ligand for the functional chemokine receptors CXCR2 and CXCR4, as well as CXCR7.[11 13 82] However, not all target cells (eg, neutrophils) express CD74.[11] MIF also binds to CXCR2 or CXCR4, or to CXCR2/CD74 and CXCR4/CD74 receptor complexes.[10 11 82] By interaction with CXCR2, MIF promotes the recruitment of monocytes and neutrophils; via CXCR4, chemotactic activity extends to T cells.[11] In intravital microscopy studies, C-C motif chemokine ligand 2-induced leukocyte adhesion and transmigration were reduced in MIF and CD74 knockdown mice.[29] Reduced migration was associated with attenuated MAPK phosphorylation, RhoA GTPase activity, and actin polymerization in MIF-deficient and CD74-deficient macrophages, which also exhibited reduced chemotaxis. Furthermore, elevated MAPK phosphatase-1 levels were present in MIF-deficient macrophages, potentially explaining the reduced MAPK phosphorylation in MIF-deficient cells. These findings indicate MIF and CD74 promote macrophage migration; however, they do so through distinct mechanisms. In studies of acute colitis-associated colorectal cancer (mouse model), CD68-positive macrophage/monocyte infiltration was decreased in MIF knockdown tumors, supporting MIF as a chemokine that facilitates macrophage recruitment.[100] In the adjacent epithelium, macrophages specifically infiltrated tumors, suggesting it regulated chemotaxis of tumor-associated macrophages to promote tumorigenesis.

In an experimental evaluation of anti-oxMIF antibodies in T-cell-mediated immune responses, ear swelling in challenged mice was significantly reduced following treatment.[93] In the aforementioned rat model of crescentic glomerulonephritis, anti-oxMIF antibody treatment resulted in fewer infiltrating macrophages, directly linking to the inhibition of MIF chemokine functions.[1] In vitro, immobilized anti-oxMIF antibody BaxB01 bound to rat oxMIF with high affinity and reduced rat macrophage migration.[109] In addition, treatment with BaxB01 in an experimentally induced rat glomerulonephritis model significantly reduced histopathological glomerular crescent formation, suggesting the anti-oxMIF antibodies altered immune cell function and/or migration.[109] Finally, oxMIF—by binding to CXCR2 on neutrophils and peripheral blood mononuclear cells—prolonged neutrophil survival by exerting anti-apoptotic effects.[111] When evaluated in murine lungs, single-point mutations in redox-modifiable (Cys-80) and redox-sensitive (Lys-66) MIF residues reduced total immune cell recruitment compared with wild-type MIF.[81]

oxMIF as a targeted therapy and diagnostic tool

Due to constitutive expression and presence in the circulation and/or tissues of healthy individuals, a targeted MIF therapy is challenging.[2] However, specific expression of oxMIF in various inflammatory diseases and cancers allows for targeted inhibition of the disease-related biological activities associated with MIF.[1 2]

oxMIF in inflammatory disease

Compared with healthy controls, overexpression of MIF and high oxMIF levels were observed in sepsis, psoriasis, asthma, inflammatory bowel disease, and SLE (figure 5).[1 19 23 24 30–32 41 44–46] For example, in patients with severe sepsis, median MIF plasma levels were approximately 4–5 times higher than in healthy blood donors.[30] Similarly, oxMIF plasma levels were significantly elevated in patients with septicemia compared with healthy controls.[1] In patients with ulcerative colitis or Crohn's disease, significantly higher levels of MIF and oxMIF were detected in biopsies from acute inflammation sites compared with non-lesion biopsy sites or healthy controls.[1] MIF also was overexpressed in patients with SLE, and elevated plasma levels of oxMIF were observed in a subpopulation of patients with SLE (with systemic exacerbations of SLE without renal involvement) but not in patients with SLE in remission or lupus nephritis.[1 23 46] Finally, in the aforementioned rat glomerulonephritis model, one dose of BaxB01 created an exposure-related, anti-inflammatory environment by inhibiting glomerular cellular inflammation.[109] Treatment significantly reduced proteinuria and diminished histopathological glomerular crescent formation, indicating BaxB01 altered immune cell function and migration.[109]

