Abstract  MIF was recently redefined as an inflammatory cytokine, which functions as a critical mediator of diseases such as septic shock, rheumatoid arthritis, atherosclerosis, and cancer. MIF also regulates wound healing processes. Given that fibroblast migration is a central event in wound healing and that MIF was recently demonstrated to promote leukocyte migration through an interaction with G-protein-coupled receptors, we investigated the effect of MIF on fibroblast migration in wounded monolayers in vitro. Transient but not permanent exposure of primary mouse or human fibroblasts with MIF significantly promoted wound closure, a response that encompassed both a proliferative and a pro-migratory component. Importantly, MIF-induced fibroblast activation was accompanied by an induction of calcium signalling, whereas chronic exposure with MIF down-regulated the calcium transient, suggesting receptor desensitization as the underlying mechanism.

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1. Introduction

Macrophage migration inhibitory factor (MIF) is one of the first cytokines discovered [1]. MIF was initially defined as a T-cell factor which inhibited the random migration of macrophages. Thus, MIF activity was implicated in “negative” regulation of cell motility. More recently, MIF was redefined as a pleiotropic inflammatory cytokine with broad target cell specificity that is secreted upon inflammatory stimulation, tissue injury, and cellular stress by a variety of immune cells, but also endothelial, pituitary, and some epithelial cells [1–3]. MIF has been recognised as a critical mediator of acute and chronic inflammatory diseases such as septic shock, Crohn’s disease, rheumatoid arthritis, and atherosclerosis [1,4]. Furthermore, MIF is overexpressed in numerous cancers, has been linked to the development of colon cancer, and has been proposed to constitute a molecular link between inflammation and cancer [5–7]. In a recent report, Hagemann et al. concluded that tumor necrosis factor-α (TNF-α) induces tumour-associated macrophages to secrete MIF, which serves to enhance the invasive capacity of the tumour cells [8]. In line with these observations, MIF was found to promote the migration of hepatic carcinoma cells through the angiogenic factors interleukin-8 (IL-8) and vascular endothelial growth factor (VEGF) [9] and to stimulate the migration of microvascular endothelial cells [10]. In elucidating the molecular mechanism underlying MIF-induced leukocyte recruitment into atherogenic vessels, Bernhagen et al. demonstrated that MIF triggers monocyte/neutrophil and T-cell arrest and chemotaxis through interaction with the chemokine receptors CXCR2 and CXCR4, respectively [11,12]. MIF-induced CXCR receptor activity involved the MIF binding protein CD74, Gα protein coupling, calcium signalling, Src-type kinase and integrin activation [11]. Together, these studies show that MIF promotes pro-migratory processes both indirectly through stimulating the release of other migratory factors and directly through an interaction with CXCR2/4.

MIF plays a key role in the aging-related attenuation of the wound healing response as demonstrated by its upregulation in wounds of estrogen-deficient mice [13]. The excessive inflammation and delayed-healing phenotype associated with reduced estrogen was reversed in MIF−/− mice. This was due to an estrogen-mediated decrease in MIF production by activated macrophages and more generally by an inhibition of the local inflammatory response by downregulating MIF [13]. Hardman et al. profiled changes in gene expression within the wounds of mice that were wild-type or null for MIF in the presence or absence of estrogen, confirming MIF as a key player in wound healing, regulating many repair/inflammation-associated genes [14].
by excision was accompanied by an accelerating effect of MIF on the wound healing process of skin tissue [15]. When comparing excision wounds from the dorsal skin of MIF+/− mice with those of wild-type animals (MIF+/+), healing was significantly delayed in MIF+/− mice, suggesting that MIF is crucial in accelerating cutaneous wound healing [16]. Thus, MIF has been proposed to both promote and attenuate wound healing processes. These seemingly contradictory data might be reconciled if one considers that the wound healing process is complex, consisting of four distinct phases. These are the homeostasis phase (immediately upon injury; mainly characterized by rapid thrombus formation), the inflammation phase (up to 4–6 days after injury; leading to the recruitment of inflammatory cells), the migration phase (up to 6–8 days upon injury; mostly featuring the accumulation of fibroblasts and the production of extracellular matrix structures), and the remodelling phase (day 8 through 1 year after injury) [17,18]. The migration phase is sometimes termed proliferation phase (day 4 through 14 after injury) [17]. Although the precise role of MIF in these phases has not yet been addressed, due to its well-known role as a key regulator of inflammation and modulator of cell migration and proliferation, MIF would be predicted to be functionally involved in at least two of the four phases.

