Macrophage Inhibitory Cytokine 1 Reduces Cell Adhesion and Induces Apoptosis in Prostate Cancer Cells

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ABSTRACT

Macrophage inhibitory cytokine 1 (MIC-1), a divergent member of the transforming growth factor-β superfamily, is linked to the pathogenesis of cancer. To delineate possible roles for MIC-1 in prostate cancer, a number of prostate epithelial cell lines have been studied, including PZ-HPV-7, DU-145, PC-3, and LNCaP cells. Factors regulating the production of MIC-1 protein by these cells and some of the effects of MIC-1 on them were investigated. Although PZ-HPV-7 and DU-145 produced no MIC-1 protein, PC-3 and LNCaP cells secreted MIC-1 protein at high levels. The secretion of MIC-1 in LNCaP cells was modulated by both androgen and estrogen. Although neither MIC-1 nor anti-MIC-1 antibody had any effect on the proliferation of epithelial cells, MIC-1 induced changes in DU-145 cells. These cells became flattened and more spread out, and this was accompanied by reduced intercellular actin filaments and intercellular junctions. The DU-145 cells then detached from their substrate and underwent caspase-dependent apoptosis. To define some of the genes responsible for these changes, cDNA microarrays, followed by confirmatory reverse transcription-PCR, was used to analyze differential gene expression induced by MIC-1. The antiapoptotic gene metallothionein 1E and cell adhesion genes RhoE and catenin δ1 were down-regulated by more than 2-fold by MIC-1, suggesting that they were, at least in part, responsible for the observed changes in the behavior of DU-145 cells. These findings suggest that although MIC-1 has no effect on cell proliferation, it reduces cell adhesion and consequently induces cell detachment. It is likely that caspase-dependent apoptosis is secondary to loss of cell adhesion and may suggest a role for MIC-1 in tumor dissemination in vivo.

INTRODUCTION

Prostate cancer is the most common noncurable male malignancy and the second greatest cause of male cancer death in most Western societies (1). The development of prostate cancer is a multistep process mediated by hormones and growth factors including those of the TGFα/β and fibroblast growth factor superfamilies. A series of molecular changes take place to promote malignant transition and then progression from being confined in the organ to an invasive and metastatic phenotype. Growth factor cytokines also make a significant contribution to the transition from androgen-dependent to androgen-independent states (2), and one of the most important of these is TGF-β.

TGF-β is a multifunctional cytokine, the biological roles of which include cell cycle control, regulation of early development, differentiation, extracellular matrix formation, hematopoiesis, angiogenesis, chemotaxis, immune regulation, and the induction of apoptosis (reviewed in Ref. 3). TGF-β is well known for its inhibition of the proliferation of epithelial cells, such as MvILu mink lung epithelial cells and prostate DU-145 cells. It has also been shown to induce apoptosis in normal prostate epithelial cells and prostatic carcinoma cells both in vivo and in vitro (4–6). This growth inhibition and apoptosis can be correlated with its function as a tumor suppressor.

Another TGF-β superfamily cytokine linked recently to epithelial malignancies is MIC-1, which is also known by other names including prostate-derived factor, growth differentiation factor-15, placentale bone morphogenetic protein (PLAB), plasental transforming growth factor β, and nonsteroidal anti-inflammatory drug-activated protein 1. It is a divergent member of the TGF-β superfamily (7, 8) and was first isolated from a subtracted cDNA library enriched for genes associated with macrophage activation (8). It is synthesized as a 62-kDa protein, which, after cleavage by a furin-like protease, is secreted as a 25-kDa, disulfide-linked dimer. MIC-1 shares relatively low sequence homology with other family members and does not cluster within existing TGF-β families (8). Under resting conditions, MIC-1 mRNA is expressed highly in placenta, moderately in adult prostate, and at lower levels in brain, liver, kidney, and pancreas (9, 10).

Relatively little is known about the function of MIC-1, but a number of factors link it to epithelial tumors. MIC-1 is powerfully induced by p53 (11), and MIC-1 concentrations in serum and MIC-1 protein production in colon and prostate cancer tissues were up-regulated compared with their normal counterparts (12, 13). Marked up-regulation of MIC-1 mRNA in the transition from normal to prostate cancer cells and from androgen-dependent to androgen-independent prostate cancer cells has been demonstrated with microarray analysis (14, 15). Additionally, nonsteroidal anti-inflammatory drugs that inhibit tumor development also induce HCT-116 colon cancer cells to undergo apoptosis, possibly mediated by autocrine/paracrine induction of MIC-1 (16). MIC-1 has also been reported to inhibit prostate epithelial proliferation (17), induce colon and mammary epithelial cancer cell apoptosis in vitro, and inhibit colon and glioblastoma tumor growth in vivo (11, 16, 18, 19).

