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REVIEW

The macrophage migration inhibitory factor protein superfamily in obesity and wound repair

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The rising number of obese individuals has become a major burden to the healthcare systems worldwide. Obesity includes not only the increase of adipose tissue mass but importantly also the altered cellular functions that collectively lead to a chronic state of adipose tissue inflammation, insulin resistance and impaired wound healing. Adipose tissue undergoing chronic inflammation shows altered cytokine expression and an accumulation of adipose tissue macrophages (ATM). The macrophage migration inhibitory factor (MIF) superfamily consists of MIF and the recently identified homolog D-dopachrome tautomerase (D-DT or MIF-2). MIF and D-DT, which both bind to the CD74/CD44 receptor complex, are differentially expressed in adipose tissue and have distinct roles in adipogenesis. MIF positively correlates with obesity as well as insulin resistance and contributes to adipose tissue inflammation by modulating ATM functions. D-DT, however, is negatively correlated with obesity and reverses glucose intolerance. In this review, their respective roles in adipose tissue homeostasis, adipose tissue inflammation, insulin resistance and impaired wound healing will be reviewed.
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INTRODUCTION

New insights into the physiology of adipose tissue have replaced the previous recognition of fat tissue functioning as inert energy storage with only a minor impact on homeostasis. Intense research efforts have revealed that adipose tissue is perhaps the largest endocrine organ of the body that influences the initiation and maintenance of different diseases.[1] Furthermore, adipose tissue is a diverse and dynamic tissue consisting of mature adipocytes as well as the stromal vascular fraction (SVF). The SVF comprises a variety of cells including endothelial progenitor cells, pericytes, lymphocytes, myocytes and preadipocytes. It is a rich source of preadipocytes, a term often used as a synonym for adipose progenitor cells or adipose-derived stem cells and adipose tissue macrophages (ATM) that exert important cellular functions.

Interest in adipose tissue as a central organ was fueled by the steadily increasing number of obese individuals and the adverse medical complications of obesity globally. Obesity has become one of the leading healthcare issues and is caused by an increase in adipose tissue mass. The incidence of obesity is on the rise worldwide. The World Health Organisation defines individuals with a body mass index (BMI) $\geqslant 30\,kg\,m^{-2}$ as obese and individuals with a BMI $\geqslant 25\,kg\,m^{-2}$ as overweight. The age-adjusted mean BMI in the United States is $28.7\,kg\,m^{-2}$ with 35% of the adult population fulfilling the World Health Organisation criteria for obesity.[2] The worldwide prevalence of overweight adults in 2013 was calculated to be 36.9% in men and 38% in women, respectively.[3] Astonishingly, the number of overweight and obese children and adolescents also is increasing in developing countries, which has emphasized the point that obesity is of global health importance.

The expansion of adipose tissue relies on both hypertrophy: the gain of adipose tissue volume by an increase in individual adipocyte size, as well as hyperplasia: the multiplication of cell number.[4] Adipogenic progenitor cells, which possess great plasticity, can differentiate into adipocytes and can contribute to adipocyte hyperplasia. It is not only the increase in adipose tissue mass but the state of the adipose tissue collectively that has a crucial impact on body homeostasis and pathologies. The adipose tissue of obese subjects contributes to a chronic inflammatory status that is characterized by increased ATM content and an altered secretome.[5] The human body comprises three distinct types of adipose tissue: brown, beige and white adipose tissue. Formerly considered to be primarily expressed

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in neonates, brown adipose tissue (high content of mitochondria causes a brown appearance) also was recently discovered at distinct locations of adult humans.[6] It has an important role in thermogenesis and its activity is reduced in obese individuals. Under special conditions brown adipocytes further can develop in white adipose tissue depots to forming so the called 'beige' adipocytes. Apart from brown and beige adipose tissue, white adipose tissue stores triacylglycerides and is the most common adipose tissue form in the adult human body and.[7] Obesity and insulin resistance lead to increased lipolysis and subsequent induction of inflammation through the release of triacylglycerides. ATMs are the predominant leukocyte fraction in adipose tissue, exert distinct functions and are a major source of soluble factors. Mature adipocytes, preadipocytes and ATM mutually interact through the secretion of soluble factors and orchestrate adipose tissue inflammation in an autocrine or parakrine manner.[8] The chronic state of adipose tissue inflammation further encompasses a pathologic glucose metabolism and contributes to wound healing disorders. Inflammation, obesity, the development of insulin resistance and wound healing are all intertwined into a highly complex network. It is evident that adipose tissue contributes to a systemic inflammatory state and thereby influences the insulin resistance, obesity and wound healing mechanisms. The identification of the relevant molecular mediators and pathways in this intricate system remains a challenge for investigation.

