Macrophage Migration Inhibitory Factor and CXC Chemokine Expression in Non-Small Cell Lung Cancer: Role in Angiogenesis and Prognosis

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ABSTRACT

Purpose: The purpose of this study was to determine whether expression of migration inhibitory factor (MIF) is increased in non-small cell lung cancer, and whether it correlates with angiogenesis and/or prognosis.

Experimental Design: We measured vessel density, and levels of MIF, angiogenic CXC chemokines, and vascular-endothelial growth factor (VEGF; by ELISA) in tumor and normal lung tissue from 87 patients after resection of lung cancer. We compared vessel density with levels of MIF, VEGF, or angiogenic CXC chemokines in the corresponding tumor homogenate. Disease-free survival was analyzed in a Cox proportional hazards model.

Results: Levels of MIF in lung cancer demonstrated a bimodal distribution, with some having “normal” values (relative to normal lung tissue) and a second cluster with markedly high values. The increased levels of MIF in lung cancer were statistically significant in both paired and unpaired comparisons (P < 0.05). The strongest correlation of vessel density was with the sum of angiogenic CXC chemokines. MIF correlated very strongly with levels of angiogenic activity in NSCLC; Ref. 18), VEGF, and tumor vessel density. We report here that levels of MIF in tumor tissue are elevated relative to normal lung tissue, and that MIF correlates highly with levels of angiogenic (glu-leu-arg, or “ELR motif”-containing) CXC chemokines. Tumors in the high MIF group had a strong correlation between MIF level and vessel density. Risk of recurrence was associated with high levels of glutamic acid-leucine-arginine amino acid motif CXC chemokines, MIF, and/or VEGF in a Cox proportional hazards model.

Conclusions: MIF expression in non-small cell lung cancer occurs in a bimodal distribution, and is closely associated with tumor levels of angiogenic CXC chemokines and with vessel density. High levels of tumor-associated CXC chemokines, MIF, or VEGF are associated with risk of recurrence after resection of lung cancer.

INTRODUCTION

Macrophage MIF is considered the first lymphokine activity to be described almost 4 decades ago (1, 2). The more recent cloning of the cDNA for human and mouse MIF permitted the discovery of a diverse array of previously unsuspected activities attributable to MIF (3, 4). This resulted in a clearer understanding of the pathobiology of MIF in conditions as varied as sepsis (5, 6), arthritis (7, 8), asthma (9, 10), wound healing (11, 12), and cancer (13, 14). MIF expression was first described in prostate cancer by Meyer-Siegler and Hudson (15), and it has since been implicated in the control of angiogenesis in animal models of cancer by Ogawa et al. (14), and Shimizu et al. (16). We have found that MIF is expressed in vitro by human NSCLC cell lines and that this expression leads to increased angiogenic activity by inducing the expression of angiogenic CXC chemokines from cocultured monocytes (17). We hypothesized that tumor-associated levels of MIF would correlate with levels of CXC chemokines and vessel density in lung cancer specimens. We measured levels of MIF in human lung cancer specimens as well as levels of the angiogenic CXC chemokines (which we have shown previously to be important regulators of angiogenic activity in NSCLC; Ref. 18), VEGF, and tumor vessel density. We report here that levels of MIF in tumor tissue are elevated relative to normal lung tissue, and that MIF correlates highly with levels of angiogenic (glu-leu-arg, or “ELR motif”-containing) CXC chemokines. Levels of VEGF were also elevated but correlated poorly with vessel density. Levels of CXC chemokines were elevated, consistent with our previous data. We additionally show that a worse prognosis is associated with high expression of CXC chemokines, MIF, or VEGF in a Cox proportional hazards model.

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3 The abbreviations used are: MIF, migration inhibitory factor; CXC, cysteine-x-cysteine motif; CXCL, CXC ligand; ELR, glutamic acid-leucine-arginine amino acid motif; ENA, epithelial neutrophil activating peptide; GRO, growth-related oncogene; NSCLC, non-small cell lung cancer; VEGF, vascular endothelial growth factor; CI, confidence interval; IL, interleukin.
MATERIALS AND METHODS

