ORIGINAL ARTICLE

Overexpression of Pim-1 during progression of prostatic adenocarcinoma

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Aims: Pim-1 is a serine/threonine kinase that has been shown to play an integral role in the development of a number of human cancers, such as haematolymphoid malignancies. Recently, evidence has shown Pim-1 to be important in prostatic carcinogenesis. In order to further our understanding of its role in prostate cancer, we investigated Pim-1 expression in normal, premalignant, and malignant prostate tissue.
Methods: Using immunohistochemistry, Pim-1 expression was analysed in prostate tissue from 120 radical prostatectomy specimens. In each case, Pim-1 staining was evaluated in benign prostatic epithelium, high grade prostatic intraepithelial neoplasia (PIN), and prostatic adenocarcinoma. The number of positively staining cells was estimated, and the intensity of staining was scored on a scale of 0 to 3+.
Results: Pim-1 immunoreactivity was identified in 120 cases (100%) of adenocarcinoma, 120 cases (100%) of high grade PIN, and 62 cases (52%) of benign glands. The number of cells staining in benign epithelium (mean 34%) was much lower than that in high grade PIN (mean 80%; $p<0.0001$) or adenocarcinoma (mean, 84%; $p<0.0001$). There was no significant difference between high grade PIN and adenocarcinoma in the percentage of cells staining positively for Pim-1 ($p=0.34$). The staining intensity for Pim-1 was significantly lower in benign prostatic epithelium than in PIN and adenocarcinoma ($p<0.001$). There was no statistically significant correlation between the level of Pim-1 expression and Gleason score, patient age, tumour stage, lymph node metastasis, perineural invasion, vascular invasion, surgical margin status, extraprostatic extension, or seminal vesicle invasion.
Conclusions: Pim-1 expression is elevated in PIN and prostatic adenocarcinoma compared with benign prostatic epithelium. This finding suggests that upregulation of Pim-1 may play a role in prostatic neoplasia.

In mammalian cells, the *Pim-1* oncogene encodes a 35 KDa cytoplasmic serine/threonine kinase that is important in the regulation of apoptosis, cell cycle progression, and transcription via the phosphorylation of target proteins.[1] The gene is located on human chromosome 6p21,[2] and belongs to a family of protein kinases that has been highly conserved throughout the evolution of multicellular organisms.[2–5] *Pim-1* gene expression is highly regulated at the transcriptional, post-transcriptional, translational, and posttranslational levels.[6] Previous studies have shown Pim-1 to be over-expressed in haematolymphoid malignancies and have proposed mechanisms by which Pim-1 may lead to tumorigenesis;[7–17] however, its expression in prostatic neoplasia has not been well studied. We therefore undertook immunohistochemical staining for Pim-1 in benign, premalignant, and malignant prostatic epithelium in a series of 120 radical prostatectomy specimens in order to determine the relationship between Pim-1 expression and prostatic carcinogenesis.

MATERIALS AND METHODS

Prostatectomy specimens

In total, 120 radical retropubic prostatectomy cases with bilateral lymphadenectomies, accessioned between 1990 and 1996, were retrieved from the surgical pathology files of Clarian Health Partners (Indianapolis, IN). No patient had received hormone therapy or radiation prior to prostatectomy. The specimens were selected to represent the full spectrum of Gleason grades and pathological stages. The majority of tumours were peripheral zone tumours, and a few cases also included transitional/central zone tumours. Because these cases were not processed using whole mount method and the detailed mapping of samples taken for processing was not available, the exact zonal distribution of tumours was not available. Sections (5 μm thick) were prepared and stained with haematoxylin and eosin, and examined under light microscopy. High grade prostatic intraepithelial neoplasia (PIN) and adenocarcinoma were diagnosed according to accepted criteria and were present in all 120 cases. Tumours were graded according to the Gleason grading system,[18] and staged according to the 1997 American Joint Committee on Cancer tumour, lymph node, metastasis staging system.[19] The pathological features are summarised in table 1. Gleason grade for the primary tumour ranged from 2 to 5, with a secondary grade ranging from 1 to 5. The prostatectomy specimens were divided into three groups, consisting of Gleason scores <7, 7, and >7. Perineural invasion was present in 104 cases (87%), and vascular invasion in 39 cases (33%). The surgical margins were positive in 56 cases (47%). Extraprostatic extension was present in 62 cases (52%), and lymph node metastasis in 22 cases (18%). Pathological stages included pT2a (11 patients), pT2b (47 patients), pT3a (42 patients), and pT3b (20 patients).