Macrophage migration inhibitory factor (MIF) was first described in the context of delayed-type hypersensitivity and has emerged as a key mediator of inflammatory pathologies.[9] Elevated MIF levels have been detected in sepsis, autoimmune diseases, different cancers and also metabolic disorders such as diabetes mellitus and obesity.[10] Recently, D-dopachrome tautomerase (D-DT or MIF-2) has been identified as a second MIF superfamily member and has been implicated in a number of pathologies.[11] Both the proteins bind to the transmembrane receptor CD74 and influence cell migration.[12] This review will highlight the role of the MIF protein superfamily in adipose tissue inflammation and its link to obesity, glucose metabolism and wound healing.

MIF IN ADIPOSE TISSUE

At the time of its discovery, MIF was considered to be a cytokine exclusively produced by immune cells. However, continued research identified MIF to be an almost ubiquitously expressed protein that acts in both an autocrine and a paracrine manner to regulate proinflammatory cytokines such as tumor necrosis factor α (TNF-α) and interferon γ.[13] A unique property of MIF is its induction by low levels of glucocorticoids with the ability to counter-regulate their immunosuppressive effects.[14]

Hirokawa *et al.*[15] initially reported MIF expression in rodent adipose tissue and in 3T3L-1 cells, a commonly studied murine preadipocyte cell line. The authors also demonstrated elevated MIF release from 3T3L-1 cells in response to TNF-α stimulation. The role of MIF in human adipose tissue was first unraveled by Skurk *et al.*[16] who analyzed cultured preadipocytes and freshly harvested adipocytes by enzyme-linked immunosorbent assay, quantitative PCR and immunohistology. Adipocytes as well as preadipocytes released significant amounts of MIF and by immunohistological staining, MIF protein could be localized in the cytoplasm of both the cell types.

The expression and secretion of MIF in human adipose tissue also varies with the location of the adipose tissue depot. Omental and subcutaneous adipocytes secrete higher concentrations of MIF than mammary adipocytes.[16] Together with the chemokine CCR2, MIF mRNA levels can serve as an indicator for the discrimination of visceral and subcutaneous adipose tissue, as reported by Alvehus *et al.*[17] Visceral adipose tissue depots consistently expressed twofold higher MIF-mRNA levels when compared with the deep and superficial fat depots. MIF also is expressed in the epicardial adipose tissue and released into its conditioned medium in considerable amounts.[18] Both MIF knockout ($Mif^{-/-}$) and wild-type mice show a similar distribution of adipose tissue in subcutaneous, intra- and retroperitoneal as well as epididymal depots.[19]

Adipogenesis is a tightly regulated process, which depends on specific adipogenic factors. Peroxisome proliferator-activated receptors (PPAR) are intracellular receptors that are mainly involved in adipogenesis. CAAT/enhancer binding proteins (C/EBP) are a transcription factor family that can induce PPARγ and are differentially expressed during adipogenesis.

The role of MIF during adipogenesis was investigated by Atsumi *et al.*[20] In primary murine embryonic fibroblasts, $Mif^{-/-}$ cells were significantly more sensitive to standard differentiation medium plus PPARγ agonist troglitazone. The expression of PPARγ isoforms was increased in $Mif^{-/-}$ cells during adipogenesis, and MIF deficiency also induced an upregulation of C/EBPα, C/EBPβ and C/EBPδ.

On the contrary, MIF knockdown by RNA interference led to a decrease in differentiation as well as disruption of clonal cell expansion and proliferation of 3T3L-1 cells.[21] Mechanistically, the MIF deficiency influenced the levels of relevant regulators of terminal adipocyte differentiation. The authors found blunted increases in PPARγ, C/EBPδ and C/EBPα mRNA levels, whereas C/EBPβ mRNA levels were elevated by MIF knockdown.

The partial discrepancies observed in these two studies appear to be a result of the different study designs. Cell type, method of MIF knockdown, differentiation medium and so on, differ in both the studies. Differential roles of C/EBP members during adipogenesis in murine embryonic fibroblasts and 3T3L-1 cells also were reported, suggesting that a careful interpretation of these results is necessary.

Lee *et al.*[22] showed that ultraviolet A (UVA) radiation, an environmental stimulus not only for skin aging but also immunomodulation and the upregulation of enzymes such as heme oxygenase-1, inhibits adipogenesis of preadipocytes. PPARγ and C/EBPα expression but not C/EBPβ and δ were ameliorated by ultraviolet A. Importantly, ultraviolet A-induced MIF expression by JNK/AMPK mediated AP-1 and NF-κB

activation as well as AMPK phosphorylation and upregulation of Kruppel-like factor-2.