MIF has been implicated in conditions with a neuroinflammatory component such as Parkinson's disease and amyotrophic lateral sclerosis, where MIF has a protective
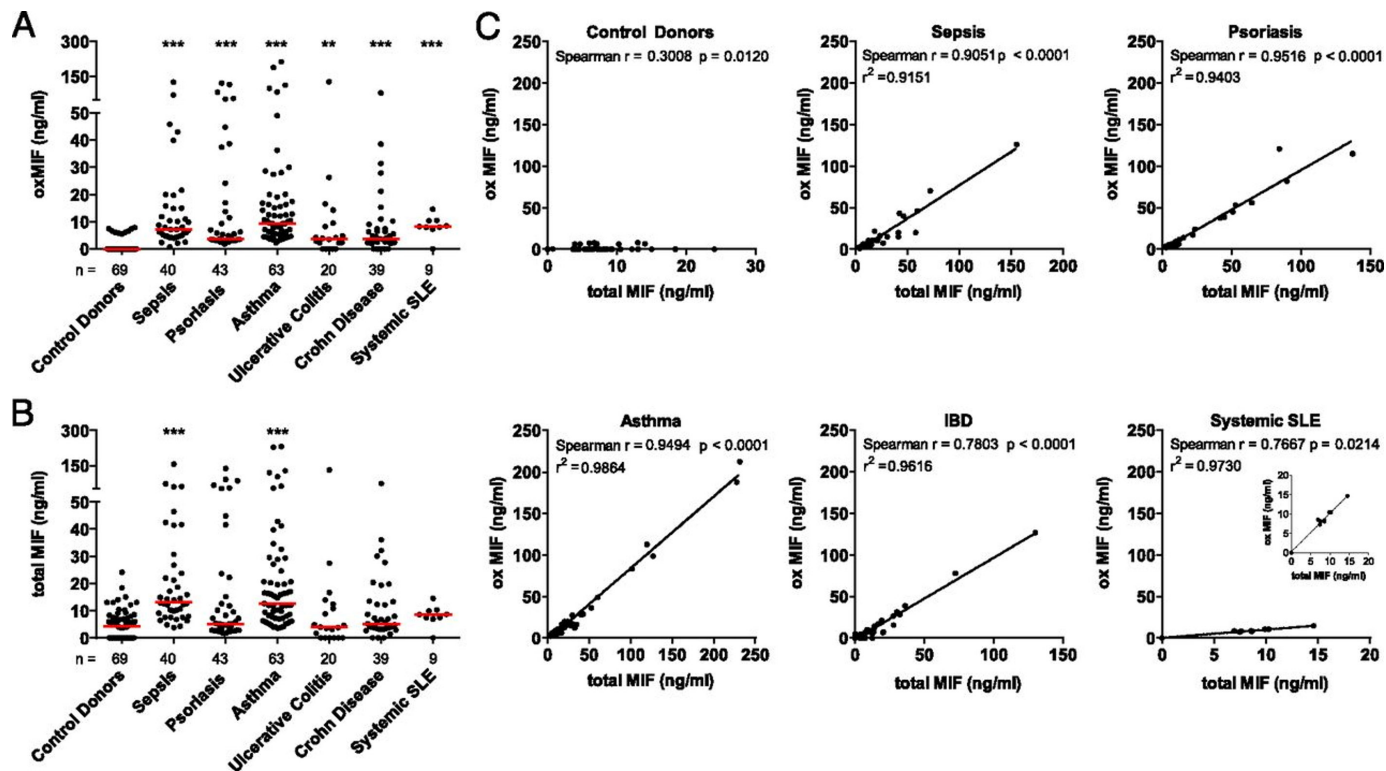
Figure 5 Presence of oxMIF in the circulation of patients with inflammatory disease and healthy controls. (A) Plasma levels of oxMIF in samples from healthy controls and patients with acute or chronic inflammatory diseases. (B) Plasma levels of total MIF in the same samples. Individual values and medians (red lines) are shown. We used the Kruskal-Wallis test followed by Dunn's multiple correction test for statistical analyses. **P≤0.01; ***p≤0.001. (C) oxMIF levels plotted against total MIF levels for each individual plasma sample. Spearman's correlation analysis showed a significant correlation between MIF and oxMIF levels for each disease, but not the healthy control group. IBD, inflammatory bowel disease; MIF, macrophage migration inhibitory factor; oxMIF, oxidized MIF; SLE, systemic lupus erythematosus. Figure has been reprinted from Thiele M, Kerschbaumer RJ, Tam FWK, et al. Selective targeting of a disease-related conformational isoform of macrophage migration inhibitory factor ameliorates inflammatory conditions. *J Immunol* 2015;195:2343–52.

