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The Significance of Metastasis Related-Factors nm23-H1 and Cathepsin D in Prostate Cancer

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ABSTRACT

Different regulators or effectors of the metastatic cascade can be of prognostic/or predictive significance. Nm23-H1 and cathepsin D operate at different levels of the metastatic process. Immunohistochemical staining of nm23-H1 and cathepsin D was performed in this study to clarify more the relation between prostatic intraepithelial neoplasia (PIN) and cancer prostate (CAP) and correlate the two markers with clinicopathological parameters in CAP to determine their prognostic significance. This study included 50 cases, 22 of them were benign prostatic hyperplasia (BPH), 5 cases of PIN and 23 cases of CAP. Cytoplasmic and nuclear nm23-H1 on one hand and tissue and stromal cathepsin D on the other were assessed separately. A high significant nm23-H1 and cathepsin D expression in PIN and CAP compared to BPH was found, while no significant difference was detected between PIN and CAP. Nuclear nm23-H1 was significantly highly expressed with advanced tumour stages (p = 0.02). High expression of stromal cathepsin D was significantly correlated with Gleason's score (p = 0.004), WHO grade (p = 0.01), Whitmore stage (p = 0.002), TNM stage (p = 0.009) and PSA level (p = 0.009). While tissue cathepsin D was significantly correlated with Whitmore stage only (p = 0.03). These findings showed a phenotypic similarity of PIN to CAP with respect to nm23-H1 and cathepsin D expression. Furthermore, strong expression of nm23-H1 likely represents an early event in the development of CAP. Presence and localization of cathepsin D suggested its involvement in tumour invasion and metastasis. It is conceivable that cathepsin D when used with current modalities such as serum PSA and Gleason's score might improve the accuracy of prostate cancer staging.

Key Words: *Prostatic intraepithelial neoplasia - Cancer prostate - nm23-H1 - Cathepsin D.*

INTRODUCTION

Invasion and metastasis can be facilitated by proteins that stimulate tumour cell attachment to host cellular or extracellular matrix, by tumour cell proteolysis of host barriers to invasion, tumour cell locomotion and by tumour cell colony formation in the target organ for metastasis [14,22]. Two classes of gene products are identified in relation to metastasis; the first are internal factors that act inside the cell in a regulatory pathway (e.g. nm23) and the second are external factors that act outside the cell to block-dissect the metastasis pathway (e.g. cathepsins) [14].

On one hand, dissemination of a tumour is regulated at a molecular level involving several genes among which the non-metastatic nm23 gene is thought to plays a critical role [42]. This putative metastasis suppressor gene has been localized to chromosome 17q21.3. Two highly homologous genes have been described so far, nm23-H1 and nm23-H2. Both proteins localize to the cytoplasm, nucleus and cell membrane. The nm23-H1 and nm23-H2 encode a protein with nucleoside diphosphate (NDP) kinase activity, associated with preservation of normal tissue architecture and down regulation of tumour progression [50]. However, a more accepted theory for its action suggested that the metastasis suppression potential depend on serine phosphorylation activity rather than on NDP kinase activity. In a number of studies of human malignant tumours such as breast carcinoma [45], malignant melanoma [11], endometrial carcinoma [38,50] an association between reduced expression of nm23 and increasing metastatic ability resulting in poor prognosis could be demonstrated. On the contrary, several studies failed to find these correlations [43,44]. High nm23 expression even could be shown to associate with tumour progression in colon [4,26,42] and bladder carcinoma [39] as well as neuroblastoma [46,48]. These contradictory results suggest that over-or underexpression of nm23 might be organ specificity [35,43,44].

The cathepsins are a large class of lysosomal enzymes, named in analogy to the Greek word meaning "to digest" and classified according to their function and the amino acids in their active centers [**14,17**]. Cathepsin D (cath D) in particular, is an aspartic lysosomal endopeptidase active at acidic pH. As a lysosomal scavenger, cathepsin D plays an important role in intracellular protein metabolism and prohormones. As a secreted enzyme, it proteolytically degrades basement membranes, extracellular matrices and proteoglycans. Therefore, it is implicated in cancer invasion and metastasis [**27**]. Three forms of cathepsin D are recognized; 52-Kd immature form, 48-Kd intermediate and 34-Kd mature stable form. It is mapped on chromosome 11 [**22**]. Numerous studies have implicated cathepsin D as a disease progression indicator based on both proteolytic function and the cell proliferation promotion aspects of the enzyme [**27**]. It also facilitates the release of the potent angiogenic factors; basic fibroblast growth factors (bFGF) and thus might stimulate neovascularization, a prerequisite for tumour growth and metastases [**10,17**].

