Expression of Macrophage Inhibitory Cytokine-1 in Benign and Malignant Prostatic Tissues: Implications for Prostate Carcinogenesis and Progression of Prostate Cancer


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Abstract: The aim of the study was to evaluate the expression of Macrophage Inhibitory Cytokine-1 (MIC-1) in benign and malignant prostate tissues and to associate its expression with clinicopathological parameters of prostate cancer. Immunohistochemical analysis of MIC-1 expression was performed on 21 benign prostatic hyperplasia (BPH), 21 prostatic intraepithelial neoplasia (PIN) and 31 prostate cancer (PCa) tissues. Expression was semiquantitatively scored by assessing both the percentage and intensity of positive staining cells. Expression levels were compared in different lesions and relations between MIC-1 expression with Gleason's grade, stage, serum MIC-1 and prostate specific antigen (PSA), measured by enzyme-linked immunosorbent assay, were investigated. Significantly higher immunostaining scores in LGPIN, HGPIN and PCA compared to BPH (p = 0.004, 0.001, <0.001, respectively) were detected. Much higher MIC-1 overexpression levels in PCA (92%), LGPIN (76.9%), LGPIN (75%) were observed than BPH (38.1%). High tissue MIC-1 expression scores were significantly associated with high Gleason grades and advanced stages. Serum MIC-1 was significantly higher in PCa patients, when compared to BPH patients and control (p<0.001). A highly significant correlation was found between tissue and serum MIC-1 in PCa cases (r = 0.713, p<0.001). These data emphasize the differential expression of MIC-1 during prostate cancer development and progression. Its upregulation from benign to malignant prostate lesions and in aggressive and advanced prostate cancer suggests that MIC-1 should be evaluated as a potential diagnostic and prognostic marker in prostate cancer.

Keywords: MIC-1, PSA, prostate lesions, immunohistochemistry, serum

INTRODUCTION

Prostate cancer is a serious health concern. Among men, cancers of the prostate, lung and colon account for about 54% of all newly diagnosed cancers while prostate cancer alone constituting about 29%of incident cases in men (Jemal et al., 2007). Very high proportion of elderly men develop prostate cancer with either clinically significant or insignificant disease (Stamatouli et al., 2007).

Despite the tremendous progress in the diagnosis and management of prostate cancer in the recent years, there is a requirement for new diagnostic markers that can accurately discriminate between indolent and aggressive variants of PCa. The underlying molecular
mechanisms that involved in prostate cancer development and progression are less clear. A better molecular understanding of the early developmental stages of prostate carcinogenesis is of critical importance. The molecular characterization of tumor cells through gene microarray studies have provided a unique opportunity to identify molecules associated with the development and progression of various cancers and offered a unique opportunity to developing potential therapeutic interventions that are aimed to selectively target specific genes and gene products (Welsh et al., 2001; Karan et al., 2002). One of the identified genes, macrophage inhibitory cytokine-1 (MIC-1), exhibited a major upregulation in prostatic cancer (Welsh et al., 2001; Igawa et al., 2002; Nakamura et al., 2003).

Several factors are involved in the pathogenesis of prostate cancer. Exposure to environmental factors or infectious agents might trigger the inflammatory states that may account for up to 20% of all human cancers (Ames et al., 1995). Inflammation and a proinflammatory microenvironment make important contributions to tumor development. MIC-1 may provide a potential link between inflammation and prostate cancer (Karan et al., 2009).

The MIC-1 gene, a divergent member of the Transforming growth factor- β (TGF- β) superfamily originally identified in the setting of macrophage activation, is located on chromosome 19 pl3.11 (Bootcov et al., 1997; Lawton et al., 1997). It has subsequently been reported under a wide variety of other names including Growth/differentiation factor 15 Precursor (GDF-15), Placental bone morphogenetic protein (PLAB), Placental transformation growth factor-β (PTGFB), Prostate differentiation factor and NSAID-activated gene 1 protein (NAG-1) (Bootcov et al., 1997; Hromas et al., 1997; Lawton et al., 1997; Paralkar et al., 1998; Bottner et al., 1999; Thomas et al., 2001; Eling et al., 2006). Its protein is synthesized as a 60 kDa dimer which is cleaved by furin-like proconvertases from its propeptide to release a 25 kDa mature protein (Bauskin et al., 2000, 2005). In tumors and tumor cell lines, MIC-1 is frequently secreted in an unprocessed, propeptide-containing form. This remains localized in tissues due to strong matrix binding mediated by its propeptide. Only processed mature MIC-1 diffuses into the systemic circulation (Bauskin et al., 2005).

