Role of Macrophage Inhibitory Cytokine-1 in Tumorigenesis and Diagnosis of Cancer

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Abstract

Macrophage inhibitory cytokine-1 (MIC-1), a transforming growth factor-β (TGF-β) superfamily cytokine, is involved in tumor pathogenesis, and its expression can be used as a clinical tool for the diagnosis and management of a wide range of cancers. Although generally considered to be part of the cell’s antitumorigenic repertoire, MIC-1 secretion, processing, and latent storage suggest a complex, dynamic variability in MIC-1 bioavailability in the tumor microenvironment, potentially modulating tumor progression and invasiveness.

Introduction

In 1997, we first reported a novel divergent member of the human transforming growth factor-β (TGF-β) superfamily, cloned based on increased expression in macrophage activation, which we named macrophage inhibitory cytokine-1 (MIC-1; refs. 1, 2). Between 1997 and 2004, it was reported by other groups and given a variety of names, including PTGF-β, PLAB, GDF15, PDF, NAG-1, and PL74. The placenta is the only tissue that expresses large amounts of MIC-1 under normal physiologic conditions (2). However, a wide variety of epithelial cells, including the neuro-epithelium express low levels of MIC-1 mRNA. Although detectible by immunohistochemistry in central nervous system epithelium, such as the choroid plexus and ependyma and the placenta, MIC-1 protein has been difficult to detect at other sites (3). In disease states, such as acute injury, inflammation, and cancer, MIC-1 expression is dramatically increased (2, 4, 5) and has been implicated as a key secretory cytokine in response to multiple cellular stressors.

MIC-1 Expression in the Antitumorigenic Response

MIC-1 overexpression by multiple tumor types coincided with the finding that p53 powerfully induced MIC-1 expression both in vitro and in vivo (6–8). Mouse xenograft studies show that measurement of circulating tumor-derived MIC-1 is a good in vivo index of p53 pathway activation, a strategy that could be used to assess therapies affecting p53 pathway activation (7). MIC-1, being one of the major secreted proteins induced by p53, is not surprisingly thought to be important in translating p53-mediated activity. Indeed, several studies show MIC-1 induction being associated with cell cycle arrest and apoptosis. In colon carcinoma cell lines, MIC-1 overexpression correlates with apoptosis, whereas the addition of WR1065, which leads to p53 dependent cell cycle arrest, also strongly up-regulates MIC-1 expression. p53 is not the only transcription factor regulating MIC-1 expression. Other transcription factors, such as Egr-1 and nuclear factor-κB, have also been implicated in this process (9). Indeed, these and other factors are likely to predominate in MIC-1 induction in p53 mutant tumors over-expressing MIC-1.

Acting largely through p53- and/or Egr-1-related pathways, the groups of Ehling and Baek (9, 10) have shown that MIC-1 expression can be stimulated in cancer cell lines by a variety of antitumorigenic agents, such as the nonsteroidal anti-inflammatory drugs (NSAID), peroxisome proliferator-activated receptor γ ligands, and genistein. Additionally, dietary compounds associated with neoplastic cell growth suppression, including retinoids, resveratrol, green tea catechins, cruciferous vegetable indole-3-carbol, and diallyl disulfide, a constituent of garlic, all induce MIC-1 expression. Other transcription factors, such as Egr-1 and nuclear factor-κB, have also been implicated in this process (9). Indeed, these and other factors are likely to predominate in MIC-1 induction in p53 mutant tumors over-expressing MIC-1.

In the presence of several growth factors, MIC-1 expression can be stimulated in cancer cell lines by a variety of antitumorigenic agents, such as the nonsteroidal anti-inflammatory drugs (NSAID), peroxisome proliferator-activated receptor γ ligands, and genistein. Additionally, dietary compounds associated with neoplastic cell growth suppression, including retinoids, resveratrol, green tea catechins, cruciferous vegetable indole-3-carbol, and diallyl disulfide, a constituent of garlic, all induce MIC-1 expression. Other transcription factors, such as Egr-1 and nuclear factor-κB, have also been implicated in this process (9). Indeed, these and other factors are likely to predominate in MIC-1 induction in p53 mutant tumors over-expressing MIC-1.