In addition, 15 cases of benign needle biopsy specimens (taken for elevated serum prostate specific antigen) and 15 cases of transurethral resection specimens (for benign prostatic hyperplasia) were examined for Pim-1 expression by immunohistochemistry.

Immunohistochemistry

The tissue blocks containing the maximum amount of tumour and the highest Gleason grade were chosen, and

Abbreviation: PIN, prostatic intraepithelial neoplasia

Table 1 Patient characteristics and Pim-1 expression in prostatic adenocarcinoma

Patient characteristic	Patients (n = 120)	Staining with antibody, % (SD)	Antibody staining intensity (SD)
Primary Gleason grade			
2	12	85.0 (12.1)	2.2 (0.4)
3	56	83.2 (13.6)	2.5 (0.5)
4	30	83.1 (11.0)	2.3 (0.6)
5	22	89.1 (7.2)	2.3 (0.6)
Secondary Gleason grade			
1	1	70.0 (0.0)	3.0 (0.0)
2	16	84.1 (15.6)	2.4 (0.5)
3	43	84.1 (11.9)	2.3 (0.5)
4	41	83.8 (11.5)	2.3 (0.5)
5	19	87.8 (10.1)	2.5 (0.6)
Gleason sum			
<7	37	84.3 (13.2)	2.3 (0.5)
7	42	81.1 (12.2)	2.6 (0.5)
>7	41	85.5 (10.1)	2.4 (0.6)
T classification			
T2a	11	83.6 (5.2)	2.2 (0.5)
T2b	47	83.4 (12.6)	2.3 (0.5)
T3a	42	85.6 (12.5)	2.5 (0.6)
T3b	20	82.0 (11.4)	2.5 (0.5)
Lymph node metastasis			
Positive	22	84.0 (13.0)	2.5 (0.6)
Negative	98	84.6 (11.7)	2.4 (0.5)
Extraprostatic extension			
Positive	62	84.0 (12.4)	2.5 (0.4)
Negative	58	85.3 (11.0)	2.4 (0.5)
Surgical margin			
Positive	56	84.3 (10.5)	2.5 (0.6)
Negative	64	85.0 (12.7)	2.4 (0.4)
Vascular invasion			
Positive	39	87.4 (8.0)	2.4 (0.6)
Negative	81	83.2 (13.2)	2.4 (0.5)
Perineural invasion			
Positive	104	84.4 (12.5)	2.4 (0.6)
Negative	16	85.4 (6.3)	2.2 (0.3)
High grade PIN			
Positive	109	84.2 (12.2)	2.4 (0.5)
Negative	11	85.3 (7.5)	2.3 (0.7)

sections (5 μm thick) were cut from these for immunohistochemical staining for Pim-1. Immunohistochemistry was performed on an automated immunostainer using a monoclonal mouse IgG1 anti-Pim-1 antibody (sc-13513; Santa Cruz Biotechnology, Santa Cruz, CA, USA; 1:30 dilution, 60 minutes at room temperature). Antigen retrieval was carried out by heating sections in 1 mmol/l ethylene diamene tetraacetic acid (pH 8.0) for 30 minutes at 95℃. Endogenous peroxidase activity was inactivated by incubation in 3% H_2O_2 for 10 minutes. Positive and negative controls were run in parallel and appropriate results obtained.

