

MINI-REVIEW

Macrophage Migration Inhibitory Factor: a Potential Marker for Cancer Diagnosis and Therapy

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Abstract

Macrophage migration inhibitory factor (MIF) is a pluripotent cytokine which plays roles in inflammation, immune responses and cancer development. It assists macrophages in carrying out functions like phagocytosis, adherence and motility. Of late, MIF is implicated in almost all stages of neoplasia and expression is a feature of most types of cancer. The presence of MIF in almost all tumors and all stages of cancer makes it an interesting candidate for cancer therapy. This review explores the roles of MIF in neoplasia.

Keywords: MIF - immune suppression - p53 - hypoxia - neoplasia - metastasis

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Introduction

Cancer is the uncontrolled proliferation of the cells (Madani et al., 2011). Mutations accumulate in the human body and hence the cancer progresses to different stages (Sen & Hopwood, 2011). Main mutations involve conversion of proto-oncogenes to oncogenes and mutations in the tumor suppressor genes. Cure or a therapy for cancer hasn't been successful because each cancer has a different combination of gene mutations which makes it difficult to make a common drug for cancer. Research has been focused on to find a common gene or protein in all types of cancer.

Macrophage migration inhibitory factor (MIF), a cytokine, has been found to be associated with most of the cancers in all stages (Bifulco et al., 2008). When discovered in 1966, MIF was thought to inhibit the migration of macrophages and hence derived its name (Bach et al., 2008). Further studies have shown that MIF is a regulator of innate immunity and helps macrophage in its functions such as phagocytosis, adherence, spreading, and metabolism (Nishihira et al., 2003). MIF is thought to be released from monocytes/macrophage in presence of glucocorticoids (Nishihira et al., 2003). MIF acts as the inflammatory mediator to stimulate the expression of the cytokines like TNF- α , IL-1, IL-6 (Calandra et al., 1995). MIF is also required for embryonic development.

Apart from the inflammatory and immunological functions, MIF is considered to play a role in cell proliferation and differentiation (Bucala & Donnelly, 2007). MIF, apart from being involved in stages of cancer, has a potential to suppress the tumor suppressor gene p53 thereby leading to uncontrolled cell growth (Hudson et al., 1999).

Our immune system is capable of fighting against the antigens which enter our body. Also the immune cells have the capacity to identify the self-cells and the damaged self-cells, which would be harmful for the human body and they eliminate such kind of cells. In case of prostate cancer, there is suppression of the immune system and also the apoptotic death of the dendritic cells (Aalamian et al., 2001). The cells in the prostate cancer have the ability to kill the dendritic cells and also inhibit their production. The dendritic cells are known to have anti-tumor property. The stimulation of dendritic cells has shown to induce apoptosis in leukemia (Nakaya et al., 2012). The tumor derived cytokines are also known to play a role in deregulating the immune system of the body. MIF is also known to suppress the functions of immune cells (Krockenberger et al., 2008). The expressions of interleukins, interferons are affected thereby altering the functions of the immune cells.

The association between inflammation and cancer is well established and it is observed that inflammation can lead to cancer development and progression (Conroy et al., 2010). Research carried out since the discovery of MIF has given more information about MIF and its role in innate and adaptive immunity. MIF has been thought to be a link to connect inflammation and cancer.

Genetics and Structure of MIF

The gene lies on chromosome 22q11.2 and regulation of the gene is by the two polymorphic sites in the promoter region (Budarf et al., 1997; Baugh et al., 2002). The first site consists of the CATT repeat at -794 which repeats 5-8 times (Baugh et al., 2002) and the site is within a potential pituitary 1 (PIT-1) transcription factor binding

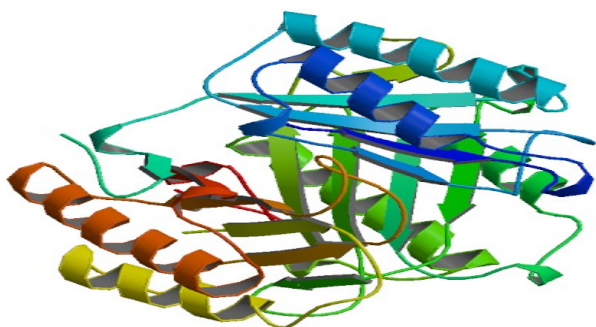


Figure 1. The Secondary Structure of Macrophage Migration Inhibitory Factor from PDB (PDB ID: 1MIF)

