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Macrophage Migration Inhibitory Factor Promotes Tumor Growth and Metastasis by Inducing Myeloid-Derived Suppressor Cells in the Tumor Microenvironment

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The macrophage migration inhibitory factor (MIF), an inflammatory cytokine, is overexpressed in many solid tumors and is associated with poor prognosis. We previously identified inhibitors of MIF within a class of natural products with demonstrated anticancer activities. We therefore sought to determine how MIF contributes to tumor growth and progression. We show in this study that in murine tumors including the 4T1 model of aggressive, spontaneously metastatic breast cancer in immunologically intact mice, tumor-derived MIF promotes tumor growth and pulmonary metastasis through control of inflammatory cells within the tumor. Specifically, MIF increases the prevalence of a highly immune suppressive subpopulation of myeloid-derived suppressor cells (MDSCs) within the tumor. In vitro, MIF promotes differentiation of myeloid cells into the same population of MDSCs. Pharmacologic inhibition of MIF reduces MDSC accumulation in the tumor similar to MIF depletion and blocks the MIF-dependent in vitro differentiation of MDSCs. Our results demonstrate that MIF is a therapeutically targetable mechanism for control of tumor growth and metastasis through regulation of the host immune response and support the potential utility of MIF inhibitors, either alone or in combination with standard tumor-targeting therapeutic or immunotherapy approaches.

we hypothesized that MIF would contribute to the interaction between the tumor and the host immune response. In this study, we provide evidence for a mechanism of MIF action in promoting tumor growth and metastasis that is unrelated to effects within the tumor cells. We demonstrate that the tumor-promoting effects of MIF require interaction with a fully competent immune system, and we characterize MIF-dependent modulation of monocytic MDSCs within the tumor microenvironment.

Materials and Methods

Cell lines, reagents, and Abs

4T1 and CT26 cells (American Type Culture Collection) were cultured at 37°C in 5% CO2 in RPMI 1640, supplemented with 10% FBS and 1% penicillin/streptomycin. Luciferase-expressing 4T1 lines (Caliper Life Sciences) were cultured with heat-inactivated FBS as recommended. Anti-MIF was from Invitrogen and anti-tubulin from Sigma. t-Dopa-chrome methyl ester and periodate were from Sigma, and sulforaphane was from LKT Labs.

Mice

Female 6- to 12-wk-old BALB/c mice and 6- to 8-wk-old SCID/bg mice were purchased from Charles River Laboratories. All animal experiments were performed with the approval of the University of Virginia Animal Care and Use Committee.

Generation of MIF-depleted and -reconstituted cell lines

For MIF-depleted cell lines, the sequences 5′-gacctATGCCATGTTCATCG-TgATTCAAGAATACAGTAAACeGCGATTCTTCCAAGCGTg3′ and 5′-aactaACGGGTAAAATGTCCCAATGTCATCTGgATCTTTGTAATAC-gCAATGAACATaGGCATg-3′ were annealed and ligated into pSiren-RetroQ plasmid (Clontech) and used to make retroviruses. Parental 4T1 cells were infected with either the targeting hairpin or an empty vector and selected with 10 μg/ml puromycin. In some experiments, a line expressing a nontargeting RNA directed against the human MIF sequence was used in place of the empty vector control. For preparation of MIF-depleted CT26, parental CT26 cells were infected with targeting or nontargeting hairpin and selected with 10 μg/ml puromycin.

For the reconstituted cell lines, the coding region for wild-type (WT) human MIF or P2G MIF, in which the codon for the proline at position 2 was mutagenized to glycine [both resistant to the short hairpin RNA (shRNA) targeting the murine MIF sequences], was inserted into PQCXI-neo vector mutagenized to glycine [both resistant to the short hairpin RNA (shRNA) targeting the murine MIF sequences], was inserted into PQCXI-neo vector and selected with 500 μg/ml puromycin. In some experiments, a line expressing a nontargeting RNA directed against the human MIF sequence was used in place of the empty vector control. For preparation of MIF-depleted CT26, parental CT26 cells were infected with targeting or nontargeting hairpin and selected with 10 μg/ml puromycin.