Evaluation of Pim-1 expression

All slides were evaluated by two investigators (TLC and LC) under multihead microscope, without prior knowledge of each patient's clinical information. In each case, Pim-1 staining was evaluated in benign epithelium, high grade PIN, and adenocarcinoma. Pim-1 is a cytoplasmic serine/threonine kinase, thus cytoplasmic staining is considered a positive result. Microscopic fields with the highest degree of immunoreactivity were chosen for analysis. The number of positively staining cells was estimated in a semiquantitative manner on a 5% incremental scale ranging from 0 to 95%. At least 1000 cells were analysed for each component. The staining intensity was classified as negative (0), weak (1+), moderate (2+), or strong (3+), as previously described.[20–23]

Statistical analysis

The mean percentage of immunoreactive cells in benign epithelium, high grade PIN, and adenocarcinoma were compared using one way analysis of variance with a random subject effect to correlate the within subject measurements. The intensities of staining in benign epithelium, high grade PIN, and adenocarcinoma were compared using the Cochran-Mantel-Haenszel test for correlated ordered categorical outcomes. Pairwise comparisons between the tissue types were made if the analysis of variance revealed significant treatment effects. A p value <0.0001 was considered significant, and all p values were two sided.

RESULTS

A cytoplasmic staining pattern was observed in epithelial cells only (fig 1). No Pim-1 immunoreactivity was seen in stromal cells. Pim-1 immunoreactivity was identified in adenocarcinoma in all 120 cases, in high grade PIN in all 120 cases, and in benign prostatic glands in 62 cases (table 2). The percentage of positively staining cells in benign epithelium (mean 34%) was much lower than that in high grade PIN (mean 80.3%; $p<0.0001$) or in adenocarcinoma (mean 84%; $p<0.0001$) (table 3). There was no significant difference between high grade PIN and adenocarcinoma ($p = 0.34$) in the percentage of cells staining positively for Pim-1 (table 3). The adenocarcinomas were grouped into low (Gleason grade <7), intermediate (Gleason grade 7), and high (Gleason grade>7) grade categories. No statistically significant difference was identified between the percentage of Pim-1-positive cancer cells and the Gleason score (table 1). In addition, there was no statistically significant correlation between the level of Pim-1 expression and Gleason score, patient age, tumour stage, lymph node metastasis, perineural invasion, vascular invasion, surgical margin status, extraprostatic extension, or seminal vesicle invasion (table 1).

The staining intensity also differed among benign prostatic epithelium, high grade PIN, and prostatic adenocarcinoma (table 2). Immunoreactivity with anti-Pim-1 antibodies was found in 62 cases of benign glands. Of these 62 positive cases, 61 showed a weak (1+) staining intensity. Moderate (2+) staining intensity was seen in the benign prostatic epithelium in only one case, and none showed strong (3+) staining intensity. In contrast, high grade PIN and adenocarcinoma showed positive staining in all cases with anti-Pim-1 antibodies, and most of these cases showed a stronger staining intensity than that seen in the benign glands (table 2). Of the 120 cases of high grade PIN, 49 (41%), 64 (53%), and 7 (6%) showed 3+, 2+, and 1+ staining intensity, respectively. Of the 120 cases of adenocarcinoma, 46 (38%), 70 (58%), and 4 (3%) showed 3+, 2+, and 1+ staining intensity, respectively. The staining intensity for Pim-1 was significantly higher in high grade PIN and adenocarcinoma compared with benign prostatic epithelium ($p<0.0001$) (table 3).

Because a significant number of cancer cases showed positive Pim-1 expression in the adjacent benign prostatic tissue, we examined benign glands from needle biopsies and transurethral resection specimens from patients with proven cancer free prostates. We found no difference in Pim-1 expression between benign prostatic tissues from cancer free prostates and those containing cancers, thus excluding the possibility of field effect.

