

Involvement of macrophage migration inhibitory factor in cancer and novel therapeutic targets (Review)

NADÈGE KINDT¹, FABRICE JOURNE^{1,2}, GUY LAURENT³ and SVEN SAUSSEZ^{1,4}

¹Laboratory of Anatomy and Cellular Biology, Faculty of Medicine and Pharmacy, University of Mons, Mons 7000;

²Laboratory of Oncology and Experimental Surgery, Institut Jules Bordet, Free University of Brussels, Brussels 1000;

³Laboratory of Histology, Faculty of Medicine and Pharmacy, University of Mons, Mons 7000;

⁴Department of Otorhinolaryngology, Faculty of Medicine, Free University of Brussels, Brussels 1000, Belgium

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Abstract. Macrophage migration inhibitory factor (MIF) was originally identified in 1966 by Bloom and Bennett as a pro-inflammatory cytokine involved in the inhibition of macrophage motility. Since then, studies have investigated the functional contribution of this pro-inflammatory cytokine in several immune diseases, including rheumatoid arthritis and lupus erythematosus. Recently, MIF has been reported to be involved in a variety of neoplastic diseases. The present review discusses previous cancer research studies that have investigated the involvement of MIF in carcinogenesis, disease prognosis, tumor cell proliferation and invasion, and tumor-induced angiogenesis. Finally, potential therapeutic approaches based on the use of MIF antagonists and neutralizing antibodies are examined. The review concludes that MIF could be a good prognostic biomarker in several types of cancer, but also that the inhibition of MIF could represent a novel therapy against cancer.

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1. Introduction

Macrophage migration inhibitory factor (MIF) is a pro-inflammatory cytokine that was first identified in 1966

in a study by Bloom and Bennett (1), which reported that T lymphocytes released a factor able to inhibit the random movement of macrophages. The *MIF* gene is localized on chromosome 22q11.2 and codes for a transcript 800 bp in length. The MIF protein is composed of 115 amino acids with a molecular weight of 12.5 kDa in the monomeric form. The active form of MIF is a homotrimer: Each monomer exhibits two anti-parallel α -helices and six β -strands (2). This cytokine shares homology with the bacterial enzyme 4-oxalocrotonate tautomerase (3). Additionally, in a study on melanin biosynthesis, MIF catalyzed the conversion of *D*-dopachrome to 5,6-dihydroxyindole-2-carboxylic acid and this was dependent on its tautomerase activity (4).

In addition to T lymphocytes, MIF is secreted by a variety of other cells, including epithelial cells, endothelial cells and macrophages (5). MIF also exhibits the properties of a stress hormone, as it is expressed at high concentrations in the anterior pituitary gland, from which its release is triggered by corticotrophin-releasing hormone (6). During inflammatory responses, MIF counterbalances the immunosuppressive activity of glucocorticoids. This effect may be explained by the fact that MIF inhibits mitogen-activated protein kinase (MAPK) phosphatase-1, which is induced by glucocorticoids (7). In addition, MIF has been implicated in several types of inflammatory diseases, including atherosclerosis, rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, psoriasis and diabetes (8,9). Therefore, at present, studies are focusing on the identification and development of pharmacological agents capable of interfering with MIF activity.

2. MIF and cancer

In addition to inflammatory diseases, MIF has also been demonstrated to be overexpressed in solid tumors, such as lung, colorectal, breast, cervical, prostate, and head and neck cancer, where it may exhibit a crucial function in tumor progression (cell proliferation and invasiveness) and tumor-induced angiogenesis (10-15). It is hypothesized that the latter processes are modulated by MIF binding to its cognate receptor, cluster of differentiation (CD)74 [also known as the invariant chain of the major histocompatibility complex class II] in association with

Correspondence to: Professor Sven Saussez, Laboratory of Anatomy and Cellular Biology, Faculty of Medicine and Pharmacy, University of Mons, 6 Avenue du Champ de Mars, Mons 7000, Belgium
E-mail: sven.saussez@umons.ac.be

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CD44. While CD74 provides the binding site, downstream signal transduction pathways [MAPK and AKT pathways] are activated via CD44.