Carcinoma of the prostate is a disease with increasing morbidity and mortality and has become the second leading cause of male cancer deaths in USA. In Egypt it constituted about 5.5% of all cancer in males [**12**]. For pathologists and urologists there are currently two main issues in prostate pathology. One involves the early detection of CAP in the preinvasive phase, understanding the biology of preinvasive or precursor lesions has, therefore become increasingly important [**7,32,33**]. Prostatic intraepithelial neoplasia (PIN) is one of these lesions [**32**]. The other, is the identification of the prognostic factors that predict the outcome of individual patients with prostatic carcinoma: the goal is to tailor the therapeutic approach to the clinical, morphological and biological features of the case. It is difficult to accurately predict the clinical course of histologically localized prostatic cancer. Tumour grade, clinical stage and serum prostatic specific antigen (PSA) levels have become the cornerstone variables in determining treatment options for patients with prostate carcinoma. However, a significant degree of heterogeneity still exists within these various prognostic subgroups, thus limiting the predictive value of these factors [**49**]. Moreover, although PSA is currently the most useful serum marker for early detection and surveillance after therapy, however a large fluctuation of the serum level can be found in patients with either localized or metastatic cancer [**8**]. These findings clearly demand an improvement in preoperative staging and a search for a more specific prognostic marker. Previous studies on prostatic carcinoma found a positive correlation between expression of nm23 protein and tumour proliferation and degree of differentiation and metastatic potential [**2,21**]. Several groups have noted a significant correlation of cathepsin D expression with prostatic cancer pathological stage and grade [**8,9,15,41**].

Therefore the aims of this study were:

- To gain a better understanding of the underlying cellular events involved in the development of prostatic intraepithelial neoplasia (PIN) and to clarify the relationship of PIN to invasive prostatic adenocarcinoma.
- Examine the expression of nm23-H1 gene products in benign prostatic hyperplasia, PIN and prostatic adenocarcinoma to clarify its metastatic or antimetastatic function.
- Study the expression of cathepsin D, to determine the involvement of proteinases in invasion and metastasis.
- Evaluate nm23 and cathepsin D expression as prognostic factors against established clinicopathologic variables in prostatic carcinoma.

PATIENTS AND METHODS

Case selection:

This study includes selected fifty prostatic specimens of Egyptian patients. They were selected on the basis of having considerable representation of the common prostatic lesions and paraffin blocks available for histopathological and immunohistochemical study. The study included 22 cases of benign prostatic hyperplasia (BPH), 5 cases of prostatic intraepithelial neoplasia (PIN) and 23 cases of prostatic adenocarcinoma (CAP).

Tumour grading and staging:

Two grading systems were used, the widely used Gleason's system and WHO system. The Gleason's system is based on the degree of glandular differentiation and the growth pattern of the tumour in relation to the stroma [**16**]. Five grades are recognized and combined to obtain

the Gleason's score (values between 2 and 10). The WHO is a three-grade system based on cellular differentiation, anaplasia and mitotic activity. Staging of the neoplastic cases was done according to the modified Whitmore-Jewett system where stage A tumours correspond to the latent neoplasms; carcinoma is unsuspected clinically and found incidentally; B stage carcinoma are clinically detectable but confined within the prostatic capsule and in stage C the disease has spread outside the capsule and in stage D there are distant metastases and the recently developed TNM final staging system [40].

Serum prostatic specific antigen determination:

Pre-operative serum prostatic specific antigen (PSA) was performed.

Immunohistochemistry:

Immunohistochemical staining was performed using a monoclonal mouse primary antibody (Cat. No. 300m, Biogenex, Clinilape. Egypt) to the nm23-H1 gene product (nucleoside diphosphate kinase A) and a polyclonal rabbit primary antibody (Code No. N1625, Dako, Life Trade, Egypt) to cathepsin D. Histostain-plus detection system was applied (Cat. NO 85-9843, ZYMED, Tabark, Egypt). Sections were dewaxed in xylene and rehydrated in graded alcohol. Peroxidase block was done by using hydrogen peroxide (3% hydrogen peroxide in absolute methanol) for 10 minutes at room temperature in humidity chamber. Then heat induced epitope retrieval (HER) procedure was done to reverse the loss of antigenicity that occurs to this epitopes in formalin-fixed, paraffin-embedded tissues for sections stained with cathepsin D. Slides were boiled in a beaker containing 0.01 M citrate buffer, pH 6.0 over a hot plate for 15 minutes at room temperature. Incubating the tissue sections with non-immune serum for 10 minutes eliminates non-specific background. Tissue sections were then incubated with the primary antibodies in the humidity chamber over night at room temperature. Negative controls were prepared by omitting the primary antibody from the staining procedure to test for non-specific background staining.Following this, was incubation with Biotinylated secondary antibody (Link) and Streptavidin-Peroxidase (enzyme conjugate) 30 minutes for each. The colour reaction was developed with DAB substrate. Mayer's Heamatoxyline was used as counter stain.

