doi:10.4149/neo_2020_190321N255

Macrophage migration inhibitory factor is differentially expressed in normal and choriocarcinoma trophoblast cells

A. VILOTIC, Z. BOJIC-TRBOJEVIC, L. VICOVAC, M. JOVANOVIC KRIVOKUCA*

Institute for the Application of Nuclear Energy, University of Belgrade, Serbia

**Correspondence: milicaj@inep.co.rs*

Received March 21, 2019 / Accepted August 11, 2019

Trophoblast cells are specific for placenta, the organ necessary for development of the fetus. Trophoblast derived choriocarcinoma is a rare cancer, with high metastatic potential, invading surrounding tissues and distant organs. Macrophage migration inhibitory factor (MIF) is a pleiotropic cytokine involved in a wide range of biological processes, which is increased in almost all human cancers. Expression of MIF in normal and choriocarcinoma trophoblast cells is investigated here, using normal extravillous trophoblast derived cell line HTR-8/SVneo, and choriocarcinoma cell lines JAR and JEG3. Expression of MIF and its receptors CD74 and CXCR2 was investigated at mRNA level using qPCR. Expression of MIF protein was studied using immunofluorescence and western blot, under reducing and native conditions, in whole cell lysates, subcellular fractions and conditioned media. The expression of MIF mRNA was similar in all three cell lines, while CD74 mRNA was more expressed in choriocarcinoma cells (14-fold for JAR, 12-fold for JEG3, $p<0.01$). CXCR2 mRNA was higher in JEG3 cell line compared to HTR-8/SVneo cells (6-fold, $p<0.01$). While the cellular level of MIF was similar, the level of secreted MIF was lower in JAR cell conditioned media compared to media of both HTR-8/SVneo (2.8-fold, $p<0.01$) and JEG3 cells (4.1-fold, $p<0.001$). Cellular distribution of MIF was similar between the studied cell types. MIF was predominantly cytoplasmic, but also detected in membrane, nuclear soluble and nuclear chromatin fraction. MIF appeared in high molecular weight complexes of >150 kDa under native conditions. A band of 140–145 kDa was consistently present in JEG3 cell lysates, while it was absent or very weak in other cell types. These results show that MIF/CD74 axis is shifted in choriocarcinoma, as previously shown for other cancers, and further justifies research towards the most effective MIF targeting therapeutics.

Key words: MIF, trophoblast, choriocarcinoma, HTR-8/SVneo, JAR, JEG3

Placenta is a highly specific organ necessary for growth and development of the fetus. Chorionic villi comprise the outermost layer of placenta, covered by syncytiotrophoblast, multinucleated layer resulting from fusion of underlying cytotrophoblast cells (CTB). Trophoblast cells that invade uterine stroma and remodel spiral arteries are called extravillous trophoblast. Despite the fact that invasive trophoblast differentiation is a physiological process, there are striking similarities between invasive CTBs and invasive cancer cells. Gestational choriocarcinoma is a highly malignant tumor characterized by abnormal trophoblastic hyperplasia, absence of chorionic villi, hemorrhage and necrosis. It is composed exclusively of cytotrophoblast and syncytiotrophoblast and is often preceded by a complete hydatidiform mole. Choriocarcinoma is relatively rare, affecting approximately 1 in 40,000 pregnancies in Europe and North America, while the rates for Southeast Asia and Japan are higher, being 9.2 and 3.3 per 40,000 pregnancies, respectively [1].

However, it has high metastatic potential and a pronounced vascular invasion, and as a result, many choriocarcinomas are hemorrhagic [2]. It often invades the uterus and surrounding organs, and usually gives distant metastasis, particularly to the lung and brain, but can also metastasize to liver, spleen, kidneys and bowels [2, 3]. Molecular mechanisms and sequential events leading to the pathogenesis of the gestational diseases remain largely unknown.

MIF is a pleiotropic cytokine that participates in a wide range of biological processes and was shown to act as a cytokine, hormone, enzyme and a chaperone [4–7]. MIF is an important regulator of both innate and adoptive immunity [8, 9] and promotes pro-inflammatory functions of immune cells [10]. Human MIF consists of 114 amino acids with a

molecular mass of 12,345 Da. The 3D structure analysis showed that MIF crystallizes as a trimer of identical subunits. Each monomer consists of a four-stranded β-sheet placed above two antiparallel α-helices [11, 12]. MIF binds to a cell surface receptor CD74 which associates with co-receptor CD44 [13], but can also bind non-cognate chemokine receptors CXCR2, CXCR4 and CXCR7 [14, 15].

MIF is constitutively expressed in many tissues and is found in plasma and, apart from infection, can be increased by stress or glucocorticoid administration [6]. MIF is expressed in placenta [16] and its secretion is regulated by estradiol in placental explants [17]. MIF is implicated in various pathological conditions including infection [18, 19], autoimmunity [20] and inflammation [21–23]. MIF was also found at high levels in almost all types of human cancers, and strongly associated with the development of tumors [24, 25]. It exerts autocrine and paracrine effects on cancer cells, which include promotion of cell proliferation, escape of the immune response, induction of angiogenesis, stimulation of cell migration and suppression of apoptosis [26, 27]. Importantly, MIF overexpression in the serum of cancer patients and in tumor biopsies has been correlated with enhanced tumor progression and metastasis [28–31].

There are no data regarding expression of MIF in choriocarcinomas. Given the fact that MIF is linked to a wide variety of cancers and its positive correlation with metastatic potential, we found it of interest to investigate the possible differences in MIF expression between normal extravillous and transformed trophoblast.

Materials and methods

Cell lines, cell culture and treatment. Normal extravillous trophoblast cell line HTR-8/SVneo was kindly provided by Dr. Charles H. Graham (Queen's University, Kingston, ON, Canada). Choriocarcinoma cell line JAR was from ATCC (Manassas, USA) and JEG3 was from ECACC (Salisbury, UK). HTR-8/SVneo cells were cultured in RPMI 1640 (Gibco, Thermo Scientific, USA), while JAR and JEG3 were cultured in DMEM/F12 (Sigma, USA), both supplemented with 10% FBS and containing 1% antibiotic/antimycotic mixture (Capricorn Scientific, Germany). For analysis of secreted MIF, cells were grown in 24-well plates in complete medium for 24 h, then rinsed and media was replaced with corresponding serum-free medium for another 24 h. JAR cells were also treated with a high affinity estrogen receptor antagonist Fulvestrant (100 and 1000 nM; Sigma, USA). For these experiments phenol red free RPMI 1640 medium (Gibco, Thermo Scientific UK) was used in order to avoid estrogen activity of phenol red.

For immunocytochemical analysis cells were seeded (6×10^5) in 3 ml of respective media into 65 mm Petri dishes and cultured on glass cover slips for 24 h. Ice-cold acetone–methanol (1:1) fixed cells were stained using anti-MIF (5 μg/ml, R&D systems, MAB289). Binding was visualized using anti-mouse IgG AlexaFluor 488 (Molecular Probes, USA). Slides were mounted with Vectashield mounting medium containing DAPI (Vector Laboratories, USA) and examined using a Carl Zeiss Axio Imager microscope with an AxioCam HR Camera (Carl Zeiss, Germany).

