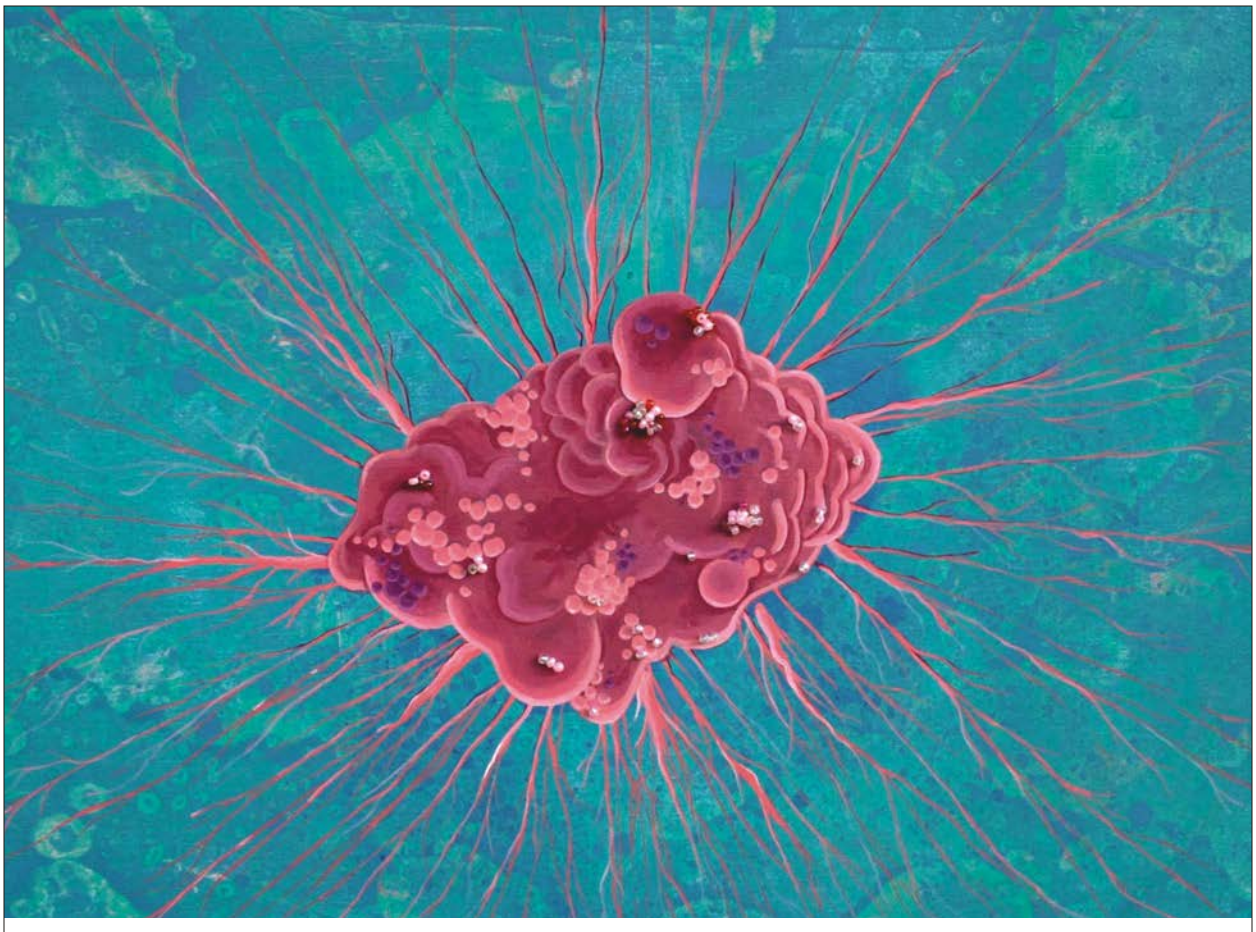

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Expression of Macrophage Inhibitory Cytokine-1 in Benign and Malignant Prostatic Tissues: Implications for Prostate Carcinogenesis and Progression of Prostate Cancer

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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%), HGPIN (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.