D-DT IN ADIPOSE TISSUE

Originally, D-DT was described as an enzyme that converts D-dopachrome into 5,6-dihydroxyindole.[23] The naturally occurring stereoisomer of dopachrome is of the L configuration, as it is derived from L-tyrosine; thus the physiologic significance of this enzymatic activity is unknown. MIF also is a D-DT, but in contrast to D-DT, which catalyzes a decarboxylation in addition to a tautomerization, MIF's catalytic activity is limited to tautomerization. D-DT acquired potential clinical significance from the recent discovery that D-DT is a functional homolog of MIF and binds to their common receptor, the CD74/CD44 complex to activate similar proinflammatory cascades including the counterregulation of glucocorticoids.[24] D-DT, also now called MIF-2, shows structural and functional overlap with MIF and both genes are located in close proximity to each other on chromosome 22q11.23. Like MIF, D-DT is stored in vesicles and rapidly released upon cell activation or cytolysis.[11] The first report portraying the relation of D-DT to inflammation was provided by Sonesson *et al.* in the context of UVB-induced inflammation of skin.[25] Fluids taken from cutaneous blisters after UVB-radiation contained appreciable levels of MIF and D-DT. Serum D-DT levels also are upregulated during systemic inflammation as occurs during sepsis or metastatic cancer.[24,26]

D-DT only recently has been described as an adipokine secreted from human adipocytes.[27] Interestingly, D-DT expression was found solely in adipocytes from subcutaneous and visceral tissue but not in SVF cells. Further *in vitro* differentiation studies with SGBS cells, a human preadipocyte cell line, showed that D-DT mRNA is expressed during the course of adipogenic differentiation and importantly, only in differentiated adipocytes but not preadipocytes. A D-DT gene knockdown led to elevated expression of lipolysis and lipogenesis genes. The activation of these genes was partially dependent on AMPK-activation or protein kinase A inhibition. Recombinant D-DT inhibits adipogenesis by reducing the expression of PPARγ2, C/EBPα, C/EBPβ, C/EBPδ, aP2 and adiponectin.[28] Moreover, D-DT induces interleukin (IL)-6 secretion in preadipocytes through the CD74/ERK1/2 MAPK pathway; MIF by contrast did not have any effect in the same experimental setting.[28] D-DT-dependent IL-6 expression was dependent on CD74 but not CD44. On the other hand, the expression of matrix metalloproteinase-1 and matrix metalloproteinase-3 were enhanced by both MIF and D-DT.

The data so far indicate that MIF and D-DT are differentially expressed in adipose tissue. Although MIF is expressed in adipocytes and SVF cells, D-DT is expressed only in mature adipocytes. Furthermore, MIF and D-DT appear to have opposing functions in adipogenesis, which is likely important for the interpretation of their respective roles in obesity.

MIF FAMILY PROTEINS IN OBESITY

Although obesity is currently under intense investigation, the underlying molecular mechanisms are far from understood and there is a need for the identification of clinical biomarkers and novel therapeutic approaches.

MIF has emerged to be an important mediator in obesity and has been proposed as a candidate biomarker for obesity.[29] A line of clinical studies have focused on this issue and showed increased plasma levels of MIF in obese compared with lean individuals.[30] In addition, MIF mRNA expression in mononuclear cells was positively correlated with the BMI and plasma free fatty acid levels. Ghanim *et al.*[31] observed higher MIF mRNA levels isolated from blood mononuclear cells and higher MIF plasma levels in 16 obese patients compared with 16 lean subjects. Church *et al.*[32] measured MIF plasma levels in 71 severely obese patients before and after undergoing a diet in combination with physical activity. MIF plasma levels were positively correlated with obesity and decreased after successful weight loss. Serum MIF levels also were evaluated in a cross-sectional study of 88 subjects with metabolic syndrome and 84 healthy subjects by Kim *et al.*[33] Patients with metabolic syndrome showed higher MIF levels, although statistical significance was reached only for female subjects. Sakaue *et al.*[34] performed MIF promoter analysis in 213 subjects and found an association between obesity and polymorphisms in the promoter gene of MIF.

Data from additional *in vitro* studies have provided further insight into the physiology of MIF in adipose tissue during obesity. A positive correlation between BMI and MIF production from omental and subcutaneous adipocytes was reported by Skurk *et al.*[16] Subsequently, Fain *et al.*[35] collected subcutaneous adipose tissue from 10 morbidly obese patients undergoing abdominoplasty and omental adipose tissue from 12 morbidly obese patients undergoing laparoscopic gastric bypass surgery. After collagenase digestion, cytokine supernatants of tissue matrix SVF and mature adipocytes were analyzed. The authors found that MIF was the only factor amongst candidate mediators (interleukin 1 receptor antagonist, cathepsin S, nerve growth factor and IL-18) that showed increased release from these sources in morbidly obese patients. The majority of MIF was released by the tissue matrix (57%) followed by adipocytes (27%) and the SVF (16%).

