Human Rhabdomyosarcomas secrete MIF that modulates metastatic behavior of tumor cells and inhibits recruitment of Cancer Associated Fibroblasts

Maciej Tarnowski¹, Katarzyna Grymula², Rui Liu¹, Joanna Tarnowska¹, Justyna Drukala³, Janina Ratajczak¹, Robert A. Mitchell¹, Mariusz Z. Ratajczak¹† & Magda Kucia¹†

¹Stem Cell Institute at James Graham Brown Cancer Center, University of Louisville, Louisville, KY, Department of Physiology Pomeranian Medical University, Szczecin, Poland and ³Department of Cell Biology, Faculty of Biochemistry, Biophysics and Biotechnology, Jagiellonian University, Cracow, Poland.

Keywords: rhabdomyosarcomas, CXCR4, CXCR7, SDF-1, MIF, stromal cells

Running Title: MIF and Rhabdomyosarcoma.

†Address for correspondence:
Mariusz Z. Ratajczak MD, PhD or Magda Kucia PhD
Stem Cell Institute at James Graham Brown Cancer Center, University of Louisville
500 S. Floyd Street, Rm. 107, Louisville, KY 40202, USA
Tel: (502) 852-1788, Fax: (502) 852-3032
Email: mzrata01@louisville.edu or mjkucio1@louisville.edu
Abstract

The overexpression of macrophage migration inhibitory factor (MIF) has been observed in many tumors and is implicated in oncogenic transformation and tumor progression. MIF activates CXCR2 and CD74 receptors, and as recently reported, may also bind to the stromal-derived factor-1 (SDF-1)–binding receptor CXCR4. Here we report that human rhabdomyosarcoma (RMS) cell lines secrete MIF and that this chemokine i) induces phosphorylation of MAPKp42/44 and AKT, ii) stimulates RMS cell adhesion, iii) enhances tumor vascularization, however surprisingly iv) decreases recruitment of cancer associated fibroblasts (CAFs). Since RMS cells employed in our studies do not express CXCR2 and CD74 receptors, the biological effects of MIF on RMS cells depend on its interaction with CXCR4 and, as we report here for the first time, MIF may also engage another SDF-1–binding receptor (CXCR7) as well. Interestingly, downregulation of MIF in RMS cells inoculated into immunodeficient mice lead to formation of larger tumors that displayed higher stromal-cell support. Based on these observations, we postulate that MIF is an important autocrine/paracrine factor that stimulates both CXCR4 and CXCR7 receptors to enhance the adhesiveness of RMS cells. We also envision that when locally secreted by a growing tumor, MIF prevents responsiveness of RMS to chemottractants secreted outside the growing tumor (e.g., SDF-1) and thereby prevents release of cells into the circulation. On the other hand, despite its obvious pro-angiopoietic effects, MIF inhibits in CXCR2/CD74-dependent manner recruitment of CAFs to the growing tumor. Our data indicates that therapeutic inhibition of MIF in RMS may accelerate metastasis and tumor growth.
Introduction

Rhabdomyosarcoma (RMS) is the most common soft-tissue sarcoma of adolescence and childhood, accounting for 5% of all malignant tumors in patients under 15 years of age. Most RMS tumors originate in the head and neck region, urogenital tract, and extremities (1-10). Based on histology, there are two major subtypes of RMS: alveolar (A)RMS and embryonal (E)RMS (11-13). Clinical evidence indicates that ARMS is more aggressive and has a significantly worse outcome than ERMS. Genetic characterization of RMS has identified markers that show excellent correlation with histological subtype. Specifically, ARMS is characterized by the translocation t(2;13)(q35;q14) in 70% of cases and the variant t(1;13)(p36;q14) in a smaller percentage of cases. These translocations generate PAX3-FKHR and PAX7-FKHR fusion genes that encode the fusion proteins PAX3-FKHR and PAX7-FKHR, which are believed to act in cell survival and deregulation of the cell cycle in ARMS cells.

As with other malignancies, the major clinical problem with RMS is its tendency to metastasize and infiltrate various organs. This unwanted process is directed by several chemokines [stromal-derived factor-1 (SDF-1), interferon-inducible T-cell alpha chemoattractant (I-TAC), and interleukin-8 (IL-8)], growth factors [e.g., hepatocyte growth factor (HGF) and insulin growth factor-1 (IGF-1)], cytokines [e.g., Leukemia inhibitory factor (LIF)] and thrombin (14-20).

The expansion of growing tumor depends on a proper vascularization as well as recruitment of tumor or cancer associated fibroblasts (CAFs) that promote both tumor expansion and metastasis (21).

It is well known that RMS cells can infiltrate the BM and, because they can resemble hematologic blasts, may sometimes be misdiagnosed as acute leukemia cells (22). In our previous work we demonstrated that metastasis of RMS cells to BM is mediated by BM stromal cells that secrete SDF-1, a major chemoattractant for hematopoietic stem cells (HSCs), that activates the CXCR4 receptor (14). Since RMS cells, similar to HSCs,
express CXCR4 on their surface, SDF-1 also mediates their recruitment/homing to BM (23).

It is known that chemokines may bind to more than one receptor and one chemokine receptor may bind more than one chemokine (24, 25). For many years it was envisioned that the SDF-1–CXCR4 axis is an exception to this rule. However as reported recently, SDF-1 may also interact with another chemokine receptor (CXCR7) and compete with another chemokine (I-TAC) for binding to this receptor (26). In our previous work we demonstrated that both the SDF-1–CXCR4 and SDF-1/I-TAC–CXCR7 axes modulate metastatic behavior of RMS cells (15, 27).

Recently it was reported that in addition to SDF-1, CXCR4 may also bind another chemokine called macrophage migration inhibitory factor (MIF) (28). MIF plays an important role in innate and acquired immune responses and was initially described as a ligand for the major histocompatibility complex class II (MHC II)-associated invariant chain, known as the CD74 receptor (29) and chemokine receptor CXCR2 (28). Monomeric MIF may also form homotrimERIC structures with homology to the enzyme D-dopachrome-tautomerase (30). Additional evidence shows that MIF is implicated in the pathogenesis of several tumors, including prostate, breast, and colon cancer as well as in melanoma and glioblastoma growth (31-34). It is accepted that MIF may affect tumor progression by i) stimulating cancer proliferation, ii) inhibiting apoptosis, iii) enhancing vascularization, or iv) inhibiting the lysis of tumor cells by natural killer cells (35-38).