Key words: 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 (Stamatiou *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

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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 p13.11 (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 epithelium, 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' diamino-benzidine 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%), 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 (Gleasons 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 sores ≤ 4 compared to those with Gleason sores 5-6 and ≥ 7 ($p = 0.004$, $p<0.001$, respectively). In Gleason score 5,6 tumors, the mean MIC-1 expression

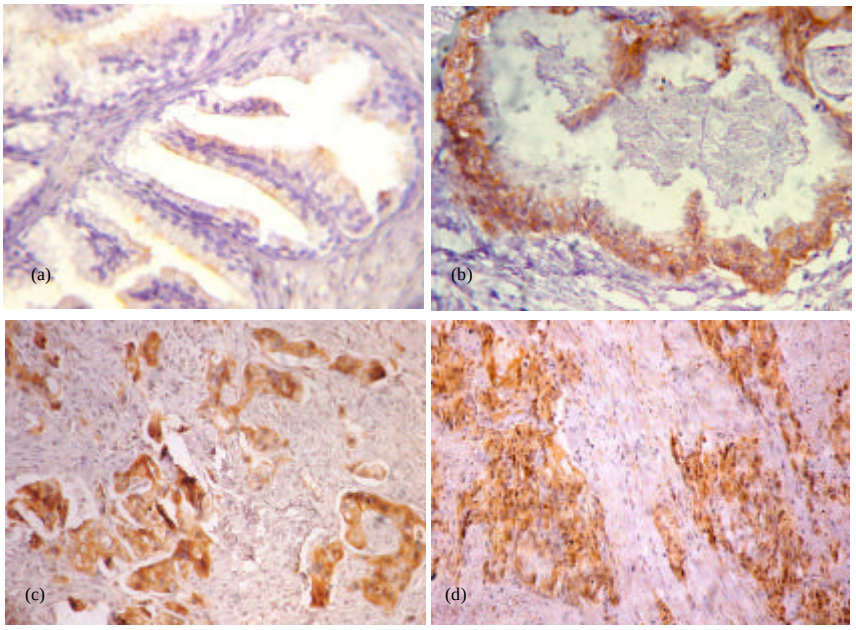

Fig. 1: Immunohistochemical staining of MIC-1: (a) weak expression in BPH, (b) high expression scores in PIN, (c, d)high expression scores in prostate cancer

Table 1: MIC-1 expression in BPH, PIN and PCa

Tissue specimen	Positive staining (%)	Staining (Mean±SD)
BPH	8/21 (38.1)	1.61±2.24
LGPIN	6/ 8 (75)	5.37±4.24
HGPIN	10/13 (76.9)	5.92±4.38
PCa	28/31 (92.3)	8.70±3.90

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

	Tissue MIC-1 expression		
Results	Positive (%)	Staining score (Mean±SD)	Serum MIC-1 concentration (pg mL^{-1})
Gleason's score			
≤4	5/7 (71.4)	4.00±3.31	1078.57±1041.57
5 -6	12/13 (92.3)	9.30±3.47	1583.46±905.96
≥7	11/ 11 (100)	11.39±1.73	2141.81±870.88
Stage			
T1	1/3 (33.3)	2.00±3.46	600.00±848.52
T2	8/9 (88.9)	6.77±4.08	1481.11±1034.08
T3	11/11(100)	10.45±1.80	1578.63±595.99
T4	8/8 (100)	11.00±1.85	2475.00±881.15

