Targeting MIF in Cancer: Therapeutic Strategies, Current Developments, and Future Opportunities

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Abstract: Strong evidence has been presented linking chronic inflammation to the onset and pathogenesis of cancer. The multifunctional pro-inflammatory protein macrophage migration inhibitory factor (MIF) occupies a central role in the inflammatory pathway and has been implicated in the tumorigenesis, angiogenesis, and metastasis of many cancer phenotypes. This review highlights the current state of the art, which presents MIF, and the second member of the MIF structural superfamily, D-DT (MIF2), as significant mediators in the inflammatory–cancer axis. Although the mechanism by which MIF asserts its biological activity has yet to be fully understood, it has become clear in recent years that for certain phenotypes of cancer, MIF represents a valid therapeutic target. Current research efforts have focused on small molecule approaches that target MIF’s unique tautomerase active site and neutralization of MIF with anti-MIF antibodies. These approaches have yielded promising results in a number of preclinical murine cancer models and have helped to increase our understanding of MIF biological activity. More recently, MIF’s involvement in a number of key protein–protein interactions, such as with CD74 and HSP90, has been highlighted and provides a novel platform for the development of anti-MIF chemotherapeutic strategies in the future. © 2016 Wiley Periodicals, Inc. Med. Res. Rev., 36, No. 3, 440–460, 2016

Key words: inflammation; cancer; MIF; D-DT; small molecule inhibitors

1. INTRODUCTION

Macrophage migration inhibitory factor (MIF) represents a well-defined, pleiotropic, pro-inflammatory mediator with an important role in the innate and adaptive immune system. Historically, MIF was one of the first functional cytokines to be identified when it was shown to inhibit the random migration of macrophages in experiments to characterize delayed type
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Figure 1. MIF and human disease. MIF has been implicated in the pathogenesis of multiple human diseases including many autoimmune and inflammatory disorders. Its role in cancer is currently being defined.

hypersensitivity. Since this discovery, MIF has been shown to play a significant role in the overall inflammatory cascade. MIF is produced and secreted by numerous cell types including macrophages, monocytes, T and B lymphocytes, eosinophils, neutrophils, and mast cells. Unusually for an immune cytokine of its type, MIF is also expressed at low levels in many endocrine, epithelial, and endothelial cell types where it is stored in cytosolic pools for recruitment at a later time in response to cellular stress and stimuli. Experimental evidence has shown that there is a direct correlation between MIF expression and the severity of numerous inflammatory and autoimmune diseases including cystic fibrosis, sepsis, atherosclerosis, rheumatoid arthritis, lupus, asthma, glomerulonephritis, and acute respiratory distress syndrome (ARDS) (Fig. 1). Recently, it has been shown that expression of MIF is driven by a novel functional polymorphism presenting in approximately 49% of the population, with carriers of the 6-, 7- and 8-CATT repeat alleles being genetically primed to produce higher levels of MIF compared to those carrying the 5-CATT repeat allele. Those patients genetically primed to produce significantly enhanced MIF have been shown to have a more aggressive clinical phenotype in a variety of inflammatory diseases such as cystic fibrosis, asthma, and rheumatoid arthritis.

A. MIF: Cytokine, Hormone, Enzyme, and Chemokine

The unique biological functions associated with MIF have led to it being described as a cytokine, enzyme, hormone, and chemokine. On a molecular level, MIF operates via CD74/CD44, initiating a sustained ERK1/2 MAPK activation (Fig. 2). It is capable of triggering significant immune responses through autocrine and paracrine loops via the induction of pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6, nitric oxide, COX2, and IFN-γ. Aside from these traditional “cytokine-like” properties, MIF also has enzymatic, chemokine, and hormone functional activity. It has shown affinity as a noncognate ligand for the CXCR receptor, facilitating chemotactic responses through the induction of the CXCR ligand IL-8. This interaction has been implicated in the pathogenesis of atherosclerosis through the recruitment of monocytes and T-lymphocytes. It has also been shown that through induction of CXCR2, MIF can recruit neutrophils to the sites of inflammation in head and neck squamous cancer.
Figure 2. MIF and its role in cell signaling. The effect of MIF on cellular signaling is initiated through receptor-mediated pathways and intracellular interactions: MIF forms a complex with CD74/CD44, leading to phosphorylation of ERK 1/2 and sustained MAPK ERK1/2 activation, this triggers downstream processes including the release of pro-inflammatory cytokines and cell proliferation. During this process, activation of phospholipase A$_2$ (PLA2) and cyclooxygenase 2 (COX2) leads to the downregulation of the tumor suppressor p53 and the inhibition of apoptosis. The Akt pathway is also activated via CD74, leading to phosphorylation of proapoptotic proteins such as BAD, this further contributes to cell survival and the inhibition of apoptosis. MIF can also play a role in the stabilization of HIF-1$\alpha$ through a p53-dependent mechanism. HIF-1$\alpha$ is a key transcription factor for angiogenic proteins such as VEGF. MIF also plays a role in chemotactic recruitment of cells to the sites of inflammation via its interaction with CXCR2/4.

