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NKX3.1 as a Marker of Prostatic Origin in Metastatic Tumors

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Abstract

NKX3.1 is a prostatic tumor suppressor gene located on chromosome 8p. Although most studies have shown that staining for NKX3.1 protein is positive in the majority of primary prostatic adenocarcinomas, it has been shown to be downregulated in many high-grade prostate cancers, and completely lost in the majority of metastatic prostate cancers (eg, in 65% to 78% of lesions). A recent study showed that NKX3.1 staining with a novel antibody was highly sensitive and specific for high-grade prostatic adenocarcinoma when compared with high-grade urothelial carcinoma. This raised the question that this antibody may perform better than earlier used antibodies in metastatic prostate tumors. However, the sensitivity and specificity for prostate carcinomas for this antibody in metastatic lesions was not determined. Although prostate-specific antigen (PSA) and prostatic-specific acid phosphatase (PSAP) are excellent tissue markers of prostate cancer, at times they may be expressed at low levels, focally, or not at all in poorly differentiated primary and metastatic prostatic adenocarcinomas. The purpose of this study was to determine the performance of NKX3.1 as a marker of metastatic adenocarcinoma of prostatic origin. Immunohistochemical staining against NKX3.1, PSA, and PSAP was carried out on a tissue microarray (TMA) (0.6-mm tissue cores) of hormone naïve metastatic prostate adenocarcinoma specimens from lymph nodes, bone, and soft tissue. To determine the specificity of NKX3.1 for prostatic adenocarcinoma, we used TMAs that contained cancers from various sites including the urinary bladder, breast, colon, salivary gland, stomach, pancreas, thyroid, and central nervous system, and standard paraffin sections of cancers from other sites including the adrenal cortex, kidney, liver, lung, and testis. Overall 349 nonprostatic tumors were evaluated. Any nuclear staining for NKX3.1 was considered positive and the percentage of cells with nuclear staining and their mean intensity level were assessed visually. Sensitivity was calculated by considering a case positive if any TMA core was positive. The sensitivity for identifying metastatic prostatic adenocarcinomas overall was 98.6% (68/69 cases positive) for NKX3.1, 94.2% (65/69 cores positive) for PSA, and 98.6% (68/69 cores positive) for PSAP. The specificity

of NKX3.1 was 99.7% (1/349 nonprostatic tumors positive). The sole positive nonprostatic cancer case was an invasive lobular carcinoma of the breast. NKX3.1 seems to be a highly sensitive and specific tissue marker of metastatic prostatic adenocarcinoma. In the appropriate clinical setting, the addition of IHC staining for NKX3.1, along with other prostate-restricted markers, may prove to be a valuable adjunct to definitively determine prostatic origin in poorly differentiated metastatic carcinomas.

Keywords

NKX3.1 protein; metastatic prostatic carcinoma; immunohistochemistry

Immunohistochemical (IHC) markers are often employed as adjunctive aids in the diagnosis of prostatic adenocarcinoma, especially in the setting of limited cancer foci on a needle biopsy.^{13,39} As the diagnosis of prostate adenocarcinoma in these difficult cases relies partly on the demonstration of the lack of a basal cell layer,^{13,18,31,39} IHC markers exclusive to basal cells, such as p63, cytokeratin 5/6, and high molecular weight cytokeratin (34βE12), are generally employed. Alpha-methylacyl-CoA racemase (AMACR), also known as P504S, which has been shown to be significantly upregulated in prostate cancer,^{19,20,26,32,42} is also used as an adjunctive aid in the diagnosis of limited primary prostate carcinoma on needle biopsy.