Since MIF may bind to CXCR4 receptor/s expressed on RMS cells (14, 15), we became interested in a potential role for MIF in RMS progression. We found that human rhabdomyosarcoma (RMS) cell lines highly express and secrete MIF, which exerts pleiotropic effects on RMS growth. Accordingly, MIF enhances adhesion of RMS cells and, since RMS cells do not express classical MIF-binding receptors (CXCR2 and CD74), this effect is mediated by SDF-1 binding to both CXCR4 and CXCR7 receptors, as we show here. We envision that MIF locally secreted in growing tumors prevents responsiveness of RMS to SDF-1 and thus release of cells into the circulation. However
MIF also enhances recruitment of endothelial cells to the growing tumor at the same time that it inhibits in CXCR2/CD74-dependent manner recruitment of CAFs and thus decreases tumor expansion. We conclude that the autocrine/paracrine MIF-CXCR4/CXCR7 axis plays an important pleiotropic role in RMS growth. However our data indicate that inhibition of MIF may lead to i) unwanted dissemination of RMS cells and ii) enhanced accumulation of CAFs that, in turn, accelerates tumor growth.
Material and Methods

Cell lines. We used human RMS cell lines (gift of Dr. Peter Houghton, World Children's Research Hospital, Columbus, OH) comprising ARMS lines (RH2, RH5, RH28, RH30, and CW9019) and ERMS lines (RH18 and RD). RMS cells used for experiments were cultured in Roswell Park Memorial Institute medium (RPMI) 1640 (Sigma, St. Louis, MO), supplemented with 100 IU/ml penicillin, 10 µg/ml streptomycin, and 50 µg/ml neomycin (Life Technologies, Inc., Grand Island, NY) in the presence of 10% heat-inactivated fetal bovine serum (FBS, Life Technologies). The cells were cultured in a humidified atmosphere at 37°C in 5% CO₂ at an initial cell density of 2.5 x 10⁴ cells/flask (Corning, Cambridge, MA) and the media were changed every 48 hours.

Recombinant MIF (rMIF). Catalytically active and 95% pure rMIF that was employed in our experiments was prepared and purified as described previously (39).

Fluorescence-activated cell sorting (FACS) analysis. The expression of CXCR2, CD74, CXCR4, and CXCR7 in RMS cell lines was evaluated by FACS analysis as previously described (14). The CXCR2 antigen was detected with FITC–anti-CXCR2 monoclonal antibody (mAb) clone no. 6C6 (BD Pharmingen, San Diego, CA) and CD74 antigen was detected with PE-conjugated mAb clone no. LN2 (BioLegend, San Diego, CA). The CXCR4 antigen was detected with APC–anti-CXCR4 mAb clone no. 12G5 (BD Pharmingen, San Diego, CA) and CXCR7 antigen was detected with PE–anti-CXCR7 mAb clone no. 11G8 (R&D Systems, Minneapolis, MN). Isotype-matched APC-, FITC- and PE-conjugated immunoglobulin (Ig) served as controls (BD Biosciences). Briefly, the cells were stained in phosphate-buffered saline (PBS, Ca²⁺- and Mg²⁺-free) supplemented with 2% bovine calf serum (BCS HyClone, Logan, UT). After the final wash, cells were re-suspended in PBS and analyzed by FACS using the LSRII instrument (Becton Dickinson, San Jose, CA).

Real-time quantitative reverse transcription PCR (RQ-PCR). Total RNA was isolated from cells treated with hypoxia and from cell controls with the RNeasy Kit (Qiagen, Valencia, CA). The RNA was reverse transcribed with MultiScribe reverse
transcriptase and oligo-dT primers (Applied Biosystems, Foster City, CA). Quantitative assessment of mRNA levels was performed by real-time RT-PCR on an ABI 7500 instrument with Power SYBR Green PCR Master Mix reagent. Real-time conditions were as follows: 95°C (15 sec), 40 cycles at 95°C (15 sec), and 60°C (1 min). According to melting point analysis, only one PCR product was amplified under these conditions. The relative quantity of a target, normalized to the endogenous control β-2 microglobulin gene and relative to a calibrator, is expressed as 2-∆∆Ct (-fold difference), where Ct is the threshold cycle, ∆Ct = (Ct of target genes) – (Ct of endogenous control gene, β-2 microglobulin), and ∆∆Ct = (∆Ct of samples for target gene) – (∆Ct of calibrator for the target gene). The following primer pairs were used:

MIF F: 5’ – CAAGGCCAACCGCGAGAAGA – 3’
MIF R: 5’ – GGATAGCACAGCCTGGATAG – 3’
IL-8 F: 5’ – CTGGCCGTGGCTCTCTTG – 3’
IL-8 R: 5’ – TTAGCACTCCTTGCAAAACTG – 3’
VEGF F: 5’ –CACCCATGGCAGAAGGAGGA – 3’
VEGF R: 5’ – GGTCTCGATTGGATGGCAGTAG – 3’
FGF2 F: 5’ – GTGTGCTAACCCTACCTGGCT – 3’
FGF2 R: 5’ – TTTCTGGCAGGTCTCTTGT – 3’

**Gene array studies.** Human bone marrow stroma-derived fibroblasts from three different donors were stimulated with MIF (100ng/ml) overnight and RNA was isolated using RNeasy Kit (Qiagen, Valencia, CA). Samples for microarray analysis were processed in triplicate. Total RNA was labeled using the IVT-Express Kit (Affymetrix, Santa Clara, CA) according to the manufacturer's instructions. The labeled RNA was hybridized to HG-U133 Plus 2.0 arrays (Affymetrix). Arrays were washed and stained using an Affymetrix FS450 Fluidics Station and scanned on an Affymetrix GeneChip Scanner 3000 7G. The resulting "cel" files were imported into Partek Genomics Suite 6.4 (St. Louis, MO) for analysis. The data were normalized using RMA and a two-way ANOVA was performed, considering treatment as well as the paired arrangement of the samples. The parameter "pair" was defined as a random parameter. Differentially expressed genes (the cut-off value was set at p<0.0012) were imported into Ingenuity pathway analysis.
software (Ingenuity, Redwood City, CA) for biological interpretation (740 mapped gene IDs were analyzed).