To better understand the hormonal factors that may modulate prostate MIC-1 expression and some of the possible consequences of MIC-1 overexpression in prostate cancer, studies of widely available prostate cancer lines were undertaken. These indicate that MIC-1 production by hormone-sensitive prostate cancer cells is modulated by hormones, and that MIC-1 has no effect on cell proliferation but reduces cell adhesion and induces apoptosis. By comparing the transcriptome of MIC-1-treated and untreated cells, we have been able to determine that these effects are likely to be at least in part mediated by MT1E, catenin δ1, and RhoE.

MATERIALS AND METHODS

Cell Culture. PZ-HPV-7 nonmalignant epithelial prostate cells (ATCC, Rockville, MD) were grown in keratinocyte, serum-free medium plus epider-
nal growth factor and bovine pituitary extract (Life Technologies, Inc.). DU-145, PC-3, and LNCaP malignant epithelial prostate cells (ATCC) were grown in RPMI 1640 (Life Technologies, Inc.) plus 10% fetal bovine serum. Mv1Lu mink lung epithelial cells (ATCC) were grown in MEM plus nonessential amino acids (Life Technologies, Inc.) and 10% fetal bovine serum.

PZ-HPV-7, DU-145, PC-3, and LNCaP cells were cultured in the standard 75-cm²-sized tissue culture flasks (Becton Dickinson, Oxford, United Kingdom). When cells reached ~90% confluency, the culture medium was exchanged for fresh medium. After an additional 24 h, conditioned medium was collected and stored at 4°C for the MIC-1 sandwich ELISA assay. To assess whether MIC-1 production was modulated by hormones, hormone-responsive LNCaP cells were plated in 96-well plates at 4500 cells/well in RPMI 1640 plus 10% fetal bovine serum for 24 h. Cells were then washed with serum-free medium and then RPMI plus 10% dextran-coated, charcoal-stripped fetal bovine serum with DHT (Sigma) or β-estradiol (Sigma) at 10⁻³ to 10⁻¹ M, or control was added in quadruplicate cultures. Cell culture medium was collected 6, 24, 72, or 96 h after treatment for estimation of MIC-1 concentration, and cells were collected for cell number determination.

Fluorescence Immunocytochemistry. PZ-HPV-7, DU-145, PC-3, and LNCaP cells were plated on sterile coverslips in 24-well culture plates. Twenty-four h later, the cells were fixed with paraformaldehyde and permeabilized with 0.1% Triton X-100 in PBS. After incubation with 2% BSA and 2% fetal bovine serum in PBS, the cells were incubated with 1:400 dilution of sheep anti-hMIC-1 polyclonal antibody 23B3 (20) or control sheep antibody. After washes with 0.1% Triton X-100 in PBS, the cells were blocked with 0.1% Triton X-100 in PBS. After incubation with 2% BSA and 0.1% Triton X-100 in PBS, the cells were blocked with 0.1% Triton X-100 in PBS. After incubation with 2% BSA and 0.1% Triton X-100 in PBS, the cells were incubated with FITC-conjugated anti-sheep antibody (Sigma) for 1 h. Immunostaining was finally visualized with a fluorescence microscope.

For cytokeratin studies, DU-145 cells were plated into 6-well plates at 2 x 10⁵ cells/well. After washing with PBS and SFM 24 h later, the cells were treated with MIC-1 or control in SFM for 3 days. After fixation, permeabilization, and blocking with fetal bovine serum and BSA, the cells were incubated with FITC-conjugated phalloidin (Molecular Probes, Eugene, OR) to label actin, or mouse anti-human vimentin (Sigma), or mouse anti-human tubulin antibodies (Sigma). Both vimentin and tubulin were visualized with Texas Red-conjugated anti-mouse IgG.