Evaluation of immunohistochemical staining:

The slide were examined by the two investigators. We used a semiquantitative method to determine immunoreactivity. For nm23-H1, the sections were graded from 1 to 4 according to the percentage of positive cells, i.e. 1 (0-25%), 2 (25-50%), 3 (50-75%) and 4 (75-100%), of cells were positive. Also, the localization of staining (cytoplasmic or nuclear) was also determined [34]. For cathepsin D epithelial tumour and stromal cell immunoreactivity were scored separately. The stromal component contained predominantly tumour-associated macrophages, fibroblasts and lymphoid cells. When reactivity was observed in less than 25% of tumour cells or in few stromal cells, the cases were scored 1, when the positivity was observed in 25-75% of tumour cells or in moderate number of stromal cells the cases were scored 2 and when more than 75% of tumour cells or many stromal cells were positive the cases were scored 3. Moreover the pattern of cathepsin D, either granular or diffuse was also assisted [8].

Statistical analysis:

Statistical analysis was performed using an Epi Info computer package for statistics. X^2 test, Fisher's exact test and Anova tests were used.

RESULTS

Morphological evaluation:

This study included 50 cases, 22 cases of benign prostatic hyperplasia (BPH), 5 cases of prostatic intraepithelial neoplasia (PIN) and 23 of them were prostatic carcinoma (CAP). In BPH group the age ranged from 41 to 72y and the mean ± SD was 62.5±9.3. In PIN group the age ranged from 59 to 75y and the mean was 64.2±6.4. The age ranged from 47-80y in CAP group with a mean of 66.2±7.4. No significant difference was found between 3 groups as regards age ($p = 0.34$). Morphological data of the studied cases are summarized in table (1).

Immunohistochemical evaluation:

Nm23-H1 expression (Table 2):

Four cases only (18.2%) of BPH group showed moderate preserved positivity while the other 18 cases showed weak poistivity. Immu-

nostaining for the nm23-H1 protein was typically moderate within the basal cell layer of the benign ducts and glands. Although the intensity and extent of immunostaining of the luminal cells varied considerably, the luminal cells showed lower immunostaining than the adjacent basal cells. The immunostaining was localized to the cytoplasm and membrane (Fig. 1).

All cases of PIN showed strong expression of nm23-H1. Although the basal cell layers of the PIN lesions were frequently discontinuous, immunostaining was typically moderate to strong. The intensity and extent of staining in the dysplastic luminal cells, however, was greater than that observed in BPH (Fig. 2). Within the dysplastic cells, immunostaining was localized also, primarily to the cytoplasm and membrane and to lesser extent to the nuclei.

Strong immunostaining was detected within the malignant cells in 15 cases (62.2%) of CAP. The staining was typically strong and localized to the cytoplasm and cell membrane (Fig. 3). Unlike BPH and PIN, strong nuclear staining was observed in 9 cases of CAP (39.1%) (Fig. 4).

In comparing nm23-H1 immunostaining of BPH, PIN and CAP, cytoplasmic staining of PIN and CAP was significantly higher than BPH (p = 0.0009). There was not, however, a significant difference between CAP and PIN (p > 0.05). On the other hand, nuclear staining in CAP was significantly higher than PIN and BPH (p = 0.001).

Relationship of nm23 expression and various clinicopathological variables:

A significant relationship was found between nuclear nm23-H1 expression and advancement in tumour Whitemore stage (p = 0.02). There was a trend to high expression of cytoplasmic nm23-H1 with increase in tumour grade and stage, however this observation was not statistically significant. Cytoplasmic and nuclear nm23-H1 expression were significantly correlated (p = 0.01) (Table 3).

Cathepsin D expression (Table 2):

Cathepsin D immunoreactivity was predominantly localized to the neoplastic acinar epithelium. Benign ductal and acinar prostatic epithelium expressed cath. D at low level (Fig. 5). In PIN and CAP staining was generally homogeneous between different microscopic fields, although heterogeneity was not uncommon. Positive reaction for cath. D was visualized as intracytoplasmic brown granules, occupying part or all cytoplasmic volume in dysplastic and tumour cells (Figs. 6,7,8). However, in BPH weak and diffuse staining for cath. D was the predominant pattern. This difference was statistically significant (p = 0.0001). Cytoplasmic staining was observed also in stromal cells. Cath. D positive stromal cells were mainly represented by reactive fibrohistiocytic cells and macrophages that were concentrated immediately adjacent to tumour cells (Fig. 7). Stroma of malignant and PIN cells showed strong cath. D expression than that in BPH (p = 0.0004).