To address the mechanism(s) through which MIF may participate in the migration (proliferation) phase, we have investigated the effect of exogenously added recombinant MIF (rMIF) on the motility of mouse embryonic fibroblasts (MEFs) in an in vitro model of artificially wounded monolayers (‘scrape wounds’) following both transient and permanent (‘chronic’) exposure with rMIF. Both MEFs from mice genetically deficient for MIF (MIF+/− MEFs) and wild-type mice were studied. Given the established effect of MIF on proliferative responses, wound closure in the presence versus absence of mitomycin C was compared and the proliferative share was assessed by Ki67 staining. Migration-associated MIF-stimulated G22-protein-coupled receptor (GPCR) activity was evaluated by measuring calcium transients. Finally, to probe for the physiological effect in the healing of skin wounds, primary human dermal fibroblasts were included in the study.

2. Materials and methods

2.1. Recombinant MIF and chemicals

Recombinant human MIF (rMIF) was prepared as described previously [19,20]. rMIF was >98% pure as analysed by SDS-PAGE electrophoresis in combination with silver staining and was biologically active as determined by its anti-apoptotic and MAPK-stimulatory activity (data not shown and [21,22]). The rMIF preparation contained negligible concentrations of endotoxin (<10 pg endotoxin/mg MIF) as measured by Limulus Amebocyte Lysate (LAL, QCL-1000) assay (Cambrex, Verviers, Belgium). Mitomycin C, miscellaneous chemicals and salts were from Sigma-Aldrich Chemicals (Taufkirchen, Germany). All reagents were of the highest grade commercially available.

2.2. Cells and cell culture

Cell culture reagents were from Invitrogen (Karlsruhe, Germany), unless stated otherwise. Primary mouse embryonic fibroblasts (MIF+/− MEFs and MIF+/+ wild-type MEFs) were prepared from MIF+/− or wild-type mice (both C57BL/6 background) as described [23]. MEFs were cultured in Dulbecco’s modified Eagle medium (DMEM), containing 10% FCS, 1% penicillin-streptomycin, and 5 mM l-glutamine. Primary MEFs were used at passage 3–6. All cell culture experiments were performed at 37 °C in a humidified incubator with 5% CO2.

Human foreskin dermal fibroblasts (HFDFs) were isolated and cultured from human foreskin of two different donors essentially as described previously [24]. Briefly, normal fibroblasts were obtained from foreskin specimen. After excision, the specimens were washed three times in sterile phosphate-buffered saline (PBS; Invitrogen, Paisley, UK) containing antibiotics (penicillin-streptomycin; Invitrogen) and antimycotics (ampicillin B, PAA, Pasching, Austria), and digested in dispase solution (50 caseinolytic U/mL; Collaborative, Bedford, MA, USA) for 20 h at 4 °C and subsequently for 2 h at 37 °C. The epidermis was removed and the dermis was digested in collagenase I (100 U/ml; Sigma, Desenzenhofen, Germany) at 37 °C for 3 h. Remaining fibroblasts were seeded into petridishes with DMEM containing high glucose and l-glutamine (Invitrogen), and 10% FCS (Biochrom, Berlin, Germany). Cells were cultured in a CO2 incubator at 37 °C in a humidified atmosphere with 5% CO2 until reaching the state of early confluence.