Normal Human Lung and NSCLC Tumor Tissue. Tissue specimens were obtained from consecutive individuals undergoing thoracotomy (n = 87) for suspected NSCLC. All of the patients provided informed consent, and tissue was obtained with approval of both the University of Michigan Tissue Procurement Core and Institutional Review Board. Standardized punch biopsy samples of tumor and normal lung distal to the tumor were homogenized and sonicated in Complete antiprotease buffer (Boehringer) on recovery from the operating room. Specimens were centrifuged at 900 g for 15 min, filtered through 0.45-μm Sterile Acrodiscs (Gelman Sciences, Inc., Ann Arbor, MI), and frozen at −70°C until thawed for assay by a specific ELISA. Additionally, an adjacent portion of tumor was fixed in 10% zinc formalin and embedded in paraffin for histological analysis. University of Michigan Hospital pathologists determined the final histological diagnosis. For each patient, smoking status was determined as current (smoking within the last 6 months), former (quit for >6 months), or never. Only cases of NSCLC were included in our analysis (squamous cell carcinoma, n = 29; adenocarcinoma, n = 50; other non-small cell or undifferentiated, n = 8). An H&E-stained section of each specimen of normal lung tissue was examined and used as a control only if the tissue was devoid of significant inflammation or malignant cell invasion (n = 60).

ELISA. Purified and biotinylated antibody for MIF, VEGF, ENA-78/CXCL5, and GROα/CXCL1 ELISAs were obtained from R&D Systems, Inc. (Minneapolis, MN). Antibodies for GRO-γ (CXCL3) were obtained from PeproTech Inc. (Rocky Hill, NJ). Purified and biotinylated antibodies for IL-8 (CXCL8) were produced as described previously (19). Aqueous tumor and normal lung extracts were subjected to specific ELISA according to our protocol published previously (17). All of the tissue samples were run in parallel for total protein content (Pierce, Rockford, IL), and results were standardized by expressing values as ng of cytokine per mg total protein.

Because the angiogenic CXC chemokines are all of equivalent potency in terms of angiogenic activity (20) and mediate their angiogenic effects via the same receptor (CXCR receptor 2; Ref. 21), we generated a combined “ELR score” for each of the tumors, representing the sum of the levels of all of the measured CXC chemokines bearing the ELR motif (glu-leu-arg, shared by the angiogenic CXC chemokines). That is total levels of IL-8/CXCL8, ENA-78/CXCL5, GROα/CXCL1, and GRO-γ/CXCL3.

Angiogenesis Bioassays. Endothelial cell chemotaxis was performed as described previously (17, 22), using various concentrations of MIF from 0.1 to 100 ng/ml as the angiogenic stimulus (120 μl). Each experiment was accompanied by a positive control (VEGF 100 ng/ml) and a negative control (nonconditioned medium) to account for variable chemotaxis from one assay to the next. Results were expressed as the mean number of endothelial cells per high-powered field ± SE. Each sample was assessed in triplicate wells. Each experiment was performed three times, and the results combined as the chemotactic index (ratio of cell migration in the experimental condition to negative control).

Microcarrier bead assays were performed using the method of Fife et al. (23). Briefly, 100 mg of Cytodex-3 microcarrier beads (Sigma) were washed three times in 20 ml PBS and resuspended in 10 ml of serum-free endothelial cell medium. Eight ml of a cell suspension containing HMEC-1 cells at 10⁵ cells/ml were suspended with 440 μl of the bead slurry. This suspension was placed in a siliconized (Sigmakote; Sigma) glass Petri dish, and the cells were allowed to adhere to beads overnight. Fresh medium was added, and the beads were allowed to incubate until the beads were coated with cells. Forty μl of beads were then added to 0.5 ml of a fibrinogen suspension (Sigma; 2.5 mg/ml in serum-free HMEC-1 medium), and placed into a well of a 24-well plate. The fibrinogen was then polymerized with thrombin (Sigma; 0.625 u/ml) and aprotinin (Sigma; 0.15 IU/ml). The bead suspensions were allowed to clot for 1 h, and test medium was added (VEGF 10 ng/ml, MIF 5 or 50 ng/ml, or negative control medium, 0.5 ml/well for a total volume of 1 ml/well). Bead formation was assessed at 4 days by counting the number of tube projections per bead. Data represents the average of 5 beads/well, and 3 wells/condition. The experiment was performed twice.