Relationship of cathepsin D expression and various clinicopathological variables:

Tissue cathepsin D was significantly more expressed with advancement in tumour stage (p = 0.03). On the other hand a significant relationship was found between stromal cath. D and Gleason's score (p = 0.004), WHO grade (p = 0.01), Whitmore stage (p = 0.002), TNM stage (p = 0.009) and PSA level (p = 0.009). Tissue and stromal cath. D were found to be significantly correlated to each other (p = 0.002) (Table 4).

Correlation between nm23 and cathepsin D:

Tissue cath.D was partially significantly correlated with cytoplasmic nm23 (p = 0.05).

DISCUSSION

The pathogenesis of metastasis consists of linked, sequential and selective steps that are regulated by transient or permanent changes in different genes **[17]**.

The nm23 gene products may play distinct and possibly opposite roles in tumours of different origin.

In the present study we noticed weak expression of nm23-H1 gene product in benign glandular epithelium of BPH with strong staining of the basal cells. These results are in agreement with previous studies **[2,23]**. Moreover, previous studies in other tissues such as beast, gastric, colon, urinary bladder and skin reported that normal and benign tissue showed weak expression of nm23-H1 compared to malignant tissue **[3,11,18,25]**.

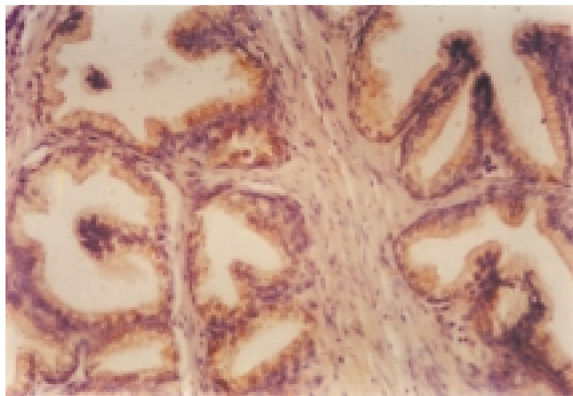

Fig. (1): Nm23-H1 immunostaining in benign prostatic hyperplasia. Weak faint expression, predominantly of the basal cell layer. Immunoperoxidase staining with Mayer's Hx counter stain X 400.

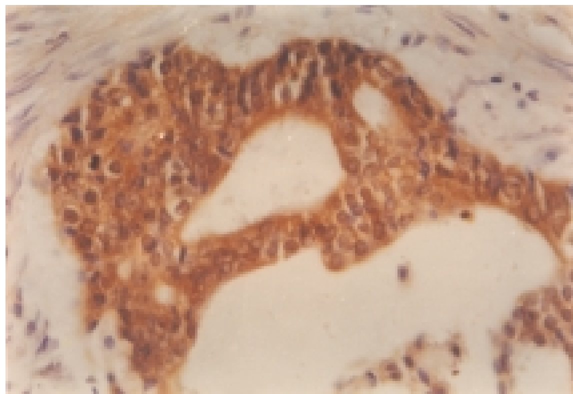

Fig. (2): Prostatic intraepithelial neoplasia, showing strong nm23-H1 expression. Immunoperoxidase staining with AMyer's Hx counter stain X 400.

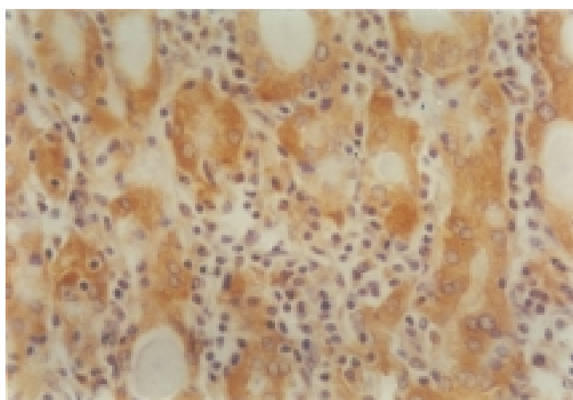

Fig. (3): Low-grade prostatic adenocarcinoma, showing moderate to strong nm23-H1 expression, mainly cytoplasmic. Immunoperoxidase staining with Mayer's Hx counter stain X 400.

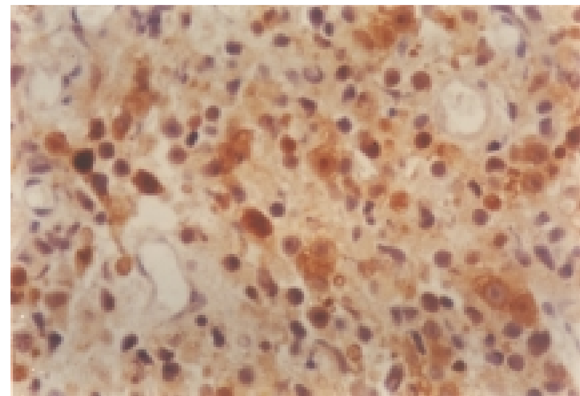

Fig. (4): High-grade prostatic adenocarcinoma with diffuse cellular infiltration. The malignant cells showing strong nuclear as well as cytoplasmic nm23-H1 expression. Immunoperoxidase staining with Mayer's Hx counter stain X 400.

