Aberrant Neuropeptide Y and Macrophage Inhibitory Cytokine-1 Expression Are Early Events in Prostate Cancer Development and Are Associated with Poor Prognosis

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Abstract

Studies to elucidate dysregulated gene expression patterns in premalignant prostate lesions have identified several candidate genes with the potential to be targeted to prevent the development and progression of prostate cancer and act as biomarkers of early disease. Herein, we explored the importance of two proteins, neuropeptide Y (NPY) and macrophage inhibitory cytokine-1 (MIC-1), as biomarkers of preinvasive prostate disease and investigated the relationship of expression to biochemical recurrence following treatment for localized prostate cancer. NPY and MIC-1 protein expression was determined by immunohistochemistry on tissue microarrays containing 1,626 cores of benign, low-grade prostatic intraepithelial neoplasia (PIN), high-grade PIN (HGPIN), and prostate cancer tissue from 243 radical prostatectomy patients. Both NPY and MIC-1 showed higher proportional immunostaining in HGPIN and prostate cancer compared with benign epithelium ($P < 0.0001$). NPY and MIC-1 immunostaining was higher in low-grade PIN compared with other benign tissues (both $P < 0.0001$) and was equivalent to immunostaining in HGPIN. NPY immunostaining of prostate cancer was independently associated with relapse, after adjusting for traditional prognostic factors, as a categorical variable in 20% intervals ($P = 0.0449$-0.0103) and as a continuous variable ($P = 0.0017$). Low MIC-1 immunostaining (20% categories) was associated with pathologic stage >2C after adjusting for predictors of pathologic stage ($P = 0.3894$-0.0176). This is the first study to show that altered NPY and MIC-1 expression are significantly associated with prostate cancer progression and suggests that these molecules be developed further as biomarkers in the management of prostate disease. (Cancer Epidemiol Biomarkers Prev 2006;15(4):711–6)

Introduction

Prostate cancer is the most commonly diagnosed cancer and a major cause of cancer death in men in western countries. Despite the prevalence of this disease, the precise mechanisms involved in prostate carcinogenesis and progression remain uncertain. Morphologic premalignant changes in prostate epithelium, such as high-grade prostatic intraepithelial neoplasia (HGPIN), precede invasive prostate cancer by several decades (1). The molecular events accompanying these changes are likely to be critical steps in the process of carcinogenesis and are therefore candidate, novel therapeutic targets for strategies, such as chemoprevention, and potential biomarkers for early detection. However, the focal distribution of precursor lesions, including HGPIN, the lack of consensus on the definition of early dysplastic changes in the prostate, and the morphologic heterogeneity associated with prostate tissue have all limited efforts to elucidate previously the *in situ* molecular alterations that occur during the progression from non-neoplastic prostate epithelium to invasive prostate cancer.

The strategy of transcript profiling has been used to characterize the gene expression profiles of clinical samples of both HGPIN and prostate cancer. The incorporation of contemporary technologies, such as laser capture microdissection and linear RNA amplification, has largely overcome the problems associated with the focal nature of preinvasive lesions and resulted in several recent studies that have identified genes that are differentially expressed in the progression from normal epithelium to invasive prostate cancer (2, 3). Two such candidates are the secreted proteins, neuropeptide Y (NPY) and macrophage inhibitory cytokine-1 (MIC-1), which were identified to be up-regulated in small cohorts of microdissected HGPIN and prostate cancer samples, findings validated by our own transcript profiling data (data not shown).

MIC-1 also known as placental transforming growth factor-β, placental bone morphogenic protein, prostate-derived factor, and growth differentiation factor-15, is a member of the transforming growth factor-β superfamily, which like other proteins in this family is synthesized as a precursor containing

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an amino-terminal propeptide and a carboxyl-terminal mature domain (4, 5). Increased MIC-1 expression is a feature of many cancers, including breast, colon, and pancreas. Several studies show an antitumorigenic role for MIC-1 where it induces apoptosis and inhibits proliferation of several tumor cell lines (6, 7).

NPY is a well-characterized neuropeptide with proposed functions in the regulation of feeding behavior, gastrointestinal motility and secretion, vasoconstriction, and inhibition of anxiety (8-10). Recent work has focused on the mitogenic and angiogenic activity of NPY, with evidence that NPY can stimulate endothelial cell proliferation and increase tumor vascularization in some solid tumor cell types (11, 12).

In the current study, we describe the validation of NPY and MIC-1 in a large, well-characterized cohort of premalignant and invasive prostate lesions and examine the potential role of these proteins in the progression of prostate cancer.

Materials and Methods

Patient Population. A cohort of archival formalin-fixed, paraffin-embedded radical prostatectomy specimens (n = 243) were selected from a previously described group of patients treated for clinically localized prostate cancer at the St. Vincent's Hospital Campus (Sydney, New South Wales, Australia) between February 1987 and June 1997 (13). Specimen and clinical data collection was with the written informed consent of patients. Follow-up data collection was prospective from 1990 with the approval of the St. Vincent's Campus Research Ethics Committee (reference no. H00/088). The date of disease relapse was defined as the date of the first increase in serum prostate-specific antigen (PSA) $\geq$ 0.4 ng/mL when it was followed by consecutive, further increases.