effect, and Alzheimer's disease and mild cognitive impairment, where conflicting data exist regarding protective versus harmful effects.[112 113] The presence of herpes simplex virus type 1 in the brain increases the risk of Alzheimer's disease.[114] Interestingly, in a study of patients with herpes and associated Alzheimer's disease, MIF had antiherpetic activity and oxMIF, which was present in postmortem brains, was postulated to be an overlapping factor, linking herpes simplex virus type 1-induced cellular changes to Alzheimer's disease-like cellular pathology.[115]

oxMIF in cancer

High levels of MIF are present in certain solid tumors (eg, colorectal cancer, gastric cancers, NSCLC, ovarian cancer, esophageal squamous cell carcinoma, pancreatic cancer, melanoma, neuroblastoma, and osteosarcoma) and are associated with high tumor burden and grade, increased metastasis risk, and poor prognosis.[2 33–40 47–51] For example, MIF was significantly overexpressed in primary ovarian cancer tissue, where its expression increased with grade of malignancy and correlated with the high MIF levels present in metastatic ascites.[37] Similarly, oxMIF was detected in primary tumor tissue and in the circulation of women with ovarian cancer, with weak-to-strong

cytoplasmic and membranous oxMIF staining in the apical papillary tumor cells, papillary projection cells, and the tumor stroma.[2] oxMIF was most prominent in adenocarcinomas, serous adenocarcinomas, and mucinous cystadenocarcinomas, and no oxMIF was detected in normal ovarian tissue (online supplemental figure 2A).[2] In addition, high levels of oxMIF were detected in ascites (online supplemental figure 2B).

High MIF levels also are present in the serum, colorectal tissue, and metastases of patients with colorectal cancer, where increased serum MIF concentrations correlated with severity of colorectal cancer and with the extent of metastasis.[35] Likewise, oxMIF was detected in primary colorectal tumors and liver metastases, with a pronounced presence in vessel-like structures (online supplemental figure 2C).[2] Moreover, in a phase I study of imalumab in patients with metastatic colorectal adenocarcinoma, plasma levels of total MIF correlated with oxMIF in oxMIF-positive patients, and significantly lower levels of oxMIF versus total MIF were detected in healthy tissue.[3]

MIF is highly expressed in lung tissue of patients with lung cancer, where high MIF levels correlated with poor prognosis.[39 52] MIF-knockdown and MIF-mutant (lacking

enzymatic activity) murine models resulted in attenuated lung tumor growth.[116] MIF knockdown also reduced cell migration in human squamous cell carcinoma and human lung adenocarcinoma cell lines.[34 52] oxMIF also was detected in lung cancer tissue and was most prominent in adenocarcinomas and squamous cell carcinomas (online supplemental figure 2D).[2]