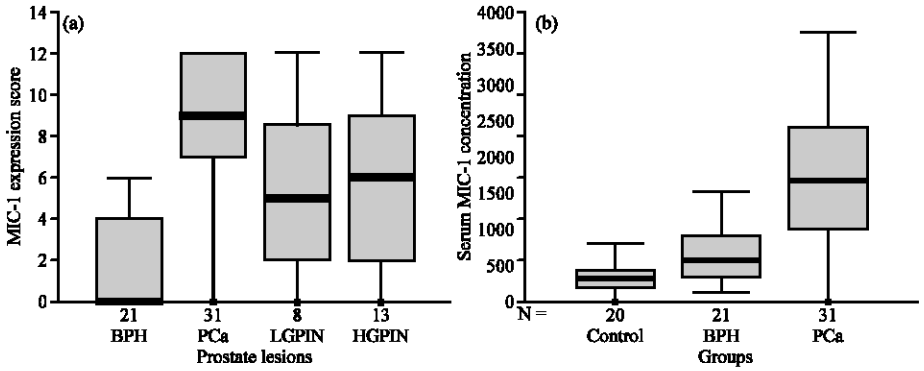

Fig. 2: MIC-1 expression box plots (a) MIC-1 expression scores in different prostatic lesions. (B) Serum MIC-1 concentration in different groups. Horizontal lines in the boxes represent the median value of each group. The top and bottom edges of the boxes indicate the score values from the 75th and the 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 PCaP

Significant differences were found among different groups (Fig. 2b). Serum level of MIC-1 was significantly higher in PCa 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 Gleasons 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 PCaP

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 ($r = 0.468$, $p = 0.008$). 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 ELIZA 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 (Chen *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 that 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 ERK-1/2 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 the current 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-1was 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*., 2003). 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 Suttles, 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; Buhtoiarov *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 identity 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

Albertoni, M., P.H. Shaw, M. Nozaki, S. Godard and M. Tenan *et al*., 2002. Anoxia induces macrophage inhibitory cytokine-1 (MIC-1) in glioblastoma cells independently of p53 and HIF-1. Oncogene, 21: 4212-4219.

Ali, S.M., 2005. Her2neu gene amplification in prostate cancer from Egyptian patients by fluorescence *in situ* hyberdization. Int. J. Cancer Res., 1: 5-9.

Ames, B.N., L.S. Gold and W.C. Willet, 1995. The causes and prevention of cancer. Proc. Natl. Acad. Sci. USA., 92: 5258-5265.

Ashida, S., H. Nakagawa, T. Katagiri, M. Furihata and M. Iiizumi *et al*., 2004. Molecular features of the transition from prostatic intraepithelial neoplasia (PIN) to prostate cancer: Genomewide gene expression profiles of prostate cancers and PINs. Cancer Res., 64: 5963-5972.

Baek, K.E., S.R. Yoon, J.T. Kim, K.S. Kim and S.H. Kang *et al*., 2009. Upregulation and secretion of macrophage inhibitory cytokine-1 (MIC-1) in gastric cancers. Clin. Chim. Acta, 401: 128-133.

Bauskin, A.R., H.P. Zhang, W.D. Fairlie, X.Y. He and P.K. Russell *et al*., 2000. The propeptide of macrophage inhibitory cytokine (MIC-1) aTGF-β superfamily member acts as a quality control determinant for correctly folded MIC-1. EMBO J., 19: 2212-2220.

Bauskin, A.R., D.A. Brown, S. Junankar, K.K. Rasiah and S. Eggleton *et al*., 2005. The propeptide mediates formation of stromal stores of PROMIC-1: Role in determining prostate cancer outcome. Cancer Res., 65: 2330-2336.

Bauskin, A.R., D.A. Brown, T. Kuffner, H. Johnen and X.W. Luo *et al*., 2006. Role of macrophage inhibitory cytokine-1 in tumorigenesis and diagnosis of cancer. Cancer Res., 66: 4983-4986.

Bootcov, M.R., A.R. Bauskin, S.M. Valenzuela, A.G. Moore and M. Bansal *et al*., 1997. MIC-1, a novel macrophage inhibitory cytokine, is a divergent member of the TGF- β superfamily. Proc. Natl. Acad. Sci. USA., 94: 11514-11519.

Bootcov, J.W., 2006. Distinct role of macrophages in different tumor microenvironments. Cancer Res., 66: 605-612.

Bostwick, D.G., 1989. Prostatic intraepithelial neoplasia (PIN). Urology, 34: 16-22.

Bottner, M., C. Suter-Crazzolara, A. Schober and K. Unsicker, 1999. Expression of a novel member of the TGF-beta superfamily, growth/differentiation factor-15/macrophage-inhibiting cytokine-1 (GDF-15/MIC-1) in adult rat tissues. Cell Tissue Res., 297: 103-110.

