ONLINE FEATURE

A Review of Macrophage Migration Inhibitory Factor in Tumourigenesis

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With its unique tautomerase activity, macrophage migration inhibitory factor (MIF) is a multi-functional pro-inflammatory cytokine implicated in the pathogenesis of numerous types of cancer. Through a variety of mechanisms MIF is tumourigenic, pro-inflammatory and pro-angiogenic. As a result of its central role in both cancer and inflammation many MIF inhibitors are currently in development, with recent studies yielding impressive results.

MIF – A Pleiotropic Molecule

Originally discovered as a cytokine which inhibits the random migration of macrophages (Bloom & Bennett, 1966), macrophage migration inhibitory factor (MIF) has been shown to also have enzymatic, hormonal and chemokine functions.

Early studies highlighted the ability of MIF to inhibit the anti-inflammatory effects of glucocorticoids, which has subsequently been implicated in the pathogenesis of numerous diseases such as acute respiratory distress syndrome (Donnelly et al., 1997), rheumatoid arthritis (Morand et al., 2006) and cancer (Conroy et al., 2010).

Crystalline structural analysis of MIF revealed it is a homotrimer containing an N-terminal catalytic site between each two sub-units, with the proline residue at position one in this hydrophobic pocket key to its activity (Swope et al., 1998). Although it has been shown that this site catalyses the isomerisation of substrates such as D-dopachrome methyl ester and phenyl pyruvate in vitro, no physiological substrate has yet been identified. Consequently, researchers have been unable to identify a reason for the evolutionary conservation of an apparently functionless catalytic site, as MIF shows >80% sequence homology across species (Esumi, et al., 1998).

Recent evidence suggests that the structural features of the catalytic site, rather than the catalytic activity itself, is essential for protein-protein interactions through which MIF becomes functionally active (Fingerle-Rowson, et al., 2009).

Although it has been unnoticed since its discovery as part of experiments to characterise delayed-hypersensitivity reactions in the 1960s (Bloom & Bennett, 1966) (David, 1966), recent evidence has generated interest in this field. MIF has been shown to be expressed by a wide variety of cells – including eosinophils (Rossi, et al., 1998), epithelial cells (Rice, et al., 2003), endothelial cells (Shimizu, et al., 2004), lymphocytes (Bacher, et al., 1996), macrophages (Calandra, et al., 1994) and platelets (Strüßmann, et al., 2013).

The Genetics of MIF

Located on chromosome 22, numerous polymorphisms in the gene are correlated with gene expression and also disease severity. A tetra-nucleotide repeat polymorphism (CATT) in the promoter of the MIF gene has been identified in human studies, with carriers of 6, 7 or 8 of these CATT repeats exhibiting higher expression of MIF than those with only 5 of these repeats. In this same study, it was found that the number of individuals carrying at least one 5-CATT allele was 50.31% of the population and that the number of repeats correlated with the severity of the clinical phenotype in rheumatoid arthritis patients (Baugh, et al., 2002).

In a study involving 131 patients with prostate cancer and 128 controls, a significant association was found between prostate cancer incidence and 7-CATT polymorphisms and it was also shown that these patients had an increased risk of recurrence at five years (Meyer-Siegler, et al., 2007). A similar association between gastric carcinogenesis and the 7-CATT polymorphism has also been identified (Arisawa, et al., 2008). That said, in all studies, a correlation - but not causation - was proven.

A second polymorphism at position -173 (G to C transition) in the MIF gene promoter was recently identified to occur in higher frequency in juvenile arthritis patients (Donn, et al., 2001). Tong et al., 2015 published a meta-analysis involving over 7000 participants from 15 studies which found the -173G/C polymorphism in the MIF gene promoter to be a risk factor for both gastrointestinal and haematological malignancies. A similar meta-analysis published in 2015 reports a moderate, statistically significant association between this polymorphism and cancer risk, with a strong association found for prostate cancer (Zhang, et al., 2015).

MIF – A p53 Inhibitor

p53 is a vital tumour suppressor genes that has been widely investigated. Owing to its essential role in maintaining the integrity of the cell it has been dubbed the Guardian of the Genome (Lane, 1992). Upon activation in response to DNA damage, hypoxia and oncogenic stress by various protein kinases such as Checkpoint Kinase 2 (Chk2) in the case of DNA damage, p53 initiates cell cycle arrest through interacting with p21, DNA repair via GADD45, and apoptosis through the up-regulation of p53-Upregulated Modulator of Apoptosis (PUMA) and Bcl-2 like protein-4 (BAX).

