Macrophage Migration Inhibitory Factor Promotes Intestinal Tumorigenesis

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Background & Aims: **The cytokine macrophage migration inhibitory factor (MIF) is expressed throughout the human gastrointestinal tract. Recently, protumorigenic activity of MIF has been described in several cancer models. Therefore, we investigated the expression and function of MIF during the early stages of intestinal tumorigenesis.** ***Methods: MIF*** **messenger RNA, protein, and tautomerase activity were measured in normal intestinal mucosa and adenomas from patients with sporadic colorectal adenomas and in the *adenomatous polyposis coli* (*Apc*)$^{Min/+}$ mouse model of intestinal tumorigenesis. MIF function was investigated by using VACO-235 human colorectal adenoma cells in vitro and by testing the effect of genetic deletion of *Mif* on *Apc*$^{Min/+}$ mouse intestinal tumorigenesis.** ***Results:*** **MIF expression and tautomerase activity were increased in human and *Apc*$^{Min/+}$ mouse intestinal adenomas compared with adjacent normal mucosa. Up-regulation of MIF occurred mainly in epithelial cells (associated with an increasing grade of dysplasia), but also in stromal plasma cells. Exogenous MIF inhibited apoptosis and promoted anchorage-independent growth of VACO-235 cells (maximal at 100 ng/mL). Homozygous deletion of *Mif* was associated with a reduction in the number and size of *Apc*$^{Min/+}$ mouse adenomas ($P = .025$ for the difference in large [>7-mm] tumors) and decreased angiogenesis (43% decrease in mean tumor microvessel density), but there was no alteration in epithelial cell apoptosis or proliferation.** ***Conclusions:*** **MIF expression is increased in sporadic human colorectal adenomas, and exogenous MIF drives tumorigenic behavior of epithelial cells in vitro. *Mif* also promotes intestinal tumorigenesis (predominantly via angiogenesis) in the *Apc*$^{Min/+}$ mouse. Therefore, MIF is a potential colorectal cancer chemoprevention target.**

The proinflammatory cytokine macrophage migration inhibitory factor (MIF) plays a critical role in host innate and acquired immunity.[1] Multiple functions have been ascribed to MIF, including activation of macrophages and T cells and antagonism of endogenous glucocorticoid activity.[1] MIF has been implicated in the pathogenesis of several acute and chronic inflammatory conditions, including septic shock and arthritis.[1] As would be expected of a critical regulator of the early innate host response to pathogens, MIF protein is expressed constitutively in the human gastrointestinal tract, primarily by epithelial cells, but also by a poorly characterized lamina propria cell population.[2] Increased plasma MIF levels are recognized in ulcerative colitis and Crohn's disease patients.[3,4] Moreover, genetic deletion of *Mif* or antibody-mediated neutralization of Mif protein abrogates the development of experimental colitis in rodents.[4,5]

More recently, MIF has been implicated in carcinogenesis in a variety of in vitro and in vivo models.[6,7] MIF abrogates p53-dependent apoptosis of macrophages and promotes RAS-mediated transformation of fibroblasts.[8–10] MIF has also been implicated in lymphoma and melanoma cell tumor growth and angiogenesis in

Abbreviations used in this paper: APC, adenomatous polyposis coli tumor suppressor; CRC, colorectal cancer; EIA, enzyme-linked immunoassay; FFPE, formalin-fixed, paraffin-embedded; HN, histologically normal; HPP, *p*-hydroxyphenylpyruvate; LPPC, lamina propria plasma cell; MEM, minimal essential medium; MIF, human macrophage migration inhibitory factor; Mif, mouse macrophage migration inhibitory factor; MVD, microvessel density; NF-κB, nuclear factor-κB; OD, optical density; $\Delta OD_{330}10-60$ s, increase in optical density at 330 nm between 10 and 60 seconds; PC, plasma cell; PCR, polymerase chain reaction; rh, recombinant human; SI, small intestine; TBS, Tris-buffered saline.

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rodents.[11,12] Therefore, MIF may be added to the increasing list of proinflammatory cytokines (eg, tumor necrosis factor α), chemokines (eg, CXCL12), and transcription factors (eg, nuclear factor-κ B [NF-κB]) now implicated in carcinogenesis.[13–15]

Overexpression of MIF has been shown in several human neoplasms,[6] including prostate,[16] breast,[17] and lung[18] cancer. One study, predating cloning and isolation of human MIF, showed that macrophage migration inhibitory activity was present in 85% of sporadic colorectal cancer (CRC) extracts and 50% of colorectal adenoma extracts, unlike normal colorectal tissue extracts, which were consistently negative.[19] More recently, increased MIF protein levels have been shown in human sporadic CRC tissue compared with normal colorectal mucosa in 1 immunohistochemical study,[20] and *MIF* expression has been detected in several human and mouse CRC cell lines, including HT29, CaCo2, LoVo, and colon 26.[2,7,20] In the last cell line, RNA interference and antibody-mediated neutralization of Mif have both been shown to impair tumor growth and neovascularization in syngeneic *BALB/c* mice.[7,21]