MIC-1 ELISA. An in-house MIC-1 ELISA assay was performed essentially as described previously (20). Briefly, ELISA plates were coated with 1:500 monoclonal mouse anti-human MIC-1 for 24 h at 4°C. Samples were diluted 1:10 to 50 in sample buffer (1% BSA, 0.05% Tween in PBS). A MIC-1 standard (1 μg/ml recombinant human MIC-1) was diluted 1:1000 in sample buffer, and eight doubling dilutions were then performed (1000 to 7.8 pg/ml). Standards or samples were run in triplicate. The plates were washed and blocked by incubation with 1% BSA for 1 h. Standards or samples were then added and incubated for 1 h at 21°C. After incubation with 1:5000 polyclonal rabbit anti-MIC-1 23B3, sheep anti-rabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, PA) for 1 h, streptavidin-horseradish peroxidase conjugate (Genzyme) for 30 min, O-phenylenediamine substrate (Sigma) was added. The reaction was terminated with H₂SO₄. Absorbance was measured at 490 nm, and a standard curve was constructed using a single binding site hyperbolic with the Graph Pad Prism program. Sample values were extrapolated from this curve. Each sample was assayed at minimum in duplicate, and the coefficients of variation were all <10%.

Cell Number Quantification with CyQuant GR Dye. Cell numbers in the 96-well microplates with LNCaP cells treated with hormones or control were determined by quantifying DNA content with CyQuant GR dye (Molecular Probes). The PCR reaction was carried out in a Rotor-Gene (Corbett Research, Mortlake, New South Wales, Australia) with 30 cycles. The specificity of the products was verified by melting curves generated by Rotor-Gene software and the sizes on electrophoretic gels. Because the kinetics of fluorescence accumulation during thermocycling are directly related to the starting number of cDNA copies, relative mRNA

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expression levels were determined on the basis of the kinetic approach using the Rotor-Gene real-time PCR software and the standard curves constructed with series purified cDNA dilutions (23). The target mRNA concentration of control cells, normalized to the level of glyceraldehyde-3-phosphate dehydrogenase mRNA, was ascribed a fold induction of 1.0.

Statistical Analysis. All data for statistical analysis were presented as mean ± SE. Differences were analyzed for significance using ANOVA among groups. A probability value of 0.05 or less was considered significant.

RESULTS

MIC-1 Is Secreted by Prostate Cancer Cell Lines. To explore the roles of MIC-1 in prostate cancer cells, nonmalignant and malignant prostate epithelial cell lines were first examined for production of significant amounts of MIC-1. Immunofluorescence revealed that the majority of LNCaP cells and PC-3 cells stained positively with sheep anti-hMIC-1 antibody (Fig. 1B). No staining was seen in LNCaP and PC-3 cells incubated with control sheep antibody. Neither DU-145 nor PZ-HPV-7 cells exhibited any intracellular MIC-1 staining (Fig. 1A).

To quantify the production of MIC-1 by these cells, cell line culture supernatants were assayed for MIC-1 content using a sensitive MIC-1 sandwich ELISA. Results were consistent with the immunocytochemistry data. Conditioned culture medium from subconfluent PZ-HPV-7 and DU-145 cells showed no detectable MIC-1 secretion over a 24-h period. In contrast, LNCaP cells grown under similar conditions secreted a huge amount of MIC-1 (>1000 ng/ml/24 h). PC-3 cells showed moderate MIC-1 production (110 ng/ml/24 h).

Both Androgens and Estrogens Inhibit MIC-1 Secretion by LNCaP Cells. Because hormones and growth factors are two of the most important factors in prostate cancer progression, the effect of hormones on MIC-1 production was investigated. To be able to correct MIC-1 secretion for cell number, the effects of hormones on this parameter were examined. Consistent with the literature, both DHT and δ-estradiol enhanced LNCaP cell proliferation. As shown in Fig. 2A, total cell numbers increased after treatment with DHT for 72 h. The highest increase, of >40%, was seen at a DHT dose of 10^{-10} M. Cell numbers were also increased by δ-estradiol, and 10^{-7} M seemed to be the most effective dosage (198,149 ± 5,3511 dpm for δ-estradiol versus 96,895 ± 16,721 dpm for control). When cell numbers were not considered, δ-estradiol had little effect on MIC-1 secretion into conditioned medium, whereas DHT, at the dose of 10^{-9} M, significantly decreased MIC-1 concentration in the culture medium by nearly 20% (Fig. 2B; P < 0.05). The inhibitory effect of DHT became more marked after MIC-1 concentration was adjusted for cell numbers (Fig. 2C). The greatest effect was again seen with 10^{-9} M DHT, which decreased MIC-1 production by 55%. Estradiol also decreased MIC-1 production per 10^4 cells. At 10^{-7} M, δ-estradiol reduced MIC-1 production per 10^7 cells to the same extent as the same dose of DHT.