Quantitative real-time PCR analysis. Total RNA was isolated from HTR-8/SVneo, JAR and JEG3 cells using TRI Reagent, as suggested by the manufacturer. First-strand cDNA was synthesized from 1 μg of total RNA, using 0.5 μg of Oligo(dT) 12–18 primers (Invitrogen, USA), 250 μM of each dNTP and 200 U of RevertAid reverse transcriptase (Fermentas, Lithuania). Real-time PCR was performed using the 7500 Real-Time PCR System (Applied Biosystems, USA). The reaction mixture contained 1 μl of cDNA, 5 μl 2x SYBR® Green PCR Master Mix (Applied Biosystems, USA) and specific forward and reverse primers in a final concentration of 0.5 μM. Reactions were run at 95 °C for 10 min, followed by 40 cycles of 15 s at 95 °C and 1 min at 60 °C. Melting curve analysis was performed to verify amplification specificity. Expression levels of *MIF*, *CD74* and *CXCR2* genes were normalized to *GAPDH*. Calculations were made by the comparative $2^{-\Delta\Delta}$Ct method. The sequences of specific primers were: *MIF*_F: CCGGACAGGGTCTACATCA; *MIF*_R: ATTTCTCCCCACCAGAAGGT; *CD74*_F: GACCTTATCTCCAACAATGAGCAAC; *CD74*_R: AGCAGAGTCACCAGGATGGAA; *CXCR2*_F: ACATGGGCAACAATACAGCA; *CXCR2*_R: GAGGACGACAGCAAAGATG; *GAPDH*_F: GAAGGTGAAGGTCGGAGT; *GAPDH*_R: GAAGATGGTGATGGGATTTC

Subcellular fractionation. Subcellular fractionation of cells from all three cell lines was carried out using the Subcellular Protein Fractionation Kit for Cultured Cells (Thermo Scientific, USA), according to the manufacturer's instructions. The procedure yields a cytosolic fraction (C), a membrane fraction (M), a nuclear soluble fraction (Ns) and a nuclear chromatin bound fraction (Nc). Protein concentration was then determined in each subcellular fraction using a BCA Protein Assay kit (Thermo Scientific, USA) and equal amounts of each fraction were loaded for native and SDS-PAGE electrophoresis and western blot.

SDS-PAGE, native electrophoresis and immunoblot. Whole cell lysates, conditioned media and subcellular fractions of HTR-8/SVneo, JAR and JEG3 cells were analyzed by western blot. For electrophoresis under reducing and denaturing conditions, all samples were prepared by boiling for 5 min in 0.125 M Tris-HCl buffer, containing 4% SDS (w/v), 20% glycerol (v/v), 0.1% bromophenol blue and 10% 2-mercaptoethanol (v/v). For native electrophoresis, cells were lysed in 20 mM Hepes containing 1% Triton X100, mixed with sample buffer (containing 0.125 M Tris-HCl buffer, 20% glycerol (v/v), and 0.1% bromophenol blue) and run under native conditions. Following electrophoresis on 6% for native conditions or 10% or 4–20% gradient polyacrylamide gel for reducing and denaturing conditions, the membranes were incubated with anti-MIF antibody

(0.5 µg/ml) overnight at 4 °C, with constant shaking. Staining for GAPDH (cell lysates) was used as the loading control. Proteins were detected with Pierce ECL Western Blotting Substrate (Pierce Biotechnology, USA). The obtained signals were scanned and analyzed by the ImageMaster TotalLab v2.01 program (Amersham Biosciences, Inc., USA).

Analysis of secreted MIF by ELISA. MIF concentration in conditioned media of HTR-8/SVneo, JAR and JEG3 cells was determined using sandwich ELISA. Polystyrene wells (Maxisorb, ThermoScientific, Denmark) were coated with anti-MIF monoclonal antibody (0.5 µg/well, MAB289, R&D systems) in 0.05 M phosphate buffer pH 7.2 with 0.15 M NaCl (PBS) and incubated at 4 °C overnight for antibody adsorption. After incubation the wells were rinsed with PBS and treated with 1% serum albumin in PBS for 1 h at 37 °C to block non-specific binding sites, washed again with PBS and used in ELISA.

Conditioned media (100 µl/well) of examined cell lines was incubated for 2 hours at room temperature (RT), with shaking. After washing with PBS, 100 µl of anti-MIF rabbit polyclonal antibody (0.02 µg/well, sc-48241, Santa Cruz Biotechnology) was added and incubated for 2 h at RT, with shaking. Wells were washed with PBS and incubated with biotinylated goat anti-rabbit IgG. After 30 min, well were washed, incubated with ABC (Vector Laboratories, USA) for another 30 min and further with substrate. The reaction was stopped with 0.2 M H_2SO_4. Absorbance was measured at 450 nm using Victor3V Multilabel Counter (PerkinElmer). Absorbances measured in assay were expressed as relative MIF secretion. Relative MIF secretion was calculated using average absorbances in conditioned of all examined cell lines compared to MIF in HTR-8/SVneo media. Secreted MIF was determined in three individual experiments.

Statistical analysis. The data were analyzed using GraphPad Prism Demo Software (GraphPad Software, Inc., USA). For statistical analyses, One-way ANOVA or Kruskal-Wallis test was used as appropriate. Values were considered significantly different when $p<0.05$.

Results

The expression of MIF in three trophoblast derived cell types was investigated at both mRNA and protein levels. *MIF* mRNA expression wasn't significantly different between these cell lines (Figure 1A). On the other hand, expression level of MIF receptor *CD74* was significantly higher in both choriocarcinoma cell lines (14-fold for JAR, 12-fold for JEG3, $p<0.01$) compared to HTR-8/SVneo cell line (Figure 1B), while non-cognate receptor *CXCR2* mRNA expression was higher in JEG3 cells (6-fold, $p<0.01$, Figure 1C) compared to HTR-8/SVneo cell line. Immunofluorescent staining for MIF showed similar pattern of expression in tested cell lines,