**Phosphorylation of intracellular pathway proteins.** Western blots were performed on extracts prepared from RMS cell lines ($2 \times 10^6$ cells) that were kept in RPMI medium containing low levels of bovine serum albumin (BSA, 0.5%) to render the cells quiescent. The cells were divided and stimulated with optimal doses of SDF-1 (300 ng/mL) or MIF (100 ng/mL) for 5 minutes at 37°C and then lyzed (for 10 minutes) on ice in M-Per lysing buffer (Pierce, Rockford, IL) containing protease and phosphatase inhibitors (Sigma). Subsequently, the extracted proteins were separated by either 12% or 15% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and the fractionated proteins were transferred to a nitrocellulose membrane (Schleicher & Schuell, Keene, NH) as previously described (40). Phosphorylation of the intracellular kinases, 44/42 mitogen-activated protein kinase (MAPK) (Thr 202/Tyr 204) and AKT, was detected using commercial mouse phospho-specific mAb (p44/42) or rabbit phospho-specific polyclonal antibodies (all from New England Biolabs, Beverly, MA) with horseradish peroxidase (HRP)-conjugated goat anti-mouse IgG or goat anti-rabbit IgG as a secondary antibody (Santa Cruz Biotech, Santa Cruz, CA). Equal loading in the lanes was evaluated by stripping the blots and reprobing with appropriate mAbs: p42/44 anti-MAPK clone no. 9102 and anti-AKT clone no. 9272 (Santa Cruz Biotech). The membranes were developed with an enhanced chemiluminescence (ECL) reagent (Amersham Life Sciences, Little Chalfont, United Kingdom), dried, and exposed to film (HyperFilm, Amersham Life Sciences).

**Chemotaxis assay.** The 8-µm polycarbonate membranes were covered with 50 µL of 0.5% gelatin. Cells were detached with 0.5 mmol/L ethylenediaminetetraacetic acid (EDTA), washed in RPMI 1640, resuspended in RPMI 1640 with 0.5% BSA, and seeded at a density of $3 \times 10^4$ in 120 µL into the upper chambers of Transwell inserts (Costar Transwell; Corning Costar, Corning, NY). The lower chambers were filled with SDF-1 (300 ng/mL), I-TAC (100 ng/mL), or 0.5% BSA RPMI 1640 (control). After 24 hours, the inserts were removed from the Transwells. Cells remaining in the upper chambers were scraped off with cotton wool and cells that had transmigrated were stained by...
HEMA 3 according to the manufacturer's instructions (Fisher Scientific, Pittsburgh, PA) and counted either on the lower side of the membranes or on the bottom of the Transwells.

**Time-lapse monitoring of the locomotion of individual cells.** The images of human RMS migrating on plastic at 37°C were evaluated with an inverted microscope using phase contrast optics. Analysis of cell migration began 18 hours after cell seeding. The locomotion images were recorded with a charge-coupled device (CCD) camera. RD, RH18, RH28, and RH30 cells were plated in Corning flasks at a density of 10^4 cells/cm² and were mock-treated or pre-stimulated by MIF (100 ng/mL) for 30 minutes before recording. The cell trajectories were constructed from 60 subsequent cell centroid positions recorded for 240 minutes at 5-minute intervals. The cell trajectories were presented in circular diagrams (41) and the length of cell tracks was calculated in addition to the final displacement. Cell tracks were recorded under the conditions described earlier for each cell line.

The following parameters characterizing cell locomotion were computed for each cell using procedures written in the Mathematica language, including: (1) total length of cell trajectory (in micrometers); (2) the trajectory as a sequence of n straight-line segments, each corresponding to cell centroid translocation within the time interval between two successive images; (3) total length of the final displacement of the cell from the starting point to the final position, i.e., distance between the first and last points of the cell track (in micrometers); (4) average speed of cell locomotion defined as total length of the cell trajectory/time of recording; and (5) the ratio of cell displacement length to cell trajectory length, called the coefficient of movement efficiency (CME) (42, 43).

**Adhesion of RMS cells to fibronectin.** Cells were made quiescent for 24 hours with 0.5% BSA in RPMI before incubation with SDF-1 (300 ng/mL) or MIF (100 ng/mL) for 5 minutes. Cells were added directly onto the protein-coated wells (5 × 10^4/well) for 5 minutes. The wells were coated with fibronectin (10 µg/mL) overnight at 4°C and blocked with BSA for 2 hours before the experiment. Following incubation at 37°C, the plates were vigorously washed 3 times and adherent cells were stained by HEMA 3 and
counted under the microscope. In some experiments, CXCR7 blocking antibody (clone 9C4, BML International, Japan) was used at 10 µg/ml

**Adhesion to HUVECs.** RMS cells were labeled before assay with the fluorescent dye calcein-AM and were subsequently stimulated with SDF-1 (300 ng/mL) or MIF (100 ng/mL) for 5 minutes and added (for 5 minutes) to the 96-well plates covered by human umbilical vein endothelial cells (HUVECs). After the non-adherent cells had been discarded, cells that adhered to the HUVECs were counted under a fluorescent microscope as described (43).

**Cell proliferation.** Cells were plated in culture flasks at an initial density of $10^4$ cells/cm$^2$ in the presence or absence of SDF-1 (300 ng/mL) or MIF (100 ng/mL). The cell number was calculated at 24, 48, and 72 hours after culture initiation. At the indicated time points, cells were harvested from the culture flasks by trypsinization and the number of cells was determined using a Bürker hemocytometer (Buffalo, NY) as described elsewhere (45).

**Human endothelial tube formation assay.** Endothelial tube formation was assessed on a synthetic basement membrane according to the manufacturer’s protocol (Matrigel, BD Biosciences). Briefly, the matrix was thawed overnight at 4°C and polymerized at 37°C for 30 min before use. HUVECs were resuspended in either reduced-serum medium with 0.5% BSA (negative control), medium supplemented with FGF2 (positive control) or conditioned media from different RMS cell lines. The cells were then seeded ($3 \times 10^3$/cm$^2$) on the Matrigel and the plates placed in a humidified atmosphere of 5% CO$_2$ at 37°C. Identical fields in each well were photographed. All conditions were tested in duplicate wells in three separate experiments using cells from different donors.

**Internalization of CXCR4 and CXCR7.** For determination of chemokine-induced receptor internalization, cells were incubated at 37°C in culture medium for 20 minutes in the presence or absence of SDF-1 or MIF (100 ng/mL). Prior to staining, cell-bound chemokine was removed by an ice-cold acidic glycine wash (50 mM glycine/HCl, pH 3, 100 mM NaCl) for 90 seconds (46). Cells were labeled with with APC–anti-CXCR4 mAb clone no. 12G5 (BD Pharmingen). CXCR7 antigen was detected with PE–anti-
CXCR7 mAb clone no. 11G8 (R&D Systems). Expression was measured by flow cytometry. Receptor internalization was evaluated by the mean channel fluorescence values.