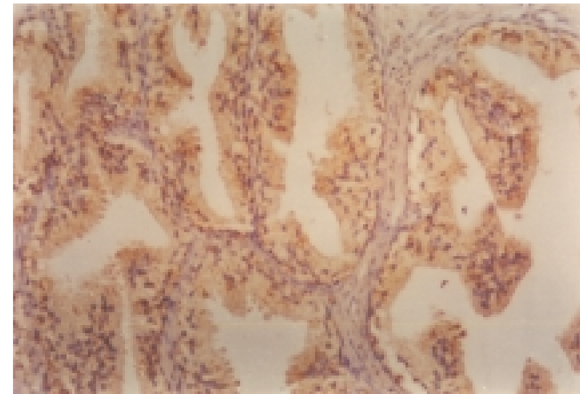

Fig. (5): Cathepsin D immunostaining in benign prostatic hyperplasia. The benign cells showing weak diffuse staining. Immunoperoxidase staining with Mayer's Hx counter stain X 400.

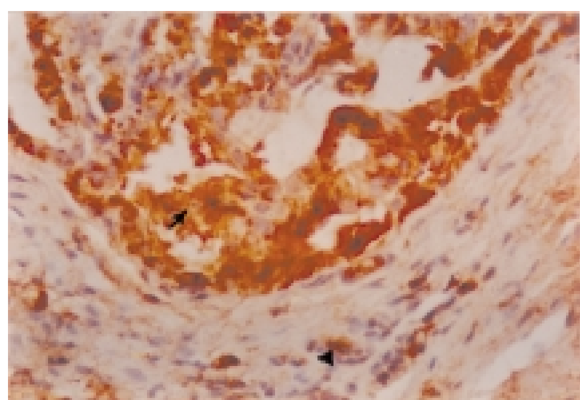

Fig. (6): Prostatic intraepithelial neoplasia, showing strong granular cathepsin D expression in tissue (arrow) and stromal cells (arrowhead). Immunoperoxidase staining with Mayer's Hx counter stain X 400.

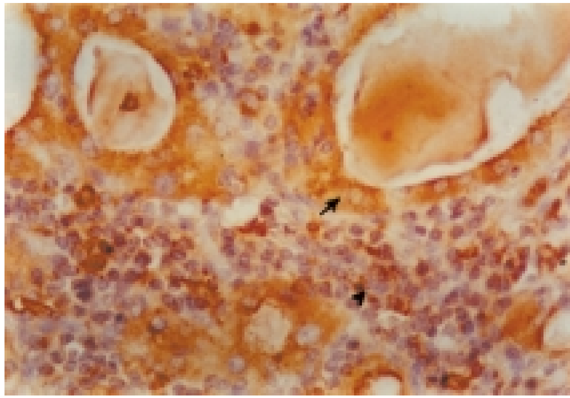

Fig. (7): Moderate granular cathepsin D immunostaining in glands (arrow) and stromal cells (arrowhead) in prostatic adenocarcinoma. Immunoproxidase staining with Mayer's Hx counter stain X 400.

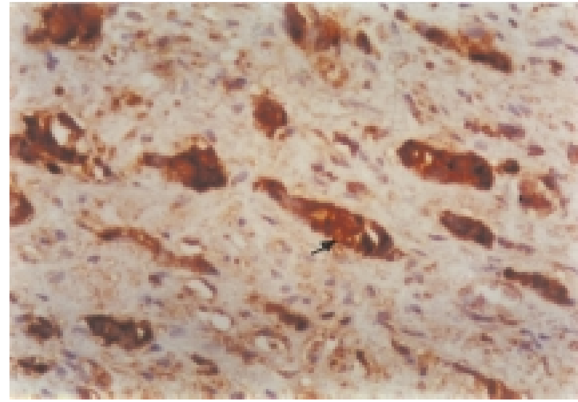

Fig. (8): Strong granular cathepsin D immunostaining in high-grade prostatic adenocarcinoma (Arrow). Immunoperoxidase staining with Mayer's Hx counter stain X 400.

Table (1): Clinicopathological data of the studied groups.