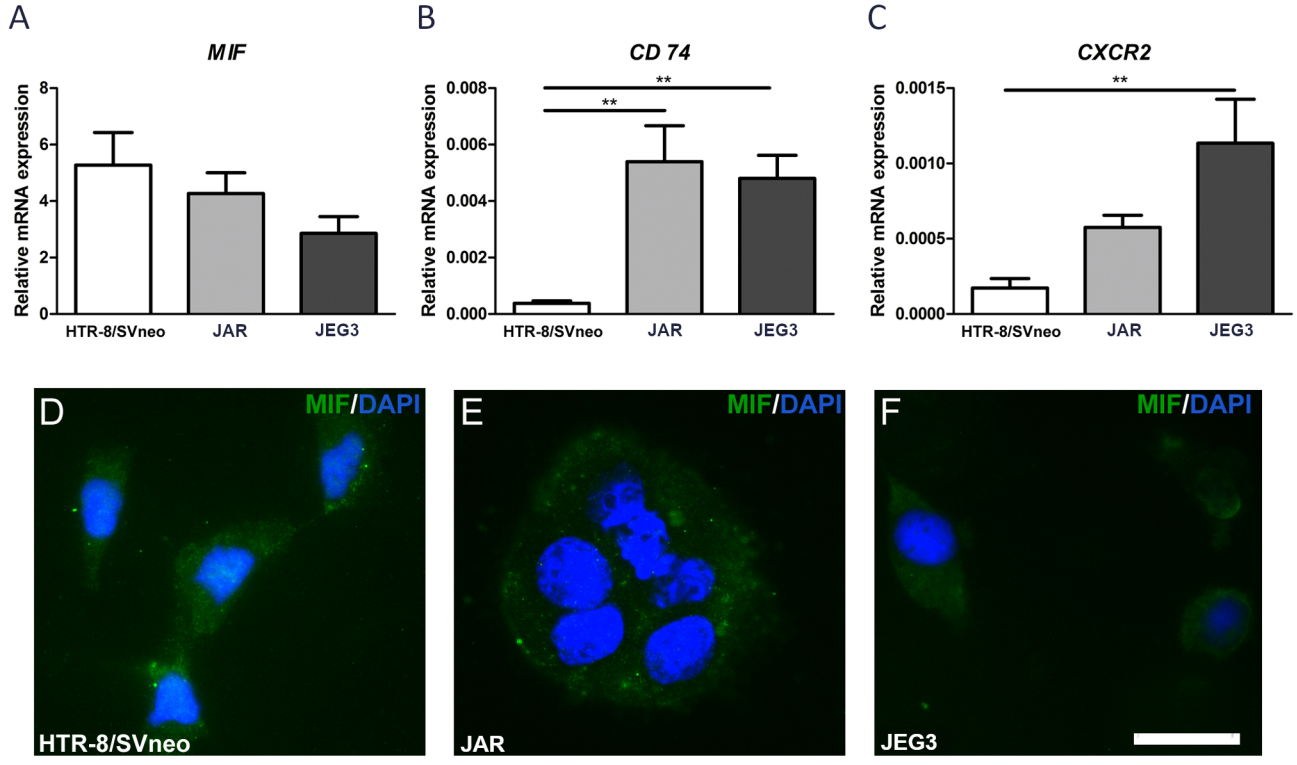

Figure 1. The expression of MIF and its receptors in normal and choriocarcinoma trophoblast. The expression of *MIF* mRNA (A), *CD74* mRNA (B) and *CXCR2* mRNA (C) in HTR-8/SVneo, JAR and JEG3 cell lines. The localization of MIF protein assessed by immunofluorescence in HTR-8/SVneo (D), JAR (E) and JEG3 (F) cells. Scale bar = 20µm. Data are expressed as mean + SEM, ** $p<0.01$, n=6.

which was predominantly cytoplasmic, with some detectable nuclear presence (Figures 1D–F).

Protein levels of MIF in whole cell lysates of the three cell types were similar, as assessed by western blot (Figure 2A). On the other hand, the level of secreted MIF was significantly lower in conditioned media of JAR cell line compared to both HTR-8/SVneo (2.8-fold, $p<0.01$) and JEG3 cells (4.1-fold, $p<0.001$, Figure 2B). The ELISA test of cell conditioned media (Figure 2C) showed that JAR cells secreted 2.43-fold ($p<0.01$) less MIF compared to HTR-8/SVneo cells. The same trend was observed when JEG3 cells were compared (2.7-fold less). This difference, however, was not significant. Cellular distribution of MIF was studied using subcellular fractionation. As expected, MIF was predominantly found

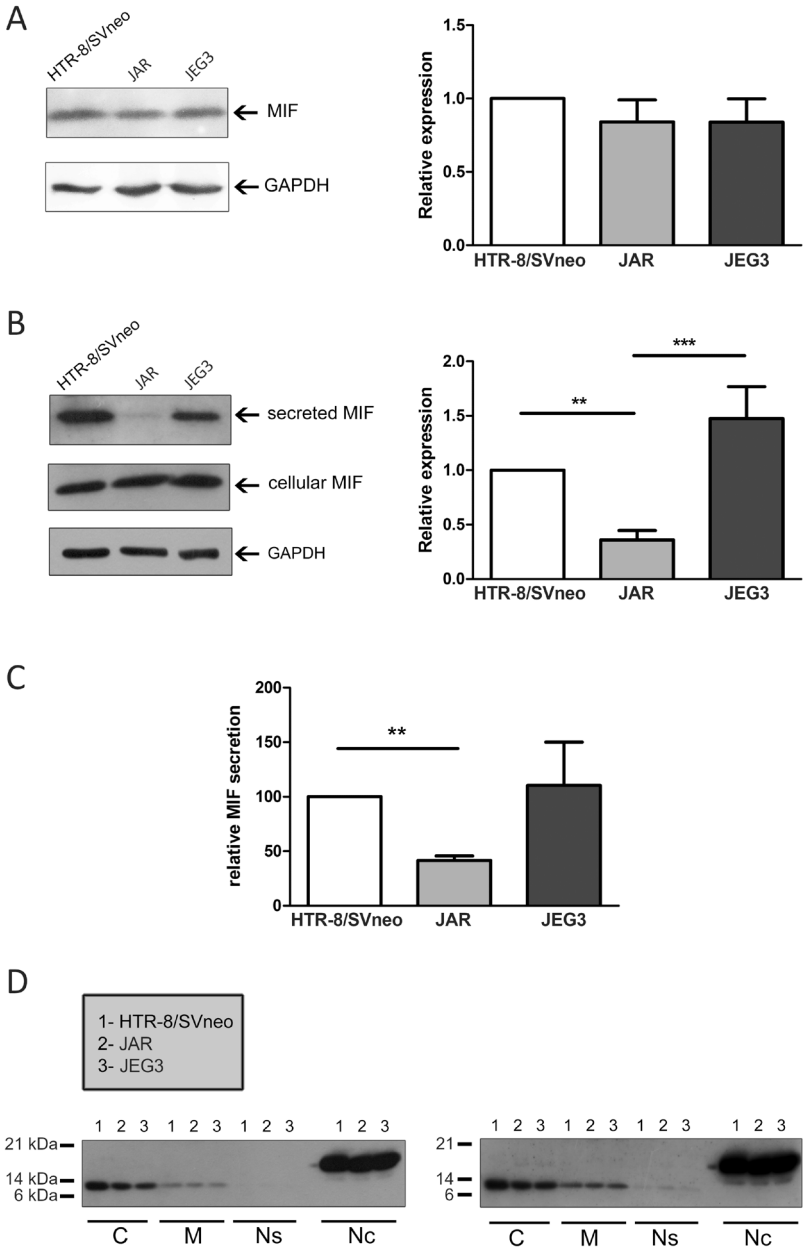

Figure 2. MIF protein expression and cellular distribution in normal and choriocarcinoma trophoblast. The expression of MIF in whole cell lysates (A), conditioned media (B, C) expressed relative to HTR-8/SVneo cell MIF level and subcellular fractions (D) in tested cell lines. The MIF level in whole cell lysates was normalized to GAPDH. Western blot of both secreted and corresponding cellular MIF expression (cells from the bottom of the same well) along with GAPDH loading control of the cell lysates are shown in panel B. The results of ELISA test of conditioned media are shown in panel C. Abbreviations in western blot samples (D): C – cytoplasmic fraction, M – membrane fraction, Ns – nuclear soluble fraction, Nc – nuclear chromatin fraction. The scans of films after shorter (3 min) and longer exposition (15 min) are shown in panel C. The charts represent relative expression compared to HTR-8/SVneo cell line, assessed by densitometric analysis. Data are expressed as mean + SEM, **$p<0.01$, ***$p<0.001$, n=4.