**Knockdown of MIF with short hairpin (sh)RNA.** In RNAi experiments, shRNA-generating plasmid pSuper (Oligoengine, Seattle, WA) was used. The oligonucleotide-targeting base sequence for human MIF was: 5’-CCTTCTGGTGAGGAAAT-3’. RMS cells were plated at 80% confluency and transfected with shRNA vector using Lipofectamine (Invitrogen, Carlsbad, CA) according to the manufacturer’s protocol. Commercially available scrambled shRNA negative control plasmid was used (Dharmacon, Lafayette, CO). For stable transfection of shRNA-producing vectors, single cell dilutions were prepared and the cells were further expanded in the presence of puromycin (1µg/ml, Invitrogen).

**Transplants of RMS cells into immunodeficient mice.** To evaluate the *in vivo* metastatic behavior of three populations of RH18 cells (RH18, RH18 scrambled, and RH18 with knockdown of MIF), cells were injected intravenously (i.v., 8 x 10⁶ per mouse) into severe combined immunodeficient (SCID)-Beige inbred mice. Marrows, livers, and lungs were removed 48 hours after injection of these cells and the presence of RMS cells (i.e., murine-human chimerism) was evaluated by the difference in the level of human alpha-satellite expression. DNA was amplified in the extracts isolated from BM-, liver- and lung-derived cells using real-time PCR. Briefly, DNA was isolated using the QIAamp DNA Mini kit (Qiagen, Valencia, CA). Detection of human satellite and murine β-actin DNA levels was conducted by real-time PCR using an ABI Prism 7500 Sequence Detection System. A 25-µL reaction mixture containing 12.5 µL SYBR Green PCR Master Mix, 300 ng DNA template, 5’-ACC ACT CTG TGT CCT TCG TTG G-3’ forward, 5’- ATC GCG CTC TCA AAA GGA GTG T -3’, and 5’-AAA CGT CCA CTT GCA GAT TCT AG-3’ reverse primers for the alpha-satellite, and 5’-GGA TGC AGA AGG AGA TCA CTG-3’ forward and 5’-CGA TCC ACA C GG AGT ACT TG-3’ reverse primers for β-actin were used. The Ct value was determined as before. The number of human cells present in the murine organs (degree of chimerism) was calculated from the
standard curve obtained by mixing different numbers of human cells with a constant number of murine cells.

In some of the experiments, cells (5 x 10⁶ per mouse) were inoculated into the hind limb muscles of SCID-Beige inbred mice. Six weeks later, the mice were sacrificed for evaluation of the RMS cells present in blood, BM, liver, and lungs. Detection of human cells was performed as described above.

Immunohistochemistry. Staining was performed in frozen 5-μm-thick histological sections. Stromal cells were identified by FAP antibody (rabbit IgG, Abcam, Cambridge, MA). Nuclei were identified with DAPI. The fluorescence images were collected with the TE-FM Epi-Fluorescence system attached to an Olympus Inverted Microscope IX81 (Olympus, Center Valley, PA).

Statistical analysis. All results are presented as mean ± standard deviation (SD). Statistical analysis of the data was performed using the nonparametric Mann-Whitney test, with p<0.05 considered significant.
Results

Human RMS cells secrete MIF, but they do not express major MIF binding receptors.

First, we evaluated whether human RMS cell lines express MIF. Figure 1 panel A shows all 5 human ARMS cell lines and both human ERMS cell lines investigated in our studies compared to normal skeletal muscles that highly express MIF mRNA. We also noticed that MIF expression was not influenced by hypoxia and by employing ELISA assays we confirmed that MIF was secreted by all of these cell lines (Figure 1 panel B).

Next, we evaluated the expression of the MIF-binding receptors CXCR2, CD74 and CXCR4 (28-31) in our RMS cell lines. Figure 1 panel C shows that all our RMS cell lines are CXCR2 and CD74 negative according to FACS. However, 4 out of 5 ARMS cell lines (RH5, RH28, RH30, and CW9019) express the SDF-1–binding receptor CXCR4 that, as reported, may also engage MIF (28). Figure 1 panel C shows that RMS also express another SDF-1–binding receptor (CXCR7) (15).

Despite a lack of CXCR2 receptor, MIF induces signaling in RMS cells.

Figure 2 panel A and B shows that MIF induces phosphorylation of AKT and MAPKp42/44 in RMS cells. Given that these cells do not express CXCR2 and CD74 receptors, this indicates involvement of other receptors in this phenomenon. Based on the pattern of kinase phosphorylation by MIF, which mimics that of SDF-1 (Figure 2 panel A and B), and because MIF may activate RH2 and RH18 cells that express CXCR7 and not CXCR4 (Figure 1 panel C), we hypothesized that another SDF-1–binding receptor (CXCR7) could be activated by MIF as well. In support of this notion, Figure 2 panel C shows that MIF internalizes CXCR7 receptor on CXCR7⁺CXCR4⁻ RH18 and RH2 cells.
MIF does not affect chemotaxis of RMS cells, but increases their adhesion by engaging either CXCR4 or CXCR7 receptors.

As we reported previously, SDF-1 increases pro-metastatic properties of RMS cells by interacting with CXCR4 (14) and CXCR7 receptors (15). Therefore, we first evaluated the effect of MIF on the spontaneous motility of RMS cells (Supplementary Figure 2) and directed Transwell migration (Figure 3 Panel A). However, we noticed that in contrast to SDF-1 (14), MIF affects neither spontaneous motility nor directed migration of RMS cells.

As we reported previously, activation of CXCR4 and CXCR7 receptors on RMS cells increases their pro-adhesive properties (15). Therefore, we next evaluated the influence of MIF on RMS adhesion to fibronectin and HUVECs. As shown in Figure 3 Panels B and C, MIF is a potent pro-adhesive factor for human RMS cells and, in particular, increases their adherence to HUVECs.

As mentioned previously, the lack of CXCR2 and CD74 expression by our RMS cell lines (Figure 2) points to the potential involvement of CXCR4 and CXCR7 in MIF signaling. Therefore, to better support the hypothesis that the biological effects of MIF are mediated by the CXCR4 and CXCR7 receptors, we focused on RMS cell lines that express only one of these receptors.