The size of adipocytes positively correlates with ATM infiltration with larger adipocytes also releasing more chemokines.[8,36] Moreover, adipocyte size is related to insulin resistance and the genesis of type 2 diabetes.[37] By histological analysis, Verschuren *et al.*[38] showed that adipocytes in $Mif^{-/-}$ $Ldlr^{-/-}$ mice were smaller than in $Ldlr^{-/-}$ mice; the double-knockout adipocytes also showed reduced c-Jun immunoreactivity. These findings were supported by Koska *et al.*[39] who documented a positive correlation between MIF and adipocyte diameter and a negative correlation with peripheral and hepatic insulin action in isolated human adipocytes and preadipocytes. MIF expression in preadipocytes also correlated with obesity, whereas comparable correlations were not observed for monocyte chemoattractant protein (MCP)-1, MCP-2, macrophage

inflammatory protein (MIP)-1α, MIP1-β, MIP-2, TNFα, IL-6, or IL-8. Kos *et al.*[40] investigated adiponectin and its receptors 1 and 2 in adipose tissue from obese patients. Adiponectin, a hormone secreted by adipocytes, is a hallmark of anti-inflammatory conditions and negatively correlates with diabetes and obesity.[41] The authors showed an inverse correlation between adiponectin and MIF in adipose tissue and a positive correlation with adiponectin receptors 1 and 2 in visceral adipose tissue as well as with adiponectin receptor 2 in subcutaneous adipose tissue.

These data were partially confirmed *in vivo*. Serre-Beinier *et al.*[19] observed a time-dependent correlation of body weight and MIF in mice. The authors showed that in MIF knockout mice bodyweight was lower at birth and in the first few months of life. After 4 months, however, MIF deficiency was linked with higher food intake and consequently higher body weight gain and increased adipose tissue when compared with wild-type mice. In Wistar rats, MIF was positively associated with visceral adipose tissue mass.[42] Verschuren *et al.*[38] reported that *Mif* deletion in $Ldlr^{-/-}$ mice had effects on macrophage infiltration and inflammation but did not affect obesity itself. However, it must be noted that Verschuren *et al.* mainly used MIF and LDL double-knockout mice in their experiments.

In contrast to MIF, D-DT mRNA expression in human adipocytes harvested from visceral fat depots showed a significant negative correlation with BMI and subcutaneous as well as visceral fat areas.[27] A similar association was observed between D-DT expression and subcutaneous fat depots but the values did not reach statistical significance. In obese *db/db* mice, D-DT expression was significantly lower in adipose tissue but not in the liver when compared with lean mice.

MIF IN ADIPOSE INFLAMMATION

Obesity is not simply an increase in adipose tissue mass but is associated with a chronic inflammatory state. Research over the last decades has solidified the concept that adipose tissue is characterized by proinflammatory humoral, cellular and stromal changes. These changes include the secretion of proinflammatory factors as well as the infiltration of inflammatory cells, which together lay the foundation for deleterious metabolic states.

Verschuren *et al.*[38] found that *Mif* deletion disrupts adipose tissue inflammation without affecting obesity or other metabolic risk factors. The main differences in gene expression observed between $Mif^{-/-}$ $Ldlr^{-/-}$ mice were in the categories of cell signaling, cell cycle control, immune response and lipid metabolism. One reasonable interpretation of these data is that MIF amplifies already existing inflammation processes rather than initiating transitions from a metabolic into an inflammatory state.

In $Mif^{-/-}$ $Ldlr^{-/-}$ mice, circulating serum amyloid A (SAA) levels, a proinflammatory marker produced by hepatic and adipose tissue, was significantly decreased early and remained low even after IL-1β challenge, which normally increases serum amyloid A expression in wild-type mice.[38] Meanwhile, the injection of recombinant MIF into $Mif^{-/-}$ $Ldlr^{-/-}$ mice resulted in serum amyloid A levels comparable to wild-type mice as well as a marked elevation of the proinflammatory marker C-reactive protein. The modulatory effect of calcitriol on adipose tissue inflammation and adipocyte-macrophage cross-talk was described by Sun and Zemel.[43] Calcitriol increased CD14 and MIF expression in adipocytes, and with the calcium-channel antagonist nifedipine having a reverse effect.

Glucocorticoids exert powerful anti-inflammatory effects and their pharmacological utility is indispensable. As mentioned earlier, MIF and D-DT share a unique counter-regulatory action on glucocorticoids that also occurs in adipose tissue. A fructose diet in rats led to increased corticosterone and MIF levels but reduced NF-κβ signaling and unchanged TNFα expression in adipose tissue, suggesting that the effect of glucocorticoid prevails over those of MIF in adipose.[42] $Mif^{-/-}$ mice have lower circulating glucocorticoid levels and Nikolic *et al.*[44,45] reported markedly reduced levels of glucocorticoid receptors in the visceral adipose tissue of $Mif^{-/-}$ mice.