Intermediate cells of a constitutively active TGF-β RI receptor in microvascular endothelial cells, both of which inhibit cell proliferation. In a synovial sarcoma line, knockdown of the SYT-SSX fusion gene, critical in sarcoma development and progression, strongly increased MIC-1 expression, whereas up-regulation of MIC-1 in an Hsp70-2 knockout breast cancer line was thought to be the mediator of the resultant G1 cell cycle arrest. Interestingly, Affymetrix Genechip analysis following neoadjuvant chemotherapy of breast cancer patients shows major up-regulation of MIC-1 (12). However, MIC-1 expression may also play a role in the anti-estrogen-resistant growth of estrogen receptor (ER)–positive breast tumors (13). Here, overexpression of AKT, which activates the ER in the absence of estrogen, strongly induced expression of MIC-1, together with other estrogen-regulated genes pS2 and Bcl-2. This conferred resistance to tamoxifen-induced apoptosis, suggesting that the context in which MIC-1 is up-regulated will dictate its final role. Indeed, this is true for many members of the TGF-β superfamily, where the effects of expression in tumors are often context specific.
MIC-1 Expression in Cancer

As well as being induced by antitumorigenic compounds, MIC-1 is overexpressed in many tumors. Many carcinoma lines secrete large amounts of MIC-1 (14) and multiple, independent differential expression studies (microarray and SAGE; refs. 4, 15) show MIC-1 expression is markedly increased in prostate and colon cancer biopsies. We have also confirmed that patients with metastatic prostate, breast, and colon cancer markedly overexpressed MIC-1 protein within tumors, and that this resulted in a large increase in serum MIC-1 levels (4). More recent studies indicate similar results for melanoma, pancreatic, and thyroid cancer (15, 16). Of these, prostate cancer has been the most extensively studied with MIC-1 overexpression documented in all (17, 18) but one study. Overall, these changes in expression suggested the use of measuring MIC-1 expression in the diagnosis and management of the disease.

Serum MIC-1 as a Clinical Tool

Increased tissue expression of MIC-1 is often associated with serum levels outside the reference range of about 200 to 1,200 pg/mL. Studies of serum MIC-1 levels in several cohorts of patients have revealed potential clinical use in the diagnosis and/or monitoring of prostate, thyroid, pancreatic, and colonic cancers. In colon cancer, there are significant increases in serum MIC-1 levels with disease progression from normal to adenoma, carcinoma, and metastatic disease (19). In colon cancer, MIC-1 serum levels reflect tumor stage and extent, and measurement at presentation is an independent predictor of metastasis and overall survival (19).

Pancreatic cancer is usually an occult disease, often leading to late diagnosis and poor survival. Historically, tumor markers have been unhelpful. However, measurement in serum of a combination of MIC-1 with CA19-9 significantly improved the diagnosis of pancreatic cancer leading to a sensitivity of 70% and specificity of 85%. Interestingly, whereas patients with pancreatic ductal adenocarcinoma had significantly higher levels of MIC-1 than those with chronic pancreatitis or healthy controls, patients with early disease had the highest serum MIC-1 levels. This may offer hope for monitoring patients at higher risk of pancreatic cancer development (16).

In prostate cancer, serum MIC-1 levels combined with total and free prostate-specific antigen measurement significantly improves...
diagnostic specificity and is potentially useful for the monitoring of disease progression (20). It is also possible that serial serum MIC-1 levels will be useful for the detection of higher grade prostate malignancy, missed due to biopsy sampling error and to indicate when patients treated with “watchful waiting” are at risk of disease progression. As serum levels of MIC-1 tend to be stable over time in otherwise healthy individuals,2 and MIC-1 is overexpressed in a wide variety of tumors, it is quite likely that, at least in at risk populations, serial measurement of MIC-1 may provide an early warning of the development of a premalignant or malignant lesion.

MIC-1 Polymorphism and Cancer

Further linking MIC-1 to cancer biology was the finding of a single nucleotide polymorphism in the MIC-1 coding region that significantly affected both on predisposition to cancer and patient survival. This polymorphism results in the change of a histidine (H) to an aspartic acid (D) residue at position 6 of the mature MIC-1 protein (19, 21). The MIC-1 D allele of the H6D polymorphism is associated with significantly better patient survival in a cohort of about 200 patients with colon cancer (19). In contrast, the presence of the H allele leads to an increased risk of prostate cancer, contributing to 7.2% of sporadic and 19.2% of familial prostate cancer cases in a Swedish study of 1383 patients and 700 controls (21). The presence of this polymorphism in the coding region of mature MIC-1, resulting in amino acids with divergent properties, suggests a functional alteration in protein rather than linkage disequilibrium with other genes.