By analysing genes which circumvented p53-mediated cell arrest, MIF was identified as an inhibitor of p53 activity in three separate biological assays (Hudson, et al., 1999). Since the publication of this information, there has been a wide research effort to uncover the exact cellular mechanisms by which MIF inhibits p53. Using MIF-Knock Out (KO) mice, it was shown that MIF inhibition of p53 coincides with Cyclooxygenase-2 (COX-2) expression in these mouse macrophage cell lines, implicating COX-2 as the intracellular mechanism by which p53-mediated cell cycle arrest and apoptosis is prevented (Mitchell, et al., 2002). However, a recent study hypothesises

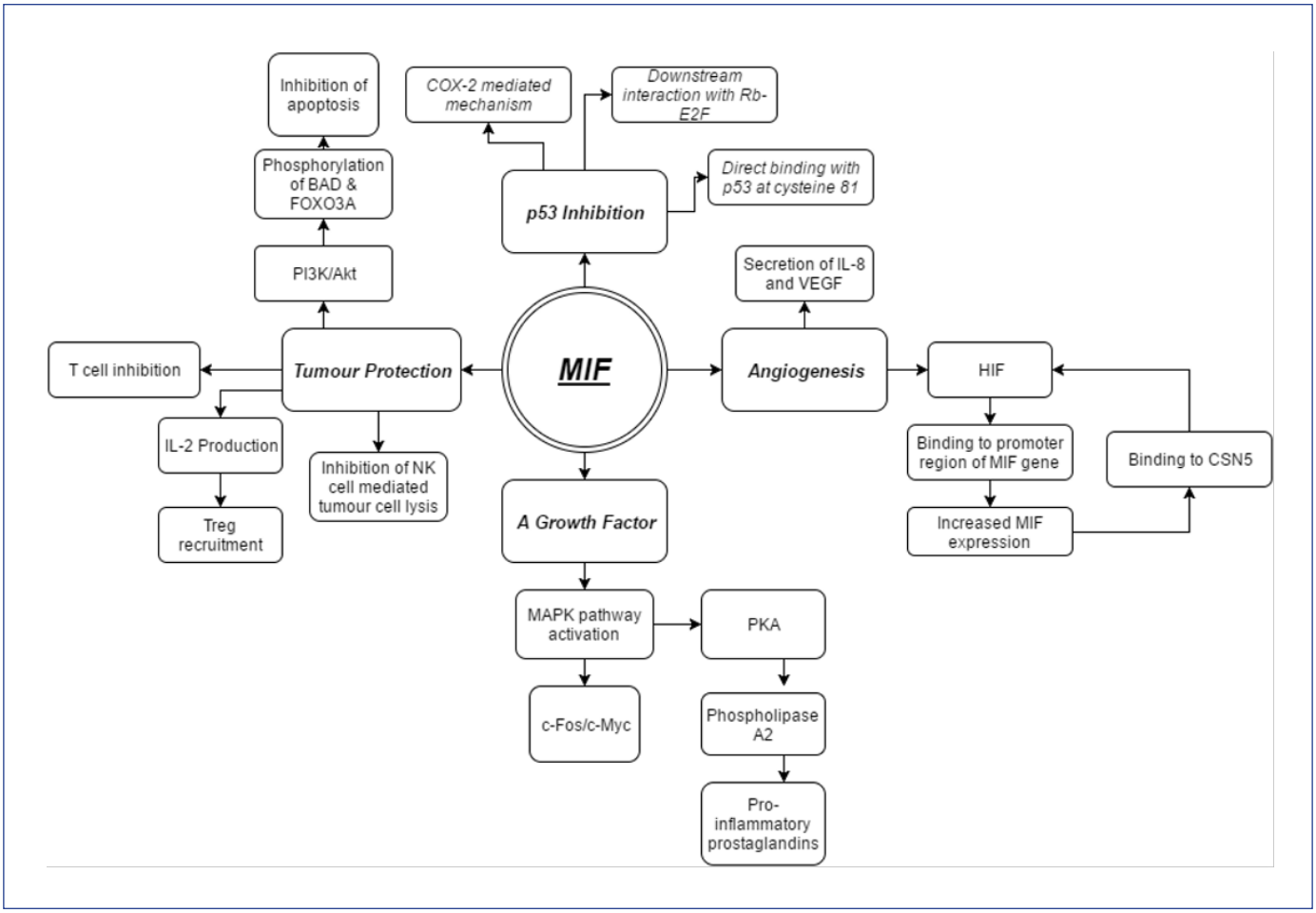

Figure 1. The pro-carcinogenic and pro-inflammatory effects mediated by MIF. *Boxes in italics represent proposed mechanisms of inhibition of p53 by MIF.*