MIF FAMILY PROTEINS AND DIABETES MELLITUS

One of the most important complications of obesity is hyperglycemia and altered glucose metabolism. Many studies support the role of MIF in insulin resistance and have correlated type 2 diabetes with increased circulating MIF levels.[46] In a healthy environment, MIF regulates physiological insulin secretion, whereas prolonged systemic inflammation disturbs glucose homeostasis and can lead to apoptosis of pancreatic islets.[20,47–49] MIF also regulates glucose metabolism by stimulating insulin release from the pancreas.[50]

In a case–cohort study based on 502 subjects with type 2 diabetes and 1632 non-diabetic individuals, Herder *et al.*[51] found an association between increased MIF serum levels and diabetes type 2 risk in women but not in men. Furthermore, this association was stronger in obese individuals and could be related to a specific *MIF* genotype. Serre-Beinier *et al.*[19] observed higher basal levels of insulin in $Mif^{-/-}$ mice, which led to an impaired glucose tolerance, especially in older mice where insulin resistance was not detected. In rats fed a high-fructose diet, which induces insulin resistance and altered glucose homeostasis, a trend toward higher MIF plasma levels and significantly higher MIF protein in adipose tissue was observed.[42]

Sakaue *et al.*[52] were the first to describe an upregulation of MIF mRNA in the presence of insulin and glucose in cultured 3T3L-1 cells. Furthermore, MIF has a role in the production of adipocytokines that mediate insulin resistance.[53] In $Mif^{-/-}$ $Ldlr^{-/-}$ mice, there was a differential expression of genes implicated in insulin sensitivity, including JAK/STAT, MAPK cascades and IGF1-R1 signaling.[38] Furthermore, higher activity of IRS1-associated phosphatidylinositol-3-kinase in $Mif^{-/-}$ $Ldlr^{-/-}$ mice with increased PI3K and phospho-AKT signaling was observed.

There also is evidence for a central role of MIF in glucose metabolism during sepsis. In contrast to wild-type mice, which experience hypoglycemia during endotoxemia, normoglycemia and preserved lactate levels was observed in $Mif^{-/-}$ mice.[20] In addition, glucose uptake into white adipose tissue was

markedly increased in *Mif*$^{-/-}$ mice. Comparable effects were not observed in brown adipose tissue, skeletal muscle and hepatic tissue, thus emphasizing the importance of white adipose tissue during septic shock.

The latest evidence for MIF's central role in obesity and glucose metabolism has been provided by Finucane *et al.*[54] Wild-type and *Mif*$^{-/-}$ mice were fed a high-fat diet over 16 weeks. When compared with wild-type mice, *Mif*$^{-/-}$ mice exhibited reduced weight and decreased insulin resistance. In wild-type mice, MIF expression was primarily increased in the SVF but not adipocytes. Furthermore, the expression of CD74, CXCR2 and CXCR4 were upregulated in epididymal adipose tissue after high-fat diet.

The role of D-DT in glucose metabolism also was investigated by Iwata *et al.*[27] in *db/db* mice. Glucose and insulin tolerance tests showed that treatment with recombinant D-DT led to a reversion of glucose intolerance. While serum insulin levels did not change, levels of free fatty acid decreased significantly. Furthermore, the expression of fatty acid binding protein-4, which is related to insulin resistance, was lower in isolated adipose tissue and in D-DT knockdown adipocytes.

MIF FAMILY PROTEINS AND MACROPHAGE MOBILIZATION

A fundamental feature of obesity is the accumulation of inflammatory cells in adipose tissue, especially ATMs, which form the predominant leukocyte population in adipose tissue.[55] MIF and D-DT are critical regulators of macrophage activation, as recently demonstrated.[11] Macrophages are both targets and active producers of MIF.[56] MIF and D-DT are present in macrophages in preformed vesicles and can be rapidly released in response to inflammatory stimuli such as lipopolysaccharide (endotoxin). Notably, lipopolysaccharide-activated macrophages produce 20 times more MIF than D-DT, and without evidence of reciprocal regulation of one mediator by the other. Both the proteins phosphorylate ERK1/2 and activate MAP kinases in a CD74/CD44-dependent fashion.

ATMs orchestrate adipose tissue homeostasis by producing copious amounts of inflammatory cytokines.[57] Although significant numbers of ATMs also are present in lean and healthy subjects, an increase in ATM number and activation state has been proposed to indicate adipose tissue inflammation.[58] As mentioned, one major cause of adipose tissue inflammation is obesity, and it has been reported that ATM accumulation occurs during diet- as well as genetic forms of obesity.[8,58,59]

A major source of ATMs is the bone marrow and the factors mediating macrophage infiltration include MCP-1, CCL-2 and other relevant cytokines.[60] MIF has a pivotal role in inflammatory cell mobilization during inflammation; for example, atherosclerosis or cutaneous inflammation via its receptors CD74, CXCR2 and CXCR4.[12,61] In *Mif*$^{-/-}$*Ldlr*$^{-/-}$ mice, macrophage accumulation was mitigated and the expression of the intercellular adhesion molecule-1 and MIF co-receptor CD44 were reduced.[38] Recently, Heinrichs *et al.*[62] observed in a study of hepatic fibrosis reduced numbers of T-cells, NK/NKT cells and ATMs in epididymal adipose tissue after a high-fat diet in *Mif*$^{-/-}$ mice. Moreover, Finucane *et al.*[54] detected decreased ATM recruitment in *Mif*$^{-/-}$ mice after a high-fat diet when compared with wild-type mice. We have found that human native adipose tissue also secretes considerable amounts of MIF and that chemotaxis of blood monocytes by adipose tissue supernatants is partially abrogated by anti-MIF antibody (unpublished data).