2.3. In vitro ‘wound healing’ assay

MIF+/− and MIF+/+MEFs were plated in 6 cm culture plates (Cellstar, Greiner, Frickenhausen, Germany) and allowed to proliferate until ~90% confluence was reached (DMEM medium containing 10% FCS, 1% penicillin-streptomycin). Some cell incubations were treated with 10 μM mitomycin C or control solvent for 2 h prior to assessing their migration behavior. For subsequent treatment of cells with rMIF, FCS content was reduced to 0.5% and rMIF added either transiently or permanently, or cells left untreated. Incubations treated with phosphate buffer instead of rMIF were designated “N”; cells transiently treated with 50 ng/mL rMIF for 2 h were labeled “+2 h” and the group of cells permanently incubated with 50 ng/mL rMIF was designated “+P”. HFDFs were plated and treated following the same scheme.

Cell cultures were ‘wounded’ (‘scraped’) (time point t = 0 h; t = 0) with a rubber policeman as described previously [25]. To measure the migratory response of the cells into the scrape wounds, microscopic photographs were taken 0 and 24 h after injury. For microscopic examination of the migration response at the indicated time intervals, cells were placed on the microscope table, examined, and returned to the incubator immediately following the measurement. The migration distances were then deduced from the compression of the 0 and 24 h photographs and expressed in arbitrary distance units.

2.4. Assessment of fibroblast proliferation by Ki67 staining

Proliferating HFDFs in the first and second passage were used. Dermal fibroblasts were cultured in chamber slides until they reached 90% confluence, the medium was changed to 0.5% FCS, and the cells incubated for another 20 h prior to the addition of rMIF. A scratch wound was produced in all chambers using a yellow Gilson pipette tip. Cells were incubated with or without 50 ng/mL rMIF for 24 h at 37 °C in a 5% CO2 incubator. Photographs were taken and 24 h after injury. For microscopic examination of the migration response at the indicated time intervals, cells were placed on the microscope table, examined, and returned to the incubator immediately following the measurement. The migration distances were then deduced from the compression of the 0 and 24 h photographs and expressed in arbitrary distance units.

2.5. Calcium signalling

MIF+/− MEFs (106 cells/mL) were labelled with the calcium-sensitive dye Fluo-4AM (Molecular Probes, Eugene, OR) at 0.9 μM in assay buffer (130 mM NaCl, 4.6 mM KCl, 1 mM CaCl2, 5 mM glucose, 20 mM HEPES, pH 7.4) for 45 min at 37 °C. To control for cellular dye loading efficiency, cells were labelled in parallel with SNARF-1 at 0.9 μM. After washing, cells were resuspended at 2 × 106 cells/mL and kept at 37 °C. Immediately after the addition of the first stimulus (rMIF at 250 ng/mL), the mean fluorescence intensity (MFI) as a measure of the cytosolic Ca2+ concentration was monitored for 120 s using
the BD FACSAria System (BD Biosciences, San Jose, CA). The second stimulus (rMIF at 250 ng/mL) was added at 130 s or after 2 h, and MFI was monitored for another 120 s. Experiments were analyzed via FlowJo Software (Tree Star, OR).

2.6. Statistical analysis

Statistical significance between two groups of data was evaluated by using Student’s t-test. P values below 0.05 were considered statistically significant. Significance between two treatment groups is indicated by *, ** or *** with * referring to P value of <0.05, ** representing a P value of <0.01, and *** indicating a P value of <0.005.

3. Results

3.1. Short-term treatment with MIF promotes the migration of wild-type and MIF-deficient mouse embryonic fibroblasts in scrape-wounded monolayers

To investigate whether MIF modulates fibroblast migration during wound healing processes, the effect of exogenously added rMIF on the motility of MEFs was studied in an in vitro model of artificially wounded monolayers (‘scrape wounds’). The apparent migration of fibroblasts into scraped spaces may both encompass a migratory and a proliferative component. As MIF has been demonstrated to differentially influence ERK1/2-MAPK-driven cell proliferation processes under transient versus permanent conditions [21,27], the potential migratory effect of MIF was studied after both transient and permanent exposure of fibroblasts to MIF. Initially, wild-type MEFs constitutively expressing MIF at appreciable levels were used.