	BPH (22)		PIN (5)		CAP (23)	
	No.	%	No.	%	No.	%
Age (y):						
≤ 60-64	8	(36.4)	3	(60)	7	(30.4)
65-69	3	(13.6)	1	(20)	8	(34.8)
≥ 70	11	(50)	1	(20)	8	(34.8)
Biopsy:						
TUR	19	(95)	5	(100)	7	(30.4)
Prostatectomy	3	(5)	-		8	(34.8)
Needle	-		-		8	(34.8)
PSA:						
≤ 4-6.9 ng/ml	-		-		8	(34.8)
≥ 7 ng/ml	-		-		15	(65.2)
WHO grade:						
I	-		-		1	(4.3)
II	-		-		16	(69.6)
III	-		-		6	(26.1)
Gleason's score:						
≤ 5	-		-		7	(30.4)
6	-		-		6	(26.1)
≥ 7	-		-		10	(43.5)
Whitmore stage:						
B	-		-		13	(56.5)
C	-		-		10	(43.5)
TNM stage:						
T2	-		-		14	(60.9)
T3	-		-		7	(30)
T4	-		-		2	(8.7)
V. invasion:						
Positive	-		-		4	(17.4)
Negative	-		-		19	(82.6)

TUR = Trans-uretheral resection.

Table (2): Expression of nm23 and cathepsin D in the different studied groups.

	BPH (22)		PIN (5)		CAP (23)		X^2	*p*-value
	No.	%	No.	%	No.	%		
Cyto. nm23:							13.9	0.0009
1 & 2	18	(81.8)	0	(0)	8	(34.8)		HS**
3 & 4	4	(18.2)	5	(100)	15	(62.2)		
Nucl. nm23:							12.8	0.001
-ve	22	(100)	5	(100)	14	(60.9)		S*
+ve	0		0		9	(39.1)		
T. cathepsin D:							3.7	0.15
1	16	(72.7)	1	(20)	11	(47.8)		NS
2 & 3	6	(27.3)	4	(80)	12	(52.2)		
S. cathepsin D:							19.9	0.00004
1	22	(100)	1	(20)	11	(47.8)		HS**
2 & 3	0		4	(80)	12	(52.2)		
P. cathepsin D:							50.0	0.0001
Granular	0		5	(100)	23	(100)		HS**
Diffuse	22	(100)	0		0			

Cyto = Cytoplasmic S = Stromal S* = Significant T = Tissue BPH = Benign prostatic hyperplasia
Nucl = Nuclear P = Pattern NS = Non significant HS** = Highly significant PIN = Prostatic intraepithelial neoplasia
CAP = Cancer prostate

Table (3): Relationship of nm23 expression and various clinicopathological variables in prostatic carcinoma group.

Variables	No.	Cyto. nm23 1 & 2 No. %	Cyto. nm23 3 & 4 No. %	*p*-value	Nucl. nm23 +ve No. %	*p*-value
Age (y):						
≤ 60-64	7	2 (28.6)	5 (71.4)		3 (42.9)	
65-69	8	3 (37.5)	5 (62.5)	*p* = 0.91	4 (50)	*p* = 0.47
≥ 70	8	3 (37.5)	5 (62.5)	NS	2 (25)	NS
PSA:						
≤ 4-6.9 ng/ml	8	3 (37.5)	5 (62.5)	0.59	3 (37.5)	*p* = 0.99
≥ 7 ng/ml	15	5 (33.3)	10 (66.7)	NS	6 (40)	NS
WHO grade:						
I & II	17	5 (29.4)	12 (70.5)	0.62	7 (41.2)	*p* = 0.81
III	6	3 (50)	3 (50)	NS	2 (33.3)	NS
Gleason's score:						
≤ 5	7	2 (28.6)	5 (71.4)	*p* = 0.88	2 (28.6)	*p* = 0.73
6	6	2 (33.3)	4 (66.7)	NS	2 (33.3)	NS
≥ 7	10	4 (40)	6 (60)		5 (50)	
Whitmore stage:						
B	13	6 (46.1)	7 (53.9)	0.37	2 (15.4)	*p* = 0.02
C	10	2 (20)	8 (80)	NS	7 (70)	S*
TNM stage:						
T2	14	6 (46.1)	8 (57.1)	0.39	3 (21.4)	*p* = 0.09
T3 & T4	9	2 (22.2)	7 (77.3)	NS	6 (66.7)	NS
V. invasion:						
Positive	4	1 (25)	3 (75)	0.56	2 (50)	*p* = 0.73
Negative	19	7 (36.8)	12 (63.2)	NS	7 (36.8)	NS

Cyto = Cytoplasmic Nucl = Nuclear S* = Significant NS = Non significant V. = Vascular

Table (4): Relationship of cathepsin D expression and various clinicopathological variables in prostatic carcinoma group.