In addition to the accumulation of ATMs in adipose tissue by the recruitment of bone marrow-derived myeloid cells, there also is evidence for an increased proliferation of these cells *in situ*.[60] Both Amano *et al.*[63] and Haase *et al.*[64] found higher proliferation rates of adipose tissue resident macrophages in obesity. Although MIF has a permissive effect on the proliferation of immune cells and prolongs survival by the inhibition of activation-induced apoptosis, the involvement of MIF in the proliferation and survival of ATMs remains to be studied.[65,66]

The inflammatory state of ATMs also was brought into focus. A widely accepted nomenclature is the distinction of 'classically activated' M1- and 'alternatively activated' M2-macrophages.[67] Proinflammatory cytokines such as TNFα, IL-6, and IL-1β are released by M1 macrophages, M2 macrophages on the other hand secrete anti-inflammatory factors including TGFβ, IL-10 or IL1-RA.[58] M2 macrophages can be further subdivided in M2a, M2b and M2c macrophages, with M2b and M2c having a regulatory phenotype and M2a macrophages having a critical role in wound healing.[68] Although this classification may be an oversimplification of the full spectrum of macrophage functional phenotypes, it appears useful for many experimental contexts.

As MIF is an upstream regulator of many proinflammatory cytokines that are characteristic for the M1 polarization status, MIF is considered a M1 macrophage-polarizing factor. Verschuren *et al.*[38] provided the first insight into MIF's involvement in ATM macrophage polarization. Whole-genome microarray analysis showed altered expression profiles of M1/2-characteristic genes in a murine obesity model. *Mif*$^{-/-}$ *Ldlr*$^{-/-}$ mice had lower M1/M2 ratios in adipose tissue when compared with wild-type mice, indicating that MIF directs ATMs into the M1 polarization status. Heinrichs *et al.*[62] observed that although a high-fat diet led to an accumulation of M2 macrophages in the liver of *Mif*$^{-/-}$ mice, ATM counts were reduced in white adipose tissue along with both M1 and M2 markers. The expression of the anti-inflammatory cytokines lL-4 and Il-10 were increased strongly, indicating that MIF deficiency leads to a reduced inflammatory environment in adipose tissue. Finally, Finucane *et al.*[54] saw that MIF deficiency led to decreased ATM recruitment with a lower M1 count after high-fat diet. The SVF of *Mif*$^{-/-}$ mice secreted significantly lower levels of TNFα and IL-1β, whereas IL-10 secretion was increased. In macrophages from tumor stroma, however, MIF appears to induce a shift toward the M2 phenotype suggesting a complex and perhaps tissue-specific role of MIF in macrophage polarization.[69]

In the case of D-DT, there is no evidence as yet for its role on ATM migration, proliferation or phenotype polarization. On the basis of the similarities between MIF and D-DT in