Immunohistochemistry. A portion of each tumor specimen was fixed in 10% zinc formalin. Paraffin-embedded tissue sections were dewaxed in xylene and rehydrated through graded concentrations of ethanol. Antigen retrieval was performed with 0.1% trypsin/ CaCl₂ for 30 min at 37°C. Samples were then stained for MIF using a modification of our technique described previously (24). In brief, using peroxidase staining kits (Vectorstain Elite, ABC Peroxidase kit for goat primary antibody; Vector Laboratories, Burlingame, CA), nonspecific binding sites were blocked. Sections were then overlaid with either antimouse MIF or control goat IgG (0.2 μg/ml in PBS; R&D Systems) for 1 h. Slides were then rinsed and overlaid with secondary biotinylated antibody (Vectastain ABC kit) and incubated for 30 min. After washing twice with PBS, slides were overlaid with ABC reagent, and incubated for 30 min. 3,3’-Diaminobenzidine substrate (Vector) was used to localize angiogenic MIF, yielding a red-brown product. After optimal color development, sections were immersed in sterile water, counterstained with Mayer’s hematoxylin for 5 min, and coverslipped with aqueous mounting solution. Sections were photographed with a Spot RT color camera coupled to a Nikon microscope, and captured on a Dell Optiplex computer using Adobe Photoshop software.

Quantitation of Vessel Density. Quantitation of vessel density was performed using the method described previously (24). In brief, tissue sections were dewaxed and rehydrated. Slides were stained for endothelial cells using antibody to Factor VIII-related antigen conjugated to horseradish peroxidase (EPOS staining reagent; DAKO Corporation, Carpinteria, CA) for 1 h at room temperature. 3,3’-Diaminobenzidine, (Vector) was used for localization of Factor VIII-related antigen. After optimal color development, sections were immersed in water and coverslipped. Tumor specimens were scanned at low magnification (×40) to identify vascular hot spots. Areas of greatest vessel density were then examined under higher magnification (×400) and counted. Each distinct area of positive staining for Factor VIII-related antigen was counted as a single vessel. Vessel density for each patient tumor was expressed as the average number of vessels per high power (×400) field from three nonoverlapping microscopic fields.
Statistical Analysis. All of the normal lung tissue samples were examined under the microscope after paraffin embedding without knowledge of the levels of cytokines in the corresponding tissue homogenate. Any normal tissue containing significant inflammation \((n = 32)\) or unsuspected tumor invasion \((n = 1)\) on histological examination was excluded from analysis of cytokine levels. Results for MIF were compared between tumor and paired normal tissue using a nonparametric analysis, the Mann-Whitney \(t\) test, because the values of MIF in tumors did not follow a normal distribution. Wilcoxon signed rank test was used to compare all of the tumors to all of the normal tissues in a nonpaired fashion (given that not all of the tumors had a paired normal lung specimen from the same patient). To determine the correlation between cytokine levels with one another and with vessel density, we performed a correlation analysis and a Fisher’s \(r\) to \(z\) translation to determine the degree of statistical significance of the correlation using the StatView statistical software package. Patients were stratified into groups with high or low levels of MIF or VEGF based on tumor-associated levels above (high) or below (low) the median for that particular cytokine. Univariate Cox proportional hazards modeling was used to determine the relation of the following variables to disease-free survival, based on their association with angiogenesis: MIF, individual CXC chemokines, total CXC chemokine levels (the ELR score), and VEGF. Kaplan-Meier survival plots were generated to describe the disease-free survival of the cohorts of patients defined by the presence of high MIF or high VEGF, both cytokines being high, or neither being high.

RESULTS

MIF Levels Are Increased in Tumor Tissue Relative to Normal Lung. We have shown recently that NSCLC cell lines express MIF constitutively and that MIF is responsible for inducing CXC chemokine-dependent angiogenic activity from monocytes in coculture. The resulting coculture-conditioned medium, when tested in angiogenesis bioassays, was markedly more angiogenic than medium generated by either monocytes or NSCLC cells cultured independently (17). Many studies have shown that CXC chemokines are major determinants of the angiogenic bioactivity in human NSCLC (24–31). Given these findings, we hypothesized that human lung tumor tissue would be associated with expression of MIF in situ. To address this question, we performed immunohistochemical staining for MIF in surgical specimens of human NSCLC. We found significant, specific staining of tumors for MIF in specimens of NSCLC (Fig. 1). To investigate this additionally, we measured levels of
MIF in aqueous tumor homogenates taken from 87 patients with lung cancer. Tumor-associated levels of MIF did not follow a normal distribution. Rather, it was apparent that there was a bimodal distribution, with approximately half the tumors having normal distribution, with approximately half the tumors having MIF in aqueous tumor homogenates taken from 87 patients with lung cancer tumors relative to normal lung tissue.