Tissue Microarray Construction. The natural history of the development of prostate cancer is putatively modeled as a morphologic progression from normal epithelium through a series of increasingly dysplastic lesions known as low-grade PIN (LGPIN) and HGPIN, culminating in invasive prostate cancer (14). A tissue microarray representation of this progression model was constructed from the paraffin specimen blocks of 243 patients who underwent radical prostatectomy. The criteria used in this study for Gleason grading were those used in standard clinical practice (15, 16). HGPIN and LGPIN were identified according to the features defined by Bostwick and Dundore (17). In total, 1,626 cores of benign (normal, hyperplasia, and LGPIN), HGPIN, and prostate cancer (Gleason patterns 1-5) were placed in 22 tissue microarray blocks using previously described techniques (18). In this cohort, 190 patients were represented by cores of prostate cancer and 189 patients were represented by HGPIN or LGPIN. Of these, 124 patients had a complete set of benign, HGPIN, and prostate cancer cores.

Immunohistochemistry. Indirect immunoperoxidase immunohistochemistry (Envision Plus, DAKO, Carpinteria, CA) was used with a 1:350 dilution of rabbit polyclonal anti-NPY antibody (AB1915, Chemicon, Temecula, CA) for NPY immunostaining of tissue microarrays. The avidin-biotin method (Vectastain, Vector Laboratories, Burlingame, CA) was used with a 1:60,000 dilution of sheep anti-human MIC-1 polyclonal antibody 233B3 for MIC-1 immunohistochemistry (19). Negative controls for NPY consisted of sections of brain and prostate incorporating NPY-containing nerve fibers incubated with primary antibody, which had been preadsorbed over 24 hours at 4°C with an excess (10 nmol/mL) of NPY (Abcam, Cambridge, United Kingdom). Sections of seminal vesicle were negative controls for MIC-1 immunostaining.

Immunostaining was scored by microscopic assessment of the percentage of the lesional cells with positive cytoplasmic staining. Staining intensity was graded between 0 and 3. Tissue microarrays were assessed independently by two observers