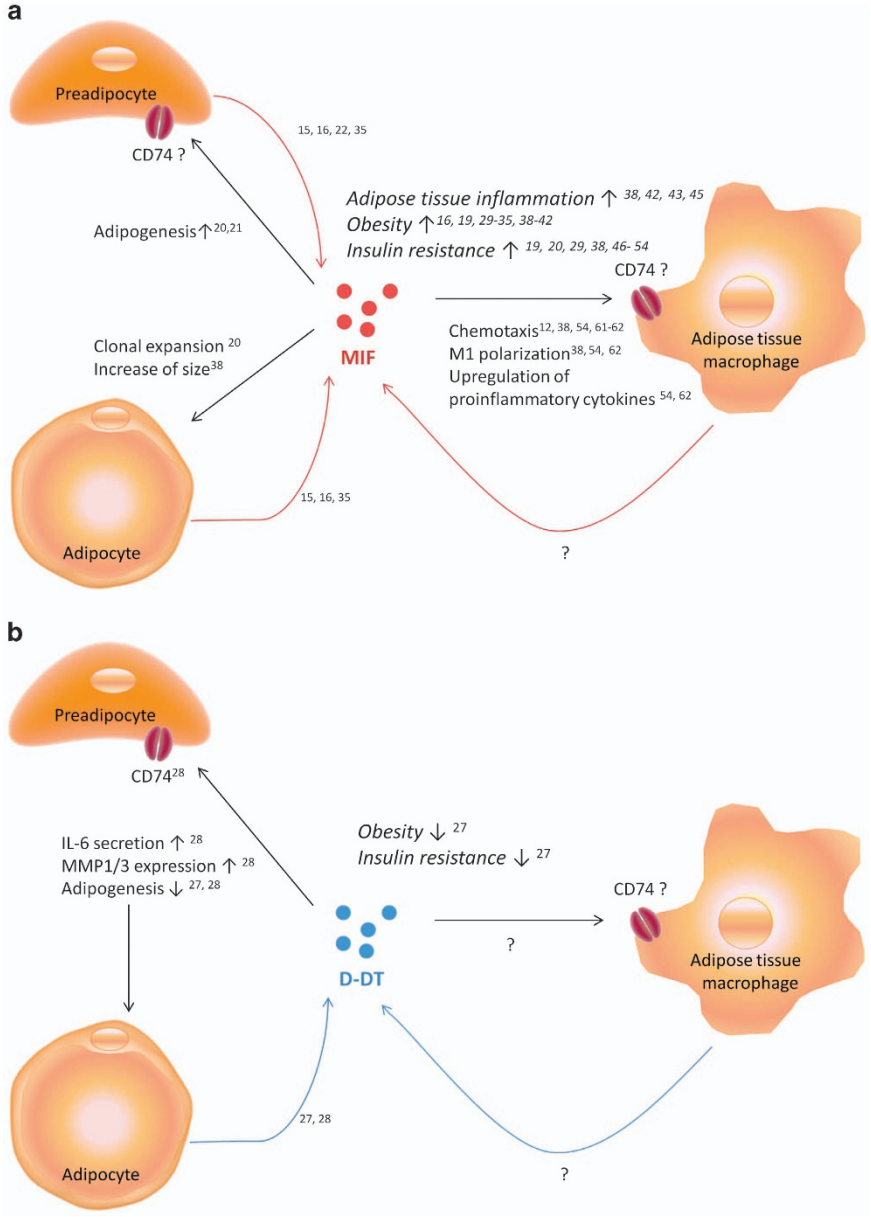

Figure 1 (**a**) MIF in adipose tissue. MIF is secreted from mature adipocytes, preadipocytes and ATMs. It promotes the adipogenesis of preadipocytes and increases the size and number of mature adipocytes. MIF further directs ATMs into an M1 polarization status and acts as a chemoattractant. MIF contributes to chronic adipose tissue inflammation, obesity and impaired glucose homeostasis. (**b**) D-DT in adipose tissue. D-DT is secreted from mature adipocytes and ATMs promotes adipogenesis of preadipocytes. D-DT is downregulated in adipose tissue from obese individuals and improves glucose intolerance. ATM, adipose tissue macrophages; D-DT, D-dopachrome tautomerase; MIF, migration inhibitory factor.

macrophage physiology, including the selective inhibition of monocyte chemoattractant protein (MCP)-1-dependent migration, a complementary effect of D-DT on ATMs is possible.[24,70] The role of MIF and D-DT in adipogenesis, obesity, inflammation, insulin resistance and their effects on adipocytes, preadipocytes and ATM are depicted in Figure 1.

In contrast, a few studies were unable to confirm a role for MIF in adipose tissue inflammation, obesity or insulin tolerance. In rats, Bertile *et al.*[71] observed that MIF expression levels in epididymal and subcutaneous adipose tissue were largely unaffected by fasting, refeeding and leptin treatment. Sakaue *et al.*[52] measured mRNA expression in adipose tissue and serum MIF levels in obese Otsuka Long-Evans Tokushima fatty and lean Wistar rats. Although mRNA expression was downregulated in adipose tissue from obese mice, there were no significant differences in circulating serum MIF levels in the two groups. Tam *et al.*[72] collected subcutaneous adipose tissue samples from 35 patients after weight loss by diet or diet plus physical exercise and compared MIF mRNA expression levels. There was no detectable effect of weight loss on MIF expression. A potential limitation of this study was that studied individuals had a BMI of only 25–30 $kg\,m^{-2}$, and adipose

tissue was collected from subcutaneous but not visceral adipose tissue depots.

Contrary to Finucane *et al.*,[54] Conine and Cross[73] did not observe MIF-dependent glucose intolerance or weight gain in obese wild-type or *Mif*$^{-/-}$ mice after a high-fat diet. These authors suggest that increased MIF levels during obesity might be a reaction to obesity rather than a cause, and increased MIF release from preadipocytes during differentiation was offered as one explanation. Conine *et al.* also did not observe any change in ATM accumulation or in adipose tissue in *Mif*$^{-/-}$ mice fed a high-fat diet. However, as a detailed analysis of ATM properties was not performed, alterations in macrophage functionality including the M1/M2-phenotype cannot be ruled out. The findings of Conine and Cross thus contrast with those of Finucane *et al.*[54] who observed alterations in glucose homeostasis and weight gain in wild-type versus *Mif*$^{-/-}$ mice. Both the groups used mice with C57Bl\6J genetic backgrounds that underwent high-fat diets by an established protocol.

OBESITY AND WOUND HEALING

Impaired wound healing is an important healthcare issue and frequently complicates the clinical course of diabetic or aged patients. Beyond financial costs it also contributes to the psychological stress of the affected patients.