However, a role for MIF during the early stages of intestinal tumorigenesis, before transition from the premalignant, benign colorectal adenoma (or polyp) to an invasive adenocarcinoma or cancer, has not yet been studied. Because these stages are relevant to the development of CRC chemoprevention strategies, we addressed the hypothesis that MIF promotes the early stages of sporadic (as opposed to inflammatory bowel disease–associated) colorectal carcinogenesis. To this end, we investigated *MIF* expression in human sporadic colorectal adenomas and in intestinal adenomas from the *adenomatous polyposis coli* $(Apc)^{Min/+}$ mouse model of familial adenomatous polyposis. We also tested the effect of exogenous MIF on human colorectal adenoma cells in vitro and the effect of genetic deletion of *Mif* on $Apc^{Min/+}$ mouse intestinal tumorigenesis in vivo.

Materials and Methods

Human Tissue

Approval for the use of archival and fresh human colorectal tissue, as well as clinicopathologic data, was obtained from the St James's and Seacroft University Hospitals Local Research Ethics Committee. Formalin-fixed, paraffin-embedded (FFPE) specimens of human sporadic colorectal adenomas were randomly selected from the Histopathology Department archives at St James's University Hospital. Tissue from patients with inflammatory bowel disease or familial adenomatous polyposis was not analyzed.

Fresh colorectal adenoma tissue and paired macroscopically normal colorectal mucosa (6 biopsy samples taken at least 2 cm away from the adenoma) were obtained by endoscopic polypectomy and biopsy. Most of each adenoma was placed in 10% (vol/vol) formalin under the supervision of a consultant histopathologist. The remaining adenoma material and paired normal colorectal mucosa were washed separately in 0.9% (wt/vol) saline and placed immediately into ice-cold phosphate-buffered saline (PBS), pH 7.4 (Sigma, Poole, UK), containing Protease Inhibitor Cocktail III (Calbiochem, San Diego, CA). Samples were then homogenized on ice by using an ULTRA-TURAX T8 homogenizer (Ika-Labortech, Dortmund, Germany) and then sonicated (10-μm amplitude for 10 seconds) 3 times (Sanyo SONIPREP 150; Sanyo Loughborough, UK) before centrifugation at 7000*g* for 20 minutes at 4°C. The supernatant was aspirated and used for total protein estimation by Bio-Rad^{D}_{C} protein assay (Hercules, CA) and assay of tautomerase activity (see below). Other fresh colorectal adenoma tissue was embedded in OCT compound (Merck Ltd, Poole, UK) and snap-frozen in isopentane cooled in liquid nitrogen before storage at −70°C.

The following clinicopathologic parameters were recorded: age and sex of the patient, adenoma size (maximum diameter in millimeters for polypectomy specimens), position (distal or proximal to the splenic flexure), histological type (tubular, tubulovillous, or villous), and the highest grade of dysplasia (mild, moderate, or severe).

Animal Studies

C57BL/6 × 129/Sv $Mif^{-/-}$ mice[22] (a kind gift from Dr John David, Boston, MA) were mated with C57BL/6 $Apc^{Min/+}$ animals (The Jackson Laboratory, Bar Harbor, ME) in specific pathogen–free conditions, as described previously.[23] Allele-specific polymerase chain reaction (PCR) genotype analysis was performed as described previously.[22,23] Comparison of the intestinal phenotype of C57BL/6 × 129/Sv $Mif^{-/-} \times Apc^{Min/+}$ vs $Mif^{+/+} \times Apc^{Min/+}$ mice of both sexes was performed between 106 and 124 days of age by a single observer blinded to the *Mif* genotype. Immediately after death, the entire gastrointestinal tract from the duodenum to the rectum was removed, opened longitudinally, and washed thoroughly with PBS. The number and size (maximum diameter in millimeters) of all adenomas in the small intestine (SI) and colon were measured separately by using a Lynx stereo dissecting microscope fitted with a graticule.[23] Intestinal tissue was fixed in 4% (wt/vol) paraformaldehyde (Sigma) in PBS at 25°C overnight before storage in 70% (vol/vol) ethanol and subsequent embedding in paraffin. Tissue sections were stained with H&E and underwent histological examination by an experienced histopathologist, who was blinded to the origin of each section. Homogenate supernatants of normal intestinal mucosa and adenomas were obtained as described previously.

Apoptotic Epithelial Cell Counting in $Apc^{Min/+}$ Mouse Intestinal Adenomas

In $Apc^{Min/+}$ mouse intestinal adenomas, 2 patterns of distribution of apoptotic bodies can be discerned, namely, scattered apoptosis within the epithelial layer and crypt lumen