Globally, the MIF effects in cancer may be mainly explained by signaling through the CD74 receptor, since we recently showed that i) CD74 is upregulated in oral cavity carcinomas compared with benign lesions, ii) knockdown of CD74 in the murine squamous cell carcinoma SCCVII cell line decreases *in vitro* proliferation, migration, MMP9 secretion and VEGF production, and iii) SCCVII CD74-knockdown cells orthotopically inoculated in mice have a weaker growth capacity than scramble cells (16).

However, additional receptors may be involved in the effects of MIF in cancer insofar as its interaction with the chemokine receptor CXCR4 may induce metastasis. Indeed, Dessein *et al* (17) showed that MIF binding to CXCR4 was associated with invasion and metastasis in human colon carcinoma cells.

MIF and carcinogenesis. Carcinogenesis refers to the processes by which normal cells are transformed into cancer cells. Several clinical studies have revealed that MIF expression is increased in cancer tissues compared with corresponding normal tissues. For example, a previous study demonstrated that in gastric cancer, positive MIF expression rates were 12, 52 and 96% in normal mucosal, gastritis and gastric cancer tissues, respectively (18). Similar observations have also been reported in pancreatic cancer, melanoma, hepatocellular carcinoma, malignant glioma and cervical adenocarcinoma (13,19-22).

Furthermore, Zhao *et al* (23) demonstrated that serum MIF levels may aid to differentiate cancer patients with hepatocellular carcinoma from individuals with other liver diseases, such as cirrhosis, when using a reference threshold of 35.3 ng/ml. Similarly, De Souza *et al* (24) reported that in oral squamous cell carcinoma patients, MIF serum levels decreased following tumor resection and thus, serum MIF was proposed as a biomarker.

In our previous studies, a significant increase in MIF immunostaining was observed in hypopharyngeal carcinoma, oral cavity carcinoma and laryngeal carcinoma when compared with normal epithelium, and low and high-grade dysplasia and carcinoma, respectively (15,25,26). In addition, our previous study also revealed that in breast cancer patients, MIF expression was increased in cancer tissues when compared with tumor-free breast tissues in glandular and stromal compartments (12). Therefore, these results provide compelling evidence that MIF is involved in tumor biology.

MIF and disease prognosis. Kamimura *et al* (27) reported that low nuclear MIF expression was correlated with a worse prognosis in lung adenocarcinoma and thus, it was postulated that the intracellular distribution of MIF has prognostic significance. By contrast, subsequent studies indicated that high MIF expression in cancer was correlated with poor patient survival. For example, Tomiyasu *et al* (28) demonstrated that high MIF expression in lung cancer tissues was correlated with heavy smoking status and a poorer prognosis. Furthermore, overexpression of MIF correlates with a worse prognosis in hepatocellular carcinoma, which is characterized

by a high frequency of recurrence, large tumor size, high tumor-node-metastasis stage and prominent vascular invasion (23,29). In oral squamous cell carcinoma, increased MIF expression correlates with a higher pathological (p)T and pN status, positive perineural invasion and tumor depth (30). In addition, in metastatic melanoma, high MIF expression is associated with faster disease progression (31). In addition, high MIF mRNA expression in pancreatic ductal carcinoma correlates with a poor survival when compared with tumors exhibiting low MIF mRNA expression (32).

With regard to circulating MIF, Zhao *et al* (23) reported that an increase in MIF serum level to 90 ng/ml (normal value, 15 ng/ml) corresponded to a poor prognosis for patients with hepatocellular carcinoma. A similar observation was reported in colorectal cancer, where serum MIF levels were elevated in patients with hepatic or lymphoid metastasis when compared with those without metastasis (11). Furthermore, gastric cancer patients with a serum MIF level of $\geq 6,600$ pg/ml exhibited a poorer prognosis than those with lower serum MIF levels (33).

In our previous study, it was demonstrated that serum MIF levels were higher in head and neck squamous cell carcinoma patients compared with healthy volunteers, and high MIF immunostaining in tumor tissues was found to correlate with a poor prognosis in terms of local tumor recurrence, nodal metastasis involvement and overall survival (15). Recently, similar results have been reported in oral squamous cell carcinoma and gastric cancer (30,34). Overall, these clinical studies indicate that MIF expression levels in serum and tumor tissue may be of prognostic value in numerous cancer types.