Wound healing is an orchestrated process of tissue restoration following damage that can be divided into hemostasis, inflammation, proliferation and remodeling phases, and is regulated by a myriad of factors.[74] The inflammation phase can be further categorized into an early and late response and is characterized by the infiltration of inflammatory cells, especially macrophages, secreting numerous cytokines in response to invading pathogens or injured tissue.[75]

Wound repair disorders describe a state of delayed wound healing in which any of the four stages of wound healing may be affected. In most cases, such disorders are characterized by a prolonged inflammatory or proliferative phase that complicates the usual physiological progression of repair. Diverse factors influence wound healing but these generally can be separated into local and systemic causes. Increased tension to the wound or bacterial infection are examples of local factors. A list of systemic factors *inter alia* comprises obesity, diabetes and a state of adipose tissue inflammation, which can jointly cause impaired wound perfusion, granulation and ultimately healing.[76] Systemic factors can have a direct influence on local factors. In wounds of patients with obesity or diabetes, a decreased vascularization or immune response can increase the risk of bacterial infections.[76–78]

ADIPOSE TISSUE AND WOUND HEALING

Expanding knowledge about wound healing physiology and adipose tissue inflammation together with the high prevalence of obesity has prompted interest in the function of adipose tissue during wound repair.[1,79] A wide body of literature indicates that obese patients have impairments in wound healing that become manifest as ulcers, septic wounds or fascial dehiscence.[79–82]

Adipose tissue affects local and systemic factors during wound healing. The systemic factors include the altered function of adipose tissue during obesity, insulin resistance and chronic inflammation, as discussed above. On the other hand, subcutaneous adipose tissue layers in close proximity to wounds participate in wound restoration via deliverance of nutrients, secretion of various factors and infiltration of inflammatory cells. Adipose tissue also contributes to cutaneous wound healing by supporting fibroblast and keratinocyte migration, proliferation and differentiation.[83–86] In fact, the transfer of adipose tissue, most commonly harvested and reinjected by thin cannulas after variable processing steps, is a common plastic surgical tool for the treatment of wounds.[87] Thus, adipose tissue undergoing chronic inflammation with altered cellular composition and cytokine expression, impaired vascularity and oxygen tension—an important factor for the susceptibility of bacterial infection, necessarily have a detrimental impact on cutaneous wound healing.

MIF FAMILY PROTEINS IN OBESITY AND WOUND HEALING

The role of MIF in cutaneous wound healing has been recently reviewedin Gilliver *et al.*[88] MIF is produced within healing skin wounds but the exact role of MIF in wound repair remains unclear. There is evidence for a beneficial as well as detrimental effect of MIF on wound healing in hitherto performed studies. On basis on the current literature, MIF's role in wound healing appears complex and a differential role of MIF in distinct phases of wound healing and as a function of systemic conditions may be reasonably assumed.

Although the underlying mechanisms may not yet be clear, in the context of diabetes and obesity it is likely that MIF overexpression exerts an unfavorable effect on the wound healing processes. In an unpublished data, we have detected that increased levels of MIF in adipose tissue harvested from wound healing disorders underscoring the fact that MIF expression is modulated in wound repair. It is reasonable to hypothesize that increased MIF production by adipose tissue during obesity promotes inflammation and tissue homeostasis to influence the outcome of wound repair.

One mechanism by which MIF or D-DT in adipose tissue might alter wound healing is via macrophage polarization. A persistence of M1 macrophages with diminished switch to the alternatively activated M2 phenotype, especially the M2c phenotype, has been reported to be a hallmark feature of non-healing wounds.[89] As mentioned earlier, recent studies indicated that MIF-deficiency influences macrophage polarization toward an anti-inflammatory or M2-phenotype.[38,54,62] A MIF-dependent alteration of macrophage phenotype in the wound or adjacent adipose tissue might therefore alter the clinical outcome in wound repair.

Another possibility is by the MIF or D-DT-dependent modification of adipose-derived progenitor cells. An increasing number of studies support the clinical effectiveness of adipose-derived stem cells in augmenting wound healing and stem cell therapy utilizing adipose-derived stem cells has become an area

of interest for regenerative tissue repair.[90] MIF and D-DT both appear to have roles in adipogenesis and an alteration in adipose-derived stem cell proliferation or differentiation may underlie their influence in the wound healing processes.

Although there are no data available to date regarding the specific role of D-DT in wound healing, Sonesson *et al.*[25] report both D-DT and MIF expression in UVB-light induced wounds, suggesting the likely participation of D-DT in repair.

CONCLUSION

In summary, accruing evidence supports the role of adipose tissue as a rich source of MIF and D-DT, with differential expression of these two mediators in distinct cellular compartments. Current literature also supports an action for MIF in the development of obesity, chronic inflammation and insulin resistance. Although investigations of D-DT are in a much earlier stage, the available data suggest that D-DT and MIF exert a specific and possible complimentary action in wound repair. As the therapies directed at the MIF/CD74 pathway reach clinical development, new opportunities may be offered for the treatment of impaired wound repair in both the healthy host and in those compromised by conditions such as obesity.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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