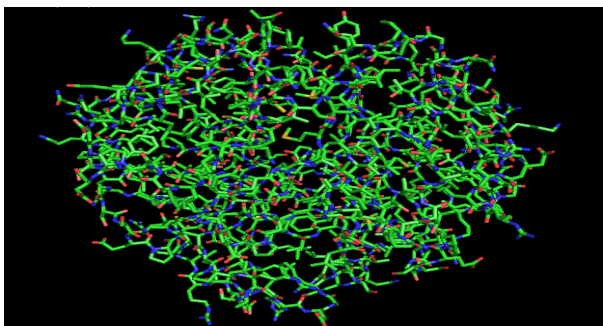


Figure 2. The Stick Model of MIF Generated using PyMOL (www.pymol.org)

site (Bifulco et al., 2008). The second site is a single nucleotide polymorphism at -173(G/C) (Xue et al., 2010). This site is associated with enhanced promoter activity in some cancer cell lines and the activity is proportional increased MIF levels in serum (Xue et al., 2010).

The gene encodes a 12.5 kDa polypeptide and the protein consists of 115 aminoacids (Sun et al., 1996a). MIF is trimer and each monomer consists of anti-parallel alpha helices against four-stranded beta sheet (Figure 1 and 2) (Sun et al., 1996a). The crystal structure shows that the active form is a 37.5 kDa homotrimer with new protein folds (Sun et al., 1996b). MIF has Pro-Pro dependent tautomerase activity and CALC dependent oxidoreductase activity (Rosengren et al., 1996; Thiele & Bernhagen, 2005). The tautomerase activity allows the MIF to catalyze D-dopachrome or L-dopachrome to its indole derivatives (Rosengren et al., 1996). Proline residue present at the N terminal mediates the tautomerase activity. MIF can exert its action by many pathways including the CD74/CD44 receptor complex (Shi et al., 2006), CXCR4 and CXCR2 (Bernhagen et al., 2007).

Using the Q-SiteFinder (<http://www.modelling.leeds.ac.uk/cgi-bin/qsitefinder/qsitefinder.cgi>), it was found that MIF has 10 binding sites (Figure 3 and 4) (Table 1). Q-SiteFinder is an online server for ligand binding site prediction. Hydrophobic probes bind to proteins and then clusters having the highest and most favorable binding energy are determined. Then the sum for each cluster is calculated to place them in a rank order.

MIF and Cell Cycle

Cell proliferation with a proper regulation is necessary

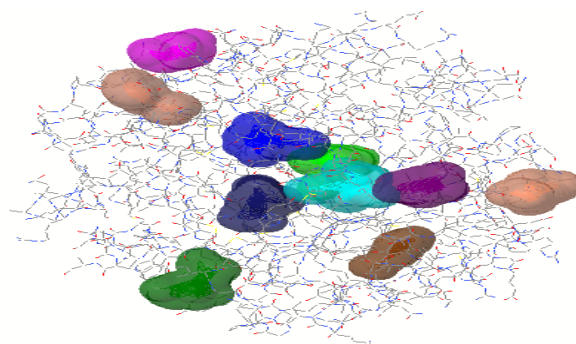


Figure 3. The Output Generated Through the Q-SiteFinder. 10 binding sites were found. Predicted binding site selection is color-coded. Green is the most favourable binding site, followed by blue, purple and orange/brown

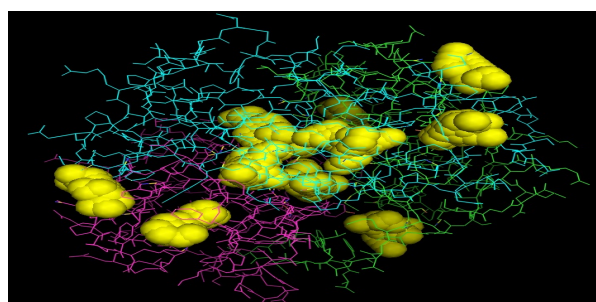


Figure 4. The Binding Sites of MIF Generated Through PyMOL. Yellow entities indicate the binding sites