In the diagnosis of metastatic carcinoma from uncertain primary sites, or poorly differentiated high-grade neoplasms involving the prostate and adjacent organs, markers that are prostate epithelium-specific/restricted, such as prostatic-specific antigen (PSA) and prostatic-specific acid phosphatase (PSAP) have been used for many years as diagnostic aids. Although PSA and PSAP are markers of prostate cancer in these settings, at times they may be only focally or weakly expressed in poorly differentiated prostatic adenocarcinomas.^{11,39} Thus, the diagnosis of prostate cancer in TUR specimens, biopsies, and in metastatic sites can be difficult at times, even with these markers, and an additional prostate-specific marker could be of diagnostic value in such cases. Newer prostate-restricted markers such as prostate-specific membrane antigen (PSMA), and prostein (also called P501S) have also been proposed as useful adjuncts in some settings, including metastatic tumors of unknown origin.^{10,21,28,29,34,35,37,44}

NKX3.1 is an androgen-regulated homeodomain gene whose expression is predominantly localized to prostate epithelium.² *NKX3.1* is located on chromosome 8p21.2, a region that shows loss of heterozygosity (LOH) in 12–89% of high-grade prostatic intraepithelial neoplasia (PIN)^{6,12,17,33} and 35% to 86% of prostatic adenocarcinomas.^{6,8,12,17,27,33,36,40} The frequency of LOH on chromosome 8p increases with advanced prostate cancer grade and stage.^{6,40} Targeted disruption of *NKX3.1* in mice results in defects in prostate branching morphogenesis, epithelial cell differentiation, growth, and protein secretion.^{3,4,7,38} Furthermore, mice deficient in *NKX3.1* have been shown to develop prostatic epithelial hyperplasia and PIN,^{4,7,22} and in mice with targeted disruption of *Pten* or *Cdkn1b* (encoding p27), loss of one or both *NKX3.1* alleles results in accelerated and more aggressive prostate tumorigenesis.^{1,15,23} As no mutations have been detected in the remaining allele of *NKX3.1*,^{30,41} it seems to function as a haploinsufficient tumor suppressor gene.

In terms of specificity of NKX3.1 for prostatic origin in tumors of unknown primary, Gelmann et al¹⁶ showed that the expression of NKX3.1 was highly specific to prostate cancer and breast cancer, with little or no staining in a large number of other tumor types. However, only 4 of 9 (44%) of untreated metastatic prostatic adenocarcinoma cases were positive using their rabbit polyclonal antibody.¹⁶ In another study of NKX3.1 IHC staining

in prostate cancer, Bowen et al⁹ showed that whereas 92/110 (83.6%) of primary prostatic adenocarcinomas were positive, 31/40 (78%) of metastatic prostatic adenocarcinomas were negative. Chuang et al¹¹ used a more recently developed anti-NKX3.1 antibody⁶ to establish the usefulness of NKX3.1 as part of a panel of IHC markers in helping to distinguish high-grade prostatic adenocarcinomas from poorly differentiated urothelial carcinomas, which is a relatively common diagnostic challenge in lesions occurring at the bladder neck. Chuang et al,¹¹ found that the sensitivity for NKX3.1 staining in high-grade prostate adenocarcinoma (Gleason score 8 to 10) ranged from 92.1% (35/38)-94.7% (36/38) depending upon how sensitivity was calculated. The specificity for prostatic versus urothelial carcinoma was 100% (0/35 of urothelial carcinomas stained positively). As the sensitivity and specificity was so high for these high-grade prostatic tumors, we surmised that this new antibody may behave differently than those reported earlier by others when applied to metastatic prostatic carcinomas. Although Chuang et al¹¹ evaluated a small number of metastatic prostate lesions (n=5), they did not systematically evaluate the diagnostic potential of NKX3.1 for metastatic prostatic adenocarcinomas in distant sites, and they did not determine the specificity of staining of this antibody beyond testing of high-grade urothelial carcinomas. Therefore, to determine whether NKX3.1 staining could add diagnostic value in challenging cases in the distinct clinical setting of a metastatic tumor of unknown primary site, in this study we determined the sensitivity of NKX3.1 staining by applying this same antibody¹¹ to a relatively large number of metastatic prostate carcinomas. As increasing sensitivity usually results in decreased specificity, we also examined a number of tumor types other than prostatic carcinoma, including adenocarcinomas of the urinary bladder, and cancers from various sites including the breast, colon, salivary gland, stomach, pancreas, thyroid, and central nervous system, adrenal cortex, kidney, liver, lung, and testis.