that MIF directly interacts with p53 via the cysteine residue at position 81 on MIF, both in vitro and in vivo. (Jung et al., 2008) Furthermore, Jung et al., showed that MIF exerts this effect by stabilising the interaction between p53 and E3 Ubiquitin Protein Ligase (MDM2) - a binding molecule which promotes the degradation of p53 via ubiquitination and subsequent proteasome degradation.

A growing body of evidence also implicates MIF in pathways downstream of p53. Knock-out of the MIF gene alters the Rb-E2F signalling complex. This prevents oncogenic transformation as the C-terminal domain of E2F4 is not able to effectively recruit histone acetyltransferase associating chromatin remodelling complexes which is necessary to promote gene transcription for cell cycle progression (Petrenko & Moll, 2005). The authors further propose that an increase in MIF – observed during chronic inflammation – may contribute to an enhanced risk of tumourigenesis by counteracting Retinoblastoma's (RB) inhibition of E2F. In a previous study, the same authors implicate this same pathway as the mechanism by which MIF-KO cells are resistant to RAS-mediated oncogenic transformation (Petrenko, et al., 2003).

MIF – A Tumour Protector

MIF exerts significant anti-apoptotic activity in both healthy and tumour cells. Following binding to CD74, with CD44 acting as a co-receptor, the activation of upstream kinases including Src and PI3K results in initiation of the Akt signalling pathway. Akt activation results in the phosphorylation and inactivation of the pro-apoptotic proteins BAD and FOXO3A, contributing to the anti-apoptotic effects of this cytokine. These functional effects of MIF were elucidated using primary fibroblasts, HeLA cervix carcinoma, and breast cancer cell lines. The importance of the PI3K-Akt pathway in the development of melanoma has been well documented (Stahl, et al., 2004). Lue et al., 2007 show that over-expression of the PI3K inhibitor PTEN attenuated the anti-apoptotic effects of MIF, providing further evidence implicating the PI3K-Akt pathway in executing this effect.

In a more recent study, siRNA knock-down of the MIF gene in melanoma cell lines caused a two-to-three fold increase in the basal level of apoptosis of control cultures treated with siRNA against MIF after three days. A 40-70% reduction in Akt phosphorylation was also seen in certain melanoma cell lines treated with siRNA against MIF after three days of transfection (Oliveira, et al., 2014). These findings highlight the significant role, at least in part, of MIF in melanoma pathogenesis.

Preventing apoptosis is not the only mechanism by which MIF inhibits tumour regression and death. MIF also aids in the suppression of the immune response to tumour cells, leading to immune tolerance and uninhibited growth of the tumour (Repp, et al., 2000) (Yan, et al., 2006) (Choi, et al., 2012). Although MIF is generally considered to be pro-inflammatory, early studies have also pointed to its anti-inflammatory and

immunosuppressive effects (Repp, et al., 2000). An unknown factor - with a sequence homology of greater than 90% with MIF - was implicated as a key inhibitor of Natural Killer (NK) cell mediated lysis of corneal endothelial cells, suggesting a role for MIF in suppressing the immune function of NK cells (Apte, et al., 1998). These findings were later confirmed in cancer cell lines by the same group (Repp, et al., 2000).

Murine models of neuroblastoma – the most common cancer in children less than one year old (Heck, et al., 2008) – show that MIF can inhibit T-cell activation and can also inhibit T cells which have already received an activation signal (Yan et al., 2006). The authors propose that via an Interferon (IFN-γ) mediated mechanism, the supra-physiological levels of MIF derived from neuroblastoma tumours results in an activation-induced T-cell death, thus suppressing the anti-tumour immune response (Yan et al., 2006). Further analysis by the same group revealed that murine models of neuroblastoma with siRNA induced knock-down of the MIF gene showed higher immune rejection against the tumour with a greater infiltration of CD8+ and CD4+ T cells, macrophages, dendritic cells, and B cells (Qiang, et al., 2008). Similar immunosuppressive effects were found in glioma cells, with PCR analysis showing that MIF mRNA expression was increased up to 800-fold in human glioma cell lines compared to controls (Mittelbronn, et al., 2011).