When transiently treated with 50 ng/mL rMIF for 2 h, a marked migratory response was observed in scraped MEF monolayers (Fig. 1). To verify that this effect was MIF-specific, migration behaviour in the presence of transiently added rMIF (“2 h”) was compared with that in the absence of rMIF (“N”) (Fig. 1B). Treatment with exogenous rMIF led to a significant MIF-dependent increase in fibroblast motility (P < 0.01). Thus, MIF was able to promote fibroblast migration in in vitro experiments mimicking wound healing situations. Moreover, this effect was only observed when MEFs were transiently but not permanently treated with rMIF, as the

Fig. 1. Short-term treatment with MIF promotes the migration of wild-type and MIF-deficient mouse embryonic fibroblasts in scrape-wounded monolayers. (A,B) Wild-type MEF monolayers were wounded by scraping and migration/wound closure followed microscopically over 24 h. Microscopic photographs of representative migration experiments at t = 0 h (upper panel) were compared with those at t = 24 h (lower panel) after scraping and following addition of 50 ng/mL rMIF for 2 h. (B) Quantitative analysis of the 24 h-migration distances of MEFs which were either not treated with exogenous rMIF (control, N), treated with 50 ng/mL rMIF for 2 h (2 h), or permanently treated with 50 ng/mL MIF over the entire migration period (P). Data are mean values ± S.D. of n = 6 independent experiments. (C) As in (A,B) except that MIF+/− MEF monolayers were used in the scrape-wound assay. Data are mean values ± S.D. of n = 5 independent experiments. Asterisks indicate statistical significance of differences (**, P < 0.01; ***, P < 0.005).
Migration response was blunted when wounded MEFs were exposed to rMIF for the entire observation time of 24 h ("P") (Fig. 1B). Migration under such conditions was not only significantly decreased compared to the "2 h" treatment \( (P < 0.005) \), but there was also a tendency, which however did not reach statistical significance, that permanent exposure to rMIF led to a slight inhibitory effect compared to migration in the absence of rMIF. This could indicate that a baseline migration response is mediated by a low-efficiency constitutive-like release of MIF from the wtMEFs, consistent with earlier reports on autocrine effects of MIF [27–29].

The MIF-induced migratory response in 'wounded' wtMEF monolayers was significant but of moderate strength (increase of \( \sim 50\% \)). Given our observation that permanent MIF treatment abolished this effect, we surmised that desensitization may occur when exposure to MIF is long-lasting as by permanent addition of exogenous MIF or due to autocrine effects. To address this issue, scrape assays were performed with MEFs derived from mice rendered genetically deficient for MIF (MIF\(^{-/-}\)). Fig. 1C demonstrates that in scratched MIF\(^{-/-}\) MEF monolayers, the migratory effect induced by 2 h treatment with exogenous MIF was much more pronounced than on cells with...
a MIF-positive background (i.e. wtMEFs) (~220% increase; \(P < 0.01\) compared to buffer-treated cells). As in the wtMEFs, the difference between transient and permanent treatment was much stronger as well (>300%; \(P < 0.005\)). Thus, MIF has a marked effect on the closure of scraped cell layers, but only when transiently added for a short time interval on fibroblasts not previously stimulated by MIF.

3.2. The MIF-stimulated 'wound-closure' response encompasses a true migratory effect and is also observed for primary human dermal fibroblasts

The migration experiments were performed in the absence of a proliferation-blocking agent. Thus, it is likely that the MIF-induced migratory response in the scraped monolayers may reflect the result of both proliferative and migratory effects of MIF, adding up to an observed overall 'migration' or 'wound-closure' effect. To distinguish between the individual components contributing to the overall effect and to verify that MIF is able to stimulate a net pro-migratory response in the scraping assay, fi