MATERIALS AND METHODS

Tissues and Tissue Microarray Assembly

This study was approved by The Johns Hopkins University School of Medicine Institutional Review Board. A high-density Tissue Microarray (TMA) was constructed, as described earlier.¹⁴ It consists of normal prostate tissue (n = 18 patients), primary prostatic carcinoma (n = 20 patients), and hormone naïve meta-static prostate carcinoma tissues. Metastatic lesions were obtained from surgical biopsies from metastatic sites in soft tissue, bone, and solid organs (n = 15 patients) or metastatic lesions within pelvic lymph nodes that were generally obtained from radical retropubic prostatectomies with pelvic lymph node dissections (n = 59 patients) carried out at the Johns Hopkins Hospital between 1987 and 2001. Four tissue cores were taken per donor block of each metastatic tumor in this TMA. However, owing to either loss of tissue or loss of tumor tissue upon block sectioning, material from 69 of 74 metastatic cases was able to be evaluated. In addition, most cases had between 1 and 3 cores that could be evaluated (Table 1). Patient ages ranged from 43 to 89 years (median = 60), the Gleason sums for the primary prostate cancers varied from 6 to 9 (mean = 7.06) and the pathologic stages ranged from T2N0Mx to T3bN1Mx. To assess the specificity of the NKX3.1 antibody, we used TMAs containing various carcinomas, such as breast, colon, salivary gland, stomach, thyroid, and adenocarcinomas of the bladder, and standard paraffin sections of other malignancies including: adrenal cortical carcinomas, renal cell carcinomas, hepatocellular carcinomas, lung carcinomas, carcinoid tumors, and testicular germ cell tumors (Table 2). The TMA containing in situ and infiltrating adenocarcinoma of the bladder specimens has been described earlier.²⁵

Immunohistochemistry

Immunohistochemistry for NKX3.1 was conducted using the EnVision + IHC kit (DAKO, Carpinteria, CA) and PSA and PSAP immunostaining was done with the Power Vision + poly-HRP IHC Kit (ImmunoVision Technologies, Co., Norwell, MA). Slides were steamed for 20 minutes in citrate antigen unmasking buffer (Vector Laboratories, Burlingame, CA) and were incubated with either rabbit polyclonal anti-NKX3.1⁶ (Athena Environmental Sciences, Inc., Baltimore MD), mouse monoclonal anti-PSA (DAKO, Carpinteria, CA, 1:500 dilution) or mouse monoclonal anti-PSAP antibody (DAKO, Carpinteria, CA, 1:50 dilution) for 45 minutes. Poly-HRP-conjugated anti-mouse/rabbit IgG antibody, as supplied in the Envision + or Powervision + kits, was used as the secondary antibody. Staining was visualized using 3,3'-Diaminobenzidine (DAB) (Sigma, Saint Louis, MO, FAST 3,3'-Diaminobenzidine Tablets) and slides were counterstained with hematoxylin.

Evaluation of Immunohistochemical Staining

The anti NKX3.1, PSA, and PSAP staining observed in the metastatic prostate cancer TMA spots were evaluated visually and the percentage of epithelial cells staining positively was assessed. In addition, every spot was assigned a mean intensity between 0 and 3 and a staining score was generated by multiplying the mean intensity score and the percent cells stained positively. To directly compare the performance of each antibody with the other, only spots that were present on the TMA slides for all 3 markers were included in the analysis. Statistical analyses were done using Stata 9.0 software (Stata, College Station, TX).

RESULTS

Staining in Normal Tissues

As we reported earlier,⁶ the intensity of staining for NKX3.1 was generally somewhat stronger in normal prostatic epithelium than in prostatic adenocarcinoma cells. In normal prostatic epithelium, as observed earlier,^{5,6,9,11,24} the staining with the anti-NKX3.1 antibody was primarily confined to the nuclei of the secretory luminal cells, with very little or no cytoplasmic staining (Fig. 1). A majority of basal cells were negative, with some weakly staining basal cells in some acini. Overall, 41 of 41 normal prostate tissue specimens stained positively with NKX3.1, which was comparable to PSA and PSAP staining, with 40 of 41 cases positive for both PSA and PSAP. Of all the nonprostatic benign tissues stained with NKX3.1, only the mucinous component of salivary gland tissue (Fig. 1) and sertoli cells showed moderate nuclear staining in infantile seminiferous tubules stained positively. Some sertoli cells in 2 adult testes were also positive for NKX3.1.