The inhibitory effect of DHT and δ-estradiol on MIC-1 production by LNCaP cells was time dependent (Fig. 2D). The effect started to be obvious at 6 h of treatment, and by 24 h, MIC-1 per 10^4 cells was reduced significantly by both DHT and δ-estradiol, although δ-estradiol was still not as effective as DHT at the same dose. At 96 h after treatment, the inhibitory effects of DHT and δ-estradiol were similar.

MIC-1 Has No Effect on Epithelial Cell Proliferation. TGF-β is well known to inhibit epithelial cell proliferation, and whether MIC-1...
were also obtained with PC-3 cells, which also secrete substantial amounts of MIC-1 (data not shown).

Although it is difficult to interpret the results on PC3 and LNCaP cells, because mutations of receptor and/or signaling pathway are common in malignant cells secreting inhibitory molecules, this is less likely to be the case with DU-145, which secretes no MIC-1. The lack of effect of MIC-1 on DU-145 growth suggests a lack of effect of MIC-1 on proliferation of prostate cancer cells.

**MIC-1 Induces Morphological Changes and Detachment in DU-145 Cells.** Because DU-145 was the only prostatic cancer cell line not secreting MIC-1, it was the most appropriate cell in which to examine, in more depth, the effect of this cytokine. After treatment with MIC-1 for 3 days in SFM, significant morphological changes could be seen. Whereas control cells looked round, cells treated with MIC-1 looked flattened and more spread out (Fig. 4A and 4.1B). This effect was visible only in a proportion of DU-145 cells located at the periphery of large clusters of cells and in very small clusters. After the 4th posttreatment day, significantly more cells treated with MIC-1 than control detached and floated in the medium. Visually, on the 4th day, the majority of the floating cells looked healthy and were viable on trypan blue staining. However, from the 5th day onward, they became smaller in size and looked unhealthy or nonviable.

To understand the basis of the morphological changes, we stained the cells with fluorescent probes for actin, vimentin, and tubulin. These showed that MIC-1 induced a dramatic reduction in intercellular actin filaments with the disappearance of intercellular junctions in about 5–10% of the total cell population. There was no effect on vimentin and tubulin distribution and no clear actin cytoskeleton change inside the cells (Fig. 4.1C and 4.1D).

To quantify and evaluate apoptosis in the DU-145 cells detached after incubation with MIC-1, flow cytometry analysis was performed on cells stained with the activated caspase marker CaspACE™ FITC-conjugated VAD-FMK in TRUCount absolute count tubes. This indicated that MIC-1 increased the total number of detached cells and the number of detached cells negative to FITC-conjugated VAD-FMK (nonapoptotic cells). Treatment with 0.1, 1.0, and 10.0 ng/ml MIC-1 for 6 days resulted in increased numbers of detached cells by 213, 354, and 565%, respectively, compared with control, and increased FITC-conjugated, VAD-FMK-negative cells by 39, 94, and 168%, respectively (Fig. 4.2).

**MIC-1 Induces Caspase-dependent Apoptosis in DU-145 Cells.** The morphological appearance of the MIC-1-treated, nonadherent DU-145 cells suggested that they might have undergone apoptosis. Assessing this with FITC-conjugated VAD-FMK showed that MIC-1 did indeed induce apoptosis in DU-145 cells. This occurred in a dose-dependent manner with maximal effect occurring at 10 ng/ml (Fig. 5), at which concentration ~10% of the cells had undergone apoptosis by 6 days. This corresponded to a more than 8-fold increase in apoptotic cells compared with controls (Fig. 5).

When adherent and detached cells were analyzed separately, it was clear that a lower proportion of the adherent cells was apoptotic compared with their nonadherent counterparts (Fig. 5). This may indicate that reduction in cell adhesion possibly plays a role in the induction of apoptosis.