Although several of its biological functions were described by Bootcov et al. (1997), Hromas et al. (1997), Paralkar et al. (1998) and Kempf et al. (2006), its principal functions, specific receptor, regulation of its expression are not well characterized nor is the molecular mechanism controlling its functions.

Under resting conditions, epithelial cells in a wide variety of organs express low amounts of MIC-1 mRNA. Thus, MIC-1 protein is difficult to detect by immunohistochemistry except in central nervous system epithelial, such as the choroid plexus and ependyma and placenta which express large amounts of MIC-1 (Bauskin et al., 2006).

Increased MIC-1 expression is a common feature of malignancy. Several studies reported a major upregulation of MIC-1 mRNA and protein in cancer biopsies including breast, colon, gastric, pancreatic and prostate cancers (Buckhaults et al., 2001; Welsh et al., 2001, 2003; Baek et al., 2009). The role of MIC-1 in cancer has been described to be of dual nature where both tumor promoting and inhibiting effects have been reported by Eling et al. (2006). It induces invasiveness (Lee et al., 2003) and tumor cells proliferation (Wollmann et al., 2005; Chen et al., 2007; Kim et al., 2008) where high MIC-1 tumor expression appeared to occur in parallel with the tumor stage, extent of metastasis and aggressive growth (Nakamura et al., 2003, Chen et al., 2007; Baek et al., 2009). Paradoxically, a number of studies reported an antitumorigenic function for MIC-1, by which it induces apoptosis and inhibits the proliferation of several tumor cell lines (Li et al., 2000; Tan et al., 2000, Albertoni et al., 2002).
Previous molecular studies revealed an up-regulation of MIC-1 in the prostatic cancer cells (Cheung et al., 2004; Nakamura et al., 2003; Igawa et al., 2002; Welsh et al., 2001). Its role in prostate cancer biology is unclear. On the one hand, MIC-1 may act as a paracrine and autocrine factor for the abnormal proliferation of androgen receptor-positive prostate cancer cells (Chen et al., 2007) and tumor dissemination through its reductive effect on cell adhesion (Liu et al., 2003). On the other hand, growth arrest in DU-145 human prostate carcinoma cells (Tan et al., 2000) and apoptosis promoting effect in MIC-1 treated prostate cancer cells were reported (Liu et al., 2003).

In order to examine the expression status of MIC-1 protein in prostate cancer and to clarify its potential connection with prostate cancer tumorigenesis and progression, we used immunohistochemistry (IHC) and conducted MIC-1 protein expression analyses in paraffin-embedded tissue specimens of benign prostatic hyperplasia (BPH), Prostate Intraepithelial Neoplasias (PIN) and prostate cancer (PCa). We also studied serum MIC-1 levels in BPH and PCa patients. Further, we evaluated the presence of possible correlation between MIC-1 expression level and tumor Gleason's grade and clinical stage, serum MIC-1 and pretreatment serum total PSA.

MATERIALS AND METHODS

Patients and Tissue Specimens

A total of 52 patients were enrolled in this study between years 2007-2009, who were referred to the Department of Urology, Minia University Hospital. Of these patients, 21 cases with BPH and 31 prostate cancers with varying tumor stages and Gleason scores. Evaluation of 8 LGPIN and 13 HGPIN lesions seen associating invasive prostate cancers were included in this study.

The patients age ranged from 43-75 years (58±8.55 mean years). Histological material was obtained by transurethral resections of the prostate (TUR-P) or represented a diagnostic biopsy. Initial sections were stained with Hematoxylin and Eosin (H and E) for pathological diagnosis and grading.

Regarding Carcinoma cases, specimens were histologically graded according to the Gleason grading system. The criteria used for Gleason grading were those used in standard clinical practice (Epstein et al., 2004). HGPIN and LGPIN were identified according to the features defined by Sakr et al. (2004). CaP cohort (n = 31 patients) was stratified further into three groups based on patients with Gleason scores ≤4 (n = 7 (22.6%) patients, Gleason scores of 5-6 (n = 13 (41.9%) patients and Gleason scores ≥7 (n = 11 (35.5%) patients. As the patients were not treated with radical prostatectomy, they were clinically and radiologically staged, according to the TNM classification of carcinomas of the prostate (Epstein et al., 2004), into T1, n = 3 (9.7%) T2, n = 9 (29%); T3, n = 11 (35.5%) and T4, n = 8 (25.8%) patients.