A, MIF levels expressed in ng MIF per mg total protein (P = 0.01 by nonparametric paired comparison, P = 0.03 by nonparametric unpaired comparison). B, frequency distribution of MIF expression in tumors (solid line) and normal lung tissue (dashed line) demonstrating the bimodal distribution of MIF levels among tumor tissue.

Fig. 2 MIF expression is increased in human lung cancer tumors relative to normal lung tissue. A, MIF levels expressed in ng MIF per mg total protein (P = 0.01 by nonparametric paired comparison, P = 0.03 by nonparametric unpaired comparison). B, frequency distribution of MIF expression in tumors (solid line) and normal lung tissue (dashed line) demonstrating the bimodal distribution of MIF levels among tumor tissue.

MIF expression is increased in human lung cancer tumors relative to normal lung tissue. A, demonstrating the bimodal distribution of MIF levels among tumor tissue (Table 1).

Previous publications have suggested that MIF can promote endothelial cell migration or proliferation (14, 16). We tested recombinant human MIF for in vitro angiogenic activity in two different assays, endothelial cell migration, and tube formation in fibrin gels. MIF did not demonstrate any angiogenic bioactivity in vitro either in endothelial cell chemotaxis assays (Fig. 3A) or in tube formation assays using endothelial cell-coated microcarrier beads embedded in fibrin gel (Fig. 3B).

MIF Levels Correlate with ELR CXC Chemokine Levels and Vessel Density. The above findings suggest that MIF does not directly promote angiogenesis in vitro. However, given that MIF induces CXC chemokine-dependent angiogenic activity by monocytes in vitro (17), we hypothesized that MIF expression by NSCLC tumors in vivo would be associated with higher levels of tumor-associated CXC chemokines. The ELR score, reflecting total levels of tumor-associated angiogenic CXC chemokines, correlated highly with the levels of MIF in the corresponding tumor (r = 0.76; P < 0.0001; Fig. 4).

To determine whether MIF levels correlated with vascular density, each tumor was subjected to staining with antibody to Factor-VIII-related antigen to delineate microvessels. We found a statistically significant positive, but weak, correlation between vessel density and levels of tumor-associated MIF (r = 0.23; P = 0.05; Table 2; Fig. 5A). Correlation analysis to determine the degree to which variation in cytokine levels between tumors accounted for the variability in vessel density was performed. Surprisingly, tumor-associated levels of VEGF had the weakest correlation with tumor vessel density (r = 0.07; P = 0.58; Table 2). With the exception of GROγ/CXCL3, levels of individual CXC chemokines correlated positively and significantly with tumor vessel density, which confirms our findings published previously (Table 2; Ref. 24). However, the strongest predictor of tumor vessel density was the ELR score (r = 0.47; P < 0.0001; Table 2; Fig. 5B).

One possible interpretation of our data is that tumors may achieve their angiogenic requirement through a variety of pathways. For example, one tumor may predominantly secrete VEGF, whereas another may secrete one or more of the CXC chemokines or one of the many angiogenic factors not measured in our study (e.g., fibroblast growth factor or platelet derived endothelial cell growth factor). Yet another type of tumor may induce the expression of angiogenic factors by infiltrating stromal cells. An example of this would be expression of MIF by tumor cells, which leads to expression of angiogenic CXC chemokines from tumor-associated monocytes. If this were true, one would expect a tumor that exploits one angiogenic mechanism might not require the presence of another angiogenic mechanism. In this scenario, vascularity in tumors in which levels of MIF are low (similar to normal tissue for example) would depend on an alternative angiogenic strategy. In these low MIF tumors, one would not expect vessel density to correlate with levels of MIF. On the other hand, in tumors with high MIF levels, one might expect a stronger correlation between MIF levels and vessel density. Consistent with this, in tumors in which MIF levels are above the median (138 ng/mg, or higher than normal lung tissue), the correlation of MIF with vessel density is stronger (r = 0.42) and statistically significant (P = 0.02; Table 2; Fig. 5A). In this group, the correlation of MIF with the ELR score is also strengthened (r = 0.79; P < 0.0001; Fig. 4, closed symbols). In the low MIF cohort, VEGF still correlated poorly with vessel density (r = 0.12; P = 0.45).