MIF and cell proliferation. Shi *et al* (35) demonstrated that MIF interacts with the CD74/CD44 receptor complex in stably transfected mammalian kidney COS-7/M6 cells, leading to MIF signal transduction via the activation of Src, Ras, MAPK kinase and extracellular-signal-regulated kinase (ERK). It is hypothesized that this pathway may account for enhanced proliferative activity (31). Indeed, the interaction of MIF with its receptor CD74 results in the stimulation of ERK1/2 leading to cyclin D1 expression (36) and enhancement of proliferative activity (35). In addition, MIF may enhance cell proliferation by activating not only the ERK1/2 pathway but also the phosphoinositide 3-kinase/AKT pathway. Notably, in a previous study, immunocytochemistry and western blotting revealed that the addition of recombinant human MIF to the cell medium of the gastric cancer MGC-803 cell line resulted in increased AKT phosphorylation (37).

MIF has been also reported to act as a potent inhibitor of the transcriptional activity of p53 by direct interaction between MIF and p53, and stabilization of the p53-MDM2 complex (38). This association prevents p53 translocation from the cytoplasm to the nucleus, and consequently represses the p53-induced cell cycle arrest and apoptosis, further supporting cell survival.

Several studies have confirmed that MIF contributes to cell proliferation in cancer. One previous study demonstrated that MIF expression was downregulated by short hairpin (sh)RNA in the murine ovarian cancer ID8 cell line, and the proliferation of inoculated tumor cells was reduced when compared with mock-transfected cells, as revealed by a decrease in the

percentage of Ki-67-immunopositive cells (39). MIF-silencing studies have revealed comparable reductions in the tumor cell proliferation of human melanoma and hepatocellular carcinoma cell lines, whereby a decrease in cyclin D1 expression was reported *in vitro* and *in vivo*, respectively (31,20). Another study reported that cyclin-dependent kinase 4, cyclin D2 and cyclin E2 expression was downregulated following MIF-knockdown in HeLa 229 cells (22). In hepatocellular carcinoma, increased MIF expression, induced by cell transfection, potentiated the promoter activity of the hepatopoiectin gene (a mediator involved in liver regeneration), leading to enhanced cell proliferation (40). Hu *et al* (41) also demonstrated that MIF potentiates cell proliferation, as the release of MIF from human hepatic sinusoidal endothelial cells (HHSECs) increased colorectal carcinoma cell proliferation. Notably, nude mice injected with colorectal cell lines mixed with mock-transfected HHSECs developed larger tumors than mice injected with cancer cells mixed with anti-MIF short hairpin (sh)RNA-transfected HHSECs (41).

Pharmacological inhibition of MIF by small molecule antagonists reduces cell proliferation. For example, in a previous study, a BrdU assay revealed that in glioblastoma cell lines treated with isoxazoline (ISO)-1, cell proliferation was inhibited in a concentration-dependent manner (42). Similarly, the use of MIF or CD74 neutralizing antibodies has been demonstrated to inhibit human prostate cancer cell (DU-145) proliferation (14).

In our previous study (15), shRNA-mediated MIF-knockdown in a murine squamous cell carcinoma cell line (SCCVII) decreased cell proliferation when compared with a control scramble shRNA cell line. These findings were confirmed *in vivo* by the observed reduction in tumor growth following injection of the SCCVII MIF-knockdown cells into C3H/Hen mice compared with mice inoculated with scramble RNA-transfected cells (15). Finally, treatment of SCCVII cells with the MIF inhibitor 4-IPP (4-iodo-6-phenylpyrimidine) resulted in a dose-dependent decrease in cell proliferation (43). Thus, these findings indicate that MIF exhibits a critical function in tumor cell proliferation via its CD74 receptor, which activates the MAPK and AKT pathways.

MIF and tumor cell invasion. Tumor invasion and metastasis are highly critical in cancer progression. Several studies have indicated that MIF contributes to cell invasiveness in cancer. Notably, Rendon *et al* (10) revealed that in lung adenocarcinoma, MIF promotes Ras-related C3 botulinum toxin substrate 1 activity and thus, tumor cell motility via lipid raft activation. Another study reported that colorectal cancer cells cultured in medium conditioned with mock-transfected HHSECs exhibit increased intracellular F-actin expression compared with cells cultured in medium conditioned by anti-MIF shRNA-transfected HHSECs, indicating that cytoskeletal remodeling promotes colorectal cancer cell migration (41). Ren *et al* (44) revealed that decreased MIF expression in a neuroblastoma cell line reduces cell invasiveness *in vitro* and metastasis formation *in vivo*: 70% of athymic nude mice injected intravenously with MIF-expressing cells developed lung metastasis, whereas only 10-20% of mice developed lung metastasis when MIF was underexpressed. Furthermore, exposure to antibodies raised against MIF or CD74 has been