In PDAC, MIF is overexpressed, and higher MIF levels correlate with lower survival—independent of tumor grade—and enhanced tumor aggression through its involvement in the epithelial-to-mesenchymal transition.[38 47] Additionally, MIF was an independent predictor of prognosis in univariate and multivariate analyses.[47] The potential for MIF as a prognostic marker is supported by the finding that MIF is highly expressed in PDAC-derived exosomes and plasma from patients with stage I PDAC before liver metastasis, and blocking MIF prevented liver premetastatic niche formation and exosome-induced PDAC metastasis.[84] oxMIF was detected in pancreatic intraepithelial neoplasia, including during early tumor stages, with a more pronounced presence at later stages of disease (online supplemental figure 2E and 2F).[2]

Finally, in a study of bevacizumab-resistant glioblastomas, high levels of oxMIF were expressed in resistant xenografts, and exposure to imalumab reduced invasion of bevacizumab-naïve and bevacizumab-resistant glioblastomas in Matrigel and bioengineered three-dimensional hyaluronic acid-based assays.[94] These findings indicate MIF oxidation was increased in the tumor microenvironment, facilitating invasion in a means targetable by imalumab.

Anti-oxMIF therapy

Fully human, monoclonal anti-oxMIF antibodies were developed, and those that did not bind redMIF and recognized distinct MIF epitopes (amino acids 50–68, including the oxidoreductase motif, and C-terminus, amino acids 86–102) were shown to neutralize the proinflammatory activities of MIF in vivo.[2 93] In addition, inhibition of oxMIF promoted cancer cell apoptosis and inhibited cancer cell proliferation in vitro and in vivo.[78] The effects of inhibiting oxMIF with an anti-oxMIF antibody were confirmed in a phase I study.[3] After treatment with imalumab, one-quarter (13/50; 26%) of heavily pretreated patients with late-stage, solid tumor cancers had stable disease (SD) based on RECIST V.1.1 criteria.[3] Furthermore, among the patients with SD, almost two-thirds (8/13; 62%) had SD lasting >4 months (NSCLC, n=2; ovarian cancer, n=2; colorectal cancer, n=1; esophageal cancer, n=1; and cancer of the parotid gland, n=1). Since many cancer treatment strategies are limited because of narrow therapeutic indices, toxicities, and development of tumor resistance to chemotherapeutic agents, regimens comprising a targeted therapy and ≥1 chemotherapeutic agent often are used. In in vitro and in vivo studies, anti-oxMIF antibodies sensitized cancer cells to cytotoxic agents and improved antitumorigenic effects (eg, decreased cell proliferation and increased apoptosis).[2] These anti-oxMIF antibodies exerted their effects by inhibiting cell proliferation and survival signaling pathways, reducing active ERK1/2 and AKT levels, and promoting activation of caspase-3.[78] Additional clinical studies are needed to further investigate the role of oxMIF as a therapeutic target.

DISCUSSION

oxMIF exhibits pathological properties of MIF (consistent with the published literature) that can be modified with oxMIF-specific antibodies (figure 4). oxMIF is specifically detected in plasma and/or tissues of diseased patients, but not in healthy individuals.[1 2] Therefore, oxMIF is a promising drug target and diagnostic biomarker in certain inflammatory diseases and solid tumors.[1 2]

Targeting MIF with biologics and small molecules is challenging because such entities are intercepted by the excess MIF protein in the circulation and in normal tissues and, therefore, are impaired in exerting beneficial effects. Furthermore, non-pathological functions of MIF, which contribute to redox-homeostasis and wound-healing processes, could be impaired by entities that do not distinguish between MIF and oxMIF.[117] Nevertheless, MIF is the precursor to oxMIF, justifying the concept that elevated MIF levels contribute to the development and maintenance of inflammatory and immunopathological processes. The identification of effective anti-oxMIF antibodies was obviously not predictable. Of the 145 anti-MIF antibodies originally analyzed from a phage display system, fewer than 10 demonstrated efficacy in in vitro and in vivo models of inflammation.[93] Subsequently, it was found that these effective antibodies only showed binding specificity for MIF under pro-oxidative conditions, which is where the name 'oxMIF' originated.[1] Unfolding of the protein under oxidizing conditions involves conformational changes that apparently expose epitopes in the MIF trimer that are not accessible to anti-oxMIF antibodies under normal (non-oxidizing) conditions.[81] Recent studies confirmed that an oxidizing environment destabilizes MIF to unfolding relative to redMIF and modulates an equilibrium between the two MIF isoforms.[81]