Table 1. The Site Volume and Number of Residues Present in the Ten Binding Sites of MIF

SITES	*Site Volume [Cubic Å]	No .Of Residues
Site 1	221	72
Site 2	218	46
Site 3	193	48
Site 4	175	66
Site 5	174	46
Site 6	142	46
Site 7	144	40
Site 8	130	30
Site 9	133	44
Site 10	119	45

*Protein Volume: 31504 Cubic Angstroms

for the normal development. The cell cycle with its various check point proteins sees to it that the abnormal cells do not divide in the body. The cell cycle is either arrested to correct the DNA repairs in the abnormal cells or the cells are forced to undergo apoptosis (Medema & Macurek, 2011). The cell cycle check points are lost in cancer and hence the cells proliferate at a very high rate (Hartwell and Kastan, 1994).

The proper functioning of cyclin dependent kinases (CDK) is critical for the cell cycle (Nemajerova et al., 2007b). CDK act in conjunction with their respective cyclins (Fingerle-Rowson & Petrenko, 2007). The cyclin proteins, CDK inhibitory proteins and other proteins involved in the cell cycle are controlled by the ubiquitin proteasome system (Yamasaki & Pagano, 2004). The proteins to be ubiquitinated and degraded are recognized by either anaphase promoting cyclosome/complex (APC/C) or Skp1-Cullin1-F-box (SCF) complex (Nakayama & Nakayama, 2006). The SCF's remain active from late G1

to early M phase and the APC/C is active from mid M phase to end of G1 phase (Fingerle-Rowson & Petrenko, 2007). The targets for SCF complex include c-jun, c-myc, p21, p27 and proteins involved in controlling the cell division (Tsvetkov et al., 1999; Welcker et al., 2004). Therefore down-regulation of SCF can promote the onset of cancer and further progression of cancer.

SCF complex is made of four components namely the Rbx1, Cul1, Skp1 and F-box. The F-box acts as the substrate recognition unit while the others are the invariable subunits. Rbx1 and Cul1 form the catalytic core of SCF (Cardozo & Pagano, 2004). The activity of the core is enhanced or stimulated by the attachment of Nedd8 proteins to conserved lysines in the domain of Cul1. The attachment of Nedd8 makes the SCF complex active (Bornstein et al., 2006). The neddylation process requires the E2 enzymes. The recruitment of E2 enzymes is decreased when deneddylation of Cullins take place with the CSN/COP9 signalosome (Lyapina et al., 2001). The CSN recruits Ubp12, a deubiquitylase, which counteracts SCF's activity. The JAB1/CSN5 is responsible for cleaving the Nedd8 from Cul1. Deneddylated cullins can displace the Skp1 and F-box proteins. Once the Nedd8 is displaced, Cand1 comes and binds to the complex thereby rendering the SCF complex inactive (Zheng et al., 2002). Through the yeast hybrid system, it was found that the binding part of MIF was JAB1/CSN5 (Kleemann et al., 2000). JAB1 is known to remove the Nedd8 from the cullins and CSN5 is involved in differentiation and morphogenesis (Fingerle-Rowson & Petrenko, 2007). Hence unbalanced activity of JAB1/CSN5 complex can prevent the ubiquitin dependent proteolysis either directly or indirectly. MIF binds to JAB1/CSN5 complex thereby preventing the deneddylation process and hence the SCF complex can remain active and perform its functions (Nemajerova et al., 2007a). Thus MIF governs the cell cycle by the activity of the SCF complex. Generally most of cancers undergo loss of p53 function and therefore G1 checkpoint is lost (Kops et al., 2005; Nemajerova et al., 2007b). Also G2M checkpoint is retained in the cancer cells which disturb the integrity of the genome (Nemajerova et al., 2007b). Hence targeting the G2M checkpoint in cancer cells along with the MIF-JAB1/CSN5 complex can also be a possible anti-cancer therapy (Kops et al., 2005).