Staining in Primary Tumors

In primary prostatic adenocarcinoma specimens (Table 3, Fig. 2), all 40 cases stained positively for NKX3.1 and the mean percent of tumor cells staining positive in their nuclei was 84.7% (range: 25% to 100%). There was some weak to moderate cytoplasmic staining for NKX3.1 in many cases, but only nuclear staining was scored as positive. Staining for PSA and PSAP was similarly positive in all 40 primary prostatic adenocarcinoma cases. The mean percentage of tumor cells staining positive in the primary tumors was 87.3% for PSA (range: 10% to 100%) and 98.6% for PSAP (range: 85% to 100%). Table 3 shows the overall mean staining scores for all 3 antibodies in all sites examined in this study.

Staining in Metastatic Tumors

Although the intensity of staining observed in lymph node metastases was somewhat lower compared with normal prostate and primary prostatic adenocarcinomas, 59 of 59 cases were

positively stained with the NKX3.1 antibody, for a sensitivity of 100% (Table 4, Fig. 3). In comparison, PSA staining was positive in 58 and PSAP was positive in 59 cases, for a sensitivity of 98.3% and 100%, respectively.

Although distant metastasis specimens stained somewhat less intensely for NKX3.1 compared with normal prostate, primary carcinoma, and lymph node metastases (Table 3), 9 of 10 cases stained positively, for a sensitivity of 90% (Fig. 4). The sensitivity of NKX3.1 in identifying metastatic prostatic adenocarcinomas overall was 98.6% (68/69 cases positive) whereas the sensitivity of PSA and PSAP staining was 94.2% (65/69 cores positive) and 98.6% (68/69 cores positive), respectively.

Looking at the different combinations of NKX3.1, PSA, and PSAP, the combination most successful in identifying prostate tissue was that of NKX3.1 and PSAP because 69 of the 69 tissue cores of prostate metastases evaluated stained positively. NKX3.1 and PSA staining combined was positive in 68 of 69; whereas PSA and PSAP combined similarly stained 68 of 69 metastases. Combining all 3 antibodies, all metastatic prostatic adenocarcinomas were positive for at least 1 marker (sensitivity of 100% using all 3 markers).

To determine the specificity of NKX3.1 in differentiating prostatic tissue and other glandular tissues, we stained TMAs and standard tissue sections composed of a number of various malignancies and matched normal tissues (Table 2). Except for one case of infiltrating lobular carcinoma of the breast (Fig. 1C), all neoplastic tissues, including all cases of in situ or infiltrating adenocarcinoma of the bladder, were negative for NKX3.1, for a calculated specificity of 99.7% (1/349 cases positive).

DISCUSSION

Studies of *NKX3.1* mRNA and protein expression in human prostate cancer and prostatic intraepithelial neoplasia (PIN) have provided somewhat contradictory results. Xu et al⁴³ reported that in prostatic adenocarcinomas *NKX3.1* mRNA was overexpressed in 31%, decreased in 21% and was similar to normal epithelium in 48% of cases. Also, a higher fraction of tumor samples showed *NKX3.1* mRNA overexpression in nonorgan confined tumors (40%) versus organ confined disease (22%). In contrast, Ornstein et al did not find a change in *NKX3.1* mRNA levels by quantitative in situ hybridization in prostatic adenocarcinomas compared with normal prostate in their study of early-stage prostate cancers.³⁰ In fact, these researchers suggested that as *NKX3.1* was expressed nearly exclusively in the prostate in adult tissues, it could prove to be a useful marker of malignant prostate epithelium.