A key aspect to the role of MIF within the inflammatory cascade is its ability to counteract glucocorticoid-induced anti-inflammatory responses.$^{27}$ To facilitate this process, MIF upregulates phospholipase A$_2$, a key target for glucocorticoid activity, while also serving to antagonize glucocorticoid activation of MAPK phosphatase 1. This, in turn, leads to a downregulation of MAPK activation responses.$^{28}$ In mice, it has been shown that administration of MIF abrogates the effect of glucocorticoids in lethal toxic shock models.$^{29}$ Furthermore, in an experimental model of a lethal form of arthritis, treatment with an anti-MIF antibody successfully protected the mice from succumbing to the disease.$^{30}$ MIF is highly expressed by the pituitary gland and consequently, plays an integral systemic regulatory role in glucocorticoid function in the body.$^{29}$ MIF expression from macrophages was stimulated by low concentration treatments of glucocorticoids; however, at higher concentrations, expression of MIF was downregulated, which suggests the presence of a negative feedback loop. These unique properties contribute to MIF being an attractive target for pharmacological intervention for the treatment of inflammatory disease. As MIF is expressed in both the intracellular and extracellular spaces, it raises the question of which type of MIF is important in the pathogenesis of these diseases. For instance, Verjans et al. have described a dual function for MIF in human breast cancer cell lines. In their study, they have shown that intracellular MIF expression by breast cancer cells was indicative of a favorable prognosis, whereas extracellular breast tissue derived MIF was pro-inflammatory and would play a pro-oncogenic role in its promotion of cancer cell–stroma interactions leading to an unfavorable patient prognosis.$^{31}$ Depending on the type of cancer in question, the specific targeting of either or both environments in which MIF circulates could lead to novel anti-MIF treatments. If a small molecule anti-MIF approach is desirable, then the membrane permeability of any potential drug molecules must be considered.
Figure 3. MIF structure and function. The remarkable similarity between the crystal structures of human MIF (A) (pdb code: 1MIF) and human D-DT or MIF2 (B) (pdb code: 3KER), and the three bacterial isomerases, chorismate mutase (CM, pdb code: 1COM), 5-carboxymethyl-2-hydroxymuconate isomerase (5-CHMI, pdb code: 4JJ9), and 4-oxalocrotonate tautomerase (4-OT, pdb code: 1OTF) when viewed down the three-fold molecular axis. (D) Close up of the hydrophobic MIF binding site (white carbon atoms) co-crystallized with the prototypical MIF inhibitor Iso-1 (cyan carbon atoms) (pdb code: 1LJT). The keto-enol tautomerase site of MIF is relatively small and contains a number of hydrophobic residues that help to lower the pKₐ of the terminal proline. (E) Although structurally similar, both MIF (tautomerization) and D-DT (decarboxylation) produce different products from the substrate D/L-dopachrome.

B. Structure of MIF

From a structural perspective, MIF does not belong to any of the known cytokine structural families and resides within its own structural superfamily. It is highly evolutionarily conserved across all species displaying sequence homology of >80% between rat, mouse, chicken, gerbil, calf, and human. MIF homologs have also been identified in many parasitic species including \textit{Plasmodium falciparum}\textsuperscript{32} and \textit{Leishmania major}.\textsuperscript{33} Recently, a number of potential MIF homologs in plants have been identified using computational models.\textsuperscript{34} Crystal structure analysis of the active form of MIF shows it exists predominantly as a symmetrical trimer, consisting of three repeating, 12.5 kDa, 114 residue subunits each with \(\beta\)-\(\alpha\)-\(\beta\) homology.\textsuperscript{35, 36} This structural motif shares characteristics analogous to another human protein, D-dopachrome tautomerase (D-DT or MIF2)\textsuperscript{37} as well as three bacterial isomerase enzymes, oxalocrotonate tautomerase\textsuperscript{38} (4-OT), 5-carboxymethyl-2-hydroxymuconate (5-CHMI), and chorismate mutase\textsuperscript{39} (CM) (Fig. 3A–C).