Wound MIF−/− MEF monolayers pre-treated with mitomycin C for 2 h were analysed for their migratory response over a 24 h time interval as before. Of note, despite the presence of a proliferation blocker, treatment of cells with rMIF promoted migration and scratch closure (Fig. 2A). As before, the effect was significant, and was only observed upon exposure to transient, short term treatment with rMIF, but not when rMIF was added permanently (\(P < 0.05\) compared to untreated control and \(P < 0.05\) compared to permanent stimulation) (Fig. 2B). Although not reaching statistical significance, permanent treatment of monolayers with rMIF showed a tendency in leading to a net reduction of the migratory response, i.e. to an apparent migration-inhibitory effect that was reminiscent of a desensitization response. As expected due to the experimental ablation of the proliferative arm of the response by the anti-proliferative chemical agent, the relative increase in MIF-induced 'wound-closure' was smaller than that seen in the absence of mitomycin C (~35%; compare Fig. 2B with Fig. 1C).

Table 1

<table>
<thead>
<tr>
<th>Donor 1</th>
<th>Width of scratch at 0 h (μm)</th>
<th>Width of scratch at 7 h (μm)</th>
<th>Closure rate (%)</th>
<th>Width of scratch at 24 h (μm)</th>
<th>Closure rate (%)</th>
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<tbody>
<tr>
<td>10% FCS</td>
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<td>797</td>
<td>15.8</td>
<td>0 (closed)</td>
<td>100</td>
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<tr>
<td>0.5% FCS</td>
<td>1071</td>
<td>1004</td>
<td>6.3</td>
<td>531</td>
<td>50.5</td>
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<tr>
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<td>1083</td>
<td>11</td>
<td>572</td>
<td>52.7</td>
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<tr>
<td>rMIF</td>
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<td>937</td>
<td>20.1</td>
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<table>
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<tr>
<th>Donor 2</th>
<th>Width of scratch at 0 h (μm)</th>
<th>Width of scratch at 7 h (μm)</th>
<th>Closure rate (%)</th>
<th>Width of scratch at 24 h (μm)</th>
<th>Closure rate (%)</th>
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<tr>
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<td>813</td>
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<tr>
<td>0.5% FCS</td>
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<td>452</td>
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<tr>
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<td>1039</td>
<td>791</td>
<td>23.9</td>
<td>115</td>
<td>89</td>
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</tbody>
</table>

Scratch widths are mean values of two experiments.

To investigate whether the observed migratory effect of MIF was limited to wounded monolayers of embryonic fibroblasts or to mouse cells, human foreskin dermal fibroblasts (HFDFs) were applied. The migration behaviour in scraped monolayers of HFDFs pre-treated with mitomycin C was analysed following a 2 h stimulation with 50 ng/mL rMIF, permanent stimulation with rMIF, or addition of control buffer, as before. In these initial studies that were performed in the presence of mitomycin C, wound closure was measured after a 24 h time interval. Short-term treatment with rMIF for 2 h led to a significant increase in the migration response both in comparison with untreated cells (\(P < 0.005\)) and permanent MIF exposure (\(P < 0.05\)) (Fig. 2C and D). As HFDFs represent a more physiological 'wound healing' model, these data confirm the notion that MIF may play an important role in the migration phase of wound healing. Together, we conclude that short-term treat-

Fig. 3. The MIF-stimulated 'wound-closure' response in primary human dermal fibroblasts encompasses a proliferative effect. Primary human foreskin dermal fibroblast (HFDF) monolayers were wounded by scraping and proliferation/wound closure followed microscopically over 20 h. Microscopic photographs of representative experiments are shown comparing the effect of a 2 h incubation with 50 ng/mL rMIF with that of control phosphate buffer. (A) Overview of the scratch area as indicated. Cells were stained with DAPI. Note that in the presence of rMIF, the wound is almost fully closed whereas spontaneous fibroblast proliferation/migration only leads to a slight narrowing of the scratch area. For quantification of these experiments, see Table 1. (B) As in (A) except that cells were additionally stained with Ki67, which specifically stains cycling/proliferating cells. Treatment of monolayers with rMIF for 2 h (upper panel) results in the appearance of numerous Ki67+ cells in the scrape area, whereas almost no Ki67+ cells are seen in control buffer-treated monolayers. The data shown are representative of two independently performed experiments with HFDFs from two donors each.
ment of artificially wounded MEF or HFDF monolayers with rMIF led to a migratory response of these fibroblast cells as part of the ‘wound healing’ process. The data also implied that MIF enhances a proliferative response when the fibroblasts are proliferation-competent, as the migratory response in the presence of mitomycin C was less pronounced than in the absence of any proliferation block.