Bowen et al, reported that loss of NKX3.1 protein expression, as assessed by immunohistochemistry (IHC), correlated with prostate cancer progression⁹; specifically, they reported complete loss of NKX3.1 staining in 20% of high-grade PIN, 6% of stage T1a/b samples, 22% of stage T3/4 samples, 34% of hormone-refractory prostate cancers, and 78% of metastases. By contrast, Korkmaz et al²⁴ conducted in situ hybridization for mRNA expression and IHC for protein staining on adjacent TMA slides and reported that a vast majority of all prostatic adenocarcinoma cases were positive for both the mRNA and protein and that there was no correlation between *NKX3.1* mRNA or protein expression and tumor grade or clinical stage. Gelmann et al,¹⁶ reported that NKX3.1 protein was present in prostatic adenocarcinomas by IHC in 66% of primary untreated tumors, 44% of untreated metastatic tumors, and 27.3% of castrate resistant/hormone refractory tumors. Asatiani et al showed that although there was reduced intensity of staining for NKX3.1 protein, quantified by image analysis, complete loss in NKX3.1 protein was observed in only 4.6% of primary tumor samples.⁵ Bethel et al observed reduced levels of NKX3.1 protein in PIN and in

primary prostatic adenocarcinomas, although virtually all cases retained moderate to high amounts of NKX3.1 staining.⁶ Using the same rabbit polyclonal antibody as used by Bethel et al⁶ (and this study), Chuang et al¹¹ recently reported that NKX3.1 protein staining was a highly specific and relatively sensitive marker when used as a diagnostic aid as part of a panel of IHC markers in helping to distinguish high-grade prostate carcinoma from high-grade bladder urothelial carcinoma. However, the study by Chuang et al¹¹ did not systematically examine the expression of NKX3.1 in metastatic prostatic adenocarcinomas, nor did it determine the specificity for prostate carcinoma beyond examination of urinary bladder cancers.

Although there is some controversy, the prevailing model of NKX3.1 protein expression in human prostatic adenocarcinoma is that the levels are reduced in primary prostate cancers and further reduced and often lost in metastatic lesions.² Contrary to this view, we found that NKX3.1 expression was retained in metastatic prostatic adenocarcinomas in the vast majority of cases. In fact, in some distant metastases, NKX3.1 staining was higher in both staining intensity and the percentage of tissue stained compared with PSA. The genetic data are consistent with retained expression of NKX3.1 in most cases—whereas loss of one allele of the *NKX3.1* gene is common, no mutations have been described in the remaining *NKX3.1* allele. It is highly likely that some of the differences found to date in the literature regarding the presence of retained NKX3.1 protein in prostatic adenocarcinomas are related to the performance of the different antibodies that have been employed. The antibody used in this study is a relatively newly developed rabbit polyclonal antibody raised against a recombinant polypeptide corresponding to the NH2-terminal 123 amino acids of human NKX3.1,⁶ and we found this antibody to be highly specific and to perform well by IHC.^{6,11} This antibody is currently undergoing licensing by a commercial vendor such that it should be available commercially (Athena Environmental Sciences Inc.) by the time this manuscript is published.

Immunohistochemical studies have been employed in two main areas in which establishing the prostatic origin of a neoplasm is imperative: transurethral resection or biopsy specimens, in which the most common scenario is the need to distinguish poorly differentiated high-grade primary prostatic adenocarcinoma from high-grade urothelial carcinoma^{11,31,39} and metastatic adenocarcinomas of unknown origin, in which the differential diagnosis is much wider.³⁹ When considering metastatic malignant neoplasms of unknown origin, PSA and PSAP are the most commonly employed markers in identifying prostatic carcinomas. However, despite their high selectivity for prostate tissue, PSA and PSAP have been known to be expressed at a significantly lesser extent in metastatic prostate carcinomas, and may at times be expressed at least somewhat in a number of non-prostate tumors.³⁹ The most well-studied examples of this are PSA expression in breast carcinomas and salivary gland neoplasms and PSAP staining in carcinoid tumors.³⁹ Nevertheless, although PSA and PSAP positivity should be interpreted somewhat cautiously when examining tumor specimens of unknown primary, and certainly in the context of histopathologic and clinical findings, most pathologists consider these markers to be relatively sensitive, specific and useful for distinguishing prostatic origin in the context of metastatic lesions. In this study, we found NKX3.1 staining to be highly specific for prostate epithelium as it was positive in only 1 of 383 nonprostatic tumors, and this tumor was a lobular carcinoma of the breast, which does not enter into the differential diagnosis when dealing with prostatic adenocarcinoma except in very rare cases of potential metastatic breast carcinoma in males. Another relatively recently discovered protein, P501S (also known as prostein), that is selectively expressed in prostatic epithelium, has also been reported to be effective in identifying prostatic origin in metastatic carcinomas.⁴⁴ In addition, a earlier study employing the metastatic prostate carcinoma TMA used in this study, found 68 of the 69 metastatic prostate carcinoma cases and 15 of the 15 distant metastasis cases to be positive for p501s.³⁴ Like PSA and PSAP,