Tumor implantation, measurement, and survival surgery

4T1 cells (1 × 10^5) were injected into the mammary fat pad of 6- to 12-wk-old female BALB/c mice. CT26 cells (2 × 10^5) were injected s.c. above the flank of 6- to 12-wk-old female BALB/c mice. Tumor volumes were estimated using the formula V = 0.4 × L × W^2 from two perpendicular measurements made with electronic calipers. In experiments with the reconstituted cell lines, tumors were removed at 18 d postimplantation. The mice were euthanized 8 d later for enumeration of metastasis.

Proliferation and colony formation assays

Cell lines, reagents, and Abs

MIF PROMOTES TUMOR PROGRESSION THROUGH MDSCs

Saluorphane treatment of mice

Mice were treated with 200 μg sulforaphane (SFN) or saline vehicle by i.p. injection, starting the day of tumor injection and continuing daily until tumor harvest.

Tissue digestion and clonogenic metastasis assay

Tumors were dissected from the mammary fat pad and digested with 10,000 U collagenase 1 (Worthington Biochemical) for 60 min at 37°C. Cell suspensions were strained through a 70-μm membrane and then stained for flow cytometry.

Isolated lungs were digested with 5 mg collagenase IV (Worthington Biochemical) and 30 U elastase (Calbiochem) for 75 min at 3°C. Strained cell suspensions were plated in IMDM plus 10% FBS, 1% penicillin/streptomycin, containing 60 μM 6-thioguanine. Plates were incubated for at least 10 d at 37°C, fixed with methanol, and stained with crystal violet for enumeration of colonies.

Imaging of lungs using luciferase

To image lung metastasis in experiments with the luciferase-expressing cell lines, mice were injected i.p. with 100 μl/10 g body weight of a 15 mg/ml solution of luciferin 5 min prior to euthanasia. Lungs were excised and immersed in a 300 μg/ml solution of luciferin in PBS for imaging on the Xenogen IVIS System (Caliper Life Sciences).

Flow cytometry

For flow cytometry analysis, cells were stained with Abs for CD45 PerCP (BD Biosciences), F4/80 aliphophycocyanin–eFluor780 (eBioscience), Ly6G FITC (BioLegend), Ly6C aliphophycocyanin (BioLegend), and CD11b Pacific blue (Invitrogen), along with Live/Dead Fixable Dead Cell Stain (Invitrogen). Live cells were gated first using Live/Dead Fixable Dead Cells Stain (negative population), then forward and side scatter, followed by CD45+ and, finally, CD11b+. A representative series of gated plots is shown in Supplemental Fig. 2A. The cells were analyzed with the Beckman Coulter Cyan ADP LX-9 Color Flow Cytometer.

Purification of MDSCs

MDSC depletion

Depletion of MIF does not alter in vitro growth properties of 4T1 cells

To address the role of MIF in tumor growth and progression and to test the hypothesis that MIF functions through impact on the immune tumor microenvironment, we used the spontaneously

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MIF promotes tumor growth and metastasis only in immunocompetent animals

To determine if MIF promotes tumor growth and/or metastasis, we implanted the MIF-containing or MIF KD 4T1 cells into the mammary fat pad of BALB/c mice. Both sets of mice developed tumors that were detectable and of similar size at approximately day 13. However, from 15 d after tumor implantation until harvest, the tumors from MIF-depleted KD 4T1 cells grew more slowly than those expressing MIF (Fig. 2A, left). Moreover, we detected fewer metastases in the lungs of mice bearing 4T1 MIF KD tumors than in the lungs of mice bearing MIF-containing tumors (Fig. 2A, right). This decrease in metastatic burden was not solely due to the difference in size of the primary tumors, as evidenced by comparing the metastasis as a function of tumor mass at the time of harvest. In this analysis, comparing tumors of similar mass, the MIF KD tumor-bearing mice still harbored far fewer metastases (data not shown).