MIF and p53

p53 is the tumor suppressor gene and it is known as the "guardian of the genome" (Lane, 1992). It is tightly suppressed in normal cells by Mdm2 which inhibits the p53 activity by degrading it (Haupt et al., 1997). When DNA mismatch is sensed by the cell cycle, the N terminal end of p53 is phosphorylated (Steegenga et al., 1996), thereby separating the p53 from Mdm2 and p53 carries out its function. Almost all tumors have a mutation in p53 gene thereby losing the apoptotic pathway and cell cycle checkpoints. MIF is known to bypass the p53 mediated growth arrest or apoptosis (Mitchell et al., 2002). In one study, MIF was shown to overcome the p53 activity in 3 biological assays (Hudson et al., 1999). The mechanism

by which MIF overcomes the p53 activity is still unclear but it is speculated that the oxidoreductase activity of MIF may have a role in this (Nguyen et al., 2003). As mentioned earlier, MIF interacts with the JAB1/CSN5 complex. The JAB modulating activities of MIF is known to be related to the cysteine 60 residue of MIF, which is redox active (Lee et al., 2006). Also p53 and JAB1 interact with each other. All these findings suggest that a MIF-JAB1-p53 complex formed can be a molecular basis for the MIF mediated suppression of the p53 functions (Lee et al., 2006).

A study carried out has shown that MIF interacts with p53 in vivo (Jung et al., 2008). The interaction is dependent on the redox status and the cysteine residues present in MIF and p53 proteins. The DNA binding domain within the residues 113 to 290 of p53 is responsible for the interaction between MIF and p53 (Jung et al., 2008). The Cysteine residue present on the 81st position of MIF interacts with the Cysteine residue at 242nd position of p53 and to some extent with cysteine at 238th position (Jung et al., 2008). Regulation of p53 by Mdm2 is critical for cellular processes. MIF stabilizes the p53 and Mdm2 binding (Jung et al., 2008). This stabilization would ensure that p53 doesn't get phosphorylated and perform its function. MIF's over-expression leads to a decrease in the expression of p21, BAX proteins and p53 itself, thereby preventing the programmed cell death to take place. Hence MIF down-regulates the activity of p53 thereby inhibiting the cell cycle arrest and apoptosis (Jung et al., 2008).

MIF and Hypoxia

Hypoxia is a condition where there is low availability of oxygen for the cells. When the cells are under stress, they adapt themselves to that condition and continue with the proliferation. Many mutations are needed for the onset of cancer (Sen & Hopwood, 2011). At some stage of changes taking place during the onset of cancer, the cells are under hypoxic condition. During hypoxic condition, many changes take place in the expression of genes like the erythropoietin (EPO), glucose transporters (GLUT), vascular endothelial growth factor (VEGF), and matrix metalloproteinase (MMPs) (Oda et al., 2008). All these changes are due to up regulation of hypoxia-inducible factor 1 (HIF-1) (Oda et al., 2008). MIF is also one of the genes found to be over-expressed under hypoxia (Bifulco et al., 2008). MIF is a target in hypoxia-induced transcription in many cancers such as the glioblastoma, neck cancer, cervical cancer (Bacher et al., 2003). This transcription is due to the presence of HIF-1 response element found on the 5' untranslated region of MIF (Baugh et al., 2006). HIF -1 is a heterodimer consisting of HIF-1 β and HIF-1 α subunits (Strieter, 2005). Over-expression of intracellular MIF or administration of extracellular MIF leads to up-regulation of HIF-1 (Oda et al., 2008). CD74 is necessary for MIF to exert its effect on HIF-1 and also the activity of ERK in MCF-7 cell lines (Oda et al., 2008). Upon binding with the type 2 receptor of CD74, MIF would induce the ERK pathway. It was shown that p53 interacts with HIF-1 α and is responsible for ubiquitin mediated degradation of HIF-1 α (Oda et

al., 2008). As MIF binds to p53 and inhibits its activity (Fingerle-Rowson & Petrenko, 2007; Jung et al., 2008), the expression of HIF-1 α is high and this would lead to the over-expression of the genes mentioned earlier thereby promoting tumor angiogenesis and metastasis (Oda et al., 2008). MIF can also stabilize HIF1 and it also prevents its degradation by the proteasomes (Winner et al., 2007a). The expression of HIF1 can also be stable with the help of prolyl hydroxylase inhibitors (Winner et al., 2007b). Jab1-CSN5 complex can also be seen to take part in this stabilization (Winner et al., 2007a).