shown to block the *in vitro* migration of prostate cancer DU-145 cells through Matrigel-coated membranes (14). In pancreatic cancer cell lines, the overexpression of MIF was associated with an increase in cell invasiveness *in vitro* and *in vivo*, with distant metastasis to the liver, spleen, lymph nodes and intestine (32). Furthermore, the MIF-associated promotion of cell invasiveness *in vitro* has also been demonstrated in colorectal carcinoma, nasopharyngeal carcinoma, adenoid cystic carcinoma and oral squamous cell carcinoma (11,30,45,46). Notably, matrix metalloproteinase-9 (MMP-9) expression appears to be correlated with MIF expression, as positive immunostaining of MIF increases with that of MMP-9, and MMP-9 is associated with cell invasion in adenoid cystic carcinoma tissue (46).

Consistent with these studies, in our previous study, SCCVII cells treated with 4-IPP exhibited a significantly lower capacity for migration through Matrigel-coated membranes when compared with untreated cells, which demonstrated the function of MIF in cell invasion (43). Taken together, these observations indicate the involvement of MIF in tumor invasion and metastasis.

MIF and angiogenesis. Angiogenesis is essential for tumor progression, as neovascularization sustains tumor cell activity, survival and dissemination (47). Hypoxia promotes vessel growth via activation of proliferation and migration of endothelial cells. This involves stabilization of hypoxia-induced factor-1 α (HIF-1 α) through two methods that link MIF and HIF1 α : i) In the extracellular environment, MIF binding to CD74 induces direct activation of HIF1 α , and ii) in the intracellular compartment, MIF can bind Jab/CSN5, which regulates the stability of HIF1 α by preventing its hydroxylation (48), leading to the expression of pro-angiogenic factors such as IL-8 and VEGF. In this context, MIF is involved in angiogenesis, demonstrated most notably by a study in breast cancer tissue, whereby a correlation was identified between MIF and IL-8 expression (49). The same study also demonstrated that increased levels of serum MIF were correlated with an increase in IL-8 serum level (49). The association between MIF and IL-8 was also confirmed *in vitro*: The addition of recombinant human MIF to breast cancer MDA-MB-231 and MCF7 cell line culture medium stimulated the secretion of VEGF and IL-8 (50). The increase in VEGF mRNA expression following exposure to recombinant human MIF was also reported in bladder cancer (51). Similarly, in non-small cell lung cancer, two studies reported that the level of CXC chemokine increased with MIF expression (51) and that MIF is required to bind its CD74 receptor to subsequently activate the transcription of *CXCL8* and *VEGF* genes (52), which confirms that MIF is implicated in angiogenesis. In addition, studies have demonstrated that MIF immunostaining correlates with tumor microvessel density in hepatocellular carcinoma, and that recombinant human MIF stimulates endothelial tube formation *in vitro*, as demonstrated by human umbilical vein endothelial cell assays (53). Similarly, addition of exogenous MIF resulted in an increase of VEGF mRNA in the colorectal LoVo cell line (11). In addition, a previous study revealed that the MIF^{-/-} mouse model of melanoma exhibits reduced vessel density compared with the wild-type model, as demonstrated by decreased immunostaining of the endothelial marker CD31 in B16-F10 tumors (54). Fu *et al* (55) reported that hypoxia in a 3% O₂ atmosphere increases MIF expression in human vascular

Table I. Overview of previous literature investigating the function of MIF and/or CD74 in carcinogenesis, prognosis, proliferation, invasion, angiogenesis and reported responses to targeted therapy.