as a band of 12.5 kDa in cytoplasm, but was also present in membrane fraction of all three cell lines, and to a lesser extent in nuclear soluble and nuclear chromatin fractions (Figure 2C). An intense band above 12.5 kDa, currently poorly understood, was also detected in nuclear chromatin fraction of all studied cell lines.

Based on the literature data regarding regulation of MIF secretion in placental explants by estradiol [17], we further investigated whether low secretion of MIF in estradiol producing JAR cells would be enhanced by treatment with estrogen receptor antagonist – Fulvestrant. The higher concentration used (1000 nM) led to a significant increase in MIF secretion (209% of control, $p<0.05$, Figures 3A, 3B).

The molecular forms of MIF were studied in whole cell lysates, conditioned media and subcellular fractions of the three cell lines under native conditions by western blot. Cellular MIF appeared in high molecular weight complexes of >150 kDa in whole cell lysates (Figures 4A, 4B), conditioned media (Figure 5A) and subcellular fractions (Figure 5B) of all three cell lines. Only JEG3 line consistently expressed a band of 140–145 kDa, which was either absent or very weak in other two cell lines (Figures 4A, 4B). Complex of the same molecular weight was also detected in conditioned media of JEG3 cells, but only in two out of four experiments (Figure 5A). This band was localized to cytoplasmic fraction of JEG3 cells (Figure 5B). A diffuse band of 140–145 kDa was detected in membrane fractions of all tree cell types (Figure 5B).

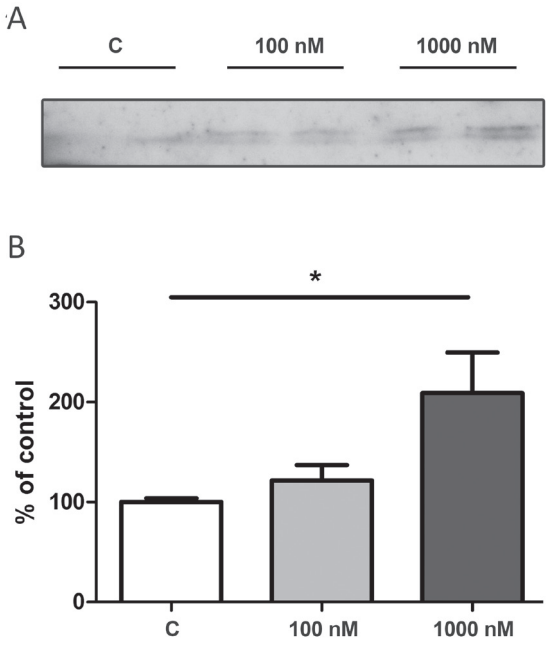

Figure 3. Increase in MIF secretion following estrogen receptor antagonist treatment in JAR cells. MIF levels in JAR cell conditioned media are increased following 24 h treatment with estradiol receptor antagonist Fulvestrant, assessed by western blot (A), followed by densitometric analysis (B). Data are expressed as mean + SEM, * $p<0.05$, n=3.

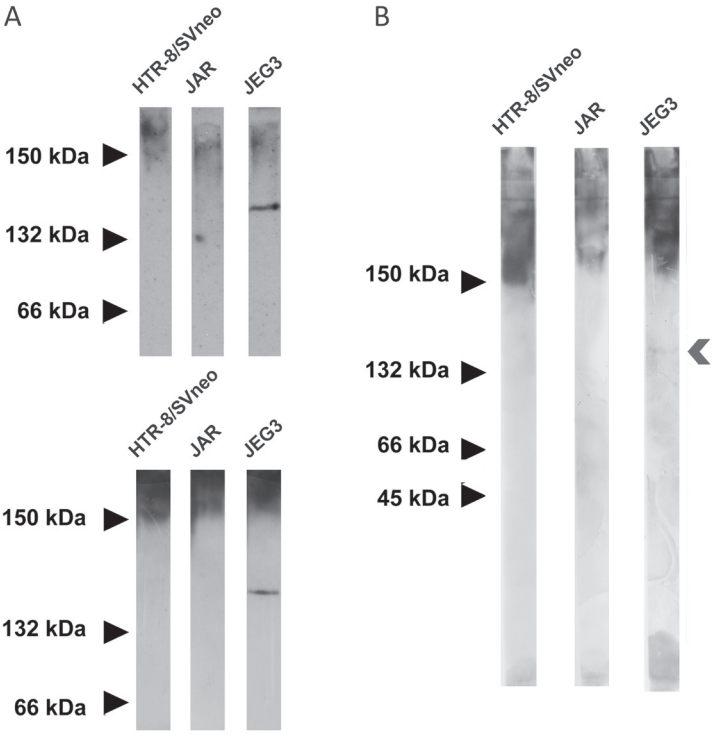

Figure 4. The molecular forms of MIF in normal and choriocarcinoma trophoblast whole cell lysates. The two representative experiments are shown for western blot following protein separation on 6% polyacrylamide gel in panel A and a representative experiment for western blot following protein separation in 4–20% gradient polyacrylamide gel in panel B. The electrophoresis was performed under native conditions.

Discussion

MIF is involved in a variety of physiological and pathological processes. In trophoblast, MIF was shown to support trophoblast invasion and migration in autocrine/paracrine manner [32]. Trophoblast and tumor cell invasion share multiple similarities including epithelial-mesenchymal transition (EMT) and degradation of extracellular matrix (ECM) by secreting proteolytic enzymes [33], the main difference being the fact that trophoblast invasion is under strict spatiotemporal control. Due to its involvement in various aspects of cancer progression, neutralization of MIF has been proposed as a potential anti-cancer therapy. Anti-MIF antibodies, small chemical compounds, as well as mRNA silencing have been used in *in vitro* studies [34–36].