MIF and Angiogenesis

Angiogenesis is a complex process involving a lot of factors for the formation of new blood vessels. Factors like basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF) and angiopoetin are necessary for formation of new blood vessels (Bifulco et al., 2008; Oda et al., 2008). The size of the tumor cannot exceed without angiogenesis (Bergers & Benjamin, 2003). As the tumor size increases, the cells which are at the periphery of the tumor do not get the proper nutrients. These cells are in stress and in hypoxic condition. The precursor endothelial cells secrete VEGF, bFGF and other pro-angiogenic factors necessary for blood vessel formation (Ichihara et al., 2011). Also there is HIF-1 α up-regulation which leads to increased expression of bFGF, VEGF, Angiopoetin (Oda et al., 2008). HIF-1 α also induces MIF and hence MIF plays a role in tumor angiogenesis (Oda et al., 2008). MIF is shown to induce a dose dependent secretion of bFGF, VEGF, IL-8 (Ren et al., 2005; Bifulco et al., 2008). MIF activates the mitogen-activated protein kinase pathway (MAPK) and phosphatidylinositol 3-kinase pathway (PI3K) which leads to the increased secretion of bFGF and VEGF (Xu et al., 2008; Veillat et al., 2010). The association of MIF with the pro-angiogenic factors has been found in many types of tumor (gastric tumor, hepatocellular cancer, glioblastoma, esophageal cancer) in vivo (Bifulco et al., 2008). ISO-1[(S,R)-3-(4-hydroxyphenyl)-4,5-dihydro-5-isoxazole acetic acid methyl ester], a prototype inhibitor of MIF (Al-Abed & VanPatten, 2011), reduced the tumor size and angiogenesis in the mouse xenograft model of DU-145 prostate cancer (Meyer-Siegler et al., 2006). MIF and CD74, through their over-expression, are known to enhance VEGF's expression in cervical cancer (Cheng et al., 2011). In neuroblastoma, N-myc expression is linked with advanced stage (Ren et al., 2004). N-myc is known to be involved in cell proliferation (Hurlin, 2005). MIF expression is associated with the grade of the tumor and N-myc expression (Ren et al., 2004; Ren et al., 2006). N-myc expression is induced via the ERK pathway activation by MIF (Ren et al., 2006). N-myc is translocated from cytosol to nucleus in neuroblastoma due to the presence of MIF (Ren et al., 2004).

MIF expression can also be induced by the clotting factors, thrombin, factor Xa indicating the presence of an endothelial autocrine loop mediated by blood clotting factors generally present during inflammatory processes (Shimizu et al., 2004).

MIF and Metastasis

The size of the tumor grows due to tumor associated angiogenesis. When there is up-regulation of HIF-1, a message is sent to the nucleus to down-regulate the expression of e-cadherin (Krishnamachary et al., 2006). E-cadherin is responsible for the formation of focal adhesion complex (Gooding et al., 2004). Due to focal adhesion complex, the cells remain intact with each other and with the basal membrane. As the e-cadherin expression is decreased, the focal adhesion complex is weak leading to epithelial mesenchymal transition (Oloumi et al., 2004). The angiogenic factors and MIF's levels are also up-regulated (Ren et al., 2004). There is increase in the expression of matrix metalloproteinases (MMPs) (Li et al., 2004; Bifulco et al., 2008). They degrade the basal membrane (Libra et al., 2009). The tumor cells enter into the blood circulation and when they receive proper homing factors, they establish secondary tumors in different organs by interacting with the homing factors. The expression of MIF is directly proportional to the expression of MMPs (Shimizu et al., 1999). MMPs have E2F binding site in the promoter region (Johnson et al., 2011). Many of these sites are present in MMPs gene of the cancer cells. Hence MMPs are regulated by the E2F transcription factors leading to metastasis. Also, MIF's receptor is CD74 and it's known for invasiveness (Meyer-Siegler et al., 2006).