Mutational analyses of latent redox-dependent, functionally important MIF residues demonstrated the propensity for MIF to toggle its confirmation and binding partners depending on the redox environment.[80 81] Similar redox-dependent conformational changes have not been demonstrated for the MIF homolog D-dopachrome tautomerase (MIF-2), which has low sequence identity (35%).[118] However, mutations in allosteric residues Pro-1, Ser-62, and Phe-100 that occupy the same positions as Pro-1, His-62, and Tyr-99 in MIF also affected structure, dynamics, and biological function of MIF-2, demonstrating a conserved control of structure-dependent functions for MIF superfamily members.[118 119] Elsewhere in nature, redox-dependent sensors exist with isoform-specific functions,[120–123] the evolutionary implications of which are yet to be understood. Redox sensitivity

likely affects both intracellular and extracellular interactions of MIF and could provide additional insight into its pro-inflammatory mechanisms and pleiotropic spectrum of functions.[19 81] Many in vitro studies on signaling, receptor binding, migration, etc, were performed using recombinant MIF, which raises the question: to what extent can these effects be attributed to oxMIF? Working with redox-sensitive proteins is complex and prone to interference. Several parameters may have unknowingly contributed to results from historical in vitro studies. The MIF protein preparation itself may have contained MIF molecules that have adopted the oxMIF structure during certain purification/refolding procedures. In cell-based assays, which may be especially complex in co-culture settings, secreted reactive oxygen species (ROS)/reactive nitrogen species or different enzyme activities may have contributed to an oxidative environment, eliciting oxMIF formation. The impact of culture media compositions should also be considered.

In acute inflammatory responses, neutrophils are activated and ROS, including MPO-derived hypochlorous acid and hypothiocyanous acid, are produced.[68] The mechanism by which MIF is converted to oxMIF in the tumor microenvironment is unknown but may involve tumor-associated neutrophils and MPO activity at inflammatory sites where both MIF and neutrophil-derived MPO are present.[67 68] Several concepts for post-translational modifications of MIF have been proposed; however, additional studies are needed to fully define the process(es).[67]

While MIF is detected at various levels in the circulation, on cell surfaces, and in the cytoplasm of cells under normal, healthy conditions, oxMIF is predominantly expressed under inflammatory conditions, such as acute and chronic inflammatory diseases or solid tumors.[1 2] Specific inhibition of oxMIF is a potent method of interfering with the biological activities of MIF (figure 4). In inflammatory diseases, the prevalence of oxMIF and evidence supporting the effects of anti-oxMIF antibodies is especially promising given the synergistic effect of oxMIF/MIF inhibition with glucocorticoids. It would be interesting to evaluate whether murine monoclonal anti-MIF antibodies that have shown beneficial effects in animal models of inflammation and cancer (eg, clone III.D.9) have a higher affinity or specificity for oxMIF relative to MIF.[32 103 124 125] Clinical evaluation of anti-oxMIF antibodies as a late-stage cancer therapeutic showed modest anti-tumor activity,[3] which could be enhanced by optimizing biophysiochemical properties and immunomodulatory functions of such antibodies and by rational combinations with standard-of-care regimens.[126] Emerging nanomedicine-based approaches[127] may also enhance therapeutic impacts of anti-oxMIF antibodies. Nanomaterials exerting ROS-scavenging and anti-inflammation effects as observed in an in vivo atherosclerosis mouse model,[128] could act synergistically with anti-oxMIF-based therapeutic approaches. However, further studies are warranted to validate these hypotheses.