Blood Sample

A control group formed of 20 apparently healthy males with matched age was included to compare the levels of serum MIC-1 and PSA within control group, BPH and PCA cases.

All blood samples were drawn at least 3 to 4 weeks after an earlier prostatic manipulation and centrifuged within 2 to 3 h after sampling. The serum samples were stored at -70°C. MIC-1 was measured using the human GDF-15 DuoSet ELISA Development kit (R and D Systems, Inc., Minneapolis, MN) and PSA serum concentration was determined using a sensitive immunoassay (Teco diagnostic CA, USA).

Pretreatment serum PSA (ng mL⁻¹) levels were determined at Minia University Hospital Laboratories. The patients did not receive any hormonal or other therapy before surgery.
The study protocol was approved by The Ethics Committee of Minia University Hospital that approved the use of these tissues and blood samples for research purposes.

**Immunohistochemistry**

Four μm-thick sections from archival paraffin-embedded tissues were cut, placed on poly-L-lysine coated slides, de-paraffinized with xylene and rehydrated through graded alcohol. Endogenous peroxidase activity was blocked by incubation with 0.3% Hydrogen peroxide/Methanol for 30 min. Antigen retrieval was achieved by microwave treatment, where the slides were placed in sodium citrate buffer (0.01 M, pH 6.0) for 10 min. A rabbit polyclonal antibody against MIC-1 was used (dilution of 1:200, Sigma). The sections were incubated with antibody overnight at 4°C. Thereafter, a biotinylated secondary antibody was applied to sections for 30 min at room temperature. Visualization of the reaction was performed with an avidin-biotin complex immunoperoxidase system using 3, 3 diaminobenzidine as a chromogen. Finally, the slides were counterstained with Mayer’s haematoxylin, dehydrated in graded alcohol, cleared in xylene and mounted with DPX.

**Positive and Negative Control**

Each staining batch included both positive and negative control sections. Negative control sections were treated with phosphate-buffered saline (PBS) instead of primary antibody. Sections of placental tissue were used as positive control.

**Scoring System**

The level of MIC-1 protein expression was evaluated using a semi-quantitative scoring system which was performed according to Karan et al. (2003).

Each slide was evaluated for both the intensity of the staining and the percentage of positive cells. The intensity and percentage of immunoreactivity were assessed independently by 2 pathologists (HMT and DMA). The correlation between them was high and when discrepancies existed, a consensus was achieved by the two observers evaluating the sections together. A final percentage of positively stained cells was calculated by averaging the lesional percent positivity across the section representing each case. The extent of the staining scored as follows: <25% of tumor cells stained (1); 25-50% of the tumor cells stained positive (2); 50-75% of the tumor cells stained positive (3) and >75% of the tumor cells stained positive (4). Final intensities of epithelial staining were similarly calculated by averaging the scores across the section representing each patient. Staining intensity was graded on 0 to 3 scale i.e., 0 for no staining, 1+ for weak immunoreactivity, 2+ for moderate immunoreactivity and 3+ for strong immunoreactivity. The composite score was calculated by multiplying the two values (intensity score X percentage score) yielding an overall score range from 0 to 12 for each specimen. In this manner, we were able to differentiate the specimens that may have had focal areas of increased staining from those that had diffuse areas of increased staining.

**Statistical Analysis**

Statistical analyses were done using the SPSS program version 11.0. Raw data were compiled and used to determine means, standard deviations (SD) and ranges of different variables. Analysis of variance (ANOVA) was employed to determine the p-values among mean measurements in order to detect differences exist among the MIC-1 means of expression in different lesions. Student’s t-test (McDonald, 2009) was conducted to compare means for two groups of cases. Correlation between MIC-1 expression and serum PSA levels was carried out using Spearman’s rank correlation test. p = 0.05 was considered significant.
RESULTS

Expression Level of MIC-1 in Prostatic Specimens

Overall, MIC-1 immunostaining was predominantly epithelial with cytoplasmic localization. Weak stromal expression was observed in some cases of PCa.