To ensure that our observations were not unique to the 4T1 cell line, we generated a MIF-depleted CT26 murine colon carcinoma cell line, along with a control line expressing a nontargeting shRNA. Effective MIF depletion was confirmed by immunoblot (Supplemental Fig. 1A). Similar to the results observed with 4T1 cells, depletion of MIF did not alter the in vitro proliferation of CT26 cells (Supplemental Fig. 1B). However, MIF depletion significantly impaired the growth of s.c. CT26 tumors in syngeneic BALB/c mice, despite the tumors appearing at similar sizes at early time points (Supplemental Fig. 1C). This suggests that similar to

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**FIGURE 1.** MIF KD cells exhibit similar grow properties in vitro. (A) Immunoblot demonstrates effective MIF depletion in the MIF KD cells compared with the vector control and the parental 4T1 cells. Lysates from cell lines were normalized by Bradford assay for total protein concentration, run on a gel, and the membrane was probed using anti-MIF and anti-tubulin Abs. (B) Effective MIF depletion is confirmed by loss of tautomerase activity. Cell lysates normalized for total protein were assayed for tautomerase activity using a colorimetric substrate. The reaction was monitored for decolorization over 5 min at 475 nm. Velocities (rate of decolorization/unit time) are indicated to the right of each kinetic plot. (C) Depletion of MIF does not alter in vitro proliferation. Cells (5 × 10^4 per well) were plated in 6-well dishes. At the indicated time points, cells were trypsinized and counted. (D) Depletion of MIF does not alter anchorage-independent growth of 4T1 cells. Cells (4 × 10^5) were seeded in soft agar in 6-well plates. The plates were incubated at 37°C for 2 wk, then the colonies counted in three randomly selected fields. For (C) and (D), n = 3, and each is representative of three independent experiments.

**FIGURE 2.** MIF-depleted 4T1 cells form slower growing tumors that are compromised in metastasis to the lung in BALB/c but not SCID/bg mice. (A) MIF depletion leads to compromised tumor growth and metastasis in BALB/c mice. *Left:* 1 × 10^4 4T1 vector control or KD cells were injected into the mammary fat pad of 6- to 12-wk-old BALB/c mice. Tumors were measured starting at day 13, and tumor volume was estimated with the formula V = 0.4 × L × W^2. *Right:* On day 21, lungs were excised and metastasis detected using the clonogenic assay. n > 8 for each group and time point and is representative of three independent experiments. (B) MIF depletion does not impact tumor growth and metastasis in immunodeficient mice. *Left:* 1 × 10^4 4T1 luciferase-expressing non-targeting shRNA (NT) or MIF-depleted (KD) cells were implanted in the mammary fat pad of 6- to 8-wk-old SCID/bg mice, and tumor growth was monitored as in (A). n = 4 for each group and time point and is representative of two independent experiments. *Right:* On day 21, lungs were excised, immersed in luciferin, and tumor metastasis detected by imaging with the IVIS system. n = 8 for each group and includes two independent experiments. *p < 0.05, **p < 0.005.
the 4T1 breast tumor model, MIF promotes CT26 tumor growth without influencing the in vitro growth properties of the cells.

Because MIF suppression did not alter cell growth in vitro but caused reduced tumor growth and metastasis in an intact animal, we hypothesized that the difference in tumor growth and metastasis observed in the BALB/c mice was due to an influence of MIF on the immune system. To test this, we implanted MIF-containing and MIF-depleted 4T1 cells expressing luciferase in the mammary fat pad of SCID/bg mice that lack T cells, B cells, and NK cells but have an intact myeloid compartment. In contrast to the results seen

![Figure 3](https://example.com/figure3.png)