C. MIF Tautomerase Activity

MIF’s relationship to bacterial isomerase enzymes led Rosengren et al. to explore the possibility that MIF possessed inherent catalytic activity akin to these enzymes.\textsuperscript{40, 41} Indeed, unusually for an immune cytokine of its type, MIF displays two distinct catalytic activities. In its homotrimeric form, three hydrophobic keto-enol tautomerase active sites exist, each spanning two subunits. The N-terminal proline (Pro1) residue of each MIF subunit resides within a
hydrophobic active site and displays a low $\text{pK}_a$ (around 5.6–6), which allows it to act as a catalytic base. The nucleophilic character of this proline residue grants MIF the ability to catalyze the isomerization of a number of substrates including L- or D-dopachrome methyl ester (Fig. 3E), phenyl pyruvate (PP), and $p$-hydroxyphenyl pyruvate (HPP) (Fig. 3F). Although these molecules are present in vivo, the relatively high binding constants associated with them suggest they are not physiological substrates for MIF and a defined physiological substrate remains undiscovered. The question of why, from an evolutionary perspective, we retain this enzymatic activity has also not been clearly defined. Historically, it has been suggested that in humans it is simply vestigial in nature; however, recent work has revealed that the catalytic Pro1 residue is necessary for many of the biological effects attributed to MIF and indeed, may have a role in important protein–protein interactions (PPI). The important observation that MIF interacts with its receptor, CD74, in the vicinity of the tautomerase site suggests that targeting this site with small molecule inhibitors may lead to disruption of this key PPI. Drug molecules, which bind tightly in MIF’s active site and possess suitable moieties that protrude from the site and into the solvent, may yield extremely potent inhibitors of MIF-related PPI. A crystal structure of the MIF/CD74 complex or a suitable MIF/inhibitor/CD74 ternary structure to confirm this hypothesis has yet to be resolved; however, strong evidence has been presented for its validity and a biochemical assay has already been developed that can be used to test putative small molecule MIF/CD74 PPI inhibitors.

D. D-DT (MIF-2) Tautomerase Activity

A second human protein, D-DT, exists that displays a structural relationship and enzymatic activity similar to MIF. Although first identified as early as 1993 as a tautomerase enzyme, little was known about this enzyme until relatively recently. Structural analysis shows D-DT has a 34% sequence homology with MIF, although not very high, the overall structural features share a striking similarity. Enzymatically, both binding pockets possess a catalytic proline residue. This structural feature allows both enzymes to catalyze D-dopachrome and HPP substrates. Interestingly, in the case of the dopachrome substrates, the two catalytic sites give rise to two different end products; D-DT produces 5,6-dihydroxyindole through decarboxylation of the substrate, whereas MIF produces the tautomerized product 5,6-dihydroxyindole carboxylic acid (Fig. 3E). However, both proteins can catalyze the keto-enol tautomerization of HPP with similar $K_m$ and $k_{cat}$ values although with a slight 12% skew in favor of D-DT over MIF. Recently, D-DT has been further characterized as a cytokine with similar functional activity to MIF making it the second member of the MIF cytokine family or MIF2. D-DT expression mirrors that of MIF in many human cancers and acts in a CD74 fashion similar to MIF. This would suggest a complementary function for MIF/D-DT in vivo and novel treatments could be identified by dual targeting these proteins. To investigate this hypothesis, Rajasekaran et al. treated D-DT with two well-known MIF inhibitors, ISO-1 and 4-IPP, to assess their ability to inhibit tautomerase activity. Although ISO-1 was designed based on its structural similarity to HPP, the authors found it had no effect on the tautomerase activity of D-DT. The covalent inhibitor 4-IPP, however, was shown to induce a significant decrease in tautomerase activity making it not only the first identified D-DT inhibitor but also the first dual inhibitor of MIF/D-DT tautomerase activity.