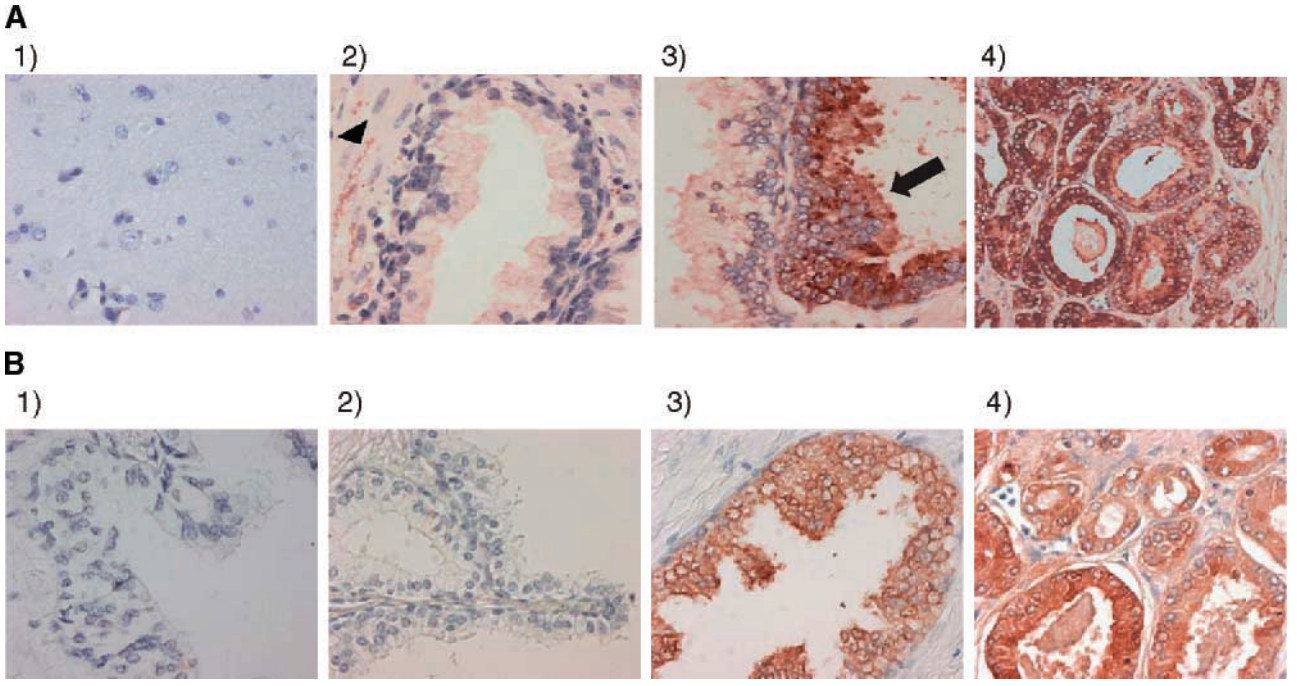

Figure 1. Photomicrographs of immunostaining for (**A**) NPY in (*1*) tissue negative control consisting of brain after primary antibody preadsorbed with NPY, (*2*) benign prostate epithelium, (*3*) HGPIN, and (*4*) Gleason pattern 3 prostate cancer and (**B**) MIC-1 in (*1*) tissue negative control consisting of seminal vesicle, (*2*) benign prostate epithelium, (*3*) HGPIN, and (*4*) Gleason pattern 3 prostate cancer. The increased expression of MIC-1 and NPY seen in the early progression model lesions on the tissue microarrays validated the up-regulation detected by the oligonucleotide microarrays. Original magnification, ×400. *Arrowhead,* intraprostatic nerve fiber positive for NPY; *arrow,* HGPIN.