**FIGURE 3.** Reconstitution with WT MIF, but not the tautomerase-deficient P2G mutant, restores tumor growth and metastasis. (A) Reconstitution of 4T1 KD cells. Immunoblot demonstrates effective reconstitution with mutant P2G MIF or WT MIF. A vector control line was prepared in parallel. (B) Tautomerase assay compares the velocities (decolorization/unit time) of the reconstituted cell lines compared with parental 4T1 cells, confirming restoration of tautomerase in the WT reconstitution. (C) WT, but not P2G, MIF restores tumor growth. Vector-, P2G-, or WT-reconstituted cells were injected into the mammary fat pad of 6- to 12-wk-old BALB/c mice and tumor monitored as for Fig. 2. *n > 8 for each group and is representative of three independent experiments. (D) WT, but not P2G, MIF restores pulmonary metastasis. Tumors were implanted as previously described and surgically removed at day 18. The mice were euthanized 8 d later, and lungs were excised and assayed for metastasis as in Fig. 2A. *n > 6 for each group and includes results from two independent experiments. *p < 0.05 (by ANOVA).

![Figure 4](https://example.com/figure4.png)

**FIGURE 4.** Tumors expressing WT MIF contain a greater proportion of monocytic MDSCs. (A) MIF expression in the tumor correlates with increased monocytic MDSCs. Vector-, P2G-, or WT-reconstituted tumors were implanted as previously described. On day 18, cell suspensions prepared from isolated tumors were stained for analysis by flow cytometry using a viability marker and the cell surface markers CD45, CD11b, Ly6G, and Ly6C. Representative plots of Ly6C/Ly6G staining from WT-, P2G-, and vector-reconstituted tumors are shown with prior gating on live, CD45+, CD11b+ cells (complete gating strategy shown in Supplemental Fig. 2A). Gated populations indicate Ly6Chigh, Ly6Clow monocytic MDSCs and Ly6Clow/int, Ly6Ghigh granulocytic MDSCs. Bottom right: Quantification of monocytic MDSCs from tumors isolated from BALB/c mice; *n = 15 for each group and includes three independent experiments. (B) SCID/bg mice were implanted with reconstituted tumors. On day 22, tumors were processed as described for (A). *n > 8 for each group and includes three independent experiments. (C) Parental 4T1 tumors were implanted into mice that received either saline or SFN (200 µg/day) starting the day of tumor cell implantation. On day 21, tumors were processed as described for (A). *n > 8 for each group and includes two independent experiments. *p < 0.05 (by ANOVA).
in the immunologically intact BALB/c mice, the resulting tumors grew at similar rates in the SCID/bg mice over the duration of the experiment (Fig. 2B, left). Moreover, ex vivo imaging of the metastatic burden in the lungs at the time of harvest demonstrated that the MIF-containing and MIF-depleted tumors were similarly metastatic in immunodeficient SCID/bg animals (Fig. 2B, right). These results suggest that the contribution of MIF to tumor growth and metastasis is due to influence over some aspect of the immune system.

**Promotion of tumor growth and metastasis is dependent on tautomerase activity of MIF**

To confirm that the effects observed in the MIF-depleted cells were due to loss of MIF, we reconstituted the KD cells with MIF using an expression plasmid encoding an shRNA resistant version of the MIF gene. In parallel, to test the importance of the tautomerase activity of MIF in promoting tumor growth and metastasis, we reconstituted the MIF KD cell line with a similar plasmid encoding a tautomerase-deficient MIF protein containing a proline to glycine substitution at residue 2 (P2G). Expression of the MIF protein in the two reconstituted cell lines was equivalent and similar to the parental cells (Fig. 3A). Tautomerase activity was restored in the WT-reconstituted line but remained absent in the P2G line, as the P2G mutant abolishes the tautomerase activity (Fig. 3B). Incipient tumors resulting from either of these cell lines were detected in the mammary glands of injected mice at the same time, but tumors derived from cells reconstituted with WT MIF grew at a faster rate (Fig. 3C) compared with those from the MIF-depleted line mock-reconstituted with an empty vector. Importantly, reconstitution with the tautomerase-defective P2G MIF did not restore the rapid tumor growth seen in WT MIF-expressing tumors. This suggests that the tautomerase activity of MIF is required for the tumor growth-promoting activity of MIF. Furthermore, the tautomerase activity was also important for promotion of metastasis by MIF, as reconstitution with WT MIF restored the ability of tumors to produce pulmonary metastases, whereas the P2G mutant MIF did not (Fig. 3D).