The distribution of positive immunostaining showed higher proportional immunostaining in LGPIN 6/8 (75%) and HGPIN 10/13 (76.9%) and PCa 28/31 (92.3%) compared to BPH 8/21 (38.1%) cases. Low to moderate expression scores was noticed in 38.1% of BPH specimens (Fig. 1a). On the other hand, higher score levels were detected in PIN (Fig. 1b) and in the great majority of prostate cancers (Fig. 1c, d).

Table 1 showed that immunoreactivity for the MIC-1 was significantly higher in prostatic adenocarcinomas as compared to benign prostatic hyperplasia tissue (p=0.001). The increase in MIC-1 immunostaining in LGPIN and HGPIN compared to BPH was statistically significant (p = 0.004, 0.001, respectively) as was the increase from HGPIN to prostate cancer (p = 0.004). No statistically significant difference in MIC-1 expression between LGPIN and HGPIN was found (p = 0.781) (Fig. 2a).

Association of MIC-1 Tissue Expression with Clinicopathological Parameters

Further, we tested whether the level of MIC-1 expression is related to clinicopathological parameters (Gleason's score and clinical stage). Overall, ANOVA identified significant differences in MIC-1 expression scores among tumors with different Gleason's scores and clinical stages (p<0.001). MIC-1 immunostaining was strongly associated with high Gleason grade and advanced tumor stage. Table 2 showed that statistically significant lower expression in tumors with Gleason scores ≤4 compared to those with Gleason scores 5-6 and ≥7 (p = 0.004, p<0.001, respectively). In Gleason score 5,6 tumors, the mean MIC-1 expression

![Image](image_url)

Fig. 1: Immunohistochemical staining of MIC-1: (a) weak expression in BPH, (b) high expression scores in PIN, (c, d)high expression scores in prostate cancer
Table 1: MIC-1 expression in BPH, PIN and PCs

<table>
<thead>
<tr>
<th>Tissue specimen</th>
<th>Positive staining (%)</th>
<th>Staining (Mean±SD)</th>
</tr>
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<tbody>
<tr>
<td>BPH</td>
<td>8/21 (38.1)</td>
<td>1.61±2.24</td>
</tr>
<tr>
<td>LGPIN</td>
<td>6/8 (75)</td>
<td>5.37±4.24</td>
</tr>
<tr>
<td>HGPIN</td>
<td>10/13 (76.9)</td>
<td>5.92±4.38</td>
</tr>
<tr>
<td>PCA</td>
<td>28/31 (92.3)</td>
<td>8.70±3.90</td>
</tr>
</tbody>
</table>

Table 2: Tissue and serum MIC-1 mean of expression in relation to Gleason score and clinical stage of the PCs

<table>
<thead>
<tr>
<th>Results</th>
<th>Positive (%)</th>
<th>Staining score (Mean±SD)</th>
<th>Serum MIC-1 concentration (pg mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gleason’s score</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤4</td>
<td>5/7 (71.4)</td>
<td>4.00±3.31</td>
<td>1078.57±1041.57</td>
</tr>
<tr>
<td>5-6</td>
<td>12/13 (92.3)</td>
<td>9.30±5.47</td>
<td>1583.46±505.96</td>
</tr>
<tr>
<td>≥7</td>
<td>11/11 (100)</td>
<td>11.39±1.73</td>
<td>2141.81±870.88</td>
</tr>
<tr>
<td>Stage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T1</td>
<td>1/3 (33.3)</td>
<td>2.00±3.46</td>
<td>600.00±849.52</td>
</tr>
<tr>
<td>T2</td>
<td>8/9 (88.9)</td>
<td>6.77±4.08</td>
<td>1481.14±1034.08</td>
</tr>
<tr>
<td>T3</td>
<td>11/11 (100)</td>
<td>10.45±1.80</td>
<td>1578.63±595.99</td>
</tr>
<tr>
<td>T4</td>
<td>8/8 (100)</td>
<td>11.00±1.85</td>
<td>2475.00±881.15</td>
</tr>
</tbody>
</table>

Fig. 2: MIC-1 expression box plots (a) MIC-1 expression scores in different prostatic lesions. (B) Serum MIC-1 concentration in different groups. Horizontal lines in the boxes represent the median value of each group. The top and bottom edges of the boxes indicate the score values from the 75th and the 25th percentile, respectively. Whiskers represent the highest and lowest values. The range is shown as a vertical line.

score was lower than that of Gleason scores ≥7 however, without statistically significant difference (p = 0.15). A significantly higher MIC-1 scores were found in T3 tumors compared to both of T1 and T2 tumors (p<0.001, p = 0.015, respectively). Also, Significant differences were found between T4 tumors and both of T1 and T2 tumors (p<0.001, p = 0.017 respectively). No significant differences were seen between T3 and T4 tumors (p = 0.52) and between T1 and T2 tumors (p = 0.10).