As discussed, MIF exerts its effects by binding to CD74, which triggers recruitment of CD44, resulting in activation of the MAPK/ERK and PI3K/AKT pathways.[10 12–14 82] MIF also binds directly to CXCR2 or CXCR4, or CXCR2/CD74 and CXCR4/CD74 receptor complexes, resulting in activation of the MAPK/ERK and PI3K/AKT pathways.[10 11 82] Effects of oxMIF could also be mediated by these same receptors. However, acknowledging that the term 'oxMIF' was originally defined in terms of antibody specificity and not in terms of an elucidated biological conversion mechanism is important.[1 93] oxMIF is detected at sites of inflammation and in the circulation of patients with chronic inflammatory diseases, suggesting the post-translationally modified form of MIF can leave inflamed tissues, depending on the disease and stage of inflammation. In contrast, oxMIF is more likely to be retained in primary tumors and corresponding metastases in most tumor types.[2 3] The soluble oxMIF form generated by neutrophil-MPO-mediated oxidation of MIF with hypochlorous acid was reported to bind to CXCR2, thereby triggering the release of IL-6 and CXCL8 by peripheral blood mononuclear cells and contributing to neutrophil survival.[111] Binding and structural analyses are needed to determine whether oxMIF also triggers cell signaling via CD74/CD44 and whether inhibitory effects of anti-oxMIF antibodies on pro-proliferative downstream signaling pathways (eg, ERK and/or AKT) are mediated by this receptor complex. The impact of oxidation of Cys-80 and/or Lys-66 on CD74 binding and cell signaling is yet to be determined.

Imalumab (first-generation anti-oxMIF antibody) showed promise in preclinical studies (in vitro and in vivo) and in a phase I study.[2 3 78 93] Imalumab neutralized the biological activity of oxMIF in relevant pathways (eg, PI3K/AKT/mTOR signaling),[3] penetrated and accumulated in tumors and metastases during treatment,[1] and colocalized with oxMIF in tumor cells and stroma, with efficient target binding by end of first cycle of treatment.[3] Clinical evaluation of imalumab was prematurely halted; however, targeted development of improved, second-generation, anti-oxMIF antibodies continues, and these antibodies are being evaluated in preclinical and clinical proof-of-concept studies.

The early phase imalumab clinical trial included patients with advanced solid tumors including metastatic lung, colorectal, and ovarian carcinomas.[3] These cancer types are newly diagnosed at annual rates of 2,200,000, 1,150,000, and 314,000[129] with 5-year mortality rates of 79%, 35%, and 51%,[130] respectively, presenting an unmet need for more effective therapies. The benign toxicity of oxMIF therapy[3] should allow for combination therapy and integration into standard-of-care regimens without exacerbating patient toxicity. In the case of inflammatory diseases, these indications are often treated with steroids. Although not yet evaluated in clinical trials, concomitant steroid and anti-oxMIF therapy would ideally capitalize on MIF's counteracting of glucocorticoids in order to provide steroid-sparing benefits.[131]

Conclusion

Pathological properties of MIF are based on biological functions, which are evidently altered by anti-oxMIF antibodies. Therefore, oxMIF reflects a druggable isoform of MIF in inflammatory diseases and cancer. Considering the versatile mechanisms of action of MIF and its role in inflammation and cancer, anti-oxMIF modalities could be applied as monotherapy and/or in combination with standard-of-care regimens (ie, immunomodulatory agents/checkpoint inhibitors, anti-angiogenic drugs, chemotherapeutics, and glucocorticoids/nonsteroidal anti-inflammatory drugs). Additional clinical assessments are necessary to validate these hypotheses.

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