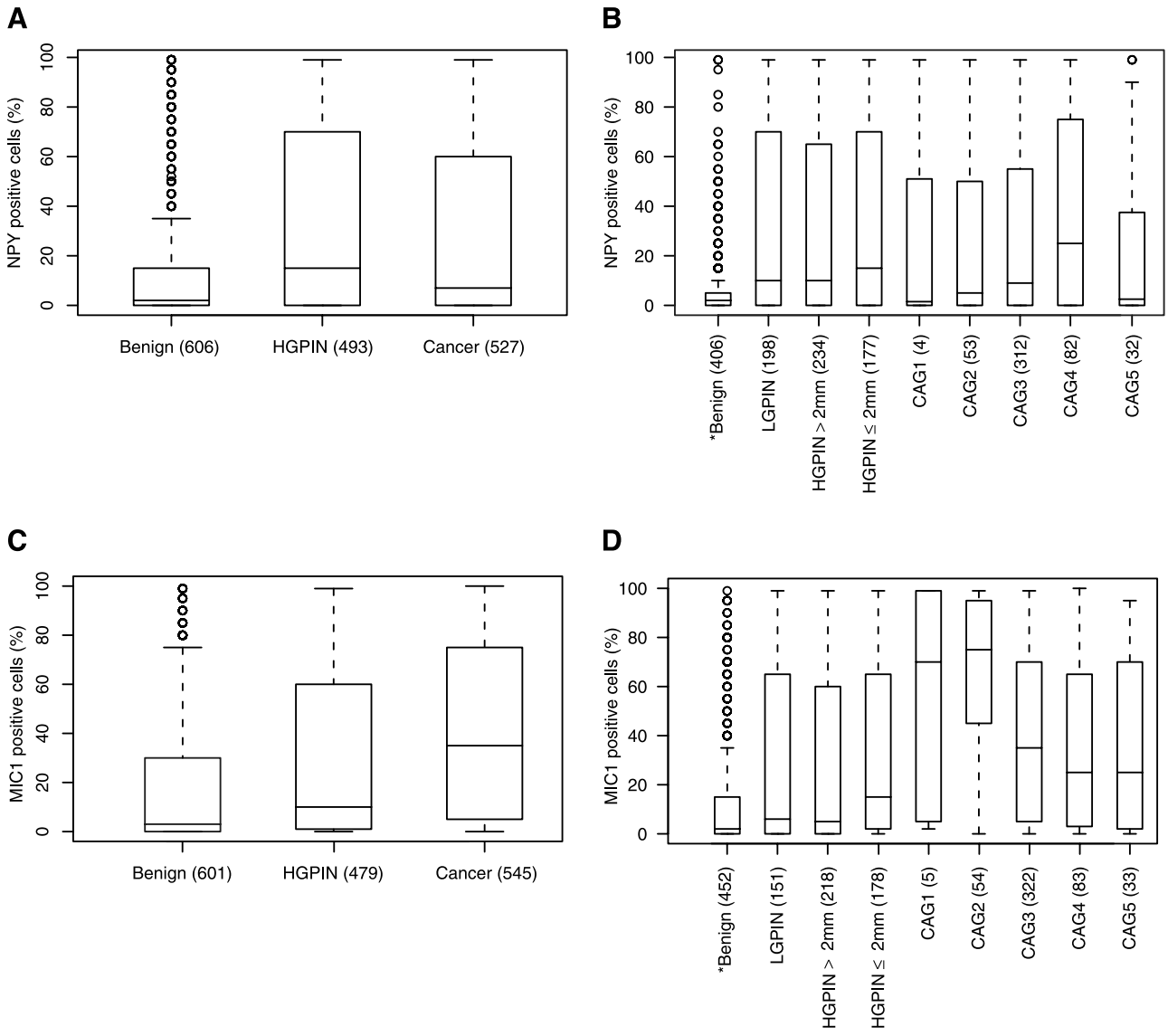

Figure 2. Box plots showing proportional NPY immunostaining in prostate tissue microarrays. **A** and **C.** Immunostaining for NPY and MIC-1 with the pathologic subgroups organized as a simplified progression model from benign prostate tissue to HGPIN and prostate cancer. **B** and **D.** Immunostaining in the pathologic subgroups benign excluding LGPIN (*Benign), LGPIN, HGPIN away from prostate cancer and close to prostate cancer (HGPIN > 2 mm and HGPIN ≤ 2 mm), and Gleason patterns 1 to 5 prostate cancer (CAG1-CAG5). *Numbers in parentheses,* number of cores for each pathology. These plots suggest that increased expression of NPY and MIC-1 occurs with the earliest morphologic changes of prostatic neoplasia. *Horizontal lines,* 25th, 50th, and 75th percentiles of immunostaining. *Bars,* 1.5 times the interquartile range; *circles,* values beyond this range.

(K.K.R. and J.G.K.) who were blinded to outcome. A final percentage of positively stained cells were calculated by averaging the lesional percent positivity across the cores representing each patient.

Statistical Analyses. The significance of any differences in immunostaining between the pathologies was assessed using a linear mixed effects model. The lesional proportion of positively stained cells was transformed using a square root arcsine function to stabilize the variance of the percentage data and modeled as a function of patient identity and pathology (''nlme'' and ''base'' packages in R, http://www.r-project.org/). The model has the following form: arcsine $\sqrt{PS} = \mu + e_i + \beta\text{pathology} + \varepsilon_{ij}$ where, i denotes patient identity and j denotes the pathology type.

Associations between immunostaining and clinical and pathologic variables were evaluated using logistic regression. Data were evaluated for associations with relapse in Cox proportional hazards models (Wald statistic). Logistic and Cox proportional hazards regression analyses were done using Statview version 4.5 software (Abacus Systems, Berkeley, CA). Statistical significance in this study was set at $P < 0.05$.

Results

Validation of Differential NPY and MIC-1 Protein Expression by Immunohistochemistry. The predominant pattern of both NPY and MIC-1 immunostaining was epithelial and cytoplasmic (Fig. 1A and B). The distribution of positive immunostaining for NPY showed higher proportional immunostaining in HGPIN (median, 15%; SD, 36%; range, 0-99%; 75th percentile, 70%) and prostate cancer (median, 7%; SD, 35%, range, 0-99%; 75th percentile, 60%) compared with benign epithelium (median, 2%; SD, 27%; range, 0-99%; 75th percentile, 15%; Fig. 2A). Comparison of NPY expression

Table 1. Cox proportional hazards analyses of NPY immunostaining and clinicopathologic predictors of relapse after radical prostatectomy

Risk factor	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	*P*	Hazard ratio (95% CI)	*P*
Pathologic stage >2C	3.2 (1.9-5.5)	<0.0001	3.1 (1.7-5.6)	0.0002
ln(PSA)*	2.1 (1.5-2.9)	<0.0001	1.9 (1.4-2.7)	<0.0001
Gleason score >6	2.0 (1.2-3.2)	0.0069	1.4 (0.8-2.3)	0.1935
Surgical margin involvement	2.3 (1.3-3.8)	0.0025	1.4 (0.7-2.5)	0.3117
NPY immunostaining† (% of cancer cells)				
80-99% (n = 15)	2.5 (1.1-5.8)	0.0303	3.1 (1.3-7.3)	0.0103
60-79% (n = 17)	1.9 (0.9-4.3)	0.1004	3.0 (1.3-6.9)	0.0096
40-59% (n = 26)	1.5 (0.7-3.0)	0.3006	1.7 (0.8-3.6)	0.1434
20-39% (n = 37)	1.3 (0.7-2.5)	0.4471	2.0 (1.0-4.1)	0.0449

*Natural log of preoperative PSA.
†Hazard ratios and *P*s are in relation to reference category 0-19% (n = 95).

levels using a linear mixed effects model showed that the increase in the proportion of cells expressing NPY in HGPIN and prostate cancer compared with benign epithelium was statistically significant (both $P < 0.0001$). The proportion of HGPIN epithelium with positive immunostaining for NPY was higher than that of prostate cancer ($P = 0.0108$; Supplementary Table S1). Examination of pathologic subgroups characterized on the tissue microarrays showed statistically significant increases in proportions of positive NPY immunostaining in Gleason pattern 4 prostate cancer compared with pattern 3 ($P = 0.042$) and pattern 5 ($P = 0.037$; Fig. 2B; Supplementary Table S2).