Serum Levels of MIC-1 in Control, BPH and PCs

Significant differences were found among different groups (Fig. 2b). Serum level of MIC-1 was significantly higher in PCs patients (1667.58±982.13 pg mL⁻¹), when compared to BPH patients (607.61±358.64 pg mL⁻¹) and normal individuals (310.25±204.95 pg mL⁻¹) p<0.001. There was a significant increase in MIC-1 level in BPH when compared to the control group (p = 0.002).
Association of Serum MIC-1 Concentration with Clinicopathological Parameters

Overall, ANOVA test showed no significant differences in serum MIC-1 concentration among tumors with different Gleason's scores (p = 0.07). Regarding tumor clinical stage, significant differences (p < 0.015) were noticed among different stages (Table 2). Significantly higher MIC-1 scores between T4 tumors and T3, T2 and T1 tumors (p = 0.017, 0.050 and 0.027, respectively).

Serum Total PSA levels in Control, BPH and PCa

The mean serum levels of PSA were 1.17±0.45 ng mL⁻¹ and 2.32±0.92 ng mL⁻¹ in control group and BPH, respectively. In PCa cases, PSA levels ranged from 3-212 ng mL⁻¹ (Mean±SD 52.43±46.65). ANOVA test identified highly significant differences among different groups (p<0.001).

Correlations Between Tissue MIC-1 Expression, Serum MIC-1 and PSA Levels in PCa Cases

A highly significant correlation was found between tissue and serum MIC-1 in PCa cases (r = 0.713, p<0.001). Also, significant positive correlations was found between tissue MIC-1 expression and serum PSA levels (r = 0.469, p = 0.008). A positive correlation of borderline significance was found between Serum MIC-1 and PSA (r = 0.340, p = 0.06).

DISCUSSION

Prostate cancer develops as a progression from normal epithelium through a series of progressively dysplastic lesions: low grade prostatic intraepithelial neoplasia, high-grade prostatic intraepithelial neoplasia and invasive prostate cancer (Bostwick, 1989).

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. Identification of molecules associated with carcinogenesis, tumor growth, invasion and metastasis has been critical to developing potential therapeutic interventions.

Increased MIC-1 expression has been documented in a variety of epithelial cancer cell lines (Liu et al., 2003) and several studies showed MIC-1 markedly increased in gastric, prostate, breast, colorectal cancers and melanoma (Welsh et al., 2001, 2003; De Wit et al., 2005; Baek et al., 2009). Patients with metastatic prostate, breast and colorectal cancers showed significantly elevated levels of serum MIC-1 (Welsh et al., 2003).

Protein profiling on microdissected samples of matched normal prostate tissue, HGPIN and PCa revealed MIC-1 expression in HGPIN and in cancer cells but not in normal prostate tissue (Cheung et al., 2004).

Present immunohistochemical and ELISA results showed significantly higher MIC-1 levels in prostate cancer tissue than that in benign tissue. Similar results were reported in prostatic tissue using microarray technology (Welsh et al., 2001), quantitative RT-PCR (Nakamura et al., 2003) analysis and immunohistochemistry (Chien et al., 2007). This was also reported in other cancers such as colon cancer, where a significant increase in serum MIC-1 levels with disease progression from normal to adenoma and carcinoma was seen (Brown et al., 2003). Therefore, the use of serum MIC-1 measurement could improve the detection of prostate cancer and could potentially lead to significant decrease in unnecessary prostate biopsies.
On the other hand, Brown et al. (2006) reported significantly lower MIC-1 serum levels in PCA cases. This was surprising however, they attributed their finding to the probable increased binding of MIC-1 to local extracellular matrix of prostate tissue which leads to decrease in serum MIC-1 levels.