Here we investigated MIF expression and subcellular localization in normal and choriocarcinoma cell lines. Similar mRNA and protein levels were observed in all three cell types. However, the

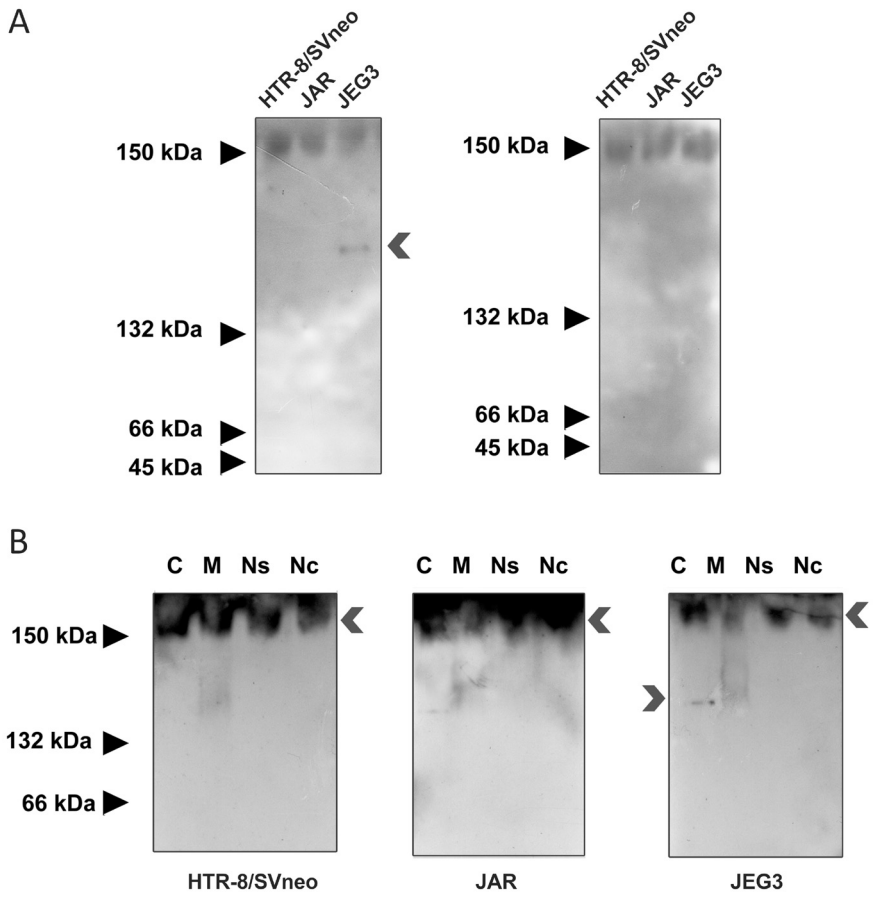

Figure 5. The molecular forms of MIF in normal and choriocarcinoma trophoblast conditioned media and subcellular fractions. The two representative experiments are shown for conditioned media (A) and a representative experiment for subcellular fractions (B) of the three tested cell lines. Western blot was performed following protein separation on 6% polyacrylamide gel under native conditions.

designated MIF receptor – CD74, and alternative receptor shared between several chemokines – CXCR2, were both significantly higher in at least one choriocarcinoma cell type compared to normal trophoblast cell line. This could point to possible excessive responsiveness to MIF and over-activation of consecutive signaling pathways. The overexpression of CD74 was also shown in various cancers [37–39] and proposed as a prognostic marker [40]. Anti-CD74 antibodies were shown to induce cytotoxic effect on B-lymphoma cells *in vitro* [41]. Blocking MIF or CD74 also attenuated growth and invasion of prostate cancer cells [34]. Non-cognate receptor, CXCR2 has in addition been implicated in various cancer types including lung cancer [42], laryngeal squamous cell carcinoma [43] and hepatocellular carcinoma [44]. Moreover, high expression of CXCR2 has been correlated with poor overall survival in cancer patients [45].

Subcellular distribution of MIF studied here was similar in all three cell types. Cytoplasmic localization was predominant, but MIF was also present in membrane fraction, and to some extent, in both nuclear chromatin and nuclear soluble fraction. Localization in membrane fraction could reflect MIF both binding to plasma membrane- or intracellular membrane-associated proteins, or MIF molecules in transit across plasma membrane. Majority of the studies showed a vesicular cytoplasmic localization of MIF. This protein has been shown to be secreted from these pools in response to a number of stimuli including lipopolysaccharide (LPS), tumor necrosis factor (TNF)-α, hypoxia, H_2O_2, etc. [46]. There are studies reporting nuclear MIF localization as well [31, 47, 48]. MIF was recently shown to exert nuclease activity, and its translocation to nucleus following treatment by cytotoxic agent has been described, implicating it in cellular death induced by DNA damage [48].

Among the data reported here we found very intriguing that, although the cellular levels of MIF were similar between investigated cell lines, JAR cells secreted dramatically less MIF. In that context it should be noted that Ietta et al. [17] found that treatment of trophoblast with estradiol modulated placental MIF secretion not affecting tissue mRNA or protein levels. Higher concentrations of estradiol led to a significant decrease in MIF secretion in their study. These findings made us wonder whether JAR cell-derived estradiol was respon-

sible for lower MIF secretion compared with the other two cell lines acting in an autocrine manner. To test this possibility, JAR cells were treated with a widely used estradiol receptor antagonist Fulvestrant. Indeed, treatment with higher concentration of Fulvestrant increased MIF secretion significantly. MIF lacks a signal sequence and is secreted by an unconventional route that requires the Golgi-associated protein, p115, and regulated MIF secretion was shown to involve ATP-binding cassette transporter protein A1 (ABC1) [49]. The underlying mechanisms were not studied here, but Ietta and coworkers [17] showed that estradiol decreased MIF secretion in placental explants by downregulating the mRNA and protein expression of the ABC1. Other mechanisms may also be involved, since JEG3 cells were shown to produce estradiol as well [50], and yet, secretion of MIF in this cell line is still functional. However, JEG3 cells were earlier shown to produce three times less estradiol compared to JAR, as reported by Bahn et al. [50] in serum free conditions, also used in our study for MIF secretion, which might partly explain this difference between the two choriocarcinoma cell lines.

Molecular forms of cellular and secreted MIF were further analyzed. Under native conditions, cellular MIF in all three cell lines appeared in protein complexes. Diffuse band of higher molecular weight detected in membrane fractions suggests that MIF associates with proteins in plasma membrane or intracellular membranes. Previously, MIF high molecular weight complexes were shown in rat bladder homogenates [51] ranging from 60–500 kDa, rat bladder intraluminal fluid [52] from 200 to 500 kDa, and human serum [53] ranging from 150–500 kDa. Similarly, data presented here show that MIF exists in trophoblast only in complexed form. Higher molecular weight forms were also observed in cell culture supernatants, suggesting MIF secretion in complexed form as well. There is a broad spectrum of proteins shown to act as MIF binding partners, which clearly points to involvement of MIF in a wide range of cellular processes. Apart from its receptors, MIF interacts with a variety of proteins including p115 [54], HIF-1α [55], insulin, HLA-DP2 β [56], p53 [57] and many other [58]. The constituents of persistent 140–145 kDa MIF complex in JEG3 cell lysates and conditioned medium remain unidentified at the moment. As pointed above, MIF is linked to a range of carcinomas, and is correlated to metastatic potential [28, 59, 60]. JEG3 and JAR are both choriocarcinoma cells, but they differ in several characteristics, including metastatic potential, which is higher in JEG3 cell line [61]. A number of metastasis-involved mediators were found expressed more in JEG3 compared to JAR cells. These include fibronectin (FN), matrix metalloproteinase-2 (MMP-2), urokinase-type plasminogen activator (uPA), Caveolin-1 (CAV-1) and VEGF-B [62]. MMP-12, another matrix metalloproteinase associated with tumor aggressiveness was detected only in JEG3 cell line [63]. Whether MIF complex, shown here to be specifically expressed in JEG3 cells, contributes to their metastatic potential remains to be elucidated. Revealing MIF binding partners in this complex will be the focus of our future research.