**MIF expression influences the prevalence of monocytic MDSCs in the tumor**

The dependence of MIF tumor-promoting activity on an intact immune system suggests that MIF does not directly impact an intrinsic property of the tumor cells but rather regulates an aspect of the tumor growth–promoting immune microenvironment. To identify MIF-dependent alterations in the immune cell infiltrates within the tumors, we examined the phenotype of cells prepared from the MIF-containing and MIF-depleted tumors using a panel of markers to detect MDSCs. Specifically, we examined the two major MDSC populations, defined as Ly6Chi/Ly6Ghigh granulocytic MDSCs and Ly6Cint/Ly6Glow monocytic MDSCs (6). The CD11b+ myeloid cells in the WT MIF-reconstituted tumors contained a significantly larger proportion of monocytic MDSCs compared with the P2G- and vector-reconstituted tumors (Fig. 4A). No differences were observed in this subpopulation in either the blood or spleen of these animals (Supplemental Fig. 2B). Because MIF is named for its effects on macrophage migration, we also examined F4/80+ macrophage populations in the 4T1 tumors. Over the same three experiments in which we detected significant differences in the monocytic MDSC population, there was no difference in the GR1–, F4/80+ macrophage population (Supplemental Fig. 2C). Therefore, MIF expression in the tumor cells acts specifically to increase the abundance of the monocytic population of MDSCs within the tumor. A reduction in monocytic MDSC abundance was also observed in the MIF-deficient CT26 tumors (Supplemental Fig. 1D). These results suggest that MIF may promote tumor growth and metastasis by enhancing the

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**FIGURE 5.** MDDCs isolated from MIF-containing 4T1 tumors more effectively suppress CD8+ T cell proliferation than MDSCs from MIF-depleted tumors. 4T1 NT or KD tumors were implanted into mice as for Fig. 2. At harvest, tumors were digested and GR1+ MDSCs were purified. Purified CD8+ T cells were stimulated with CD3/CD28 in the presence or absence of MDSCs at ratios of 1:1 and 1:4 (T cell/MDSC). The graph shows combined results from two independent experiments. *p < 0.05 (by ANOVA).

**FIGURE 6.** Conditioned media from MIF-expressing 4T1 cells induces differentiation of monocytic MDSCs. CD11b+ cells were isolated from the spleen of WT tumor-bearing mice and incubated with conditioned media from MIF nontargeting (NT) control cells or MIF KD 4T1 cells with or without SFN as indicated for 5 d (schematic of experiment in Supplemental Fig. 3A). (A) Phenotype of purified CD11b cells at start of experiment, stained as for Fig. 4A. n = 3. (B) Phenotype of cells after 5 d of culture. Cells were stained for analysis by flow cytometry as for Fig. 4A. n > 12 for each group and includes results of four independent experiments. *p < 0.05 (by ANOVA).
immune-suppressive properties of the tumor microenvironment through influence over MDSC populations.

The differences in MDSC abundance were not simply the result of differences in tumor size, as MDSC populations characterized in WT MIF-expressing tumors in SCID/bg mice also displayed a greater fraction of monocytic MDSCs than those from MIF-depleted or P2G-reconstituted tumors (Fig. 4B), despite all of the tumors being of similar size. Significantly, although the MIF-dependent differences in monocytic MDSC populations were observed in the immunocompromised mice, tumors that express MIF do not display accelerated tumor growth and metastasis in these mice (Fig. 2B). Therefore, although MIF is still able to influence the abundance of monocytic MDSCs in the SCID/bg hosts, in the absence of an antitumor immune response mediated by T cells and NK cells, there is no impact of MIF on tumor growth and metastasis.

Parallel to this genetic demonstration of the importance of the tautomerase activity of MIF in regulation of MDSC populations, we used SFN, a natural product inhibitor of MIF (26), to suppress pharmacologically the MIF tautomerase. Treatment of 4T1 tumor-bearing mice with SFN reduced the prevalence of monocytic MDSCs in tumors, similar to the effects of MIF depletion from tumors (Fig. 4C). Together with the failure of the tautomerase-deficient P2G mutant to rescue MDSC recruitment, this suggests that MIF controls the prevalence of monocytic MDSCs through a mechanism that requires its tautomerase activity.