3.3. The MIF-stimulated ‘wound-closure’ response in primary human dermal fibroblasts encompasses a proliferative effect

The application of mitomycin C indirectly showed that MIF also modulated fibroblast proliferation. To directly test whether MIF-induced ‘scratch wound closure’ included a proliferation-activating effect of MIF, scratched MIF-treated HFDF monolayers in the absence of mitomycin C were analysed further. These experiments also included a more detailed quantification of the MIF-induced scratch wound-closure effect. Quantification was performed by measuring the width of the scratch in the MIF- vs. buffer vs. FCS-treated monolayers at various time intervals following DAPI staining of cell nuclei. HFDFs from two different donors were analysed.

The width of the scratch at 0 h was ~1000 μm in each specimen and was considered 0% closure, with widths ranging from 946 to 1231 μm. Full closure of the scratch (0 μm) was observed by treatment of cells with 10% FCS for 24 h for one donor (Table 1). Addition of recombinant exogenous MIF for 2 h led to marked scratch closure (Fig. 3 A). The MIF-induced effect represented a relative closure rate of 86.6 ± 2.4% and was specific, as buffer or 0.5% FCS alone only led to spontaneous closure rates of 54.7 ± 4.2% or 55.6 ± 2.9%, respectively (Table 1). Thus, MIF potently promotes closure of scratch wounds of human dermal fibroblasts.

As no mitomycin C was added to the cells, the MIF-induced closure effect likely encompassed both proliferation and migration processes. To directly evaluate the effect of MIF on proliferation following ‘wounding’, we performed Ki67 stains of the fibroblasts in the scratch region, a staining method specifically detecting cycling cells. Fig. 3B shows that stimulation with rMIF led to a marked increase inKi67+ cells, while DAPI counterstaining confirmed that migration also contributed to MIF-induced wound closure. Quantitative analysis (ratio: Ki67+/DAPI-positive cells; 5 fields each; 2 time points) revealed that 62.3 ± 12.5% of the DAPI-positive cells were Ki67+, indicating that proliferation contributed by roughly two-third to the observed apparent wound-closure effect, while migration accounted for approximately one-third.

3.4. MIF-stimulated calcium signalling is down-regulated by chronic exposure to MIF

We recently demonstrated that MIF binds to CXCR2 and CXCR4 and promotes the chemotactic migration of leukocytes through a GPCR-mediated receptor pathway involving CD74 and calcium signalling [11]. Fibroblasts express low but measurable levels of CXCR4 and CD74 as evidenced by flow cytometry analysis (data not shown) and we therefore