First, we selected CXCR7^+^CXCR4^-^CXCR2^-^ RH18 cell line (Figure 1 Panel B and) that binds both SDF-1 and I-TAC (15, 27). Figure 4 Panel A shows that SDF-1-, I-TAC- and, more importantly, MIF-mediated adhesion of these cells to fibronectin was inhibited by blocking the CXCR7 receptor with 9C4 antibody. This indicates that CXCR7 is involved not only in SDF-1 and I-TAC signaling (15, 27) but also in MIF signaling as well.

Next, we employed RH30 cells that highly express CXCR4, and very low-level CXCR7 (<8%). Figure 4 Panel B shows that both SDF-1- and MIF-mediated adhesion of CXCR4^+^CXCR7^{low} RH30 cells to fibronectin was inhibited in the presence of the CXCR4 antagonist AMD3100, but was not significantly influenced by CXCR7 blocking
antibody 9C4. This indicates that in RH30 cells, MIF enhances adhesion by engaging the highly expressed on their surface CXCR4 receptor.

In another type of assay, we noticed that when added to the upper chamber of the Transwell system, MIF retains the RMS cells in the upper chamber and thus significantly inhibits chemotaxis of CXCR7+ RH18 and CXCR4+ RH30 cells in response to the SDF-1 gradient (Supplementary Figure 3). This further supports the notion that MIF interacts with both SDF-1–binding receptors.

**Conditioned media from RMS cells enhance vasculogenesis in a MIF-dependent manner.**

MIF is a known stimulator/modulator of tumor angiogenesis (36, 37, 47). Figure 5 Panel A shows that conditioned media harvested from RMS cell lines strongly promote tube formation by HUVECs. This effect is at least partially dependent on MIF (Figure 5 Panel B) as CM from RH18 cells in which we downregulated MIF or CM from RH18 cells + MIF inhibitor ISO-1 are less efficient in this assay.

Figure 5 Panel C shows MIF influence on expression of other pro-angiogenic factors, such as VEGF, IL-8, and FGF in RH18 wt and RH18 MIF-kd cells. Accordingly, overnight stimulation of RH18 wt by MIF slightly upregulated expression of VEGF and IL-8. Basic expression of these two gene products in RH18 MIF-kd cells was significantly lower; however, stimulation by exogenous MIF restored expression of VEGF and IL-8 to the level observed in RH18 wt cells. At the same time, FGF expression was not affected by MIF downregulation and, moreover, stimulation by exogenous MIF slightly increased its expression (Figure 5 panel C).

**Downregulation of MIF in human RMS cells leads to unexpected enhanced tumor growth.**

In a set of *in vitro* experiments, we noticed that exogenously added MIF did not influence proliferation or survival of RMS cells cultured under serum-free conditions (data not
shown). However, to exclude the possibility that autocrine-secreted MIF may already regulate RMS cell proliferation, we downregulated MIF expression in RH18 cells that highly express this chemokine by employing an shRNA strategy (Figure 6 Panel A inset), but no difference in proliferation between MIF knockdown (MIF-kd) and wild type RH18 cells was observed (Supplementary Figure 3).

Next we tested two models in RH18 and RH18 MIF-kd cells. In a first set of experiments, RMS cells were injected intravenously into immunodeficient SCID/Beige mice and 24 hours later we estimated the number of tumor cells in different organs of the transplanted animals by quantifying the amount of human DNA in murine tissues as described (15). However, in this type of short-term transplantation assay, no differences were noticed between wt and MIF-kd RH18 cells (Supplementary Figure 4 panel A).

In another long-term type of assay, RH18 MIF-kd and wt cells were inoculated into skeletal muscles of the tibia of immunodeficient SCID/Beige mice and 4 weeks later animals were sacrificed and the tumors measured. Figure 6 Panel B shows that RH18 cells with downregulated MIF grew much larger tumors. Histological analysis revealed that these tumors had a higher content of stromal elements (Figure 6 panel C).

Interestingly, we also noticed a higher number of circulating RMS cells in the peripheral blood of mice bearing tumors initiated by MIF-kd RMS cells (Supplementary Figure 4 panel B). This suggests that MIF secreted by RMS cells may somehow “immobilize” the cells within the growing tumor, preventing their egress from the tumor.

**MIF inhibits recruitment of stromal fibroblasts by growing RMS cells.**
Based on the observation that RH18 MIF-kd cell-derived tumors are highly enriched in stromal elements (Figure 6 Panel C), we became interested in the potential influence of MIF on stromal fibroblasts. First we noticed that MIF does not affect proliferation of these cells (data not shown); however, as shown in Figure 7 panel A, it inhibits migration of BM stromal-derived fibroblasts in 10% serum. Moreover, CM harvested from RD cells that express MIF at low levels (Figure 1 Panel A) displays strong chemoattractive activity...
against BM-derived fibroblasts as compared to RH18 cells that highly express MIF (Figure 1 Panel A). Furthermore, chemotaxis of stromal cells toward CM harvested from RH18 cells was significantly upregulated when MIF was knocked down by an shRNA strategy (Figure 7 Panel A). We also observed that in human fibroblasts MIF activates phosphorylation of MAPKp42/44 and p38 as well as AKT (Figure 7 Panel B). This supports involvement of MIF signaling in inhibition of the recruitment of fibroblasts (32) by RMS tumors cells. Interestingly, we noticed that MIF inhibits migration of RMS cells to IL-8 gradient (Supplementary Figure 5) that as we reported previously is secreted by RMS cells during hypoxia and may strongly chemoattract CAFs (17).

Finally, our gene array data for human stromal cells unstimulated or stimulated with MIF reveals that MIF may affect expression of several genes i) involved in chemotaxis, ii) adhesion, iii) chemokines and iv) growth factors (Supplementary Figure 6). Thus, MIF may affect crosstalk between CAFs and the growing tumor.
Discussion

RMS is the most common soft-tissue sarcoma of adolescents and children and frequently infiltrates the BM to the degree that it mimics acute lymphoblastic leukemia (22). The prognosis is poor in particular for the more aggressive and metastatic ARMS type (12, 13). In our previous reports, we focused on the role of chemokines (14, 15, 17), selected growth factors and cytokines (18, 20), as well as the coagulation cascade (16) in RMS metastasis. In particular, we were interested in involvement of the SDF-1–CXCR4/CXCR7 axis in this process (15, 27).

However, since it was recently demonstrated that the CXCR4 receptor also binds MIF (28), we became interested in the role of the MIF-CXCR4 axis in RMS progression. Accordingly, the biological effects of MIF migration on monocyte and granulocyte migration are mediated by MIF binding to CXCR4 in addition to the CXCR2 and CD74 receptors (28, 29). Moreover, CXCR4 and CXCR2 form heteromeric receptors with CD74 that are involved in optimal MIF signaling (48).