**DNA Microarray Analysis of MIC-1-induced Changes in DU-145 Gene Expression Profile.** To understand, on a more global basis, the alterations that MIC-1 was inducing in DU-145 cells, its effects on a large proportion of the transcriptome were assessed using cDNA microarrays. A set of cDNA microarray slides with 19k cDNA clones purchased from Ontario Cancer Institute were hybridized with cDNA synthesized from RNA isolated from cells treated with MIC-1 or control. About 30% of the cDNA clones showed signals at least 2-fold higher than background. At 1 and 3 h after treatment with 10 ng/ml...
MIC-1, only MT1E (GenBank accession number H93127) consistently showed a difference of >2-fold in gene expression. MIC-1 decreased MT1E mRNA by 0.41. Several other genes were downregulated by MIC-1 treatment by just under 2-fold. These included expressed sequence tags, highly similar to SMHU1B, human metallothionein 1B (0.52; GenBank accession number R99207); 60S acidic ribosomal protein P1 (0.54; GenBank accession number R99207); metallothionein 1H (0.55; GenBank accession number R99207); and other expressed sequence tags, similar to RNA helicase-related protein (0.55, GenBank accession number R99207).

Microarray data showed more gene expression changes after 9 h of MIC-1 treatment. At this time point, MIC-1 increased gene expression of complement component 5 (GenBank accession number 114869) by 2.3-fold and decreased gene expression of THO2 (GenBank accession number AA044429) by 0.48, RhoE (GenBank accession number N35505) by 0.47, integrin α3 (GenBank accession number H41926) by 0.46, and catenin δ1 (GenBank accession number T95499) by 0.46.

Real time RT-PCR of total RNA from three separate experiments at each time point was used to validate selected microarray data. Specific primers for MT1E, complement component 5, THO2, RhoE, integrin α3, and catenin δ1 were designed and synthesized. Real-time RT-PCR confirmed that MIC-1 treatment for 1 h decreased MT1E gene expression by 0.369 ± 0.050 (P < 0.05) and 3 h MIC-1 treatment by 0.423 ± 0.030 (P < 0.05). At 9 h after treatment, MIC-1 decreased catenin δ1 expression by 0.348 ± 0.071 (P < 0.05) and RhoE expression by 0.463 ± 0.026 (P < 0.05). THO2 mRNA was decreased by MIC-1 to a lesser extent, 0.734 ± 0.08, whereas a decrease of integrin α3 mRNA and an increase of complement component 5 mRNA could not be confirmed in RNA samples not used in microarray experiments. One possible reason for the inconsistency was contamination of cDNA clones on the microarray slides. Inconsistency is known to be an issue with DNA microarrays, and this is largely thought to be attributable to quality control issues associated with their preparation (24). For example, ~38% of cDNA clones from IMAGE Consortium, which was one of the main suppliers of cDNA clones for Ontario Cancer Institute microarray slides, were proved to be contaminated (25).

**DISCUSSION**

This study shows that MIC-1 secretion is markedly increased in the malignant prostate LNCaP and PC-3 cell lines compared with malignant DU-145 and nonmalignant PZ-HPV-7 prostate cell lines. The reason why the brain-derived metastatic prostate cancer cell line DU-145, unlike lymph node- and bone marrow-derived PC-3 and LNCaP cells, did not produce a detectable amount of MIC-1 is unknown. However, overall, these observations are similar to those for secretion of TGF-β1 superfamily members such as TGF-β1, TGF-
\[ \beta_2 \text{ and activin, which are produced in both malignant and nonma-} \]
\[ \text{lignant prostate epithelial cell lines as well as in human prostatic} \]
\[ \text{tissues (26). However, malignant cells produce higher levels than their } \]
\[ \text{normal counterparts (26). Our data, as well as already published} \]
\[ \text{reports, suggest that MIC-1 falls into the same pattern. MIC-1 protein} \]
\[ \text{is overexpressed in colon and prostate cancer tissues compared with} \]
\[ \text{normal neighboring areas (12, 13), and MIC-1 is one of the most} \]
\[ \text{significantly overexpressed genes in prostate cancer tissues compared} \]
\[ \text{with normal prostate (14).} \]

MIC-1 secretion in hormone-sensitive LNCaP cells is down-regulated by both androgen and estrogen. Although the presence of estrogen receptors in LNCaP cells has been reported, LNCaP cells contain a mutated androgen receptor, which is capable of responding to estrogen and adrenal steroids as well as androgen (27, 28). Therefore, it is likely that in these cells, estrogen is reducing MIC-1 production through the same receptor and signal pathway as androgen. Because androgens are an important factor in prostate cancer development and progression, the interaction between MIC-1 and hormones may well be important and might even be part of the mechanism by which androgens exert their effects in this disorder.