Fig. 4. MIF triggers rapid calcium transients in mouse embryonic fibroblasts and MIF-stimulated calcium signalling is down-regulated by chronic exposure to MIF. (A) Fluo-4- and SNARF-1-stained MIF+/− MEF were treated with 250 ng/mL rMIF and calcium signals immediately measured by flow cytometry (first arrow). Cells were desensitized thereafter, as re-stimulation with rMIF after 120 s (second arrow) did not induce a calcium response. (B) Desensitization lasts for a prolonged time period in the permanent presence of MIF. Cells were treated as in (A) except that re-stimulation with rMIF was performed 2 h and 2 min after the initial calcium signal had declined. The initially added rMIF was not removed. The calcium measurements shown are representative of 2–3 independent experiments performed.
surmised that the MIF-induced wound-closure effect involves calcium signalling. In fact, treatment of MIF−/−MEFs with rMIF induced a rapid calcium transient within a few seconds that also desensitized fibroblasts for a subsequent stimulation with rMIF after 120 s (Fig. 4A), comparable to what had been previously observed by us in leukocytes [11]. To begin to explore the mechanism underlying the inhibition of fibroblast-induced scrape wound-closure under chronic exposure to MIF, MIF-induced calcium responsiveness was then examined over a more ‘chronic’ time period. A fibroblast calcium signal was triggered as before by addition of rMIF, but the MIF-containing medium was not removed. When the cells were re-challenged with rMIF 2 h and 2 min after the initial MIF-triggered calcium signal had declined, mimicking chronic exposure to MIF in the scratch wound monolayers, no anew rise in calcium was measurable (Fig. 4B). This indicated that prolonged exposure of fibroblasts to exogenous MIF leads to a desensitization of MIF receptor activity and calcium signalling with a subsequent inhibition of fibroblast-mediated scrape wound closure.

4. Discussion

Recent data have implicated MIF as a key regulator of wound healing responses [13,14,16]. Depending on the model, MIF inhibited or promoted wound healing. In mouse models of estrogen-depletion, mimicking aging-related reductions of estrogen levels in women, MIF was found up-regulated in cutaneous wounds but functioned to attenuate wound healing [13,14]. Estrogen-mediated down-regulation of MIF and protection from MIF-dependent inflammatory processes was recently confirmed in a model of colon inflammation [30]. On the other hand, Abe and colleagues observed that an upregulation of MIF in skin wounds of rats was accompanied by an accelerating effect of MIF on the wound healing process of skin tissue [15]. On first sight contradictory, these observations may suggest that MIF influences more than one mechanism contributing to wound healing outcome. In fact, MIF is a potent inflammatory mediator, suggesting that it could play a role in the inflammation phase of wound healing, in line with the findings by Ashcroft and colleagues [13]. MIF activates matrix-degrading enzymes such as MMP-1, -9, and -13 [31–34] and contributes to the mobilization of the extracellular matrix in vivo wound situations [14], supporting the notion that overexpression of MIF in the inflammation phase encompasses a matrix-turnover function. Of note, recent findings suggest that MIF, contrary to its historic name, promotes the chemotactic recruitment of inflammatory leukocytes into sites of injury such as atherogenic arteries or the inflamed microvasculature [11,35] and can activate the migration of smooth muscle cells [36]. Together this would suggest that MIF, along with cytokines such as PDGF or VEGF, could play an important role in the early inflammation phase of wound healing.

While fibroblast recruitment occurs to only a smaller extent in the inflammation phase, pronounced pro-migratory and proliferative responses of this cell type are induced in the migration/proliferation phase. MIF has been observed to enhance the proliferation rate of several cell types including fibroblasts [1,27,37]. However, the effect of MIF on fibroblast proliferation in wound situations has not been studied. Also, it is unknown whether stimulation of cell migration by MIF may extend to fibroblasts and could occur during wound healing processes. We hypothesized that MIF promotes fibroblast proliferation and migration during the ‘migration phase’ of wound healing. If true, this could mean that MIF participates in the regulation of wound healing through two distinct mechanisms in two different phases of the process. To probe for this possibility, we examined the effect of MIF on wound closure in a well-defined in vitro model of wounded fibroblast monolayers.

Our data unanimously show that MIF promotes closure of artificial scrape wounds in fibroblast monolayers. This effect encompasses the expected pro-proliferative effect on the fibroblasts and our mitomycin C blockade experiments and Ki67 stains suggest that MIF-induced fibroblast proliferation also occurs in wound situations. Importantly, we also show that MIF promotes fibroblast migration upon wounding of the monolayers. MIF-triggered fibroblast migration and wound closure only occurred, when cell layers were treated with rMIF for short time intervals (2 h), but not upon sustained exposure. This probably excludes the intermediate expression and release of MIF-stimulated pro-migratory molecules such as chemokines or migration-regulating cytokines. In fact, in light of recent findings showing that MIF can not only indirectly activate cell migration, but can directly trigger migration through interaction with CXCR chemokine receptors [11], our experiments indicate that MIF enhances fibroblast migration by direct means.