Since MIF is a new potential CXCR4 ligand, we became interested in a role for MIF in human CXCR4+ rhabdomyosarcomas. MIF is a critical mediator of acute and chronic inflammatory diseases, including septic shock. It also plays a role in several types of cancers, including prostate, breast and colon cancer as well as melanoma and glioblastoma (49). We report here for first time that human RMS cell lines highly express MIF mRNA and secrete this chemokine into the culture medium. This suggests that MIF may exert autocrine/paracrine effects on RMS cells. It is known that RMS cells also secrete other factors such as, for example, IL-8 (17) and VEGF (50). However, since they do not express the corresponding receptors, these factors are not involved in autocrine regulatory loops. On the other hand, while human RMS cells express CXCR4 and CXCR7 receptors, they do not express either SDF-1 nor I-TAC (14, 15). Therefore, both of these chemokines are similarly not involved in autocrine regulation of RMS cells.
By contrast, in the autocrine secretion by RMS cells, MIF may interact with CXCR4 and thus establish an autocrine regulatory loop. The presence of such an autocrine MIF-CXCR4 loop was recently described for human drug-resistant metastatic colon carcinoma cell line HT-29 (51). In HT-29 cells that do not express either CXCR2 or CD74, the autocrine MIF-CXCR4 loop enhances the invasive potential of cancer cells and, as demonstrated by employing specific inhibitors, this axis promotes cell proliferation. However in our hands, exogenous- or autocrine-secreted MIF did not affect either RMS cell proliferation or cell survival. Thus, in a similar way as we reported for other chemokines such as SDF-1 (14) and I-TAC (15), MIF does not affect proliferation of RMS cells, despite the fact that both binding receptors CXCR4 and CXCR7 are expressed by RMS cells.

We noticed, however, that stimulation of RMS cells by MIF increases their pro-adhesive properties. By employing internalization and signaling studies, blocking antibodies and small molecular inhibitors we demonstrated that this effect is mediated by MIF interaction with CXCR4 and, as we show here for the first time, could also depend on activation of CXCR7. To support this notion i) CXCR7 was internalized on RMS cells after exposure to MIF, ii) MIF stimulated MAPKp42/44 and AKT phosphorylation in CXCR7+CXCR4-CXCR2- RMS cell lines and iii) responsiveness of CXCR7+CXCR4-CXCR2- RMS cells to MIF was inhibited in a presence of CXCR7 blocking antibodies or CXCR7 receptor antagonist. However, further studies are needed to confirm that both SDF-1–binding receptors potentially bind MIF. Furthermore, additional studies will be required to see if MIF interacts with CXCR7 on other cell types.

To our surprise, the MIF-CXCR4/CXCR7 interaction did not influence spontaneous or gradient-dependent migration of RMS cells. This suggests that a primary role of autocrine-secreted MIF is the promotion of local adhesion of tumor cells and high levels of MIF secreted by tumor cells may somehow decrease their ability to leave the primary tumor in response to other exogenous chemoattractants. In fact, we noticed that downregulation of MIF in RH18 cells by shRNA decreased their spontaneous adhesion to fibronectin. Overall, we envision that the autocrine MIF-CXCR4/CXCR7 loop may
decrease chemotactic responsiveness of RMS cells to SDF-1 or I-TAC gradients in other tissues. In support of this, we observed a higher number of RMS cells circulating in peripheral blood in mice that were bearing tumors with MIF-kd RH18 cells. Thus, in contrast to other tumors (35), MIF is not a pro-migratory chemokine for RMS cells, but rather plays an important role in retention of cells within the primary tumor.

As expected, we found that MIF chemoattracts endothelial cells that promote vascularization of growing tumor tissue (37, 52). In addition to MIF, RMS cells also secrete other pro-angiopoietic factors such as IL-8 (17) and VEGF (50). However, as we noticed, the expression of these factors in RMS cells is somehow upregulated by the autocrine MIF loop. In support of this notion, mRNA for IL-8 and VEGF become downregulated in MIF-kD RH18 cells.

In addition to blood vessels, another important component of the growing sarcoma are cancer associated fibroblast (CAFs) (21). Theoretically, CAFs could be created in endothelial-cell–derived tumors by epithelial mesenchymal transition (EMT), but as in sarcomas of mesenchymal origin, the major mechanism seems to be their recruitment from circulating peripheral blood or surrounding tissues (21).

Our data indicate that MIF strongly inhibits chemoattraction of CAFs in vitro and in vivo in growing RMS tumors inoculated into skeletal muscles of immunodeficient SCID/Beige mice. In support of this notion, it was reported recently that MIF-enriched pituitary extracts and epithelial serum-free conditioned media inhibited migration of mesenchymal stem cells in a MIF-dependent manner (53). Accordingly, chemotaxis of stromal-derived fibroblasts was enhanced after these extracts were pre-cleared with ISO-1, a small molecule inhibitor of MIF (54). Furthermore, as demonstrated in another study, this inhibitory effect of MIF against stromal cells was not mediated by the CXCR2 receptor, which is abundantly expressed on stromal fibroblasts (55), but by MIF interaction with the CD74 receptor (29).
We envision that MIF mainly modulates tumor size by inhibiting recruitment of CAFs to the growing cancer (Figure 7 panel C). This notion is supported by the recent report on breast tumors indicating that expression of MIF in tumor tissues is inversely correlated with tumor size (32). Based on our data, we envision that the small size of breast tumors in cancer cells expressing high levels of MIF could be explained by a decrease in the incorporation/expansion of stromal cells. CAFs are chemoattracted by several factors including SDF-1. However, since RMS cells do not secrete SDF-1 (14) other factor/s must be involved in this process. As demonstrated previously RMS cells in hypoxic conditions highly secrete IL-8 that is a strong chemoattractant for fibroblasts (17). Thus, based on our current observation that MIF inhibits chemotaxis of stroma cells to various chemoattractants including IL-8, we postulate that autocrine secreted by RMS cells MIF can effectively counteract IL-8 effect and inhibit recruitment of CAFs to expanding tumor.