The distribution of positive immunostaining for MIC-1 showed higher proportional immunostaining in HGPIN (median, 10%; SD, 33%; range, 0-99%; 75th percentile, 60%) and prostate cancer (median, 35%; SD, 35%; range, 0-99%; 75th percentile, 75%) compared with benign epithelium (median, 3%; SD, 28%; range, 0-99%; 75th percentile, 30%; Fig. 2C). The increase in MIC-1 immunostaining in HGPIN compared with benign epithelium was statistically significant ($P < 0.0001$) as was the increase from HGPIN to prostate cancer ($P < 0.0001$; Supplementary Table S3). Examination of the pathologic subgroups revealed a statistically significant ($P = 0.0031$) increase in the proportion of cells staining positively in HGPIN ≤ 2 mm from invasive prostate cancer compared with HGPIN > 2 mm from prostate cancer (Fig. 2D; Supplementary Table S3). Gleason pattern 2 prostate cancer showed higher proportional immunostaining for MIC-1 than the other Gleason patterns ($P < 0.0001$; Supplementary Table S4).

We sought to examine the association of NPY and MIC-1 with the appearance of the earliest morphologic features of neoplasia by examining immunostaining in LGPIN lesions. LGPIN was diagnosed by a pathologist (J.G.K.) in 198 cores immunostained for NPY and 151 cores immunostained for MIC-1. Interestingly, immunostaining of LGPIN for NPY and MIC-1 showed higher median and 75th percentiles of positive staining than benign epithelium excluding LGPIN (Fig. 2B and D). The higher immunostaining for NPY and MIC-1 in LGPIN compared with other benign tissues was highly significant (both $P < 0.0001$; Supplementary Tables S2 and S4). The NPY and MIC-1 immunostaining showed no statistically significant difference in immunostaining between LGPIN and HGPIN > 2 mm from prostate cancer (NPY, $P = 0.92$; MIC-1, $P = 0.2825$) or HGPIN ≤ 2 mm from prostate cancer (NPY, $P = 0.34$; MIC-1, $P = 0.0862$; Supplementary Tables S2 and S4).

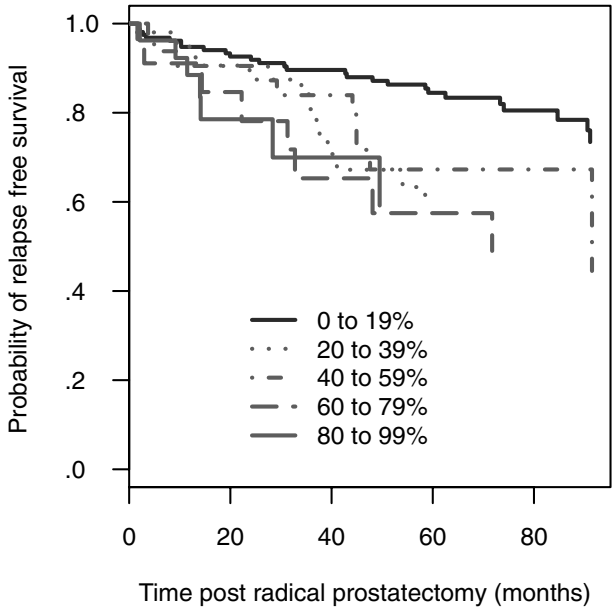

Figure 3. A Cox proportional hazards graph showing the predicted relapse free survival with time postradical prostatectomy for each 20% category of proportional NPY immunostaining of prostate cancer when adjusted for the clinicopathologic variables listed in Table 2. Increasing NPY expression is associated with increased risk of relapse.

Prognostic Value of NPY and MIC-1 Expression. The prognostic value of NPY and MIC-1 immunostaining in prostate cancer was evaluated in 190 patients treated for early prostate cancer with radical prostatectomy. The mean age at surgery was 63 years (SD, 6; range, 46-76). The mean and median follow-up postsurgery was 81 months (SD, 24; range, 1-160), and 65 (34%) patients suffered relapse of their disease in the study period. Mean and median preoperative PSA levels were 15.6 ng/mL (SD, 15.1; range, 1-97) and 10.2 ng/mL, respectively. The mean and median Gleason scores were 6 (SD, 1; range, 4-10) and pathologic stage >2C was present in 93 (49%) patients. Pelvic lymph node metastases were present in 3 (1.6%) patients, and 43 (22%) patients received postoperative adjuvant antiandrogen or radiation therapy.

To assess whether NPY provided independent prognostic information when considered with other established markers of relapse after radical prostatectomy, Cox proportional hazards analyses were used to examine the association of proportional immunostaining of prostate cancer specimens with risk of relapse. When modeled as a continuous variable, each increase in NPY immunostaining of 1 SD (27.8%) resulted in an increased risk of relapse of 1.3-fold [95% confidence interval (95% CI), 1.1-1.6; $P = 0.0206$] in univariate analysis and 1.5-fold (95% CI, 1.2-1.8; $P = 0.0017$) in multivariate analysis after adjusting for the traditional prognostic indicators modeled in Table 1. Consideration of NPY immunostaining

Table 2. Logistic regression analysis of MIC-1 immunostaining and clinicopathologic predictors of extraprostatic invasion in radical prostatectomy specimens