Cancer type	Carcinogenesis ^a , refs.	Prognosis ^b , refs.	Proliferation ^c , refs.	Invasion ^d , refs.	Angiogenesis ^e , refs.	Therapy ^f , refs.
Adenoid cystic carcinoma				4		45
Bladder					50	
Breast	12				49	
Cervical	13					
Colorectal		11	41	11,41	11	56
Gastric	18	33,34	37			
Glioma	22		42			
Head and neck	24,25,26,15	30,15		30,43,45		43
Liver	21,23	23,29	21,40		53	
Lung		28		10	51,52	57
Melanoma	20	31	31		54	56
Multiple myeloma						59
Neuroblastoma				44		
Pancreatic	19	32		32		
Prostate			14	14		58

^aHigh MIF expression in tumor tissue or high MIF level in serum associated with carcinogenesis. ^bHigh MIF level (tumor or serum) correlated with short patient survival time. ^cFunction of MIF in proliferation, as reported using MIF-silencing strategies (21,31,41), MIF inhibition by antagonist (42,43), MIF or CD74 neutralizing antibodies (14), MIF knock-in (40) and recombinant MIF (40). ^dInvolvement of Ras-related C3 botulinum toxin substrate 1 (10), F-actin (41), N-Myc, Ras, cMet, tropomyosin receptor kinase B (44) and matrix metalloproteinase-9 (46) in MIF-induced invasion. ^eFunction of interleukin-8 (49), vascular endothelial growth factor (50,52), CXC (51), chemokine (C-X-C motif) ligand 8 (52) and hypoxia-induced factor-1 α (55) in the MIF-mediated angiogenesis. ^fInhibition of the MIF/CD74 signaling pathway using small molecule inhibitors (43,46,56,57) MIF (58) and CD74 (59) neutralizing antibodies. MIF, migration inhibitory factor; CD, cluster of differentiation.

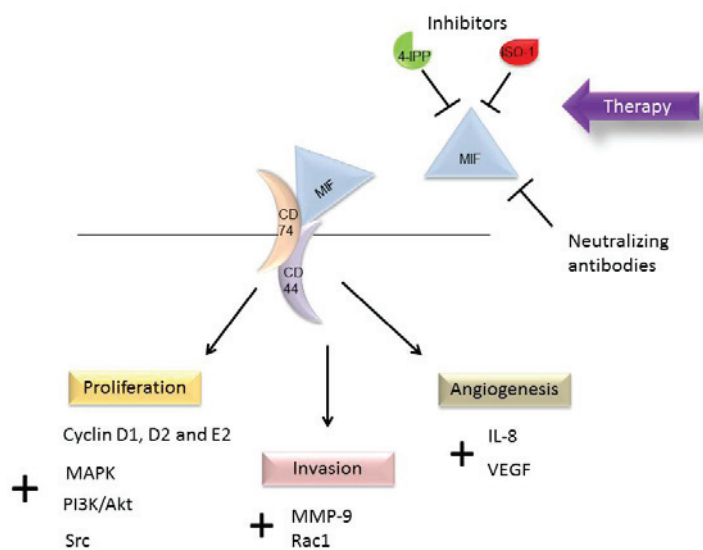


Figure 1. Schematic representation of the involvement of MIF in cancer, which affects several pathways in proliferation, invasion and angiogenesis, via interaction with its membrane receptor, CD74. Small molecule inhibitors, 4-IPP and ISO-1, and neutralizing antibodies have demonstrated an inhibitory effect on MIF. MIF, migration inhibitory factor; CD, cluster of differentiation; 4-IPP, 4-iodo-6-phenylpyrimidine; ISO-1, isoxazoline-1; IL, interleukin; MMP-9, matrix metalloproteinase-9; MAPK, mitogen-activated protein kinase; PI3K, phosphoinositide 3-kinase; VEGF, vascular endothelial growth factor; Rac1, Ras-related C3 botulinum toxin substrate 1.

smooth muscle cells, further supporting the hypothesis that MIF and HIF-1 α mediate the response to hypoxia in such a model.

Therefore, these results demonstrate the function of MIF in angiogenesis.

MIF and cancer therapy. MIF may present a novel therapeutic target in the field of oncology. The inhibition of the effect of MIF on tumor cells may be achieved by the use small molecule inhibitors, such as ISO-1 and 4-IPP, with high bioavailability and low toxicity, by the inhibition of MIF activity by neutralizing antibodies and by targeting its receptor, CD74.