Variables	No.	Tissue cath. D		p-value	Stromal cath. D		p-value
		1 No. %	2 & 3 No. %		1 No. %	2 & 3 No. %	
Age (y):							
≤ 60-64	7	2 (28.6)	5 (71.4)		2 (28.6)	5 (17.4)	
65-69	8	5 (62.5)	3 (38.5)	0.41	4 (50)	4 (50)	0.41
≥ 70	8	4 (50)	4 (50)	NS	5 (62.5)	3 (38.5)	NS
PSA:							
≤ 4-6.9 ng/ml	8	6 (75)	2 (25)	0.08	7 (87.5)	1 (12.5)	0.009
≥ 7 ng/ml	15	5 (33.3)	10 (66.7)	NS	4 (26.7)	11 (73.3)	HS**
WHO grade:							
I & II	17	10 (58.8)	7 (41.2)	0.15	11 (64.7)	6 (35.3)	0.01
III	6	1 (16.7)	5 (83.3)	NS	0	6 (100)	S*
Gleason's score:							
≤ 5	7	5 (71.4)	2 (28.6)		6 (85.7)	1 (14.3)	
6	6	3 (50)	3 (50)	0.24	4 (66.7)	2 (33.3)	0.004
≥ 7	10	3 (30)	7 (70)	NS	1 (10)	9 (90)	HS**
Whitmore stage:							
B	13	9 (64.3)	4 (30.8)	0.03	10 (76.9)	3 (23.1)	0.002
C	10	2 (20)	8 (80)	S*	1 (10)	9 (90)	HS*
TNM stage:							
T2	14	9 (64.3)	5 (35.7)	0.08	10 (71.4)	4 (28.6)	0.009
T3 & T4	9	2 (22.2)	7 (77.3)	NS	1 (11.1)	8 (88.9)	HS**
V. invasion:							
Positive	4	1 (25)	3 (75)	0.59	1 (25)	3 (75)	0.59
Negative	19	10 (52.6)	9 (47.4)	NS	10 (52.6)	9 (47.4)	NS

V. = Vascular S* = Significant NS = Non significant HS** = Highly significant

In recent years, attention has been focused on the potential role of prostatic intraepithelial neoplasm as a preinvasive lesion of the prostate [32]. The development of PIN is characterized by increased expression of several biomarkers, which may influence the proliferative potential of the dysplastic cells [36]. PIN is frequently observed in the peripheral zone of the prostate, the area from which most of the adenocarcinomas develop. In addition, the frequency of PIN in prostates with adenocarcinoma is higher than in benign prostate [37]. Several studies have shown a concordance between the ploidy status of PIN and the adjacent malignancy [35,36]. In addition, chromosomal loss of heterozygosity on chromosome 8p12-21, a common finding in CAP, also occurs in PIN lesion [37]. The most interesting observation of the present study was the detection of strong nm23-H1 expression in dysplastic prostatic epithelium. Nm23-H1 gene product was reported to be strongly expressed in dysplastic and malignant cells [35,37]. The strong immunostaining in PIN cases was similar to that of adenocarcinoma cases which suggested that PIN is a preinvasive lesion and so strong expression of nm23-H1 is likely represent an early event in the development of CAP [36]. Therefore, the strong expression of nm23-H1 in PIN and CAP represents an additional phenotypic similarity of the two lesions.

The initial hypothesis that decreased nm23-H1 expression in primary tumour correlates with increased frequency of metastasis and thus, decreased patient survival, could not be confirmed in all tumour systems. Although several studies have shown the expression of nm23 in prostatic adenocarcinomas, the results in some cases seem contradictory. Igawa et al. [19] showed increased immunostaining for nm23-H1 in poorly differentiated as well as advanced stage prostatic adenocarcinomas. In contrast, Konishi et al. [23] showed decreased expression of nm23-H1 in metastatic compared with localized prostatic lesions. The reason for these apparently contradictory findings is unclear. It is possible that this disparity is the result of different antibodies to nm23-H1 in these studies. In the present study, we used a monoclonal antibody directed against nm23-H1 gene product only. Moreover, localization of prostate cancer metastasis suppressor activity on human chromosome 17, revealed absence of nm23 in the conserved metastasis suppressor region, which

further support the concept that nm23-H1 does not act as a metastatic suppressor gene in cancer prostate [6,7].

The role of increased nm23-H1 expression in prostatic carcinogenesis is not fully understood. High nm23 expression appears to be associated with rapid cell proliferation instead of its antimetastatic activity. Tissue specific factors may be involved in the dissociation of nm23 expression from its antimetastatic activity [25]. Nm23 expression has been shown to be proportional to proliferation, growth factors receptor levels and signal transducing protein [29]. In this respect, the increased nm23-H1 expression detected in PIN and prostatic adenocarcinoma may be associated with the increased proliferative potential of both of these lesions [20,47].