MIC-1, unlike TGF-\( \beta \), did not have an effect on the proliferation of Mv1Lu epithelial and various prostate epithelial tumor cell lines. In the case of the former, this is contradictory to a previous report, which used partially purified MIC-1 protein from conditioned medium from DLD-1 colon tumor cells transfected with a MIC-1-expressing plasmid. In this construct, MIC-1 was Flag tagged at the COOH terminus. However, in TGF-\( \beta \) superfamily proteins, the COOH terminus is buried in the dimer interface, and hence addition of a Flag tag in this location may well result in the expression of misfolded MIC-1. Our data, however, are consistent with a recent report that LN-Z308 glioblastoma cells and primary neuronal cells do not show growth inhibition by MIC-1 (19), whereas glioblastoma cells could be growth inhibited by TGF-\( \beta \). Because MIC-1 does not inhibit cell proliferation, it is likely to have a different receptor activation and/or signal pathway from that of TGF-\( \beta \).

This study further supports existing data (11, 16, 18) that MIC-1 is proapoptotic in at least some epithelial cancer cell lines. However, this proapoptotic effect may be secondary to altered adhesion of DU-145 cells. It was strikingly obvious that prior to undergoing apoptosis, MIC-1-treated DU-145 cells showed significant morphological changes, followed by reduced cell-cell adhesion and finally cell detachment. Because cell detachment preceded apoptosis, it is likely that cell detachment, at the very least, contributed to the development of apoptosis. If a similar loss of adhesion was stimulated by MIC-1 in vivo, it would suggest that MIC-1 might facilitate cancer cell detachment, migration, and metastasis.

Although MIC-1 clearly reduced DU-145 cell adhesion and induced cell apoptosis, these processes seemed to be confined to a small proportion of the total cell population (<10%). The small number of these apparently responsive cells may relate to particular cell-cell and cell-matrix contacts or a particular stage of the cell cycle. This is supported by the finding that morphological changes, cell detachment, and apoptosis occurred at the periphery of large clusters of cells and in cells in very small clusters.

Whatever the reason was for the effect being observed only in a minority of cells would significantly decrease the capacity of any microarray-based strategy to detect affected gene transcripts. This may help explain why only a limited number of altered gene transcripts were detected. However, the changes noted in MT1E, catenin \( \delta 1 \), and RhoE gene expression levels are remarkably consistent with the functional and morphological data described and suggest likely mechanisms.

MT1E is the only metallothionein family member that is expressed in estrogen receptor-negative cells but not in estrogen receptor-positive cells (29). Metallothionein has been documented extensively to protect cells from apoptosis and reactive oxidative stress by stabilizing DNA binding of nuclear factor-xB and inhibiting caspase activation (30, 31). Catenin \( \delta 1 \) and RhoE are both related to cadherin. Cadherins are cell-cell adhesion receptors essential for the establishment of the epithelial cell shape, maintenance of the differentiated phenotype, and formation of adherence junctions between cells (32). Inhibition of endogenous Roh in epithelial cells dismantles adherence junctions by removing cadherin receptors and decreasing cell-cell adhesions and actin accumulation at the site of cell contact (32). RhoE
especially has been suggested to have a role in epithelial cell adhesion—
because of its cellular localization at the lateral membranes of these
cells (33). Catenin δ1 localizes at both cellular junctions and the
nucleus. Similar to other catenins, catenin δ1 interacts with cadherin
at intercellular junctions. Although most results suggest that it anchors
cadherin complex to the actin cytoskeleton and enhances cell-cell
adhesion and intercellular junctions, there has also been evidence
indicating that it inhibits anchoring under certain experimental condi-
tions (34).

In summary, MIC-1 is overexpressed in malignant PC-3 and
LNCaP cells but not in nonmalignant PZ-HPV-7 and malignant DU-
145 cells. The production of MIC-1 in hormone-sensitive LNCaP cells is
down-regulated by both androgen and estrogen. Although MIC-1
does not inhibit proliferation of prostate epithelial cancer cells, it
induces caspase-dependent apoptosis that, at least in part, is likely to
be attributable to down-regulation of MT1E gene expression. Further-
more, MIC-1 reduces cell-matrix and cell-cell adhesion, at least partly
through decreasing RhoE and catenin δ1 gene expression. Although
cell detachment may partly contribute to apoptosis in vitro, it does not
always do this in vivo, where detachment is necessary for migration,
which in cancer often leads to metastasis in vivo. Additional studies of
the interaction of hormones and MIC-1, and the effect of MIC-1 on
prostate cancer metastasis in vivo, will provide important information
for the management of prostate cancer.

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