Boyle, G.M., J. Pedley, A.C. Martyn, K.J. Banducci and G.M. Strutton *et al*., 2009. Macrophage inhibitory cytokine-1 is overexpressed in malignant melanoma and is associated with tumorigenicity. J. Invest. Dermatol., 129: 383-391.

Brown, D.A., R.L. Ward, P. Buckhaults, T. Liu and K.E. Romans *et al*., 2003. MIC-1 serum level and genotype: Associations with progress and prognosis of colorectal carcinoma. Clin. Cancer Res., 9: 2642-2650.

Brown, D.A., C. Stephan, R.L. Ward, M. Law and M. Hunter *et al*., 2006. Measurement of serum levels of macrophage inhibitory cytokine 1 combinedwith prostate-specific antigen improves prostate cancer diagnosis. Clin. Cancer Res., 12: 89-96.

Buckhaults, P., C. Rago, B. St Croix, K.E. Romans and S. Saha *et al*., 2001. Secreted and cell surface genes expressed in benign and malignant colorectal tumors. Cancer Res., 61: 6996-7001.

Buhtoiarov, I.N. P.M. Sondel, J.C. Eickhoff and A.L. Rakhmilevich, 2007. Macrophages are essential for antitumour effects against weakly immunogenic murine tumours induced by class B CpG-oligodeoxynucleotides. Immunology, 120: 412-423.

Chen, S.J., D. Karan, S.L. Johansson, F.F. Lin and J. Zeckser *et al*., 2007. Prostate derived factor as a paracrine and autocrine factor for the proliferation of androgen receptor-positive human prostate cancer cells. Prostate, 67: 557-571.

Cheung, P.K., B. Woolcock, H. Adomat, M. Sutcliffe and T.C. Bainbridge *et al*., 2004. Protein profiling of microdissected prostate tissue links growth differentiation factor 15 to prostate carcinogenesis. Cancer Res., 64: 5929-5933.

De Wit, N.J.W., J. Rijntjes, J.H.S. Diepstra, T.H. van Kuppevelt and U.H. Weidle *et al*., 2005. Analysis of differential gene expression in human melanocytic tumour lesions by custom made oligonucleotide arrays. Br. J. Cancer, 92: 2249-2261.

Dumont, N. and C.L. Arteaga, 2003. Targeting the TGF beta signaling network in human neoplasia. Cancer Cell, 3: 531-536.

Eling, T.E., S.J. Baek, M. Shim and C.H. Lee, 2006. NSAID activated gene (NAG-1), a modulator of tumorigenesis. J. Biochem. Mol. Biol., 39: 649-655.

Epstein, J.I., F. Algaba, J.R. Allsbrook, S. Bastacky and L. Boccon-Gibod *et al*., 2004. Acinar Adenocarcinoma. In: Pathology and Genetics of Tumours of the Urinary System and Male Genital Organs, Eble, J.N., G. Sauter, J.I. Epstein and I.A. Sesterhenn (Eds.). IARC Press, Lyon, France, ISBN 10: 9283224159, pp: 359.

Hernes, E., S.D. Fossa, A. Brener, B. Otnes and J.M. Nesland, 2004. Expression of the epidermal growth factor receptor family in prostate carcinoma before and during androgen-independence. Br. J. Cancer, 90: 449-454.

Hromas, R., M. Hufford, J. Sutton, D. Xu, Y. Li and L. Lu, 1997. PLAB, a novel placental bone morphogenetic protein. Biochim. Biophys. Acta, 1354: 40-44.

Igawa, T., F.F. Lin, M.S. Lee, D. Karan, S.K. Batra and M.F. Lin, 2002. Establishment and characterization of androgen-independent human prostate cancer LNCaP cell model. Prostate, 50: 222-235.

Jemal, A., R. Siegel, E. Ward, T. Murraya, J. Xu and M.J. Thun, 2007. Cancer statistics 2007. CA: Cancer J. Clin., 57: 43-66.