Due to involvement of MIF in many pathological conditions therapeutic approaches aimed at inhibiting its enzymatic and biologic activities or functional blocking of its receptors have been widely explored. A number of MIF inhibitors have been and are being developed, and some of them are in the process of clinical trials (Clin.Trials.gov ID: NCT01765790). CD74 has been proposed as target for multiple myeloma therapy [64] and humanized anti-CD74 mAb was shown to have therapeutic activity in B-cell malignancies [65]. Hertzer et al. [66], proposed CXCR2 as a target for pancreatic cancer treatment.

The current findings of various research groups, together with our results show that MIF/CD74 axis is shifted in various cancers, including choriocarcinoma, and further justifies the research towards the most effective MIF targeting therapeutics.

Acknowledgements: This work was funded through project 173004 of the Ministry of Education, Science, and Technological Development, Republic of Serbia.

References

[1] LURAIN JR. Gestational trophoblastic disease I: Epidemiology, pathology, clinical presentation and diagnosis of gestational trophoblastic disease, and management of hydatidiform mole. Am J Obstet Gynecol 2010; 203: 531–539. https://doi.org/10.1016/j.ajog.2010.06.073

[2] Benirschke, Kurt, Burton, Graham J., Baergen, Rebecca N. in book Pathology of the human placenta. chapter Maternal Diseases Complicating Pregnancy: Diabetes, Tumors, Preeclampsia, Lupus Anticoagulant. Springer-Verlag, New York, Inc. 2000; 495–555. ISBN 978-3-642-23941-0

[3] NGAN HYS, SECKL MJ, BERKOWITZ RS, XIANG Y, GOLFIER F et al. Update on the diagnosis and management of gestational trophoblastic disease. Int J Gynaecol Obstet 2018; 143: 79–85. https://doi.org/10.1002/ijgo.12615

[4] BERNHAGEN J, CALANDRA T, MITCHELL RA, MARTIN SB, TRACEY KJ et al. MIF is a pituitary-derived cytokine that potentiates lethal endotoxaemia. Nature 1993; 365: 756–759. https://doi.org/10.1038/365756a0

[5] ROSENGREN E, BUCALA R, AMAN P, JACOBSSON L, ODH G et al. The immunoregulatory mediator macrophage migration inhibitory factor (MIF) catalyzes a tautomerization reaction. Mol Med 1996; 2: 143–149.

[6] CALANDRA T, BERNHAGEN J, METZ CN, SPIEGEL LA, BACHER M et al. MIF as a glucocorticoid-induced modulator of cytokine production. Nature 1995; 377: 68–71. https://doi.org/10.1038/377068a0

[7] CHEREPKOVA OA, LYUTOVA EM, ERONINA TB, GURVITS BY. Chaperone-like activity of macrophage migration inhibitory factor. Int J Biochem Cell Biol 2006; 38: 43–55. https://doi.org/10.1016/j.biocel.2005.07.001

[8] BACHER M, METZ CN, CALANDRA T, MAYER K, CHESNEY J et al. An essential regulatory role for macrophage migration inhibitory factor in T-cell activation. Proc Natl Acad Sci 1996; 93: 7849–7854. https://doi.org/10.1073/pnas.93.15.7849

[9] CALANDRA T, ROGER T. Macrophage migration inhibitory factor: a regulator of innate immunity. Nat Rev Immunol 2003; 3: 791–800. https://doi.org/10.1038/nri1200

[10] MITCHELL RA, LIAO H, CHESNEY J, FINGERLE-ROWSON G, BAUGH J et al. Macrophage migration inhibitory factor (MIF) sustains macrophage proinflammatory function by inhibiting p53: Regulatory role in the innate immune response. Proc Natl Acad Sci 2002; 99: 345–350. https://doi.org/10.1073/pnas.012511599

[11] SUN H-W, SWOPE M, CRAIG C, BEDARKAR S, BERNHAGEN J et al. The subunit structure of human macrophage migration inhibitory factor: evidence for a trimer. Protein Eng 1996; 9: 631–635. https://doi.org/10.1093/protein/9.8.631

[12] Bucala R (Eds.). The MIF Handbook. World Scientific Publishing Co Pte Ltd, Singapore, 2012, p 400. ISBN: 978-98-143-3535-5

[13] SHI X, LENG L, WANG T, WANG W, DU X et al. CD44 Is the Signaling Component of the Macrophage Migration Inhibitory Factor-CD74 Receptor Complex. Immunity 2006; 25: 595–606. https://doi.org/10.1016/j.immuni.2006.08.020

[14] BERNHAGEN J, KROHN R, LUE H, GREGORY JL, ZERNECKE A et al. MIF is a noncognate ligand of CXC chemokine receptors in inflammatory and atherogenic cell recruitment. Nat Med 2007; 13: 587–596. https://doi.org/10.1038/nm1567

[15] ALAMPOUR-RAJABI S, EL BOUNKARI O, ROT A, MÜLLER-NEWEN G, BACHELERIE F et al. MIF interacts with CXCR7 to promote receptor internalization, ERK1/2 and ZAP-70 signaling, and lymphocyte chemotaxis. FASEB J. 2015; 29: 4497–4511. https://doi.org/10.1096/fj.15-273904

[16] ARCURI F, CINTORINO M, VATTI R, CARDUCCI A, LIBERATORI S et al. Expression of macrophage migration inhibitory factor transcript and protein by first-trimester human trophoblasts. Biol Reprod 1999; 60: 1299–1303. https://doi.org/10.1095/biolreprod60.6.1299

[17] IETTA F, BECHI N, ROMAGNOLI R, BHATTACHARJEE J, REALACCI M et al. 17{beta}-Estradiol modulates the macrophage migration inhibitory factor secretory pathway by regulating ABCA1 expression in human first-trimester placenta. Am J Physiol Endocrinol Metab 2010; 298: E411–418. https://doi.org/10.1152/ajpendo.00522.2009

[18] DAS R, KOO M-S, KIM BH, JACOB ST, SUBBIAN S et al. Macrophage migration inhibitory factor (MIF) is a critical mediator of the innate immune response to Mycobacterium tuberculosis. Proc Natl Acad Sci U S A 2013; 110: E2997–3006. https://doi.org/10.1073/pnas.1301128110