Risk factor	Univariate analysis		Multivariate analysis	
	Odds ratio (95% CI)	*P*	Odds ratio (95% CI)	*P*
ln(PSA)*	1.5 (1.0-2.2)	0.0483	1.3 (0.8-1.9)	0.2825
Gleason score >6	2.8 (1.6-5.1)	0.0006	2.4 (1.3-4.6)	0.0060
MIC-1 immunostaining† (% of cancer cells)				
0-19%	4.4 (1.5-13.0)	0.0068	3.8 (1.3-11.5)	0.0176
20-39%	3.7 (1.2-11.4)	0.0223	3.8 (1.2-12.1)	0.0237
40-59%	6.0 (1.6-21.9)	0.0067	5.2 (1.4-19.7)	0.0158
60-79%	1.7 (0.6-5.0)	0.3257	1.6 (0.5-4.9)	0.3894

*Natural log of preoperative PSA.
†Hazard ratios and *P*s are in relation to reference category 80-99%.

as more clinically interpretable 20% categories resulted in smaller subgroups in the analysis that, nevertheless, trended toward and achieved statistical significance in univariate analysis when the majority of prostate cancer cells stained positive (Table 1). In multivariate analysis with traditional prognostic indicators, patients with increasing proportional NPY staining of their prostate cancers (20-39%, 40-59%, etc.) had an increased risk of relapse compared with patients with low staining (reference range, 0-19%; Table 1; Fig. 3). No significant associations were detected between the NPY immunostaining (continuous) and pathologic stage >2C (P = 0.1905), Gleason score >6 (P = 0.7127), or surgical margin involvement (P = 0.9067) in logistic regression analyses or with ln(PSA) (P = 0.5579) in simple regression analysis.

MIC-1 immunostaining was also associated with a poor clinical outcome after radical prostatectomy. When MIC-1 immunostaining was modeled as a continuous variable, each decrease in MIC-1 positivity of 1 SD (28.3%) was associated with a 1.5-fold (95% CI, 1.1-2.0; P = 0.0027) increase in risk of relapse in univariate analysis. As a categorical variable, only very low MIC-1 immunostaining (0-19%) showed a statistically significant association with relapse in univariate analysis (hazard ratio, 2.8; 95% CI, 1.1-7.4; P = 0.0335) and no independent association was found as either a continuous variable (P = 0.0904) or as a categorical variable (P = 0.7261-0.2058) in multivariate analysis adjusting for traditional predictors of relapse.

Logistic regression analyses were used to examine for associations between MIC-1 immunostaining and clinicopathologic variables and showed that MIC-1 immunostaining of prostate cancer was associated with the pathologic stage of radical prostatectomy specimens (Table 2). Univariate analysis and a multivariate analysis adjusting for predictors of pathologic stage revealed that patients with decreasing proportions of MIC-1 immunostaining (20% categories) had increased odds of pathologic stage >2C compared with patients with high staining (79-99%; Table 2).

Discussion

These results provide the first comprehensive validation in an independent cohort of the relevance of NPY and MIC-1 in early preinvasive prostate disease. In addition, further insights into the morphologic progression from benign epithelium to poorly differentiated cancer were gained here by examination of immunostaining in the pathologic subgroups classified on the tissue microarrays. In particular, the similar immunostaining of LGPIN to HGPIN suggested that overexpression of NPY and MIC-1 could be observed in lesions displaying the earliest morphologically identifiable features of the neoplastic phenotype. The interobserver variation reported previously in the diagnosis of LGPIN necessitates a degree of caution in the interpretation of this result (20). However, the potential of these findings to facilitate a more precise morphologic characterization of early prostatic dysplasia based on these markers suggests that they warrant confirmation in an independent cohort of LGPIN lesions.

This is also the first comprehensive analysis of the role of NPY and MIC-1 expression in prostate cancer progression. Of particular note was the strength of independence of NPY immunostaining from established prognostic factors that represent malignant processes, such as loss of differentiation (Gleason pattern) and invasiveness (pathologic stage). An apparent connection with neuroendocrine differentiation, previously associated with the development and progression of prostate cancer (21), was suggested by the wide expression throughout the central and peripheral nervous system of this highly conserved 36–amino acid protein. Preliminary data assessing the nominal link between the NPY overexpression and neuroendocrine differentiation found similar patterns of chromogranin A and serotonin immunostaining in our tissue microarrays to those reported previously (data not shown; ref. 22). However, the distinct differences in both pattern and localization within corresponding tissue microarray cores to NPY suggest that the overexpression of NPY is a different process from the traditional concept of neuroendocrine differentiation.

The pathway most consistently implicated in the proliferative actions of NPY is the mitogen-activated protein kinase signaling pathway (23, 24) As mitogen-activated protein kinase signaling has recently been shown to be associated with proliferation of prostate cancer cells and progression in clinical specimens (25, 26), the overexpression of NPY in neoplastic prostate tissue may represent a novel autocrine stimulus of this pathway in prostate cancer. There is also increasing attention on the potential role of NPY in angiogenesis (12). Certainly, the possibility that NPY acts as a stimulator of tumor vascularization in early disease warrants further investigation.