E. MIF, a Key Player in the Inflammation–Cancer Axis

There is currently an expanding body of evidence linking chronic inflammation with cancer progression, severity, and a negative disease prognosis. The immune systems natural...
heightened defensive capabilities against microbial invasion have, to a degree, contributed to the development of a microenvironment favoring tumor growth. This is further enhanced by the propensity for tumor cells to selectively induce specific strands within the inflammatory web, contributing to tumor survival and proliferation. Epidemiological studies have shown a direct link between chronic inflammatory diseases and the subsequent development of cancer; for example, patients with inflammatory bowel disease have an increased chance of developing colon cancer. A list of inflammatory disorders and their related cancers is provided in Figure 4. MIF possesses a number of key functional pro-tumor characteristics that allow it to play a central role in this phenomenon. Overexpression of MIF has been identified in numerous cancer cell types including squamous carcinoma, colon cancer, lung cancer, breast cancer, prostate cancer, glioblastomas, cervical adenocarcinoma, malignant melanoma, and nasopharyngeal cancer with a direct correlation found between increasing MIF expression levels and a more aggressive cancer phenotype. Experimental evidence in recent years has demonstrated defined roles for MIF in angiogenesis, metastasis, and tumorigenesis. First, MIF influences cellular homeostasis through the downregulation of the tumor suppressor protein p53. This leads to the prevention of p53-induced apoptosis causing increased survival and proliferation of malignant cells (Fig. 5). It has also been shown to activate the PI3K/Akt pathway, promoting cell survival of tumor cells through the phosphorylation of proapoptotic proteins BAD and Foxo3a. Likewise, in cervical adenocarcinoma cells, MIF knockdown induced apoptosis and inhibited cell proliferation via the upregulation of proapoptotic proteins such as Bax and caspase-3 and the downregulation of antiapoptotic proteins of the Bcl-2 family. Second, MIF can mirror the effects of the oncogenic protein, Ras, via a sustained ERK1/2 MAPK activation. Ras mutations occur in high percentages in many tumors and sustained MAPK activation is a typical feature of this process. MIF has also been shown to have an effect on anticancer immunity by inhibiting tumor-specific NK and CTL cellular activity. Furthermore, MIF production is upregulated in hypoxic conditions, a characteristic often associated with tumor development and progression. During hypoxia, MIF contributes to the stabilization of hypoxia inducible factor 1-alpha (HIF1α), a key transcription factor involved in the expression of angiogenic proteins such as a vascular endothelial growth factor (VEGF), lysyl oxidase (LOX), and connective tissue growth factor (CTGF). Within the cell, MIF can bind to JAB1/CSN5 and has a key role in the cells response to DNA damage and repair.

F. MIF’s Tautomerase Active Site and Cancer

The importance of MIF in cancer has been demonstrated in a number of clinically relevant cancer models where deletion of MIF has shown a significant decrease in the progression
The role of MIF in cancer. MIF promotes angiogenesis, downregulates p53 expression, stimulates a sustained ERK activation, and induces COX-2/PGE-2 production.

and mortality of disease. MIF deletion in Eμ-Myc mice led to protection from lymphoma. Similarly, MIF deletion is related to less progressive disease in models of fibrosarcoma, lung cancer, and skin cancer. The role MIF’s enzymatic active site plays in cancer progression is currently being defined. The development of a novel transgenic tautomerase null mouse (P1G) has been key in elucidating the potential role of the enzymatic site of MIF. The P1G knock in mouse expresses a mutated MIF in which the terminal proline is replaced by a glycine residue rendering the protein catalytically inactive. The P1G protein showed significant but reduced binding to both MIF receptors CD74 and the intracellular JAB1, however, displayed an intermediate phenotype to the wild-type and MIF knock out in tumor induction and growth control assays. In models of skin cancer, and more recently Lewis lung cancer, tumor volume was significantly reduced in P1G mice compared to controls. These observations suggest that the structural features of the MIF tautomerase active site and not its intrinsic catalytic activity are important for PPI. This finding adds considerable weight to the argument that all putative MIF inhibitors should be assayed for their ability to disrupt PPI.
G. d-DT (MIF-2) and Cancer

As previously stated, MIF has been shown to have a role in angiogenesis through the induction of angiogenic factors such as VEGF and IL-8. In lung adenocarcinoma cells, MIF and d-DT were shown to have an additive effect on the induction of these oncogenic factors, suggesting a possible cooperative role for MIF and d-DT in malignant disease.77 In the same cancer model, d-DT and MIF were shown to cooperatively antagonize tumor suppressor pathways including AMP78, 79 and p53.79 In an in vivo model of melanoma, B16F10, neutralization of d-DT with anti-d-DT antibodies reduced tumor progression, showing a role for d-DT in cancer progression analogous to MIF.80 Pasupuleti et al. has shown that in clear cell renal cell carcinomas (ccCRCs), d-DT and MIF expression share comparable levels.81 Notably, inhibition of d-DT had a greater therapeutic effect than that of MIF on tumor growth. d-DT appears to compensate for MIF in areas where MIF is inhibited or downregulated and may explain some of the failure to dampen the effects of MIF signaling through therapeutic options thus far explored. This adds further strong evidence to suggest dual targeting of MIF/d-DT could lead to novel potent anticancer therapeutics.

H. Strategies for MIF Inhibition in the Treatment of Cancer

The idiosyncratic nature of MIF coupled with the considerable evidence surrounding its involvement in the pathogenesis of disease makes it an attractive target for therapeutic intervention. Anti-MIF strategies are an active area of research in the area of oncology and MIF has been highlighted as a highly relevant target for anticancer therapies. Thus far, a number of approaches have been exploited in order to inhibit the biological functions of MIF in cancer studies. These approaches (Fig. 6) include the following:

1. Small molecule disruption of MIF activity.
2. Indirect destabilization of MIF.
3. Anti-MIF antibodies.

In this review, we examine these approaches and summarize recent developments with a focus on small molecules, which have gone into preclinical in vivo studies.