By interacting with CXCR4, CXCR2 and CD74, MIF can arrest the macrophage at sites of malignant tumors (Bernhagen et al., 2007; Bifulco et al., 2008). A population of macrophages within the tumor population are termed as tumor-associated macrophages (TAM) (Colombo & Mantovani, 2005). These macrophages, instead of performing the immune functions, help in the proliferation of the tumor cells. The TAM can produce growth factors required for proliferation, stimulate angiogenesis (Pollard, 2004). Through the treatment of anti-MIF antibodies, the invasiveness of murine colon cancer cell line was reduced (Sun et al., 2005). In the in vivo murine model, transfection of MIF RNAi to the cells suppressed the liver metastasis (Sun et al., 2005).

Pathways and Interactions of MIF

Earlier it was mentioned that MIF can interact with CD74/CD44 receptor complex as well as with CXCR4, CXCR2 (Bernhagen et al., 2007). MIF can bind to the extracellular domain of CD74. CD74 is involved in transporting proteins from the endoplasmic reticulum to places/compartments where class II MHC loading takes place (Ong et al., 1999). The cascade doesn't start just with the binding of MIF to CD74. CD44 binding is necessary for the MIF mediated signaling cascade to start along with Src-tyrosine kinase (Shi et al., 2006). This leads to phosphorylation of ERK1/2 which further trigger off various effector proteins involved in inflammatory processes and cell proliferation. Some examples include C-myc, NF-k β . The ERK1/2 remains phosphorylated for many hours and hence this cascade continues for a longer

time (Mitchell et al., 1999; Lue et al., 2006). Along with this AKT pathway is also activated by MIF (Lue et al., 2007). This leads to phosphorylation of BAD and BAX proteins (proteins involved in apoptosis) and the cells acquire signal to withstand apoptosis (Lue et al., 2007).

The nuclear factor – kappa beta is a transcription factor which plays a role in cell cycle, cell proliferation, inflammation and carcinogenesis (Miller et al., 2010). In HeLa cells, inhibition of this pathway leads to reduced cell growth (Miller et al., 2010). In medulloblastoma, inhibition of NF- κ B pathway can reduce the growth of the tumor and its viability (Spiller et al., 2011). In MIF deficient cells, downregulation of fibroblast growth factor-inducible 14 (Fn14) leads to NF- κ B pathway inhibition. Fn14 is a TNF like weak inducer of apoptosis (TWEAK) receptor which could activate the NF- κ B pathway thereby leading to cell proliferation (Liu et al., 2008). Hence one may conclude that MIF plays a role in NF- κ B pathway but the exact role of MIF in this pathway is still to be elucidated. Another gene involved in cell cycle, cell proliferation is the c-myc gene. C-myc is a proto-oncogene which upon mutation gets converted to oncogene. The c-myc gene represses the functions of CDK inhibitors like p15, p21 and p27 (Vlach et al., 1996; Claassen & Hann, 2000; Staller et al., 2001). The activity of c-myc is sustained for a longer time due to presence of MIF (Liu et al., 2008). When MIF levels are high, the transforming growth factor beta (TGF- β) levels are low. When MIF expression is brought down, the TGF- β 3, TGF- β 2 are up-regulated and they start the signal transduction (Liu et al., 2008). The activation of TGF- β pathway would lead to cell cycle arrest via the TGF- β pathway.

CD44 is a known cancer stem cell marker (Dhingra et al., 2011). CD44 is a co receptor of MIF which upon binding starts a downstream signaling pathway involving the ERK1/2 pathway and AKT pathway (Shi et al., 2006). It is speculated that CD44 plays a role in invasion and its being considered as a metastasis promoting molecule (Marhaba & Zoller, 2004; Jang et al., 2011). CD44 helps in the attachment of stem cells and circulation of the tumor cells to the bone marrow endothelial cells (Lapidot et al., 2005). The interaction between MIF and CD44 needs to be evaluated further to find out more information which can be useful in treatment of cancer and development of new drugs.