The small inhibitory agents ISO-1 and 4-IPP have been demonstrated to inhibit the effects of MIF in cancer. ISO-1 exerts an inhibitory action on cell migration and invasion *in vitro* in adenoid cystic carcinoma, as demonstrated by a decrease in MMP-9 expression (46). Additionally, an analog of ISO-1, ISO-66, has recently been reported to be active in melanoma and colon cancer models, where it decreases tumor growth by stimulating an antitumor immune response *in vivo* (56). Regarding head and neck carcinoma, our previous study investigated the effect of 4-IPP on SCCVII cells and reported that the pharmacological inhibition of MIF resulted in impaired proliferation and invasiveness *in vitro* (43). Recently, Mawhinney *et al* (57) developed a novel inhibitor of MIF enzymatic activity (SCD-19), which was demonstrated to markedly inhibit the growth of nascent and established murine Lewis lung carcinoma.

The efficacy of human anti-MIF antibodies in human carcinoma has been reported by Hussain *et al* (58). The study revealed that the anti-MIF antibodies, BaxG03 and BaxB0, inhibit cell proliferation via the reduction of the phosphorylation of ERK1/2 and AKT in prostate cancer cell lines. It was also revealed that these antibodies decreased the migration of the PC3 cell line, with a half-maximal inhibition in the range of 2-10 nm/l. In addition, the study evaluated the *in vivo* efficacy of several anti-MIF antibodies (BaxG03, BaxB01 and BaxM159) in a xenograft model of prostate cancer and observed that the tumor size was reduced in mice that were administered antibodies for 30 days when compared with the control. Furthermore, the number of Ki-67-positive cells diminished after treatment compared with the control. In this study, the median effective dose was 8-14 mg/kg (58).

Regarding the mechanism of action of MIF, its receptor, CD74, may also present a promising therapeutic target to inhibit MIF signaling. In this context, a CD74 specific monoclonal antibody, milatuzumab, has been developed, which has been reported to enhance the action of doxorubicin in multiple myeloma cell lines (59). At present, a multiple myeloma phase I clinical trial combining milatuzumab and doxorubicin is ongoing (clinicaltrials.gov identifier, NCT01101594).

In our previous study, the effect of MIF underexpression on mouse survival following inoculation with SCCVII cells and subsequent treatment with cisplatin or 5-fluorouracil was evaluated (15). The results revealed that the tumors growing after the injection of MIF-knockdown cells were more sensitive to cisplatin (4 mg/kg twice a week for 2 weeks) and 5-fluorouracil (20 mg/kg daily for 4 days) treatment than tumors developing from control cells, which further supports the hypothesis that MIF downregulation may potentiate the effect of chemotherapy agents.

Furthermore, a recent study reported that pharmacological inhibition of the ATPase activity of heat shock protein 90 (HSP90) resulted in the degradation of MIF in numerous types of cancer cell (60). Thus, targeting HSP90 may present a novel strategy for the inhibition of MIF in cancer.

3. Conclusion

In this review, the effect of MIF on tumor cell proliferation, migration and tumor-induced angiogenesis in clinical and experimental studies was discussed (Table I). Overexpression of MIF in tumor tissue, as well as high levels of MIF in patient serum, have demonstrated prognostic value associated with a short survival time. The poor clinical outcome of cancer patients exhibiting high MIF expression may be explained by its potentiating effects on proliferation, invasion and angiogenesis (Fig. 1). Indeed, binding of MIF to its receptor may stimulate the MAPK pathway and inhibit p53, each positively impacting tumor cell proliferation and survival. In addition, the overexpression of MIF was correlated with the overexpression of MMP-9, further supporting its role in invasiveness. Furthermore, the action of MIF on angiogenesis may occur through the activation of HIF1 α , and subsequently, the production of pro-angiogenic factors. A number of the effects of MIF may be driven by interaction with its membrane receptor CD74, while interaction with the chemokine receptor CXCR4 has also been proposed. Significantly, MIF also inhibits p53, inhibiting cell cycle arrest and apoptosis (Fig. 1). Altogether, such data provides a novel understanding of the potential role of MIF in the mechanisms of resistance to cancer therapy.

Thus, as MIF is implicated in numerous aspects of cancer progression, via direct activation of its CD74 receptor and direct inhibition of the tumor suppressor p53, inhibition of this factor and/or of its receptor may present a novel treatment strategy against cancer, alone or in combination with conventional, as well as targeted therapies.

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