In addition to inhibiting the action of both NK cells and activated T cells, MIF has also been shown to recruit regulatory T cells (Tregs) to the tumour micro-environment (Choi et al., 2012). In a study investigating a murine model of colon carcinoma, flow cytometry analysis determined that lower levels of CD4+ Regulatory T cells (Tregs) were present in the tumour micro-environment of MIF-/- mice than MIF+/+ mice, suggesting a potential role for MIF in recruiting Tregs. Further analysis revealed that MIF exerts this effect through mediation of IL-2 production (Choi, et al., 2012).

MIF also mediates myeloid-derived suppressor cells (MDSCs) in the tumour micro-environment (Simpson, et al., 2012). MDSCs are a haematopoietic stem cell derived lineage of cells known to have strong immunosuppressive effects (Gabrilovich & Nagaraj, 2009). For example, MDSCs have been shown to inhibit T cells by attenuating the main downstream signalling pathways of IL-2 receptor binding (Mazzoni, et al., 2002).

MIF – A Growth Factor

MIF binds to tumour cells via CD74 (Leng, et al., 2003) with CD44 being required as a co-receptor (Shi, et al., 2006). Subsequent initiation of the ERK1/2 pathway by the receptor complex, results in activation of pro-growth transcription factors such as c-Myc and c-Fos (Zhang & Liu, 2002). Through both autocrine and paracrine activation of this pathway, MIF can cause significant tumour growth. Furthermore, in an ERK-dependent signalling pathway, MIF stimulates activation of Protein Kinase A (PKA) and subsequent activation of phospholipase A2, causing release of arachidonic acid from the inner leaflet of the plasma membrane (Mitchell, et al., 1999). Via COX-2 activity, pro-inflammatory prostaglandins are formed from this arachidonic acid, further implicating MIF in mediating cancer-promoting inflammation.

Reduced expression of microRNA-451 (miRNA-451) in nasopharyngeal carcinoma cell lines is associated with a poorer prognosis than cells in which this miRNA is highly expressed. It was further shown that miRNA-451 targets MIF expression —concluding that miRNA-451 inhibits cell growth and tumour invasion by preventing MIF translation (Liu, et al., 2013). Similar results, with respect to miRNA-451, have been found in renal cell carcinoma cell lines (Tang, et al., 2015) and in lesions of endometrial epithelial cells (Graham, et al., 2015). The inverse correlation between miRNA-451 expression and cell survival and growth in numerous cancer types is indicative of the importance of MIF in promoting cancer cell viability.

MIF – A Pro-angiogenic Factor

Early studies involving the use of anti-MIF monoclonal antibodies (mABs) in tumour cells first implicated MIF in tumour angiogenesis (Chesney, et al., 1999). More recent studies suggest that MIF has a pro-angiogenic potency similar to that of basic fibroblast growth factor (bFGF) (Amin, et al., 2003). The exact molecular mechanisms involve activation of MAPK and PI3K intracellular signalling pathways in endothelial cells (Amin, et al., 2003). In numerous cancer types, MIF stimulates secretion of the pro-angiogenic factors; vascular endothelial growth factor (VEGF) and interleukin-8 (IL-8), contributing to its ability to promote angiogenesis. For example, MIF induces VEGF and IL-8 secretion via an autocrine mechanism in human oesophageal carcinoma cells obtained from patients (Ren, et al., 2005). Similar results have been found in human breast cancer cells, with respect to VEGF and IL-8 expression (Xu, et al., 2008).