1. Small Molecule Disruption of MIF Biological Activity

The most widely adopted approach in MIF drug discovery involves targeting the enzymatic active site of MIF with small molecule competitive inhibitors; this area has been extensively reviewed elsewhere.82–84 Thus far, approaches have relied heavily on computer-aided drug design (CADD) and virtual screening. Although these approaches have has led to the discovery of a myriad of hits, with the exception of a few notable examples, very little hit to lead and lead optimization studies have been conducted. Further synthetic efforts are required to maximize biological activity and fully map the structure activity relationships of these putative inhibitors. Ouertatani-Sakouhi et al. has stated that there are a number of mechanistic ways in which MIF antagonists can interact with the tautomerase active site,85 these include: (i) binding in the active site (competitive inhibition); (ii) covalent linkage to Pro1 (irreversible inhibition); (iii) allosteric inhibition; (iv) stabilization of the MIF monomer, in turn, preventing active trimer re-association; and (v) compound-induced dissociation of the MIF homotrimer. Of these strategies, the two most widely used to study the effects of MIF and cancer in vivo involve competitive inhibition of MIF and covalent linkage of small molecules to Pro1. A small number of allosteric inhibitors of MIF have been identified such as ebselen,85 ibudilast,86 and 6'-[(3,3-dimethoxy[1,1'-biphenyl]-4,4'-diyl)bis(azo)]bis[4-amino-5-
Figure 6. Strategies for MIF inhibition in the treatment of cancer. MIF's functional activity can be targeted in a number of ways. Allosteric, covalent, and competitive inhibitors of MIF prevent interactions with the MIF receptor CD74 thereby inhibiting downstream cell signaling effects, such as MAPK activation, p53-dependent apoptosis, and Akt activation. Anti-MIF antibodies can also disrupt interaction with CD74 and elicit a similar response. MIF inhibitors, which are cell membrane permeable, can bind to intracellular MIF and block protein–protein interactions inside the cell. Finally, MIF is a client protein for HSP90, which stabilizes intracellular MIF. A HSP90 inhibitor can interfere with this PPI leading to MIF degradation. Similarly, an HER2 inhibitor can block HSP90 expression and in turn destabilize MIF.

hydroxy-1,3-napthalenedisulphonic acid] tetrasodium salt (p425). Although p425 showed significant effects on cytokine signaling and reversed MIF’s suppressive effects on p53-dependent apoptosis, to the best of our knowledge, none of these allosteric inhibitors have been examined in an in vivo cancer model. It would be interesting to see if the promising biological activity of such inhibitors is maintained in an in vivo cancer setting.

Competitive inhibitors
The isoxazoline class of compounds represents the most extensively studied class of competitive MIF inhibitors. Initially discovered by Lubetsky et al., lead optimization efforts led to the identification of the gold standard prototypical MIF inhibitor S,R-3-(4-hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid methyl ester (Iso-1, Fig. 7). This inhibitor has been a key tool in elucidating the complex biological role that MIF occupies in cancer and inflammation. (An excellent review of Iso-1 as a proof of concept inhibitor can be found here.) For instance, it has been shown that inhibition of MIF by Iso-1 leads to a decrease in cell migration, proliferation, and invasion in multiple human cancer cell lines including A549, DU145, LN229, and HS683e. In vivo, Iso-1 has shown significant efficacy in models of prostate and colorectal cancer in which treatment with the inhibitor led to a decrease in tumor growth and vascularization. Although highly promising results were obtained, the in vivo enzymatic kinetics of Iso-1 and its route of intraperitoneal administration represent a significant challenge with regard to the design of human clinical trials.

In an effort to resolve these issues, an Iso-1 derivative, Iso-66 (Fig. 7), has recently been developed by the same group. This compound has shown enhanced stability and lower
Figure 7. Chemical structures of MIF inhibitors. A number of MIF inhibitors have been synthesized and tested. A selection of the MIF inhibitors used to elucidate the role of MIF in cancer is shown including competitive, allosteric, and covalent inhibitors.

Toxicity than Iso-1, however, critically, maintains the significant in vivo efficacy of the parent compound. Iso-66 is a fluorinated oxazoline derivative showing strong tautomerase activity with a $K_i$ of around 1.5 $\mu$M and high chemical stability. In in vivo models of melanoma and colorectal cancer, Iso-66 was shown to be nontoxic and led to decreased tumor burden in diseased animals. Ex vivo analysis showed that this was due to expansion of antitumor-specific effector cells. As MIF inhibits the activity of tumor-specific CTL and NK cells, blocking MIF activity led to restoration of their tumor killing functionality.