Karan, D., D.L. Kelly, A. Rizzino, M.F. Lin and S.K. Batra, 2002. Expression profile of differentially-regulated genes during progression of androgen-independent growth in human prostate cancer cells. Carcinogenesis, 23: 967-975.

Karan, D., S.J. Chen, S.L. Johansson, A.P. Singh, V.M. Paralkar, M.F. Lin and S.K. Batra, 2003. Dysregulated expression of MIC-1/PDF in human prostate tumor cells. Biochem. Biophys. Res. Commun., 305: 598-604.

Karan, D., J. Holzbeierlein and J.B. Thrasher, 2009. Macrophage inhibitory cytokine-1: Possible bridge molecule of inflammation and prostate cancer. Cancer Res., 69: 2-5.

Kempf, T., M. Eden, J. Strelau, M. Naguib and C. Willenbockel *et al*., 2006. The transforming growth factor-b superfamily member growth-differentiation factor-15 protects the heart from ischemia/reperfusion injury. Cir. Res., 98: 351-360.

Kim, I.Y., D.H. Lee, D.K. Lee, B.C. Kim and H.T. Kim *et al*., 2003. Decreased expression of bone morphogenetic protein (BMP) receptor type II correlates with insensitivity to BMP-6 in human renal cell carcinoma cells. Clin. Cancer Res., 9: 6046-6051.

Kim, K.K., J.J. Lee, Y. Yang, K.H. You and J.H. Lee, 2008. Macrophage inhibitory cytokine-1 activates AKT and ERK-1/2 via the transactivation of ErbB2 in human breast and gastric cancer cells. Carcinogenesis, 29: 704-712.

Lawton, L.N., M.F. Bonaldo, P.C. Jelenc, L. Qiu and S.A. Baumes *et al*., 1997. Identification of a novel member of the TGF-beta superfamily highly expressed in human placenta. Gene, 203: 17-26.

Lee, M.S., T. Igawa, T.C. Yuan, X.Q. Zhang, F.F. Lin and M.F. Lin, 2003. ErbB-2 signaling is involved in regulating PSA secretion in androgen independent human prostate cancer LNCaP C-81 cells. Oncogene, 22: 781-796.

Li, P.X., J. Wong, A. Ayed, D. Ngo and A.M. Brade *et al*., 2000. Placental transforming growth factor-b is a downstream mediator of the growth arrest and apoptotic response of tumor cells to DNA damage and p53 overexpression. J. Biol. Chem., 275: 20127-20135.

Liu, T., A.R. Bauskin, J. Zaunders, D.A. Brown, S. Pankhurst, P.J. Russell and S.N. Breit, 2003. Macrophage inhibitory cytokine 1 reduces cell adhesion and induces apoptosis in prostate cancer cells. Cancer Res., 63: 5034-5040.

McDonald, J.H., 2009. Handbook of Biological Statistics. 2nd Edn., Sparky House Publishing, Baltimore, Maryland.

Nakamura, T., A. Scorilas, C. Stephan, G.M. Yousef and G. Kristiansen *et al*., 2003. Quantitative analysis of macrophage inhibitory cytokine-1 (MIC-1) gene expression in human prostatic tissues. Br. J. Cancer, 88: 1101-1104.

Nowroozi, M.R., S. Zeighami, M. Ayati, H. Jamshidian and A.R. Ranjbaran *et al*., 2009. Prostate-specific antigen doubling time as a predictor of Gleason grade in prostate cancer. Urol. J., 6: 27-30.

Paralkar, V.M., A.L. Vail, W.A. Grasser, T.A. Brown and H. Xu *et al*., 1998. Cloning and characterization of a novel member of the transforming growth factor-β/bone morphogenetic protein family. J. Biol. Chem., 273: 13760-13767.

Patrikainen, L., K. Porvari, R. Kurkela, P. Hirvikoski, Y. Soini and P. Vihko, 2007. Expression profiling of PC-3 cell line variants and comparison of MIC-1 transcript levels in benign and malignant prostate. Eur. J. Clin. Invest., 37: 126-133.

Polascik, T.J., J.E. Oesterling and A.W. Partin, 1999. Prostate specific antigen: A decade of discovery what we have learned and where we are going. J. Urol., 162: 293-306.