MIF interacts as a non-cognate ligand with CXCR2 on monocytes and neutrophils and with CXCR4 on T cells [11]. Surface-expressed CD74/invariant chain serves as a MIF binding site on B cells and some fibroblast and tumour cell types [37,38]. The fibroblasts applied in our current study express low but measurable levels of surface CXCR4 and CD74. While no information is yet available about the role of the MIF/CXCR4 axis in fibroblast migration and wound healing, CXCR4 in conjunction with its cognate ligand SDF-1 (CXCL12) have been implicated in fibroblast migration, both through chemotaxis and chemokinesis, as well as in dermal wound healing responses [39,40]. GPCR-mediated cell migratory responses lead to a rise in intracellular calcium as part of the signalling process and MIF-triggered leukocyte migration involves calcium signalling [11]. We show that MIF induces rapid calcium signalling in mouse embryonic fibroblasts and that long-term treatment of fibroblasts with MIF leads to desensitization of the calcium response, providing a mechanism for the observed failure of fibroblasts to migrate upon chronic exposure to MIF. CXCR2 and CD74 form a receptor signalling complex [11], but it is unknown whether CXCR4 can also engage in a complex with CD74. It will thus be important in the future to elucidate the precise contributions of these MIF receptor proteins in MIF-mediated wound closure responses both in vitro and in vivo, including the potential role of endogenously produced CXCL12 [40].

It may be speculated that MIF-mediated fibroblast migration might be connected to the recently uncovered migratory function of cytosolic p27Kip1 [41]. p27 is a cell cycle inhibitor protein acting in the nucleus of all cells [42], but it has been recognized that growth factor signalling can result in nuclear export of Ser-10-phosphorylated p27 to the cytoplasm, where it induces actin cytoskeletal rearrangement and subsequent migration of cells. This ‘cell-scattering activity’ of p27 occurs independently of its cell cycle arrest functions. MIF is known
to interact with JAB1/CSN5 [20], a coactivator protein and component of the COP9 signalosome that is involved in the nuclear export of p27 [43,44].

The pro-migratory and wound-closure effect of MIF was not only observed in MEFs but also in primary HFDFs. HFDFs represent an excellent model of wound healing processes and disorders [24,45,46], suggesting that the observed effects are relevant for wound healing in vivo.

In summary, judging from the previous data by the Ashcroft and Nishihira groups [13–16] in conjunction with our current study, we predict that MIF contributes to both the inflammatory and migration/proliferation phases of wound healing. We propose the following chronology of events involving MIF. Upon injury, it is likely that there will be a rapid and transient release of preformed MIF from mesenchymal cells, contributing to leukocyte recruitment and, to a minor extent, recruitment of fibroblasts in the inflammatory phase, representing necessary pro-healing processes. However, in situations and models, where MIF is chronically expressed in the inflammatory phase, such as under estrogen depletion, MIF, due to its potent inflammatory potential, would serve to attenuate wound healing progression, in which case therapeutic targeting of MIF appears desirable. Interestingly, blocking the interaction of the MIF receptor CXCR4 with its cognate ligand CXCL12 has been shown to significantly improve skin recovery after burn wounds [40], implying that blockade of the MIF/CXCR4 axis could be equally beneficial. On the other hand, MIF expression in the migration/proliferation phase would contribute to improving wound healing outcome due to its pro-proliferative activity and, as recognized for the first time in our current study, through its pro-migratory activity on fibroblasts. While these findings unravel a role of MIF in the migration phase of wound healing and underpin the notion that targeting MIF may represent a promising therapeutic option in diseases with impaired or unbalanced wound healing, the possible interplay and mechanisms of MIF’s contribution to the inflammation and migration phases certainly need further exploration.


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