Consequently, Cytokine PharmaSciences, Inc. has built on this work by developing two orally available oxazoline derivatives for the treatment of MIF-related diseases. These compounds, CPSI-2705 and CPSI-1306 (Fig. 7), initially developed for use in the treatment of autoimmune disorders such as multiple sclerosis (MS) and diabetes mellitus, potently block not only MIF tautomerase activity but also the key MIF/CD74 PPI showing activity of up to 100 times that of Iso-1. Further evaluation of these compounds has generated significant and promising results in murine bladder and skin cancer models. Bladder cancer is one of the most lethal forms of cancer and modern treatments are inadequate and often associated with a poor patient prognosis. Novel treatments for bladder cancer and methods for the early diagnosis of this form of cancer are a significant current clinical unmet need. MIF is highly expressed in bladder cancer and in vitro studies have shown a significant reduction in cell proliferation of bladder cancer cells when treated with anti-MIF antibodies. An in vivo study was carried out using the murine $N$-butyl-$N$-(4-hydroxybutyl)-nitrosamine (BBN) bladder cancer model in which treatment with either CPSI-2705 or CPSI-1306 led to a significant reduction in tumor growth, angiogenesis, and cell proliferation. Of the two inhibitors tested, CPSI-1306 showed a much more pronounced effect than that of CPSI-2705, the authors of the study suggest that this is possibly due to its longer in vivo half-life. Following on from this, CPSI-1306 has also been used to study squamous cell carcinomas and the effect of UVB light induced inflammation. In an experimental model using Skh-1 mice, the effect on UVB-induced inflammation was examined. CPSI-1306-treated mice were shown to have a significant decrease in skin thickness, increased p53 expression, decreased cell proliferation, and reduced myeloperoxidase (MPO)
activity, a characteristic product produced by neutrophils during periods of acute inflammation, when compared to untreated animals. An extension of this study based around a more persistent UVB exposure model in a squamous cell carcinoma showed similar results, which included decreased tumor burden in treated mice.

Mawhinney et al. have recently reported the discovery of a novel MIF inhibitor that leads to a significant decrease in tumor volume in a murine lung cancer model. The research group has established a model of Lewis lung cancer in wild-type, MIF knock out and the tautomerase null P1G mutant. Upon measurement and comparison of tumor growth in each animal, a significant decrease in growth was noticed in both the MIF knock out and the P1G for this form of cancer. Through a number of biochemical screens, a novel isocoumarin compound, 3-(2′-methylphenyl)-isocoumarin (SCD-19, Fig. 7), was identified as a potential lead compound. In the model, treatment with the inhibitor at the time of tumor inoculation showed a 90% reduction in tumor volume compared to the control. Lung cancer is an aggressive disease and often can be diagnosed at a late stage when the tumor has already grown significantly. To further this work, a model, which better replicates the human condition, was used; namely, treatment with the inhibitor was commenced when the tumor was palpable in the mouse (ca. Day 7). The investigators found a significant reduction in tumor volume (mean of 81%) when compared to untreated mice. This strategy involved the use of a novel MIF inhibitor to target both the tumor and host-derived MIF to maximize the effects of anticancer therapy.

In 2012, Choi et al. showed that anti-MIF treatment could aid the adaptive immune response to colon cancer. In a study using CT26 colon carcinoma, they examined the effect of MIF on tumor progression and the production of tumor regulatory T cells (Tregs) in mice. Tumor-derived CD4+ Tregs play an important role in suppressing the immune response to tumor cells, which leads to the development of immune tolerance, local tumor growth, and systemic progression of tumors into the blood and lymphatic systems. In this study, tumor growth was significantly lower in MIF knockout mice compared to the wild-type mice and the authors concluded that MIF promotes tumor progression in mice by increasing Tregs in the tumor environment through the upregulation of IL-2 production. Using the MIF inhibitor 3-(3-hydroxybenzyl)-5-methylbenzooxazol-2-one (Debio1036) (Fig. 7) and an anti-MIF monoclonal antibody, the authors showed it was possible to block inducible CD4+ Treg generation in vitro through blocking of anti-CD3-induced IL-2 production by the splenocytes of wild-type mice.

Irreversible inhibitors.

Although medicinal chemists generally discount irreversible inhibitors during drug discovery projects due to their susceptibility to off-target effects and toxicity issues, a recent resurgence in this “nonconventional” class of inhibitor has led to new avenues for exploration. The presence of a terminal nucleophilic proline residue makes MIF an ideal candidate for modification with a covalent inhibitor and, indeed, a number of potent irreversible MIF inhibitors have already been identified including 2-oxo-4-phenyl-3-butanoate, phenylpyrimidines, acetaminophen analogs, and epicatechins.