In the present study, staining for nm23-H1 usually cytoplasmic but nuclear staining was also seen in some cases. Strong expression of cytoplasmic nm23-H1 was observed with advancement in tumour grade, however this trend was not significant. While, nuclear nm23-H1 was significantly related to progression in tumour stage (p = 0.02). The heterogeneous pattern of immunoreactivity for nm23-H1 may reflect their diverse biological significance by accumulation of the nm23-H1 gene product in different cellular compartments [11]. Nuclear nm23-H1 protein staining was reported to identify a category with worse prognosis in thyroid carcinoma [31]. This goes with our results, where nuclear staining was expressed in advanced tumour with highly atypical and singly infiltrated cells.

In the present study, cathepsin D, an aspartyl proteolytic enzyme, has been evaluated in tumour and host cells separately. A polyclonal antibody, which demonstrates the active mature form of cath. D, was used. We selected this method because it has the advantage of precise tissue localization of the enzyme as compared with cytosolic immunoenzymatic assays [28]. This is particularly important with cath. D, which is highly expressed by infiltrating inflammatory cells [28]. Tumour samples, which contain invading stromal and inflammatory cells contaminant, express high levels of cath. D and so possibly leading to an inappropriate estimation of the role of this protease [13,17].

Our results of cath. D in BPH, PIN and CAP were in agreement with previous studies [5,24,30]. Cath. D were significantly more expressed in PIN and CAP than BPH (p = 0.0004) and the expression is mainly granular, while in benign epithelium it was weak and diffuse. This finding is proposed to reflect the predominantly lysosomal localization of cath. D in the cell and a potential change in cath. D function [1].

In the present study a significant relation has been observed between cath. D and PSA level (p = 0.009). PSA is a serine protease with chymotrypsin-like activity and has been shown to hydrolyze some of the insulin-like growth factor binding proteins (IGFBPs), the inhibitors of insulin-like growth factors (IGFs). The resulting increase in free IGFs level with a mitogenic activity, thereby stimulating cell proliferation [8]. However, the IGFBPs secreted from prostatic cancer cells were also found to be hydrolyzed by cath. D. Moreover, cath. D was found to be capable of hydrolyzing PSA, indicating that it could modulate PSA action as well. Taken together, both PSA and cath. D appear to act upon the IGFs system, one of the major regulatory systems in prostate growth suggesting that they play an important role in the growth regulation of prostate cancer [9].

High stromal cath. D expression was significantly correlated to Gleason's score (p = 0.004) and WHO grading system (p = 0.01) in the present study. In agreement with our results Maygarden et al. [30] found a significant association of cath. D with Gleason's score. Strong expression of cath. D was related to cell proliferation. The higher proliferation rate of strongly positive tumours may be partly related to a mitogenic effect of cath. D [30,33,34].

Our results showed a significant relation between stromal cath. D and whitmore (p = 0.002) and TNM (p = 0.009) tumour staging systems. The role of cath. D in invasion and metastasis has been largely studied [28]. Although the mechanism of metastatic stimulation by mature cath. D is not fully known at present, these studies at least suggest that an increase in mature cath. D may be a prerequisite for facilitating progression and metastasis [8]. Increased expression of the lysosomal proteases; cath. D has been implicated in metastatic progression of CAP [5,36]. Moreover, it has been postulated that neoplastic cells produce paracrine growth factors that upregulate host-cell expression of

matrix proteinase enzymes facilitating the process of invasion and vessel formation [10]. In consistent with our finding, Kuczyk et al. [24] compared low stage and grade to higher stage and grade prostatic adenocarcinoma and found significantly higher cath. D in the later group.

An important finding in this study is that stromal cath. D and not tissue cath. D that was significantly associated with various clinicopathological parameters. Tissue cathepsin D was significantly related to tumour stage only (p = 0.03). It is known that in certain neoplasms, the number of tumour-associated histiocytes correlates with tumour invasiveness and positive staining of non-neoplastic host cells was associated with high grade tumour and high cell mitotic rate [28]. This observation directs the attention to the importance of separate evaluation of stromal and tissue positivity.

Collectively, both nm23-H1 and cath. D was significantly correlated. Both markers seem to be related to more aggressive tumours with advancement in stage and grade.

In conclusion, the similar expression of nm23-H1 in PIN and invasive prostatic adenocarcinoma continues to support the previous observation of the close phenotype similarity of these two lesions. Thus, strong expression of nm23-H1 likely represents an early event in prostatic carcinogenesis that remains throughout the progression of malignancy. Moreover, enhanced expression of proteolytic enzymes including cath. D by dysplastic cells in PIN may represent an initial event in the development of invasive CAP. In prostate nm23-H1 does not behave as a metastatic suppressor gene, in the contrary it is more correlated to metastasis, so the search for a marker of antimetastatic potential in CAP must be continued. On the other hand, presence and localization of cath. D suggested its involvement in invasion and metastasis in CAP. It is conceivable that cath. D when used with current modalities such as serum PSA and Gleason's score might improve the accuracy of prostate cancer staging.

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