[19] BARBOSA BF, PAULESU L, IETTA F, BECHI N, ROMAGNOLI R et al. Susceptibility to Toxoplasma gondii proliferation in BeWo human trophoblast cells is dose-dependent of macrophage migration inhibitory factor (MIF), via ERK1/2 phosphorylation and prostaglandin E2 production. Placenta 2014; 35: 152–162. https://doi.org/10.1016/j.placenta.2013.12.013

[20] STOSIC-GRUJICIC S, STOJANOVIC I, NICOLETTI F. MIF in autoimmunity and novel therapeutic approaches. Autoimmun Rev 2009; 8: 244–249. https://doi.org/10.1016/j.autrev.2008.07.037

[21] AL-ABED Y, DABIDEEN D, ALJABARI B, VALSTER A, MESSMER D et al. ISO-1 Binding to the Tautomerase Active Site of MIF Inhibits Its Pro-inflammatory Activity and Increases Survival in Severe Sepsis. J Biol Chem 2005; 280: 36541–36544. https://doi.org/10.1074/jbc.C500243200

[22] BUCALA R, DONNELLY SC. Macrophage Migration Inhibitory Factor: A Probable Link between Inflammation and Cancer. Immunity 2007; 26: 281–285. https://doi.org/10.1016/j.immuni.2007.03.005

[23] COX GM, KITHCART AP, PITT D, GUAN Z, ALEXANDER J et al. Macrophage Migration Inhibitory Factor Potentiates Autoimmune-Mediated Neuroinflammation. J Immunol 2013; 191: 1043–1054. https://doi.org/10.4049/jimmunol.1200485

[24] GRIEB G, MERK M, BERNHAGEN J, BUCALA R. Macrophage migration inhibitory factor (MIF): A promising biomarker. Drug News Perspect 2010; 23: 257–264. https://doi.org/10.1358/dnp.2010.23.4.1453629

[25] BABU SN, CHETAL G, KUMAR S. Macrophage Migration Inhibitory Factor: a Potential Marker for Cancer Diagnosis and Therapy. Asian Pacific J Cancer Prev 2012; 13: 1737–1744. https://doi.org/10.7314/apjcp.2012.13.5.1737

[26] KROCKENBERGER M, ENGEL J, KOLB J, DOMBROWSKY Y, HÄUSLER S et al. Macrophage migration inhibitory factor expression in cervical cancer. J Cancer Res Clin Oncol 2010; 136: 651–657. https://doi.org/10.1007/s00432-009-0702-5

[27] RICHARD V, KINDT N, SAUSSEZ S. Macrophage migration inhibitory factor involvement in breast cancer (Review). Int J Oncol 2015; 47: 1627–1633. https://doi.org/10.3892/ijo.2015.3185

[28] MEYER-SIEGLER K, HUDSON PB. Enhanced expression of macrophage migration inhibitory factor in prostatic adenocarcinoma metastases. Urology 1996; 48: 448–452. https://doi.org/10.1016/S0090-4295(96)00207-5

[29] HIRA E, ONO T, DHAR DK, EL-ASSAL ON, HISHIKAWA Y et al. Overexpression of macrophage migration inhibitory factor induces angiogenesis and deteriorates prognosis after radical resection for hepatocellular carcinoma. Cancer 2005; 103: 588–598. https://doi.org/10.1002/cncr.20818

[30] XIA HH-X, YANG Y, CHU K-M, GU Q, ZHANG Y-Y et al. Serum macrophage migration-inhibitory factor as a diagnostic and prognostic biomarker for gastric cancer. Cancer 2009; 115: 5441–5449. https://doi.org/10.1002/cncr.24609

[31] KAMIMURA A, KAMACHI M, NISHIHIRA J, OGURA S, ISOBE H et al. Intracellular distribution of macrophage migration inhibitory factor predicts the prognosis of patients with adenocarcinoma of the lung. Cancer 2000; 89: 334–341.

[32] JOVANOVIC KRIVOKUCA M, STEFANOSKA I, ABU RABI T, AL-ABED Y, STOSIC-GRUJICIC S et al. Pharmacological inhibition of MIF interferes with trophoblast cell migration and invasiveness. Placenta 2015; 36: 150–159. https://doi.org/10.1016/j.placenta.2014.12.003

[33] MURRAY M, LESSEY B. Embryo Implantation and Tumor Metastasis: Common Pathways of Invasion and Angiogenesis. Semin Reprod Med 1999; 17: 275–290. https://doi.org/10.1055/s-2007-1016235

[34] MEYER-SIEGLER KL, ICZKOWSKI KA, LENG L, BUCALA R, VERA PL. Inhibition of macrophage migration inhibitory factor or its receptor (CD74) attenuates growth and invasion of DU-145 prostate cancer cells. J Immunol 2006; 177: 8730–8739. https://doi.org/10.4049/jimmunol.177.12.8730

[35] RENDON BE, ROGER T, TENENG I, ZHAO M, AL-ABED Y et al. Regulation of human lung adenocarcinoma cell migration and invasion by macrophage migration inhibitory factor. J Biol Chem 2007; 282: 29910–29918. https://doi.org/10.1074/jbc.M704898200

[36] AL-ABED Y, VANPATTEN S. MIF as a disease target: ISO-1 as a proof-of-concept therapeutic. Future Med Chem 201; 3: 45–63. https://doi.org/10.4155/fmc.10.281

[37] LAZOVA R, MOYNES R, MAY D, SCOTT G. LN-2 (CD74). A marker to distinguish atypical fibroxanthoma from malignant fibrous histiocytoma. Cancer 1997; 79: 2115–2024. https://doi.org/10.1002/(sici)1097-0142(19970601)79:11<2115::aid-cncr8>3.0.co;2-n

[38] DATTA M, SHAHSAFAEI A, NADLER L, FREEMAN G, DORFMAN D. Expression of MHC class II-associated invariant chain (Ii;CD74) in thymic epithelial Neoplasms. Appl Immunohistochem Mol Morphol 2000; 8: 210–215. https://doi.org/10.1097/00129039-200009000-00007

[39] KOIDE N. Establishment of Perineural Invasion Models and Analysis of Gene Expression Revealed an Invariant Chain (CD74) as a Possible Molecule Involved in Perineural Invasion in Pancreatic Cancer. Clin Cancer Res 2006; 12: 2419–2426. https://doi.org/10.1158/1078-0432.CCR-05-1852

[40] ISHIGAMI S, NATSUGOE S, TOKUDA K, NAKAJO A, IWASHIGE H et al. Invariant chain expression in gastric cancer. Cancer Lett 2001; 168: 87–91. https://doi.org/10.1016/s0304-3835(01)00503-1

[41] GRIFFITHS GL, GOVINDAN S V., SGOUROS G, ONG GL, GOLDENBERG DM et al. Cytotoxicity with Auger electron-emitting radionuclides delivered by antibodies. Int J Cancer 1999; 81: 985–992. https://doi.org/10.1002/(sici)1097-0215(19990611)81:6<985::aid-ijc23>3.0.co;2-w