Recent studies in small numbers of benign and malignant prostate samples have implicated a role for MIC-1 in the progression from benign to invasive prostate epithelium (2, 3, 27). Our data provide the first comprehensive validation study supporting these findings and are essential for the further development of MIC-1 as a marker of early disease. The likely importance of MIC-1 in clinical prostate cancer is highlighted further by the results of a recent large Swedish case-control study that implicates *MIC-1* as a susceptibility gene (28). In this study, an association between a single nucleotide polymorphism in exon 2 of the *MIC-1* gene and overall prostate cancer risk was shown as well as, importantly, the risk of diagnosis of advanced disease (28). Indeed, on a population basis, it was estimated that the proportion of prostate cancer cases attributable to the polymorphism was 7.2% for sporadic cancer and 19.2% for familial cancer. Our finding that higher levels of MIC-1 protein and RNA are detectable in the earliest stages of prostate disease development further promotes the potential of an early functional

change predisposing individuals to the development of aggressive prostate cancer and suggests that detection of such changes may be possible before the development of invasive disease.

A potential mechanism by which MIC-1 may be involved in the biology of invasive cancer is by serving as a biomarker for activation of the tumor suppressor p53, because previous studies have shown that MIC-1 is induced by p53 (29). Indeed, loss of p53 function has been associated with increasing Gleason grade (30) and may explain the decreased expression of MIC-1 seen in higher Gleason pattern cancer compared with Gleason pattern 2 cancer in our cohort (Supplementary Table S4). The loss of p53 activation and its tumor suppressive actions may explain the worse outcomes of those patients with lower MIC-1 immunostaining. Alternatively, MIC-1 expression has been linked with tumor apoptosis and it is possible that it helps limit tumor growth by this mechanism (6). Other potential mechanisms may involve the p53-independent regulation of MIC-1 by cellular stressors associated with cancer, such as anoxia and DNA damage (7, 31). This is supported by a recent report that MIC-1 was significantly induced in cancer cells depleted of the stress-inducible heat shock protein 70-2 with a concomitant antiproliferative effect (32).

In a recent study designed to investigate the role of the propeptide in regulating the secretion of MIC-1, we showed that prostate cancer cell lines secrete MIC-1 predominantly in an unprocessed form, which associates with the extracellular matrix via the propeptide (33). Using a nude mouse tumor xenograft model, we then showed that the presence of this propeptide is an important *in vivo* mechanism for regulating the relative distribution of MIC-1 between the circulation and tissue extracellular matrix stores. Indeed, we found that, in prostate cancers taken from men treated by radical prostatectomy, increased stromal stores of MIC-1 conferred a better prognosis. These data suggest that the occurrence of localized stromal stores of MIC-1 is likely to play a central role in modulating local bioavailability of MIC-1, which then in turn may affect patient outcome.

In summary, we have confirmed a role for NPY and MIC-1 in the earliest stages of prostate disease and showed for the first time that aberrant expression of NPY is associated with biochemical recurrence after treatment for localized prostate cancer. Future work will need to determine if the measurement of NPY and MIC-1 in tissue and/or serum can be applied in the preoperative setting for the detection and monitoring of early prostate disease.