Role of MIC-1 in Tumor Development

Although there is strong linkage of MIC-1 expression to epithelial tumors, less is known about its role and the manner in which it exerts its effect. Most studies point to an antitumorigenic role for MIC-1. In particular, a number of reports suggest a role in induction of apoptosis via both p53-dependent and p53-independent mechanisms. MIC-1 overexpression in HCT-116 colon and MDA-MB-468 breast carcinoma lines resulted in reduction in cell viability, and nude mice xenograft models of the HCT-116 transfectants showed a significant reduction in tumor size (6, 22). These findings suggest that MIC-1 may negatively affect tumor growth. Interestingly, a similar study using a glioblastoma cell line, which unlike the HCT-116 line, was unresponsive to MIC-1 in vitro, completely failed to grow as a tumor xenograft in nude mice when transfected with MIC-1 (8). This suggests MIC-1 has significant paracrine effects, which modulate the tumor environment. Further supporting a role for MIC-1 in the development and evolution of tumors is data showing that MIC-1 has antiangiogenic activity both in vitro and in vivo (23).

Recent studies shed light on the possible mechanisms of tumor growth modulation. MIC-1 inhibits the expression of the cyclin D1 oncogene that promotes breast and other solid tumors (24). Additionally, inhibition of MIC-1 expression with small interfering RNA prevented NSAID-induced cell cycle arrest of ovarian cancer cells, in part by down-regulation of p21WAF1 (25). Our studies, using DU-145 prostate cancer cells treated with recombinant MIC-1, show loss of adhesion and induction of apoptosis mediated by a reduction in the expression of antiapoptotic gene metallothionein 1E and genes RhoE and catenin δ1. The latter two, being involved in cell adhesion, may also point to a possible role in tumor dissemination (14).

Adding to the potential of MIC-1 as a tumorigenic protagonist is the finding that higher MIC-1 expression in gastric cancer cell lines has been associated with a more invasive phenotype. This seems to be due to MIC-1 increasing expression of the urokinase type plasminogen activator (uPA) and the uPA receptor, an effect mediated through extracellular signal-regulated kinase 1/2 (ERK1/2; ref. 26). Thus, whereas most studies highlight an antitumorigenic role for MIC-1, these suggest that this may not always be the case. Such apparently contradictory effects are typical of TGF-β and are mediated by a variety of factors including the nature of the tumor and its environment. Furthermore, tumor progression is often associated with accumulated genetic alterations that can erode its sensitivity to pro-apoptotic molecules. Thus, it is possible that overexpression of MIC-1 in early cancer induces apoptotic or other pathways that serve to limit tumor growth (Fig. 1).

Adding another level of complexity to its role in tumor development is the finding that MIC-1 is commonly secreted from tumors in its unprocessed, propeptide containing precursor form, with the degree of processing varying widely from tumor to tumor. The MIC-1 propeptide mediates binding to extracellular matrix, creating latent stromal stores of proMIC-1. Intracellular processing of MIC-1, thus, ultimately determines the proportion of MIC-1 that remains localized in the tumor microenvironment versus that diffusing into the systemic circulation. The biological relevance of these latent stromal stores to tumor progression was highlighted when examination of a prostate cancer tissue array revealed considerable patient variability in the degree of stromal staining. Decreasing tumor stromal staining for proMIC-1 was an important independent predictor of disease relapse (27). As unprocessed proMIC-1 is secreted by tumors other than prostate, it seems likely that modulating local MIC-1 bioavailability will affect patient outcome not only in prostate cancer but also other epithelial tumors.

A lot remains to be uncovered on the roles of MIC-1 and its biology, and whereas the evidence linking MIC-1 with cancer is compelling, it is still incomplete. Its receptor is unknown, and its signaling pathways are as yet to be delineated. Activation of Akt and ERK pathways has been reported, and there is some evidence for SMAD pathway activation, suggesting MIC-1 may act through a TGF-β superfamily receptor. The effects of MIC-1 can sometimes be apparently contradictory, and in differing circumstances, MIC-1 can exhibit tumorigenic and antitumorigenic activity. More recent studies suggest that this is very likely a function of the tumor stage, tissue of origin, and the interaction of the tumor with its local microenvironment. However, what is clear, is that there is strong evidence for MIC-1 measurement in blood and tissues to detect and monitor cancer.

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