DISCUSSION

Pim-1 is a serine/threonine kinase that has been shown to play an integral role in the development of a number of human cancers. In this study, we analysed the expression of Pim-1 in benign epithelial epithelium, high grade PIN, and prostatic adenocarcinoma in 120 radical prostatectomy

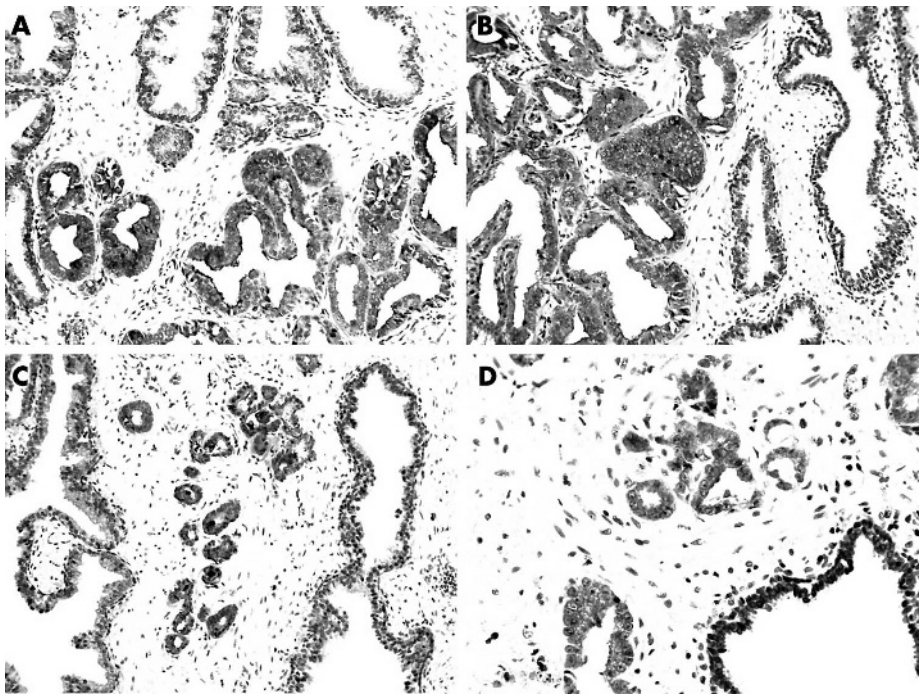

Figure 1 Pim-1 immunohistochemistry in the prostate (A–D) Normal prostate glands show no or minimal staining in the secretory cells lining the lumen of the gland. Adjacent cancer cells show strong Pim-1 expression. Pim-1 expression in high grade prostatic intraepithelial neoplasia (PIN) was evident (A and B). Original magnification×20.

specimens using immunohistochemistry. Our finding of significantly higher Pim-1 levels in neoplastic prostate tissue (high grade PIN and adenocarcinoma) than in benign prostatic epithelium suggests that increased expression of the *Pim-1* oncogene is a frequent occurrence. The observation of no statistical difference between high grade PIN and adenocarcinoma in Pim-1 expression suggests that Pim-1 overexpression begins early in the development of prostate cancer. Pim-1 overexpression as an early step in prostatic carcinogenesis would also explain the lack of a statistically significant correlation between the level of Pim-1 expression and numerous adverse pathological features, such as high Gleason score, lymph node metastasis, vascular invasion, extraprostatic extension, seminal vesicle invasion, and positive surgical margins.

Overexpression of the Pim-1 protein has been reported in haematolymphoid malignancies.[8 10 12] Pim-1 has been asserted to promote tumorigenesis through multiple mechanisms, including its interaction with other proteins such as *c*-myc, Cdc25A dual specificity phosphatase, and androgen receptors, and its ability to induce genomic instability.[16 24–26] Pim-1 was originally discovered as a preferential locus for proviral integration by Moloney murine leukaemia virus in viral induced T cell lymphomas in mice and has been shown to cooperate with c-myc in lymphoid cell transformation.[2 8] Subsequently, Pim-1 was found to be overexpressed in B cell lymphomas, erythroleukaemias, and various human leukaemias.[2]