References

1. Sakr WA, Haas GP, Cassin BF, Pontes JE, Crissman JD. The frequency of carcinoma and intraepithelial neoplasia of the prostate in young male patients. J Urol 1993;150:379–85.
2. Ashida S, Nakagawa H, Katagiri T, et al. Molecular features of the transition from prostatic intraepithelial neoplasia (PIN) to prostate cancer: genome-wide gene-expression profiles of prostate cancers and PINs. Cancer Res 2004;64:5963–72.
3. Cheung P, Woolcock B, Adomat H, et al. Protein profiling of microdissected prostate tissue links growth differentiation factor 15 to prostate carcinogenesis. Cancer Res 2004;64:5929–33.
4. Bootcov MR, Bauskin AR, Valenzuela SM, et al. MIC-1, a novel macrophage inhibitory cytokine, is a divergent member of the TGF-β superfamily. Proc Natl Acad Sci U S A 1997;94:11514–9.
5. Paralkar VM, Vail AL, Grasser WA, et al. Cloning and characterization of a novel member of the transforming growth factor-β/bone morphogenetic protein family. J Biol Chem 1998;273:13760–7.
6. Liu T, Bauskin AR, Zaunders J, et al. Macrophage inhibitory cytokine 1 reduces cell adhesion and induces apoptosis in prostate cancer cells. Cancer Res 2003;63:5034–40.
7. Albertoni M, Shaw PH, Nozaki M, et al. Anoxia induces macrophage inhibitory cytokine-1 (MIC-1) in glioblastoma cells independently of p53 and HIF-1. Oncogene 2002;21:4212–9.
8. Michel MC, Rascher W. Neuropeptide Y: a possible role in hypertension? J Hypertens 1995;13:385–95.
9. Sheikh SP. Neuropeptide Y and peptide YY: major modulators of gastrointestinal blood flow and function. Am J Physiol 1991;261: G701–15.
10. Pedrazzini T, Seydoux J, Kunstner P, et al. Cardiovascular response, feeding behavior and locomotor activity in mice lacking the NPY Y1 receptor. Nat Med 1998;4:722–6.
11. Zukowska-Grojec Z, Karwatowska-Prokopczuk E, Fisher TA, Ji H. Mechanisms of vascular growth-promoting effects of neuropeptide Y: role of its inducible receptors. Regul Pept 1998;75–76:231–8.
12. Kitlinska J, Abe K, Kuo L, et al. Differential effects of neuropeptide Y on the growth and vascularization of neural crest-derived tumors. Cancer Res 2005;65:1719–28.
13. Quinn DI, Henshall SM, Haynes A-M, et al. Prognostic significance of pathological features in localized prostate cancer treated with radical prostatectomy: implications for staging systems and predictive models. J Clin Oncol 2001;19:3692–705.
14. McNeal JE, Bostwick DG. Intraductal dysplasia: a premalignant lesion of the prostate. Hum Pathol 1986;17:64–71.
15. Foster C, Bostwick D. Grading prostate cancer. In: Deshmukh N, Foster C, editors. Pathology of the prostate. Philadelphia: WB Saunders; 1998. p. 191–227.
16. Epstein J, Yang X. Grading of prostatic adenocarcinomas, the Gleason system. In: Epstein J, Yang X, editors. Prostate biopsy interpretation. 3rd ed. Philadelphia: Lippincott Williams & Wilkins; 2002. p. 154–76.
17. Bostwick DG, Dundore PA. Biopsy pathology of the prostate. 1st ed. London: Chapman and Hall Medical; 1997.
18. Henshall SM, Afar DE, Rasiah KK, et al. Expression of the zinc transporter ZnT4 is decreased in the progression from early prostate disease to invasive prostate cancer. Oncogene 2003;22:6005–12.
19. Brown DA, Ward RL, Buckhaults P, et al. MIC-1 serum level and genotype: associations with progress and prognosis of colorectal carcinoma. Clin Cancer Res 2003;9:2642–50.
20. Epstein JI, Grignon DJ, Humphrey PA, et al. Interobserver reproducibility in the diagnosis of prostatic intraepithelial neoplasia. Am J Surg Pathol 1995;19:873–86.
21. Bostwick DG, Qian J, Pacelli A, et al. Neuroendocrine expression in node positive prostate cancer: correlation with systemic progression and patient survival. J Urol 2002;168:1204–11.
22. Abrahamsson PA. Neuroendocrine differentiation and hormone-refractory prostate cancer. Prostate Suppl 1996;6:3–8.
23. Hansel DE, Eipper BA, Ronnett GV. Neuropeptide Y functions as a neuroproliferative factor. Nature 2001;410:940–4.
24. Ekstrand AJ, Cao R, Bjorndahl M, et al. Deletion of neuropeptide Y (NPY) 2 receptor in mice results in blockage of NPY-induced angiogenesis and delayed wound healing. Proc Natl Acad Sci U S A 2003;100:6033–8.
25. Aguirre-Ghiso JA, Estrada Y, Liu D, Ossowski L. ERK(MAPK) activity as a determinant of tumor growth and dormancy; regulation by p38(SAPK). Cancer Res 2003;63:1684–95.
26. Uzgare AR, Kaplan PJ, Greenberg NM. Differential expression and/or activation of P38MAPK, ERK1/2, and JNK during the initiation and progression of prostate cancer. Prostate 2003;55:128–39.
27. Welsh JB, Sapinso LM, Kern SG, et al. Large-scale delineation of secreted protein biomarkers overexpressed in cancer tissue and serum. Proc Natl Acad Sci U S A 2003;100:3410–5.
28. Lindmark F, Zheng S, Wiklund F, et al. H6D polymorphism in macrophage-inhibitory cytokine-1 gene associated with prostate cancer. J Natl Cancer Inst 2004;96:1248–54.
29. Yang H, Filipovic Z, Brown D, Breit SN, Vassilev LT. Macrophage inhibitory cytokine-1: a novel biomarker for p53 pathway activation. Mol Cancer Ther 2003;2:1023–9.
30. Quinn DI, Henshall SM, Head DR, et al. Prognostic significance of p53 nuclear accumulation in localised prostate cancer treated with radical prostatectomy. Cancer Res 2000;60:1585–94.
31. Li PX, Wong J, Ayed A, et al. Placental transforming growth factor-β is a downstream mediator of the growth arrest and apoptotic response of tumor cells to DNA damage and p53 overexpression. J Biol Chem 2000;275: 20127–35.
32. Rohde M, Dausgaard M, Hartvig Jensen M, Helin K, Nylandsted J, Jaattela M. Members of the heat-shock protein 70 family promote cancer cell growth by distinct mechanisms. Genes Dev 2005;19:570–82.
33. Bauskin AR, Brown D, Junankar S, et al. The propeptide mediates formation of stromal stores of promic-1: role in determining prostate cancer outcome. Cancer Res 2005;65:2330–6.