Hypoxia is a common feature of most tumour micro-environments and is essential for tumour growth (Harris, 2002). High intra-tumoural hypoxia is considered an adverse prognostic factor (Vaupel, 2008). A candidate gene study for hypoxia-induced genes implicated upregulation of the MIF gene and subsequent Northern Blot analysis in squamous cell carcinoma cell lines confirmed this finding (Koong, et al., 2000). Further studies using HeLa cells determined that expression of MIF is controlled via co-operative activity of both cAMP response element binding protein (CREB) and the hypoxia inducible factor 1-α (HIF1-α), via a hypoxia response element (HRE) in the 5'untranslated region (5'UTR) of the MIF gene (Baugh et al., 2006). Furthermore, it was found that over-expression of CREB blocks the increase in MIF promoter activity seen in hypoxic cellular conditions (Baugh, et al., 2006). The authors suggest that over-expression of CREB may cause HIF-1α to compete with CREB for transcriptional activators. Increased MIF expression under hypoxic conditions has also been shown to occur in human vascular smooth muscle cells (VSMCs) (Fu, et al., 2010). Knock-down of the MIF gene via siRNA resulted in reduced proliferation and migration of the VSMCs due to hypoxia (Fu, et al., 2010), further highlighting the importance of MIF in angiogenesis in a hypoxic environment.

The activity of HIF-1α is also dependent on basal levels of MIF in the tumour cell, suggesting a feedback mechanism may be at play (Winner, et al., 2007) (Oda, et al., 2008). MIF interacts with COP9 signalosome sub-unit 5 (CSN5), which also interacts and aids in the stabilisation of HIF-1α. Consequently, one study found that MIF-deficiency in pancreatic adenocarcinoma cells resulted in reduced CSN5-HIF-1α interacts and a resulting defects in HIF-1α in the tumour cells (Winner, et al., 2007). Using lysophosphatidic acid (LPA) it has been suggested that MIF, HIF-1α, and CSN5 form a ternary complex which stabilises HIF-1α (No, et al., 2015). However, other evidence suggests that CSN5 is not critical to the MIF-induced HIF-1α activation seen in hypoxic breast cancer cells. This promotion of HIF-1α is p53-dependent and also requires the binding of MIF to its cell surface receptor CD74 (Oda, et al., 2008).

There seems to be a conflict in the literature as to whether MIF is endocytosed by tumour cells and subsequently interacts with CSN5 to stabilise HIF-1α ((No, et al., 2015) or whether MIF

binds to CD74 and promotes HIF-1α activity via a p53-mediated mechanism (Oda, et al., 2008).

MIF – A Clinical Biomarker

A growing body of evidence suggests the potential of MIF as a biomarker for tumours, especially prostate cancer (Meyer-Siegler, et al., 2002)(Muramaki, et al., 2006). Using Polymerase Chain Reaction (PCR) and laser capture microscopy analysis of invasive prostate cancer cells, MIF mRNA levels were 6.5 times greater than normal prostate epithelial cells and ELISA analysis of serum samples found a positive association between serum concentration of MIF and prostate cancer diagnosis (Meyer-Siegler, et al., 2002). The same group reported similar findings in a study involving 115 prostate cancer patients and 158 controls (Meyer-Siegler, et al., 2005). MIF has also been positively correlated with prostate specific antigen (PSA), Gleason score and percentage positive biopsy score (PPBC) of prostate cancer (Muramaki, et al., 2006).

Similarly, MIF concentrations in serum but not in saliva are reduced in patients with oral squamous cell carcinoma following surgical tumour resection (De Souza, et al., 2014). There is elevated secretion of MIF from ovarian cancer cells compared to healthy aged-matched controls, signifying a potential role for MIF as both a prognostic indicator and therapeutic target in the future (Agarwal, et al., 2007). Analysis of the expression of six separate genes, including MIF, can distinguish malignant from non-malignant bladder epithelial cells obtained via surgical biopsy with 100% accuracy (Dong, et al., 2009). Soft tissue sarcoma patients, with tumour cells positive for MIF and stearoyl-CoA desaturase 1 (SCD1) gene expression, show significantly lower survival rates than in MIF and SCD1 negative patients (Takahashi, et al., 2013).

The MIF receptor, CD74, which forms a receptor complex with CD44 upon MIF binding to activate intracellular signalling pathways such as the ERK1/2 pathway, has also been identified as a potential biomarker. Using clinical data from 135 patients with malignant pleural mesothelioma, it was shown that both CD74 and MIF were expressed in over 95% of the samples. However, CD74 but not MIF was identified as an independent prognostic marker for this condition (Otterstrom, et al., 2014). A similar positive relationship between CD74 expression and tumour staging was found in B cell chronic lymphocytic leukaemia (Butrym, et al., 2013) and gastric carcinoma (He, et al., 2015).