Rasiah, K.K., J.G. Kench, M. Gardiner-Garden, A.V. Biankin and D Golovsky *et al*., 2006. Aberrant neuropeptide Y and macrophage inhibitory cytokine-1 expression are early events in prostate cancer development and are associated with poor prognosis. Cancer Epidemiol. Biomarkers Prev., 15: 711-716.

Sakr, W.A., R. Montironi, J.I. Epstein, M.A. Rubin and A.M. De Marzo *et al*., 2004. Prostatic Intraepithelial Neoplasia. In: Pathology and Genetics of Tumours of the Urinary System and Male Genital Organs, Eble, J.N., G. Sauter, J.I. Epstein and I.A. Sesterhenn (Eds.). IARC Press, Lyon, France, ISBN-10: 9283224159, pp: 360.

Schiemann, W.P., D. Rotzer, W.M. Pfeifer, E. Levi, K.R. Rai, P. Knaus and M.E. Kadin, 2004. Transforming growth factor- β (TGF- β)-resistant B cells from chronic lymphocytic leukemia patients contain recurrent mutations in the signal sequence of the type I TGF-β receptor. Cancer Detection Prev., 28: 57-64.

Selander, K.S., D.A Brown, G.B Sequeiros, M. Hunter and R. Desmond *et al*., 2007. Serum macrophage inhibitory cytokine-1 concentrations correlate with the presence of prostate cancer bone metastases. Cancer Epidemiol. Biomarkers Prev., 16: 532-537.

Stamatiou, K., V. Papadimitriou, E. Michail, D. Delakas, E. Michalodimitrakis and F. Sofras, 2007. Identification of insignificant prostate cancers. Int. J. Cancer Res., 3: 162-166.

Stout, R.D. and J. Suttles, 2004. Functional plasticity of macrophages: Reversible adaptation to changing microenvironments. J. Leukocyte Biol., 76: 509-513.

Tan, M., Y. Wang, K. Guan and Y. Sun, 2000. PTGF- β, a type beta transforming growth factor (TGF-beta) superfamily member, is a p53 target gene that inhibits tumor cell growth via TGF-beta signaling pathway. Proc. Nat. Acad. Sci., USA., 97: 109-114.

Thomas, R., L.D. True, P.H. Lange and R.L. Vessella, 2001. Placental bone morphogenetic protein (PLAB) gene expression in normal, premalignant and malignant human prostate: Relation to tumor development and progression. Int. J. Cancer, 93: 47-52.

Vollmer, J., 2006. CpG motifs to modulate innate and adaptive immune responses. Int. Rev. Immunol., 25: 125-134.

Welsh, J.B., L.M. Sapinoso, A.I. Su, S.G. Kern and J. Wang-Rodriguez *et al*., 2001. Analysis of gene expression identifies candidate markers and pharmacological targets in prostate cancer. Cancer Res., 61: 5974-5978.

Welsh, J.B., L.M. Sapinoso, S.G. Kern, D.A. Brown and T. Liu *et al*., 2003. Large-scale delineation of secreted protein biomarkers overexpressed in cancer tissue and serum. Proc. Natl. Acad. Sci. USA., 100: 3410-3415.

Wollmann, W., M.L. Goodman, P. Bhat-Nakshatri, H. Kishimoto and R.J. Goulet *et al*., 2005. The macrophage inhibitory cytokine integrates AKT/PKB and MAP kinase signaling pathways in breast cancer cells. Carcinogenesis, 26: 900-907.

Yang, H., Z. Filipovic, D. Brown, S.N. Breit and L.T. Vassilev, 2003. Macrophage inhibitory cytokine-1: A novel biomarker for p53 pathway activation. Mol. Cancer Ther., 2: 1023-1029.

Zhang, Q., J.N. Rubenstein, T.L. Jang, M. Pins and B. Javonovic *et al*., 2005. Insensitivity to transforming growth factor- β results from promoter methylation of cognate receptors in human prostate cancer cells (LNCaP). Mol. Endocrinol., 19: 2390-2399.