We found that MIC-1 protein expression was either absent or weakly expressed in BPH. However, MIC-1 protein was more highly expressed in PIN, the putative precursor of invasive PCA. Rasiah et al. (2006) also, reported higher levels of MIC-1 protein and RNA in the earliest stages of prostate cancer development Rasiah et al. (2006) suggesting that up-regulation of MIC-1 is an early event in the genesis of prostate cancer.

Keeping up with previous studies (Ashida et al., 2004; Cheung et al., 2004), we noticed significantly higher MIC-1 expression levels in PCA compared to LGPIN and HGPIN cases. Our data provide further validation supporting previous finding, implicating a potential role for MIC-1 in the pathogenesis of prostate cancer and raise the possibility to use increased MIC-1 expression level in the prostate samples as a marker to predict the potential onset and development of prostate cancer.

Although there is a strong correlation between MIC-1 expression and epithelial tumors, less is currently known regarding its role and the signaling pathways by which it exerts its functions. The effects of MIC-1 can sometimes be apparently contradictory and in differing circumstances, MIC-1 can exhibit antitumorigenic and tumorigenic functions. Some studies suggested an antitumorigenic role for MIC-1 where MIC-1 induced apoptosis via both p53-dependent and p53-independent mechanisms (Li et al., 2000; Tan et al., 2000; Albertoni et al., 2002; Liu et al., 2003). While others (Chen et al., 2007; Kim et al., 2008) provided evidence for its tumorigenic role where MIC-1 operate as a mediator of tumor progression and as a positive regulator of tumors via the ERK1/2 signal pathway in androgen receptor (AR)-positive prostate cancer (Chen et al., 2007) and via PI3K/Akt/mTOR and ERK1/2 signaling pathways in certain ErbB2-overexpressing tumors, such as breast and gastric cancers (Kim et al., 2008). Interestingly, ErbB2 gene amplification (Ali, 2005) and protein over expression (Hernes et al., 2004) were reported in prostate cancer. Furthermore, MIC-1 reduced cell-matrix and cell-cell adhesion and induced cell detachment partly through decreasing RhoE and catenin gene expression in prostate cancer cells (Liu et al., 2003).

In this study, we found that the great majority of prostate cancer cases showed high tissue MIC-1 expression score and serum levels that were significantly associated with aggressive features of the tumors. Higher Gleason's score and advanced cancers showed significantly higher tissue MIC-1 scores compared to those with lower Gleason's score and earlier clinical stages. These findings are in line with a previous study assessed the quantitative expression of MIC-1 mRNA using Quantitative RT-PCR (Nakamura et al., 2003). They reported a significantly higher expression of MIC-1 mRNA in higher Gleason score tumors. Also, a previous immunohistochemical study reported high MIC-1 expression scores in 100% of high grade Gleason score tumors (Karan et al., 2003). Interestingly, MIC-1 mRNA level in benign prostatic tissue seemed to reach that in paired prostate cancer tissue simultaneously with the increasing Gleason score (Patrikainen et al., 2007). These data connect MIC-1 expression to prostate cancer progression and support its tumorigenic role in prostate cancer.

We also found an increase in serum MIC-1 values in relation to tumor clinical stage and Gleason's score. A finding that was statistically significant with clinical stage only. Serum MIC-1 was an independent marker of the presence of PCa and tumors with a Gleason score of ≥7 (Brown et al., 2006) and showed a striking correlation with the metastatic progression of tumors (Brown et al., 2003; Welsh et al., 2003) and advanced prostate cancer together with presence of bone metastases (Selander et al., 2007).
The association of MIC-1 expression with tumor progression has been recently reported in other tumors such as gastric cancer (Baek et al., 2009) and melanoma (Boyle et al., 2009).

In addition to MIC-1 tumorigenic function seen in various tumors and tumor cell lines, acquired insensitivity to its apoptosis-inducing effects may be developed owing to genetic alterations associating tumor progression. Similar insensitivity to the growth-inhibitory effects of other members of the transforming growth factor-β family has been detected in various cancer cells and this effect has been attributed to mutations in their receptors (Zhang et al., 2005; Schiemann et al., 2004; Kim et al., 2008). This will be elucidated once the currently unknown cellular receptor for MIC-1 is characterized.

On the other hand, others suggested an anti-tumorigenic role for MIC-1 in prostate cancer where decreased expression was seen in higher Gleason grade cancer (Rasiah et al., 2006). Being a p53 target (Li et al., 2000; Yang et al., 2003), therefore, loss of p53 activation may explain the association of lower MIC-1 immunostaining with increasing Gleason grade with in this group of cases (Rasiah et al., 2006).