MIF, ELR Score, and VEGF Levels Are Associated with Worse Prognosis. In the course of follow-up of the cohort in this study (median, 16 months; range, 2–32), 27 of the 87 patients have had recurrence of their lung cancer. In a univariate Cox proportional hazards model, factors associated with recurrence of lung cancer after resection included MIF, the ELR score, and VEGF levels above the median (Table 3). When both VEGF and MIF were elevated above the median, disease-free survival was significantly worse (Fig. 6).
Table 1  Levels of the angiogenic CXC chemokines and VEGF (expressed as ng per mg total protein) are increased in tumor relative to normal lung tissue

Only GROγ/CXCL3 levels were unchanged between tumor and normal tissue. ELR score represents the sum of the levels of each angiogenic (ELR motif containing) CXC chemokine in the tumors (Ps represent Bonferroni corrected comparisons between tumor and normal tissue; n = 87 tumors, and 60 normal specimens).

<table>
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<tr>
<th></th>
<th>GROα CXCL1</th>
<th>GROγ CXCL3</th>
<th>ENA78 CXCL5</th>
<th>IL-8 CXCL8</th>
<th>ELR score</th>
<th>VEGF</th>
</tr>
</thead>
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<tr>
<td>Tumor</td>
<td>0.75 ± 0.2</td>
<td>2.0 ± 0.4</td>
<td>3.7 ± 2.8</td>
<td>1.34 ± 0.3</td>
<td>7.8 ± 2.9</td>
<td>5.5 ± 2.1</td>
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<tr>
<td>Normal</td>
<td>0.25 ± 0.05</td>
<td>1.2 ± 0.2</td>
<td>0.35 ± 0.09</td>
<td>0.7 ± 0.1</td>
<td>1.2 ± 0.2</td>
<td>0.5 ± 0.07</td>
</tr>
<tr>
<td>( P )</td>
<td>0.05</td>
<td>0.20</td>
<td>0.05</td>
<td>0.05</td>
<td>0.02</td>
<td>0.02</td>
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**DISCUSSION**

Our study of MIF levels in NSCLC contains several novel findings. This is the first report to demonstrate and quantify significant protein levels of the cytokine MIF in human lung cancer, a finding that has implications for the biology of these tumors. Another important finding of this study is that shorter disease-free survival after surgical resection of lung cancer was associated with higher levels of MIF and/or VEGF in the resected tumor. MIF was discovered 4 decades ago as a factor in mixed lymphocyte cultures that inhibited the random migration of guinea pig peritoneal macrophages (1, 2). However, since the cloning of MIF in 1989, an array of activities has been associated with this cytokine that implicate it in the pathogenesis of diverse disease states, including cancer. MIF mRNA has been found in prostate cancer tumors and cell lines, and has been associated with increased metastatic capacity of these tumors (15, 32, 33). MIF has several properties that are most relevant to cancer and metastases, including promotion of angiogenesis (14, 16, 17), induction of matrix metalloproteases (33), and inhibition of p53-mediated apoptosis (34, 35).

Ogawa et al. (14) and Shimizu et al. (16) showed independently that inhibition of MIF reduced angiogenesis using \textit{in vivo} models of colon cancer and melanoma, respectively. Our findings differ somewhat from the findings of these authors in that, in our study, MIF did not promote endothelial cell migration or tube formation directly \textit{in vitro}. The reasons for this discrepancy are not immediately clear, but may relate to either differences in the target cells used or to other technical aspects of the assay. We have confirmed our findings with two different sources of endothelial cells (HMEC-1 cell line as reported herein, and primary human dermal microvascular cells; data not shown). Our previous findings, suggest that MIF indirectly promotes the angiogenic response by inducing the expression of angiogenic CXC chemokines \textit{in vitro} (17). This would explain differences between neutralization studies of MIF \textit{in vivo versus in vitro}.

Therefore, a second important aspect of the current study is to confirm these findings by showing a strong correlation between tumor-associated levels of MIF and angiogenic CXC chemokines \textit{in vivo}. A number of studies have confirmed the central role of angiogenic CXC chemokines in promoting angiogenesis in lung cancer (18, 24–26, 29–31). Our previous studies have suggested, and this study confirms, a strong correlation between levels of the angiogenic CXC chemokines and tumor vessel density in NSCLC (24). The correlation of MIF with angiogenic CXC chemokine levels suggested that MIF would also correlate with vessel density. Whereas this was statistically significant, the association between MIF levels and
vessel density was weak. For tumors in which MIF levels were similar to those found in normal lung tissue, there was no relation between MIF levels and vessel density. However, for tumors in the high-MIF group, the correlation of MIF with both the ELR score and with vessel density was markedly strengthened.