Finally, to confirm that the MIF status of the tumor and the resultant shift in MDSC populations resulted in functional changes within the tumor microenvironment, we isolated GR1+ cells from MIF-containing and MIF-depleted tumors and evaluated their ability to suppress CD8+ T cell proliferation in vitro. The purified GR1+ cells isolated from MIF-expressing tumors suppressed T cell proliferation significantly more than those from the MIF-depleted tumors (Fig. 5) when comparing an equivalent number of cells. In fact, at a 1:1 ratio of MDSCs to T cells, the MDSCs isolated from the MIF-depleted tumor failed to suppress proliferation significantly, whereas the cells from the MIF-containing tumor were effective. These results demonstrate that the MIF-expressing tumors have a more highly suppressive MDSC infiltrate and therefore likely benefit from a more immunosuppressive microenvironment.

**MIF promotes the differentiation of CD11b+ cells into monocytic MDSCs in vitro**

The MIF-dependent increase in prevalence of monocytic MDSCs in the 4T1 tumors could result from either increased recruitment of these cells into the tumor or differentiation of the MDSCs toward the monocytic phenotype, perhaps within the tumor. Others have reported that factors such as GM-CSF can control differentiation between the two subsets of MDSCs (36). Using a similar approach (diagrammed in Supplemental Fig. 3), we evaluated the effect of MIF on MDSC differentiation in vitro by flow cytometry. After purification of CD11b+ MDSCs from the spleen of a tumor-bearing mouse, the majority of cells were of the less suppressive granulocytic phenotype, with the monocytic subset composing <10% of the total population (Fig. 6A). Tumor-conditioned media from MIF-containing 4T1 cells induced differentiation of CD11b+ myeloid cells into monocytic MDSCs (Fig. 6B). Conditioned medium from MIF-depleted (KD) 4T1 cells resulted in a significantly reduced proportion of the CD11b+ cells differentiating into monocytic MDSCs. Cell viability in the cultures at the end of the incubation ranged between 25 and 50% (Supplemental Fig. 3B). Notably, there was no difference in cell viability between the cultures treated with the conditioned media from the MIF-containing versus the MIF-depleted 4T1 cells, suggesting that the differences seen in monocytic MDSCs cannot be solely attributed to differences in survival. These observations suggest that
either MIF itself or some MIF-induced secreted factor is responsible for the accumulation of monocytic MDSCs in this assay.

Addition of the MIF inhibitor SFN to the supernatant from the MIF-expressing 4T1 cells significantly decreased the induction of monocytic MDSCs (Fig. 6B). In contrast, SFN did not alter the induction of monocytic MDSCs in the cultures containing MIF KD supernatants. These results suggest that the differing prevalence of MDSCs in the MIF-containing versus MIF-depleted 4T1 tumors results from MIF-induced differentiation of myeloid cells in the microenvironment into the monocytic MDSC population. Inhibition of this process by SFN suggests that it occurs through a mechanism dependent on MIF tautomerase activity.

**MIF promotion of tumor growth and metastasis requires MDSCs**

To verify that the contribution of MIF to tumor growth and metastasis was due to the reduction of MDSC abundance in the tumor, we depleted MDSC populations from mice and determined the effect on tumor growth and metastasis. In a pilot experiment, we demonstrated that i.p. delivery of three doses of GR1 Ab on days 11, 14, and 17 after tumor implantation resulted in a near complete depletion of MDSCs from the tumor when assayed on day 17 (Supplemental Fig. 4B). Subsequently, mice bearing either MIF-containing or MIF-deficient tumors were treated with anti-GR1 Ab to deplete MDSCs using the same dosing regimen (Supplemental Fig. 4A) with depletion initiated at day 11 postimplantation, before the presence of a measurable tumor. The experiment was harvested at day 20. Depletion of MDSCs in mice bearing MIF WT tumors resulted in impaired tumor growth (Fig. 7A) and inhibition of metastasis to lung (Fig. 7B). In contrast, depletion of MDSCs in mice bearing MIF KD tumors had no further impact on tumor growth (Fig. 7A) compared with MIF KD tumors in mice administered an isotype control Ab. Because MIF KD tumors fail to metastasize to lung (Fig. 7B), it was not possible to determine if MDSC depletion had any further impact on pulmonary metastasis in the absence of MIF. From this, we conclude that the contribution of MIF to 4T1 tumor growth and metastasis can be attributed to the effects of MIF in controlling MDSCs in the tumor microenvironment. Taken together, our results lead us to propose the model shown in Fig. 8.