The studies on the biological role of MIC-1 in prostate cancer, however, are far behind and apparently conflicting. Such apparently contradictory effects of MIC-1 resemble that of TGF-β superfamily, which behaves as a tumor suppressor during the early stages of tumor development and a growth/metastasis enhancer as the tumor progresses to a malignant one (Dumont and Arteaga, 2003).

To the best of our knowledge, an explanation for this change in MIC-1 biological activity is not clearly understood. However, its contradictory effects in differing circumstances could be related to the nature of the tumor, tumor stage, tissue of origin and the interaction of the tumor with its local microenvironment (Bauskin et al., 2006). Variations in MIC-1 biological activity may also be related to different forms of MIC-1 that may vary according to tumor and tumor progression (Eling et al., 2006) and the intracellular processing of MIC-1 that ultimately controls the proportion of MIC-1 remaining localized in the tumor microenvironment and that diffusing into circulation (Bauskin et al., 2005, 2006).

The presence of MIC-1 in the tumor microenvironment can inhibit the secretion of tumor necrosis factor-α by activated macrophages, therefore, reducing the tumor killing activity of macrophages (Bootcov et al., 1997). These cells show a remarkable degree of plasticity during tumor progression with a switch in macrophage phenotypes occurring during tumor progression. The distinct role of macrophages in either tumor progression (macrophage phenotype: M2 or type II alternatively activated) or suppression (macrophage phenotype: M1 or classically activated) based on the influence of tumor microenvironment (Stout and Suttes, 2004, Bootcov, 2006).

Macrophages may play a key role in regulating the level of MIC-1 in the prostate. The tumorigenic function of MIC-1 could be modified by educating the macrophages (Karan et al., 2009). Targeting macrophages via Toll-Like Receptor (TLR) agonists (Vollmer, 2006; Bulthoirav et al., 2007) might regulate the physiologic environment leading to modulation of MIC-1 function for tumor disadvantage and tumor growth inhibition (Karan et al., 2009).

On studying the association between tissue and serum MIC-1 in PCa cases, highly significant correlation between both forms were found that may explain the dependency of processed MIC-1 on unprocessed MIC-1 levels.

Measurement of total serum PSA has been the most widely used tool for early detection, staging, grading and monitoring of PCa (Polascik et al., 1999; Nowroozi et al., 2009). Serum MIC-1 combined with prostate-specific antigen has been shown to improve the specificity of prostate cancer diagnosis (Brown et al., 2006). In the current study, we identified a significant positive correlation between MIC-1 expression and pretreatment serum total PSA.
levels. A previous cell line study (Chen et al., 2007) found that forced expression of MIC-1 induces PSA by LNCaP cell and reported a positive correlation between MIC-1 and secreted PSA by these cells. The mechanisms underlying the association between MIC-1 and PSA expression in prostate cancer is still not fully characterized. MIC-1 activates ERK1/2 pathway (Chen et al., 2007) which was shown to upregulate PSA secretion in human prostate cancer LNCaP C-81 cells (Lee et al., 2003). Therefore, MIC-1 may induce PSA secretion via ERK1/2 pathway activation.

In summary, we speculate that MIC-1 expression correlates with prostate carcinogenesis and positively associates adverse tumor characteristics. We have shown that MIC-1 protein expression increased from BPH to PIN and through different grades and stages of PCa associating high Gleason grade and advanced clinical stage tumors. Serum MIC-1 was markedly increased in PCa and was significantly associated with advanced stage. MIC-1 may possess prognostic utility and may be a promising molecular marker for diagnosis and monitoring of PCa. Our finding that significant correlation between MIC-1 tissue and serum levels exists warrants more investigation on a larger scale to study the combined expression of unprocessed MIC-1 staining of PCa biopsies and processed MIC-1 serum levels in order to identify their differential expression and functions in prostatic cancer.

A lot remains to be uncovered on the roles of MIC-1 in cancer and its biology. Thus, additional studies to define the roles of MIC-1 in tumor biology are clearly warranted. Further studies of signaling pathways of MIC-1 are needed for the elucidation its biological significance in association with PCa development and carcinogenesis and for prospective targeted therapy of prostatic cancer.

REFERENCES