An unexpected finding of our data is the weak correlation between tumor-associated levels of VEGF and tumor vessel density. Despite this, VEGF remains a predictor of recurrence of disease after resection. The reason(s) for this apparent discrepancy are unknown, but might imply a "cofactor" required for VEGF-mediated angiogenesis in lung cancer, which we have not measured. Alternatively, angiogenesis inhibitors present but not accounted for in the tumors may mask an association between a potent angiogenic factor like VEGF and histological vessel density.

One interpretation of our data is that the apparently absolute requirement for angiogenesis in solid tumors can be met through a variety of mechanisms. For example, the angiogenic switch, which occurs early in the life cycle of a tumor, might entail the spontaneous secretion of angiogenic cytokines such as IL-8/CXCL8, ENA-78/CXCL5, VEGF, or any number of other angiogenic factors that have been implicated in tumor angiogenesis. Alternatively, the transformed cell population may elicit the production of angiogenic factors from nontransformed

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<th>Variable</th>
<th>n</th>
<th>Relative risk (95% CI)</th>
<th>P</th>
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<tbody>
<tr>
<td>ELR score (risk per 1 ng/mg increase)</td>
<td>87</td>
<td>1.012 (1.003–1.020)</td>
<td>0.006</td>
</tr>
<tr>
<td>MIF (risk per 10 ng/mg increase)</td>
<td>87</td>
<td>1.01 (1.00–1.01)</td>
<td>0.04</td>
</tr>
<tr>
<td>VEGF high (&gt;0.67 ng/mg)</td>
<td>44</td>
<td>2.54 (1.21–5.31)</td>
<td>0.01</td>
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host cells in the tumor stroma, as we and others have shown (17, 22, 36–39). One example of the latter mechanism is the expression of MIF by NSCLC cell lines and tumors, leading to the production of angiogenic CXC chemokines within the tumor, which then promote tumor growth. It should be noted that angiogenic CXC chemokine expression within tumors does not require the expression of MIF, because many low-MIF tumors in our study expressed appreciable levels of angiogenic CXC chemokines. Also, we have previously demonstrated significant expression of angiogenic CXC chemokines in lung cancer cell lines as well as tumor specimens (24, 26, 40). Nevertheless, our current data suggest that tumor-associated levels of MIF can account for a significant proportion of the variability in CXC chemokine levels between tumors.

We have hypothesized from our data that tumors may induce angiogenesis by preferentially secreting (or inducing tumor-infiltrating host cells to secrete) one type of angiogenic factor over another. If this “selection” of angiogenic strategy occurs in a random fashion in a developing tumor, it is also likely that some tumors may exploit more than one angiogenic pathway. One might expect that such tumors would carry a worse prognosis, a contention that is supported by our findings. The cumulative disease-free survival of patients with both high MIF and high VEGF (with “high” defined as a level above the median) was significantly worse than patients in whom both variables were low or in whom either variable was low.

In addition to promoting angiogenesis, MIF may play a role in tumors by virtue of its ability to regulate p53 activity. Two groups of investigators independently identified MIF in assays seeking clones that inhibited p53-mediated growth arrest and apoptosis (34, 35). These studies suggest that MIF could provide both angiogenic and antiapoptotic activity within a tumor. Additionally, MIF-dependent inhibition of p53-mediated apoptosis in the tumor and its stroma may extend the life span and prolong the tumor growth-promoting activity of tumor-associated macrophages (34), a possibility that has been has been proposed by other authors as well (41).

In summary, we have found that a subset of human lung cancer tumors is associated with high levels of MIF, which correlated strongly with levels of angiogenic CXC chemokines, with vascularity, and with increased risk of recurrence of lung cancer. The strongest predictor of tumor vessel density in our study was the overall levels of angiogenic CXC chemokines, which we refer to as the ELR score. Our data also suggest that as a tumor develops an angiogenic phenotype, it may segregate along a pathway that exploits one angiogenic strategy in preference over others. Additional studies are required to test this hypothesis.

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