MIF and Tumors

MIF expression is seen in almost all cancers. Here are some of the cancers where MIF's role in the progression of cancer is observed. MIF is overexpressed in esophageal squamous cell carcinoma lesions (Ren et al., 2005). Marred function of MIF is observed in non-small cell lung cancer, lung adenocarcinoma and squamous cell lung cancer (Rendon et al., 2007; Liu et al., 2010). Glioblastoma, neuroblastoma, colonic cancer and colorectal cancer have elevated levels of MIF (Shkolnik et al., 1987; Bacher et al., 2003; Ren et al., 2006; Lee et al., 2008). Through ELISA, it was found that the intratumoral level of MIF is indirectly proportional to the tumor involved loco-regional nodes (Bando et al.,

2002). In bladder cancer, MIF expression stimulates the proliferation of urothelial tumor cells (Guo et al., 2011). In the endometrial cancer, MIF induces VEGF thereby promoting tumor angiogenesis (Bondza et al., 2008). The high levels of MIF in prostate cancer correspond with the metastatic potential of the cancer cells (Tang et al., 2011). In lymphoproliferative disorders, MIF's binding to CD74 and CD44 provided a signal which sees to it that B cells do not enter the apoptotic pathway. The expression of MIF, which leads to c-met activation, is necessary for the B cell survival in lymphocytic leukemia cells (Cohen et al., 2012). MIF secretes IL-8 which provided a signal for the cells to survive in chronic lymphocytic leukemia (Binsky et al., 2007). The lipopolysaccharides induce the expression of MIF in HL-60 cells, a leukemic cell line (Nishihira et al., 1996). In mouse model, loss of MIF delayed the onset of B-cell lymphoma (Talos et al., 2005). Also even in *Helicobacter pylori* induced gastric cancer, MIF-TLR4 activity promotes the production of factors necessary for cancer progression (Bifulco et al., 2008; El-Omar et al., 2008). MIF's role in many cancers and in the different stages is becoming clearer with the studies being carried out across the globe.

Conclusions and Prospects

MIF, as it's implicated in all stages of tumor, becomes a potential target for future cancer treatments or therapy. MIF expression is found in all types of tumors. MIF expression corresponds with the tumor aggressiveness and its metastatic potential. The production of MIF through autocrine signals from ovarian cancer cell has shown to stimulate the production of cytokines, chemokines and angiogenic factors which lead to growth of the tumor (Hagemann et al., 2007). MIF knock down in the syngeneic ovarian cancer model is shown to change the ascetic microenvironment. The expression of TNF- α , IL-6, IL-10 was decreased drastically in the microenvironment (Hagemann et al., 2007). Also ISO-1 and other small inhibitory molecules of MIF are being developed and tested in cancers with higher MIF levels. A humanized antibody developed against MIF's receptor is in clinical trials for lymphoproliferative disorders (Stein et al., 2007; Bifulco et al., 2008). In silico studies can also prove to be helpful in finding new targets for MIF. The site inhibiting the tumor growth can be known through the in silico studies. These studies will help to narrow down the molecules to be tested against cancer. MIF is shown to have 10 binding sites. Further studies can be done to know which one among the ten sites is the tumor inhibitory site. Many anti-MIF antibodies have been treated in many animal models of cancer and all these have shown to decrease the tumor growth.

MIF has a very interesting relationship with p53. It antagonizes the activity of p53 (Hudson et al., 1999; Jung et al., 2008). If the interaction between MIF becomes even clear, MIF would surely be "miracle target" in developing drugs and designing new therapies.

Most of the research focuses on the role of MIF in cancer. The signal cascade of MIF needs to be studied more to know if there are any other proteins interacting

with MIF which lead to cancer progression. Research should now be focused on immune suppression and change in macrophage functions due to elevated expression of MIF. It's been said that MIF arrest macrophage at sites of malignant tumors and that the production of cytokines involved in immune system are elevated thereby suppressing the immune system. But what and how is the effect of increased MIF levels in cancer on macrophages and other immune cells is not known clearly. Macrophages are antigen presenting cells. They help in presenting the fragment of antigens (processed through phagocytosis) to the MHC class II molecule which then helps in destruction of antigen by the T cells. But how the function of macrophage is altered is still to be known. What changes take place in macrophages when MIF protein is present in high levels needs to be known. The macrophage cells can be cultured in the presence of high levels of MIF and changes in the macrophage cells can be monitored. These MIF exposed macrophage cells can be injected into mouse models to see how the macrophages function and how they lead to onset of cancer and progression.

In conclusion, MIF due to its interesting properties, is now viewed as a new and potential target against cancer. All these findings along with the available knowledge on MIF in cancers would provide new insights and this would lead to making better anti- cancer strategies and synthesizing new drugs for cancer treatment which would complement the present therapies for cancer.

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