The oncogenic effect of Pim-1 on non-haematopoietic malignancies is currently under investigation.[13 16 25–27] Ellwood-Yen *et al* demonstrated that Pim-1 overexpression, in cooperation with increased levels of c-myc, could lead to murine prostatic intraepithelial neoplasia and invasive adenocarcinoma in c-myc transgenic mice.[25] The study demonstrated that the oncogenic potential of the Pim-1 gene in cooperation with c-myc was dose dependent with regard to disease progression. The progression from benign to neoplastic epithelium was evident within 3–6 months in the high c-myc group, and 10–12 months in the low c-myc group.[25]

The proposed role of Pim-1 in the development of human cancers, especially its cooperativity with c-myc, suggests that Pim-1 is important during early tumorigenesis. Consistent with this notion, our data demonstrate that high levels of Pim-1 are found early in prostatic carcinogenesis, as early as the premalignant high grade PIN stage. The role of Pim-1 in early prostatic tumorigenesis is further supported by the evidence that overexpression of this protein induces chromosome mis-segregation as a result of defects in the mitotic spindle checkpoint and centrosome amplification.[16] Consequently, polyploidy and aneuploidy in these prostatic epithelial cells may contribute to carcinogenesis, as aneuploidy and chromosomal aberrations are hallmarks of most

Table 2 Intensity of Pim-1 staining of benign and neoplastic prostate in radial prostatectomy specimens

	Staining intensity grade			
Cell type	**0**	**1+**	**2+**	**3+**
Normal	58 (48%)	61 (51%)	1 (1%)	0 (0%)
High grade PIN*	0 (0%)	7 (6%)	64 (53%)	49 (41%)
Cancer*	0 (0%)	4 (3%)	70 (58%)	46 (38%)

*Indicates percentage of staining intensity was statistically higher compared with that of the normal cells with $p<0.0001$ using a Cochran-Mantel-Haenszel test. High grade PIN and cancer are not different.

Table 3 Comparison of staining percentage of Pim-1 immunoreactivity of cells in prostate tissues in radical prostatectomy specimens

	Mean (SD)	**p**
Normal	33.6 (35.2)	
High grade PIN	80.3 (15.8)	
Cancer	84.1 (12.1)	
Normal *v* PIN		<0.0001
Normal *v* cancer		<0.0001

The p values were calculated using the paired Wilcoxon rank sum test.

TAKE HOME MESSAGES

- Pim-1 is a serine/threonine kinase shown to play an integral role in the development of a number of human cancers, which may include prostate cancer.
- Pim-1 expression was analysed in prostate tissue from 120 radical prostatectomy specimens.
- Pim-1 immunoreactivity was identified in 120 cases (100%) of adenocarcinoma, 120 cases (100%) of high grade prostatic intraepithelial neoplasia (PIN), and 62 cases (52%) of benign glands.
- The number of cells staining in high grade PIN and in adenocarcinoma was significantly higher than in benign prostatic epithelium.
- This study suggests that upregulation of Pim-1 may play a role in prostatic neoplasia.

epithelial malignancies, including prostate cancer.[16] Further studies on the regulation of the Pim-1 signalling transduction pathway may provide insights for prostate cancer prevention and treatment.

There is emerging evidence that central zone and transitional zone tumours, while morphologically similar, may exhibit differing genetics and clinical behaviour compared with peripheral zone tumours. Although we used radical prostatectomies for our study, differentiating the exact origin of the tumours is still fraught with difficulties. Numerous studies have shown that prostatic adenocarcinoma is a multifocal disease.[28–30] The majority of prostate have more than one tumour. Noguchi *et al* found that out of 148 transition zone tumours, 52% also had a secondary tumour in the peripheral zone.[31] The use of the whole mount technique may be useful in addressing the issue of zonation and prostatic carcinogenesis.

The significance and the exact biological role of Pim-1 overexpression in neoplastic prostate tissue is unclear. The overexpression of Pim-1 in high grade prostatic intraepithelial neoplasia and in prostatic adenocarcinoma is consistent with emerging evidence supporting a role of Pim-1 dysregulation in tumorigenesis.

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