**Discussion**

Our results demonstrate a link between the proinflammatory cytokine MIF and the prevalence of monocytic MDSCs in the tumor microenvironment, leading to promotion of tumor growth and metastasis. This differs from previous reports that attribute the tumor growth–promoting effects of MIF to activities within the tumor cells (30–32, 37). Our work reveals a new mechanism for MIF as an immune modulator in the tumor microenvironment.

Significantly, we demonstrate that the tautomerase activity of MIF is required for promotion of tumor growth and metastasis. Using mutation of the catalytic proline and/or inhibition of MIF tautomerase with the enzyme inhibitor SFN, we further demonstrate that this enzymatic activity is important for inducing monocytic MDSCs both in tumors and in our in vitro differentiation assay. The importance of the MIF tautomerase activity has been debated (28, 38, 39). However, other inhibitors of the MIF tautomerase also influence downstream biological outcomes (28, 29, 40). Our current findings lend support to the conclusion that, although a physiological target has yet to be identified, the tautomerase activity is important for functions of MIF that contribute to its role in tumor growth and metastasis.

In this study, we observed MIF-dependent differences in the prevalence of monocytic MDSCs in the tumor microenvironment. As previously described, the monocytic MDSC subset is thought to have a higher capacity to suppress host anti-tumor response (9), ultimately supporting increased tumor growth and metastasis. Therefore, our demonstration that MIF contributes to the prevalence of these cells within the tumor suggests a possible mechanism through which MIF promotes tumor progression by influence over the tumor microenvironment.

Given that human cancers demonstrate increased MIF expression (17–21) and that MDSCs have been observed in the blood of human cancer patients (3–5, 41, 42), MIF is potentially a valuable therapeutic target for efforts aimed at inhibiting the tumor-supportive host immune response. Specifically, inhibition of MIF could block the accumulation of immune-suppressive MDSCs in the tumor microenvironment and restore effective anti-tumor immunity. This strategy, either alone or in combination with immunotherapy approaches designed to boost anti-tumor immune responses, offers an alternative to therapies that directly target the tumor cells. These approaches might be particularly useful in cancers that do not have recognized molecular therapeutic targets but that exhibit a strong inflammatory response or for those that are resistant to currently available targeted therapies. Finally, as tumors can recur and/or reappear as metastatic disease even years after primary tumor resection, our observation that MIF is important for spontaneous pulmonary metastasis suggests that MIF inhibition could represent a key approach not only for preventing primary tumor outgrowth but also for prevention of metastatic disease.

Our work not only furthers our understanding of the role of MIF in tumor progression but also suggests a previously unappreciated mechanism of action for cancer chemopreventives. We characterized the isothiocyanate chemopreventives, including SFN, as effective, covalent, natural product inhibitors of MIF (26). Epidemiological evidence and experimental models have long supported the conclusion that isothiocyanates exhibit anticancer activities (43–46). However, much of the function of isothiocyanates was thought to be through the detoxification of carcinogens (43–46), preventing the very earliest steps in tumor formation. Alternative models have focused on the ability of isothiocyanates to decrease proliferation of the cells (47, 48) or induce apoptosis (49, 50). Our study provides a novel molecular mechanism to explain isothiocyanate chemoprevention through inhibition of MIF and the resulting influence over the tumor microenvironment through specific impact on the immunosuppressive immune infiltrate.

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**Disclosures**

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