Modern pharmacology is developing small molecular inhibitors of the receptor-ligand axes involved in cancer metastasis. Such strategies are proposed, for example, to inhibit SDF-1–CXCR4 (55-58) and SDF-1–CXCR7 (26) signaling. However, our data indicate that in contrast to other malignancies (35), inhibition of MIF expression could have the opposite effect on RMS tumor growth. Our results indicate that the high expression of MIF by RMS cancer cells may prevent their egress from the primary tumor and decrease tumor size by inhibiting recruitment of stromal cells. This also explains why breast tumors with high MIF expression are smaller in size and patients have a favorable prognosis according to both tumor-specific overall survival and recurrence-free survival (32).

In conclusion, we have provided evidence for the first time that RMS cells highly express MIF, which in RMS cells is an autocrine/paracrine factor that interacts with CXCR4 as well as with other SDF-1–binding receptors such as CXCR7. However, we found that while MIF does not affect RMS cell proliferation, survival or chemotaxis, it induces intercellular signaling and increases adhesiveness of cells. Furthermore, while MIF may enhance vascularization of the growing tumor, its inhibitory effect on chemoattraction of
fibroblasts results in formation of smaller tumors, demonstrating the importance of stromal recruitment in sarcoma growth. On the other hand, we did not find any difference in MIF expression between ARMS and ERMS cells. Further studies are needed to see if MIF could be a prognostic factor and its expression correlated with a less metastatic tumor phenotype. Finally, in contrast to other tumor types, inhibition of MIF (e.g. by small inhibitory molecules) may lead to enhanced metastatic spread and accelerated tumor growth. Thus, an involvement of MIF in tumor growth varies with the type of tumor and the potential pros and cons of anti-MIF treatment should be considered individually based on tumor type.

Acknowledgments. This work was supported by NIH P20RR018733 from the National Center for Research Resources to MK and NIH R01 CA106281-01, NIH R01 DK074720, EU structural funds, Innovative Economy Operational Program POIG.01.01.01-00-109/09-01 and the Henry M. and Stella M. Hoenig Endowment to MZR.
Literature:


53. Fischer-Valuck BW, Barrilleaux BL, Phinney DG, Russell KC, Prockop DJ, O'Connor KC. Migratory response of mesenchymal stem cells to macrophage migration...


Figure Legends

Figure 1. Expression of MIF and MIF receptors by human RMS cell lines. Panel A. MIF expression was evaluated by real-time PCR and the fold difference was calculated on the basis of 2ΔCt values normalized by gene expression in normal muscle cells. Data from three independent experiments are pooled together. Panel B - ELISA data showing secreted MIF present in CM from RMS cells. Data from three independent experiments are pooled together. Panel C. Flow cytometry was performed for detection of CXCR2, CXCR4, CD74 and CXCR7 receptors. The experiment was repeated three times with similar results. A representative study is shown.

Figure 2. SDF-1 and MIF interact with CXCR4 and CXCR7 receptors and activate intracellular signaling in human RMS cell lines. Panel A - Phosphorylation of MAPK p42/44 and AKT in human RMS cell lines stimulated by SDF-1 (300 ng/mL for 5 min) and MIF (100 ng/mL for 5 min). The experiment was repeated three times with similar results. A representative study is shown. Panel B – The densitometric analysis of blots shown in Panel A. Panel C - Internalization studies. RH30 cells (upper panel) and CXCR7^CXCR4^- RH2 and RH18 cells (lower panel) were detached from plastic culture flasks with cell stripper, washed, and resuspended in serum-free medium. Cells were stimulated with MIF (100 ng/mL) for the indicated periods of time in normal culture conditions. Prior to staining, cell-bound chemokine was removed by an ice-cold acidic glycine wash. Cells were labeled with APC–anti-CXCR4 and PE–anti-CXCR7 mAbs. Expression was measured by flow cytometry. Receptor internalization was evaluated by the mean channel fluorescence values and calculated as the percentage of control (unstimulated). Data from three separate experiments are pooled together.

Figure 3. Effect of MIF on the motility and the adhesiveness of RMS cell lines. Panel A. Chemotaxis of RMS cells across Transwell membranes covered with gelatin in response to SDF-1 or MIF gradients. Gray bars show chemotaxis in control medium (no SDF-1 or MIF in upper and lower chambers), white bars show chemotaxis in response to
SDF-1 (300 ng/mL) present in the lower chamber, and black bars show chemotaxis response to MIF (100 ng/mL) present in the lower chamber. Data from 5 separate experiments are pooled together. * p<0.05. **Panel B and C.** Adhesion of human RMS cells to fibronectin (Panel B) and to HUVECs (Panel C). RMS were not stimulated (control, gray columns) or stimulated with SDF-1 (white columns) or MIF (black columns). Data from 4 separate experiments are pooled together. * p<0.05.

**Figure 4. Effect of CXCR7 blocking antibody (9C4) on adhesion of RMS cell lines to fibronectin after stimulation with SDF-1, I-TAC and MIF.** Panel A – CXCR7⁺CXCR4⁻CXCR2⁻ RH 18 cells RMS cells and Panel B – CXCR4(high)CXCR7(low) RH30 cells were not stimulated (control) or stimulated with SDF-1, I-TAC or MIF. Prior to stimulation, cells were incubated for 1 h with 10 µg/ml blocking antibody 9C4. Data from 4 separate experiments are pooled together. * p<0.05.

**Figure 5. Effect of RMS-derived MIF on angiogenesis.** Panel A – Tube formation assay. In the tube-formation assay, HUVECs were stimulated with 0.5% BSA (negative control), FGF or MIF alone (positive controls, both 100 ng/ml) and with conditioned media from different ARMS and ERMS cell lines. The experiment was repeated three times and representative pictures are shown. **Panel B** - Tube-formation assay was performed on HUVECs after stimulation with conditioned media from RH18 cells (CM), MIF knock-down RH18 cells (CM MIF kd) and RH18 cells conditioned media + MIF inhibitor (ISO-1). As positive control we employed recombinant fibroblast growth factor-2 (FGF-2). Data are pooled together from three independent experiments p<0.05. **Panel C** - Real-time PCR analysis of expression of mRNA for VEGF, IL-8, and FGF in RH18 and RH18 MIF-kd cells. RMS cells were either kept overnight in serum starvation with RPMI+0.5% BSA (−) or stimulated with MIF (100 ng/ml). The expression of mRNA was measured by real-time PCR. Fold difference was calculated on the basis of 2ΔCt values normalized by the gene expression level in unstimulated RH18 cells. Data from 4 separate experiments are pooled together. * p<0.05.
Figure 6. Influence on tumor growth by RH18 and RH18 MIF-kd cells inoculated in immunodeficient SCID/Beige mice. Panel A. MIF expression by RQ-PCR in wild type RH18 and RH18 transfected with scrambled shRNA vector or RH18 transfected with shRNA vector downregulating MIF expression (RH18 MIF-kd). Panel B. Tumor formation by RH18 wild type and MIF-kd RH18 cells inoculated into the hind limb muscles of SCID/Beige inbred mice. Four weeks later, mice were sacrificed and femora were harvested to evaluate the size of the growing tumor. Data are pooled together from three independent experiments (five mice per experiment). * p<0.001, ** p<0.05. Panel C. Identification of stromal-fibroblast cells (CAFs) in RMS tumors growing in immunodeficient SCID/Beige mice by employing FAP antibodies (rabbit IgG, Abcam, Cambridge, MA). Nuclei were identified with DAPI. Representative tissue sections are shown.