In 2008, Winner et al. identified 4-Iodo-6-phenylpyrimidine (4-IPP, Fig. 7) as a potent inhibitor of MIF tautomerase activity. This compound binds irreversibly to the Pro1 residue of MIF through nucleophilic displacement of an aromatic iodo group. 4-IPP has an IC₅₀ value that is ten times lower than that of ISO-1. In addition, 4-IPP showed a reduction in the motility and growth of lung cancer cells in vitro. In a recent in vivo study, the same group has identified a central role for MIF in the alternate activation of tumor-associated macrophages (TAMS). They conducted a detailed study using a model of melanoma in which it was demonstrated that tumor growth was decreased and survival rates were increased in both MIF null cohorts.
and 4-IPP-treated cohorts when compared to wild type. 4-IPP was further evaluated in head and neck squamous cell carcinoma cell line SCCVII. Treatment of the cells with the inhibitor inhibited cell proliferation and invasiveness.\(^{104}\)

More recently, it has been demonstrated that 4-IPP is the first identified dual D-DT/MIF inhibitor.\(^{44}\) In an in vivo model of *Pseudomonas aeruginosa* infection, D-DT and MIF act in an additive manner to recruit neutrophils to the sites of infection; this can cause significant lung damage to immunocompromised patients. When treated with the 6-PP analogs of MIF, neutrophil recruitment to the lungs was reduced by up to 50%. This adds significant weight to the expanding body of evidence that the terminal proline residue appears to have a role in PPI and must remain unsubstituted. While irreversible inhibitors historically have not been favored as therapeutic candidates, the 4-IPP published work highlights that a dual strategy of targeting MIF/D-DT enzymatic activity represents a valid therapeutic strategy for selected diseases.

Naturally occurring dietary isothiocyanates (ITCs) have also been identified as potent covalent inhibitors of MIF. ITCs are a well-known class of electrophilic compounds, present in abundance in cruciferous vegetables such as broccoli, watercress, kale, wasabi, brussel sprouts, and cauliflower. These compounds have long been associated with significant anticancer and anti-inflammatory properties;\(^{105}\) however, the intracellular targets of these compounds have remained elusive. Using a combination of proteomic approaches as well as site-directed mutagenesis and mass spectrometry, the \(N\)-terminal proline residue has been identified as a key site for covalent modification with ITCs.\(^{106}\) Although MIF contains a number of cysteine residues, addition of phenethyl ITC (PEITC, Fig. 7) led to modification exclusively at Pro1 and the formation of thiourea adducts.\(^{107}\) Indeed, a number of ITCs, including sulforaphane, benzyl, \(n\)-hexyl, and phenethyl ITCs, have demonstrated potent MIF tautomerase inhibition via covalent modification of Pro1.\(^{108}\) It has been shown that a diet involving high levels of consumption of ITCs is protective against lung, breast, colon, and skin cancer.\(^{109,110}\) Although there are a number of mechanisms by which ITCs can elicit an anticancer effect, its ability to bind to MIF and modulate its activity is an interesting prospect and would provide an explanation for many of these effects. A recent report has shown that celecoxib, a COX-2 selective NSAID, in combination with allyl ITC (AITC, Fig. 7), had a significant effect on the growth of bladder cancer in vivo.\(^{111}\) In a rat model of bladder cancer, AITC and celecoxib at low doses had a synergistic effect on the inhibition of tumor growth (up to 63% in combination compared to 17% in celecoxib alone and 23% in AITC alone). The effect was attributed to the depletion of prostaglandin E2 (PGE-2). As MIF has been shown to upregulate COX-2 and, in turn, PGE-2 production, the inhibition of MIF by AITC is probably responsible for this anticancer effect.