MIF – A therapeutic target

The importance of MIF in cancer, as well as a host of inflammatory diseases, has made the multi-functional cytokine an attractive target as a potential therapeutic agent.

As previously mentioned, the N-terminal catalytic site, although enzymatically redundant, is vital to MIF-protein interactions. Replacement of the proline at position one at the N-terminus with glycine causes reduced tumour growth in mice with this knock-down MIF gene (Fingerle-Rowson, et al., 2009). Addition of an alanine residue between the proline at position one and the methionine at position two causes almost complete attenuation of the biological activity of MIF (Lubetsky, et al., 1999). This strong evidence, implicating the importance of the tautomerase catalytic site, has made the N-terminal hydrophobic pocket a target for MIF inhibitors —both competitive (Mawhinney, et al., 2015) and irreversible (Winner, et al., 2008)

Iso-1 is the most common experimentally used inhibitor of MIF. This isoxazoline drug has been shown to reduce tumour proliferation and invasion in numerous human cancer types including colorectal cancer (He, et al., 2009) androgen-independent prostate cancer (Meyer-Siegler, et al., 2006) and gallbladder cancer (Subbannayya, et al., 2015). Using Iso-1 as a building block, Iso-66 was designed and tested in murine melanoma and colon carcinoma models, causing a 45% and 60% reduction in tumour growth respectively, compared to controls when the drug was injected once daily for 20 consecutive days. Interestingly, Iso-66 did not attenuate cancer cell proliferation, but rather enhanced the cytotoxic activity of natural killer cells, lymphokine-activated killer cells and CD8+ T cells. (Ioannou, et al., 2014)

Other novel competitive inhibitors have yielded stronger results in other cancer cell lines. An isocoumarin drug, SCD-19, reduced tumour volume in murine models of small cell lung cancer by 81% compared to controls when the inhibitor was given intraperitoneally once the tumour became palpable seven days post-transfection (Mawhinney, et al., 2015).

The development of virtual screening programmes has rapidly enhanced the identification of tautomerase-activity enhancers, with a recent study identifying 10 novel drugs with an IC50 below 10μM and one drug with an IC50 less than 1μM, which is 26 times more potent than the gold standard Iso-1 (Xu, et al., 2014).

Irreversible inhibitors—which covalently bind to the tautomerase catalytic site—have also proven effective as anti-tumour agents. 4-IPP covalently binds to the hydrophobic enzymatic pocket of MIF, causing irreversible inhibition of the cytokine, and is up to 10 times more potent than the competitive inhibitor Iso-1 in human lung adenocarcinoma cell lines (Winner, et al., 2008).

P425, an allosteric inhibitor of MIF tautomerase activity, is also currently in development. It binds at the interface between two MIF trimers via hydrophobic interactions. P425 has proven to be more potent than Iso-1, which the authors attribute to its unique binding site on MIF. They suggest that inhibiting the tautomerase catalytic site alone may not be enough to completely attenuate the pro-inflammatory activity of MIF (Bai, et al., 2012) because several studies have found that a second catalytic site on MIF – thiol-protein oxidoreductase – may mediate some of the pro-inflammatory effects (Nguyen, et al., 2003) (Thiele & Bernhagen, 2005).

The use of antibodies (Ab) as a potential cancer therapeutic has come to the fore recently as a result of a phase I clinical trial involving an anti-MIF Ab (ClinicalTrials.gov identifier: NCT01765790). Completed in November 2015, the results of the trial are expected to be published within the coming months. Earlier studies involving murine models of colon adenocarcinoma found that injection of an anti-MIF antibody at specific points throughout the experiment attenuated tumour growth and angiogenesis by day 22 (Ogawa, et al., 2000).

Conclusion

MIF is a pleiotropic cytokine, hormone, enzyme and chemokine. Research has highlighted the significant role MIF plays in promoting both inflammation and tumourigenesis—extending from directly enhancing tumour cell proliferation to promoting tumour angiogenesis and inhibiting the anti-tumour immune response. Further knowledge into both the exact molecular structure of MIF and the pathways through which MIF exerts these effects is required before effective MIF inhibitors, with the potential to progress from the bench to the bedside, can be developed.

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