Figure 7. Effect of RMS-derived MIF on chemotraction of stromal cells. Panel A. Chemotaxis of BM-derived fibroblast cells across Transwell membranes covered with gelatin in 10% FBS, 10% FBS supplemented with 100 ng/ml MIF, conditioned medium from RD cells, RH18 cells, RH18 MIF-kd cells, and RH18 transfected with scrambled vector. The experiment was repeated three times with different batches of mesenchymal cells. Data from three separate experiments are pooled together. * p<0.05. Panel B. Bone marrow-derived fibroblasts were stimulated with MIF and activation of MAPKp42, p38, and AKT were evaluated by western blot. The experiment was repeated two times and results are quantified by densitometry (right part of panel). A representative blot is shown. Panel C. Pleiotropic effects of MIF on RMS growth. MIF secreted by RMS cells i) activates adhesiveness of tumor cells via CXCR4 and CXCR7 receptors, ii) decreases responsiveness to SDF-1 secreted by distant tissues, and iii) as an end result, somehow prevents egress of sarcoma cells from the primary tumor. On the other hand, despite its strong angiopoietic effects, MIF decreases recruitment of stromal cells (CAFs) and thus may negatively affect tumor expansion.
Legends to Supplementary Figures.

Supplementary Figure 1. Effect of MIF on motility of RMS cells. The composite trajectories of RD, RH18, RH28, and CW9019 cells migrating without (control) or with the addition of 100 ng/mL (MIF) are shown in circular diagrams drawn with the initial point of each trajectory at the origin of the plot.

Supplementary Figure 2. MIF interferes with the SDF-1–CXCR4 axis in Transwell chemotaxis assay. Chemotaxis of RH18 (Panel A) and RH30 (Panel B) cells across Transwell membranes covered with gelatin with 0.5% BSA: (control [-]); SDF-1 in lower chamber; MIF in lower chamber; and SDF-1 in lower chamber and MIF in upper chamber. Data from three separate experiments are pooled together. * p<0.05.

Supplementary Figure 3. Effect MIF kd on proliferation of RH18 cells. RH18 cells (scr), and RH18 cells (MIF kd) stimulated or non-stimulated by exogenous MIF were tested in serum free and FBS containing medium.

Supplementary Figure 4. Influence of MIF on the metastatic potential of RMS cells in vivo. RH18, RH18 with scrambled shRNA vector, and RH18 with downregulation of MIF were inoculated intravenously (Panel A – short term assay) or intra-muscularly (Panel B – long term assay) into SCID/Beige mice. 48 hours latter (short term assay) or four weeks later (long term assay), organs were harvested and DNA isolated. The number of human cells in BM, lung, liver and peripheral blood was estimated per 10^6 murine cells by real-time RT-PCR. Real-time PCR data are pooled together from three independent experiments (five mice per experiment). * p<0.001.

Supplementary Figure 5. Influence of MIF on migration of BM-derived stroma fibroblasts. All chemokines were employed at optimal concentrations (SDF-1 -300 ng/ml, IL-8 – 100 ng/ml and I-TAC 100 ng/ml) and chemotaxis was evaluated in presence or absence of MIF (100 ng/ml) Data from three separate experiments are pooled together. * p<0.05.
Supplementary Figure 6. Panel A and B. Gene array data representing changes in gene expression profile in human BM-derived fibroblasts after stimulation with MIF. 740 genes were analyzed (cut-off value p<0.0012) by Ingenuity (Redwood City, CA). Depending on their involvement in different cellular processes, changes in expression of 92 genes are presented.
Figure 1 AB
CXCR4        CXCR2        CD74         CXCR7
RH2          <1%                       0%                         0%                         31%
RH5          50%                        0%                         0%                          5%
RH28         30%                        0%                         0%                          50%
RH30         99%                        0%                         0%                           7%
CW9019       11%                       0%                         0%                         15%
CXCR4        CXCR2        CD74         CXCR7
RH18         <1%                       0%                         0%                         55%
RD            2%                        0%                         0%                         52%

Figure 1C
Figure 2A
**Figure 2B**

The figure shows two bar graphs comparing the relative phosphorylation of MAPK42/44 and AKT between SDF-1 and MIF for different cell lines. The bars represent the mean ± standard error. The cell lines are labeled as RH2, RH5, RH28, RH30, CW9019, RH18, RD, and ARMS ERMS. The x-axis represents the cell lines, and the y-axis shows the relative phosphorylation levels.
CXCR4 Internalization

% of expression

RH30

0 10 20 30
Time (min)

CXCR7 Internalization

% of expression

RH2
RH18

0 15 30 45
Time (min)

Figure 2C
Figure 3
Figure 4
Figure 5
Figure 6
Effects of MIF

1. Increased adhesiveness of RMS cells
2. Autocrine desensitization of responsiveness of RMS expressed CXCR4 and CXCR7 to external sources of SDF-1 that results decreased egress of cells from primary tumor
3. Inhibition of retirement of CAFs

Prevents accumulation and expansion of stroma cells (CAFs) in the growing tumor

Figure 7C
Human Rhabdomyosarcomas secrete MIF that modulates metastatic behavior of tumor cells and inhibits recruitment of Cancer Associated Fibroblasts

Maciej Tarnowski, Katarzyna Grymula, Rui Liu, et al.

Mol Cancer Res  Published OnlineFirst September 22, 2010.

Updated version

Access the most recent version of this article at:
doi:10.1158/1541-7786.MCR-10-0288

Supplementary Material

Access the most recent supplemental material at:
http://mcr.aacrjournals.org/content/suppl/2021/03/13/1541-7786.MCR-10-0288.DC1

Author Manuscript

Author manuscripts have been peer reviewed and accepted for publication but have not yet been edited.