[42] SAINTIGNY P, MASSARELLI E, LIN S, AHN Y-H, CHEN Y et al. CXCR2 Expression in Tumor Cells Is a Poor Prognostic Factor and Promotes Invasion and Metastasis in Lung Adenocarcinoma. Cancer Res 2013; 73: 571–582. https://doi.org/10.1158/0008-5472.CAN-12-0263

[43] HAN L, JIANG B, WU H, WANG X, TANG X et al. High expression of CXCR2 is associated with tumorigenesis, progression, and prognosis of laryngeal squamous cell carcinoma. Med Oncol 2012; 29: 2466–2472. https://doi.org/10.1007/s12032-011-0152-1

[44] LI L, XU L, YAN J, ZHEN Z-J, JI Y et al. CXCR2–CXCL1 axis is correlated with neutrophil infiltration and predicts a poor prognosis in hepatocellular carcinoma. J Exp Clin Cancer Res 2015; 34: 129. https://doi.org/10.1186/s13046-015-0247-1

[45] QIAO B, LUO W, LIU Y, WANG J, LIU C et al. The prognostic value of CXC chemokine receptor 2 (CXCR2) in cancers: a meta-analysis. Oncotarget 2017; 9: 15068–15076. https://doi.org/10.18632/oncotarget.23492

[46] JANKAUSKAS SS, WONG DWL, BUCALA R, DJUDJAJ S, BOOR P. Evolving complexity of MIF signaling. Cell Signal 2019; 57: 76–88. https://doi.org/10.1016/j.cellsig.2019.01.006

[47] PYLE M. Macrophage migration inhibitory factor expression is increased in pituitary adenoma cell nuclei. J Endocrinol 2003; 176: 103–110. https://doi.org/10.1677/joe.0.1760103

[48] WANG Y, AN R, UMANAH GK, PARK H, NAMBIAR K et al. A nuclease that mediates cell death induced by DNA damage and poly(ADP-ribose) polymerase-1. Science 2016; 354: aad6872. https://doi.org/10.1126/science.aad6872

[49] FLIEGER O, ENGLING A, BUCALA R, LUE H, NICKEL W et al. Regulated secretion of macrophage migration inhibitory factor is mediated by a non-classical pathway involving an ABC transporter. FEBS Lett 2003; 551: 78–86. https://doi.org/10.1016/s0014-5793(03)00900-1

[50] BAHN RS, WORSHAM A, SPEEG K V, ASCOLI M, RABIN D. Characterization of Steroid Production in Cultured Human Choriocarcinoma Cells. J Clin Endocrinol Metab 1981; 52: 447–450. https://doi.org/10.1210/jcem-52-3-447

[51] VERA PL, MEYER-SIEGLER KL. Substance P induces localization of MIF/α1-inhibitor-3 complexes to umbrella cells via paracellular transit through the urothelium in the rat bladder. BMC Urol 2006; 6: 24. https://doi.org/10.1186/1471-2490-6-24

[52] VERA PL, ICZKOWSKI KA, LENG L, BUCALA R, MEYER-SIEGLER KL. Macrophage migration inhibitory factor is released as a complex with alpha1-inhibitor-3 in the intraluminal fluid during bladder inflammation in the rat. J Urol 2005; 174: 338–343. https://doi.org/10.1097/01.ju.0000161606.15696.79

[53] MEYER-SIEGLER KL, ICZKOWSKI KA, VERA PL. Further evidence for increased macrophage migration inhibitory factor expression in prostate cancer. BMC Cancer 2005; 5: 73. https://doi.org/10.1186/1471-2407-5-73

[54] MERK M, BAUGH J, ZIEROW S, LENG L, PAL U et al. The Golgi-associated protein p115 mediates the secretion of macrophage migration inhibitory factor. J Immunol 2009; 182: 6896–6906. https://doi.org/10.4049/jimmunol.0803710

[55] NO YR, LEE SJ, KUMAR A, YUN CC. HIF1α-Induced by Lysophosphatidic Acid Is Stabilized via Interaction with MIF and CSN5. PLoS One 2015; 10: e0137513. https://doi.org/10.1371/journal.pone.0137513

[56] POTOLICCHIO I, SANTAMBROGIO L, STROMINGER JL. Molecular Interaction and Enzymatic Activity of Macrophage Migration Inhibitory Factor with Immunorelevant Peptides. J Biol Chem 2003; 278: 30889–30895. https://doi.org/10.1074/jbc.M302854200

[57] JUNG H, SEONG H-A, HA H. Critical Role of Cysteine Residue 81 of Macrophage Migration Inhibitory Factor (MIF) in MIF-induced Inhibition of p53 Activity. J Biol Chem 2008; 283: 20383–20396. https://doi.org/10.1074/jbc.M800050200

[58] KLUG J, MEINHARDT A. Towards the MIF Interactome, pp 77–99. In: R Bucala (Eds.). World Scientific Publishing Co Pte Ltd, Singapore, 2012, p 400. ISBN: 978-98-143-3535-5. https://doi.org/10.1142/9789814335362_0004

[59] SUN B, NISHIHIRA J, YOSHIKI T, KONDO M, SATO Y et al. Macrophage migration inhibitory factor promotes tumor invasion and metastasis via the Rho-dependent pathway. Clin Cancer Res 2005; 11: 1050–1058.

[60] SHUN CT. Expression of macrophage migration inhibitory factor is associated with enhanced angiogenesis and advanced stage in gastric carcinomas. World J Gastroenterol 2005; 11: 3767–3771. https://doi.org/10.3748/wjg.v11.i24.3767

[61] JINGTING C, YANGDE Z, YI Z, HUINING L, RONG Y et al. Heparanase expression correlates with metastatic capability in human choriocarcinoma. Gynecol Oncol 2007; 107: 22–29. https://doi.org/10.1016/j.ygyno.2007.05.042

[62] HUINING L, JINGTING C, KEREN H. Metastasis gene expression analyses of choriocarcinoma and the effect of silencing metastasis-associated genes on metastatic ability of choriocarcinoma cells. Eur J Gynaecol Oncol 2011; 32: 264–268.

[63] POLAKOVA K, BANDZUCHOVA E. Comparison of HLA-G and MMP transcription in human tumor cell lines. Neoplasma 2018; 65: 943–951. https://doi.org/10.4149/neo_2018_180409N230

[64] BURTON JD CD74 Is Expressed by Multiple Myeloma and Is a Promising Target for Therapy. Clin Cancer Res 2004; 10: 6606–6611. https://doi.org/10.1158/1078-0432.CCR-04-0182

[65] STEIN R, QU Z, CARDILLO TM, CHEN S, ROSARIO A et al. Antiproliferative activity of a humanized anti-CD74 monoclonal antibody, hLL1, on B-cell malignancies. Blood 2004; 104: 3705–3711. https://doi.org/10.1182/blood-2004-03-0890

[66] HERTZER KM, DONALD GW, HINES OJ. CXCR2: a target for pancreatic cancer treatment? Expert Opin Ther Targets 2013; 17: 667–680. https://doi.org/10.1517/14728222.2013.772137