2. **Destabilization of MIF**

An intriguing recent discovery related to MIF involves the chaperone protein heat shock protein 90 (HSP90). Schulz et al. have demonstrated an alternative way in which the biological activity of MIF can be targeted.\(^{112,113}\) The mechanism by which MIF is stabilized in cancer cells has only recently begun to be identified. MIF has been identified as a novel client of HSP90 in cancer cells, protecting it from degradation. The use of HSP90 inhibitors in cancer cell lines showed significant levels of MIF degradation, which in turn has a knock-on effect in cell proliferation and apoptosis. In their study, they also examined the role played by MIF in an in vivo model of human breast cancer ErbB2. Genetic analysis confirmed MIF stabilization by HSP90 occurs in vivo and the use of the HSP90 inhibitor 17-(alkylamo)-17-(demethoxygeldanamycin) (17AAG) showed reduced levels of MIF protein and cell proliferation. As considerable levels of HSP90 and MIF are expressed in cancer cells compared with normal cells, it is quite logical that the targeting of HSP90 with small molecules could be an alternative therapeutic target in an anti-MIF cancer strategy. Indeed, a number of HSP90 small molecule inhibitors are in clinical
trials for cancer and perhaps it is their effect on MIF stabilization that is responsible for their potent anticancer activity. The same group has also recently shown that HER2/Erb2 overexpression in breast cancer controls the major oncogenic growth factor HSF1.\textsuperscript{114} This protein controls the fate of HSP90 and in turn, the clients of HSP90. In vitro and in vivo studies have demonstrated that inhibition of HER2 blocks activation of HSP90 leading to destabilization of MIF. This result suggests that depending on the type of cancer encountered, a targeted therapeutic strategy can be developed; in HER2 overexpressing breast cancer, an HER2 inhibitor can be used to block the action of HSP90 leading to MIF degradation, whereas in non-HER2 expressing cancers, an HSP90 inhibitor can be used to destabilize MIF.

3. Anti-MIF Antibodies

Although possessing a number of disadvantages, the utility of monoclonal antibodies in the treatment of cancer has recently been demonstrated. Traditionally, antibodies are considered undesirable for a number of reasons including their relatively short half-lives, the high costs associated with their production, undesirable routes of administration, and their potential for immunogenicity. The recent clinical success using monoclonal antibodies such as Milatuzumab and Rituximab has changed this view. MIF monoclonal antibodies have been around for a number of years, however their application in cancer has only recently been explored. A number of monoclonal antibodies developed by scientists at Baxter have shown potent in vitro and in vivo anti-MIF activity in human PC3 prostate cancer cell lines.\textsuperscript{115} Three monoclonal antibodies, BaxG03, BaxB01, and BaxM159, were shown to inhibit MAPK/ERK1/2 activation in vitro leading to a reduction in cell growth and viability of the cancer cell lines. They also showed effects on MIF-induced cellular invasion and chemotaxis. In an in vivo PC3-Xenograft model, treatment with the antibodies led to decreased tumor volumes in a dose-dependent manner. A subsequent Phase 1 trial has been initiated using anti-MIF monoclonal antibodies to treat patients with solid tumors (clinicaltrials.gov.NCT01765790). Similarly, in a CT26 colon cancer model, treatment with anti-MIF neutralizing antibodies showed a significant reduction in tumor growth when compared to controls.\textsuperscript{116}

**Perspective**

The complex, enigmatic nature of MIF and its involvement in cancer pathogenesis is an ongoing productive area of research. Its exact role in every cancer phenotype still requires further elaboration and indeed, the nature of its role may be situation dependent. Nevertheless, the recent results demonstrate that for numerous cancer phenotypes, MIF offers a potentially valid and important therapeutic target, especially due to its pro-inflammatory nature. The problem, thus far, has been translating the positive results obtained from small molecule approaches and in vivo studies into a suitable clinical candidate to progress into human trials. To this end, further work in the area of small molecule research is ongoing. As demonstrated in the case of the oxazoline class of MIF inhibitors, extensive lead optimization efforts have been able to produce orally available potent MIF inhibitors that are efficacious in vivo and have good safety profiles. It would be interesting to see further development and in vivo studies being pursued for other classes of MIF inhibitor. As it is now widely accepted that disrupting enzymatic activity alone is not sufficient to fully abrogate MIF’s biological effects, future inhibitors should also be screened for their ability to disrupt MIF/CD74 interactions as well as tautomerase activity. Interestingly, anti-MIF monoclonal antibodies interact with specific sequences on MIF and do not interfere with tautomerase activity placing more importance on developing small molecule inhibitors that disrupt MIF-related PPI. Obtaining an X-ray crystal structure of the MIF/CD74/inhibitor ternary complex would further enhance our
understanding of the interactions between these two proteins and aid in the discovery process. The intriguing prospect of discovering novel dual inhibitors of D-DT and MIF is a realistic target for researchers in this area over the next few years and computational chemistry and protein X-ray crystallography should be utilized to identify novel putative inhibitors of both active sites. Alternatively, dual D-DT/MIF monoclonal antibodies could also be developed and may lead to promising results. Probing the biological relationship of these two cytokines could be key in unlocking the complex nature of MIF biology. The recent, important, discovery that MIF is a client of HSP90 and is affected by HER2 in breast cancer offers a unique additional biological explanation for targeting HSP90/HER2 in cancer. In the future, targeted delivery of MIF inhibitors to specific cancer within specific organs will allow enhanced delivery of active compounds specifically to the cancer—while limiting systemic side effects.

REFERENCES


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