however, P501s staining is exclusively cytoplasmic. Thus, the potential usefulness of NKX3.1 is also highlighted by the finding that its localization is predominantly nuclear, which can add additional diagnostic confidence in cases in which there is only relatively weak staining for one or more of the cytoplasmic markers such as PSA, PSAP, or P501s.

In summary, we report for the first time that IHC staining for NKX3.1 protein is retained in most primary untreated metastatic carcinomas of prostatic origin. The major difference in our study that would seem to account for the higher sensitivity for metastatic prostatic adenocarcinoma is the use of a relatively novel antibody against NKX3.1.^{6,11} Taking this study together with another recent study also showing strong staining for NKX3.1 in the majority of poorly differentiated primary prostate cancers,¹¹ the addition of NKX3.1 protein staining to a panel of markers, if applied in the appropriate clinicopathologic context, may add diagnostic value in the diagnosis of metastatic lesions of unknown primary origin.

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FIGURE 1.

A, NKX3.1 staining in normal prostate tissue. The staining is mainly restricted to the nuclei of the luminal epithelial cells. B, NKX3.1 Staining in normal salivary gland tissue. Note that the staining is limited to the nuclei of the mucinous glands. C, NKX3.1 staining in the one positive case of invasive lobular carcinoma of the breast.

FIGURE 2.
A primary prostatic adenocarcinoma TMA spot stained with (A) NKX3.1 (B), PSA (C) and PSAP (D).

FIGURE 3.
A prostate carcinoma metastasis to a lymph node stained with H&E (A), NKX3.1 (B), PSA (C) and PSAP (D).

FIGURE 4.

A soft tissue metastasis stained with H&E (A), NKX3.1 (B), PSA (C) and PSAP (D). Note the lack of staining with PSA, while NKX3.1 and PSAP still stain strongly.

TABLE 1

Distribution of the Number of Spots Per Case That Were Able to be Evaluated For All 3 Prostate Markers Tested in Metastatic Prostate Cancer TMAs

No. Cores Per Case	Frequency	Percent
1	26	37.68
2	15	21.74
3	25	36.23
4	3	4.35
Total Cases	69	
Total Cores	143	

TABLE 2

Summary of Tumors Stained With NKX3.1

TMA's Stained	No. Cases	Positive
Metastatic prostate	69	68
Breast		
Invasive ductal carcinoma	14	0
Invasive lobular carcinoma	4	1
Bladder		
Villous adenoma	6	0
In situ adenocarcinoma	1	0
Infiltrating adenocarcinoma		
Moderately differentiated	22	0
Poorly differentiated	4	0
High-grade papillary Urothelial carcinoma	2	0
High-grade invasive Urothelial carcinoma*	35	0
Periurethral (Skene's Gland) adenocarcinoma	1	0
Colon		
Infiltrating adenocarcinoma	11	0
Central nervous system		
Glioblastoma multiforme	20	0
Anaplastic astrocytoma	20	0
Astrocytoma	20	0
Oligodendroglioma	20	0
Salivary gland		
Adenoid cystic carcinoma	18	0
Mucoepidermoid carcinoma	12	0
Acinic cell carcinoma	3	0
Adenocarcinoma, NOS	2	0
Pleomorphic adenoma	3	0
Stomach		
Intramucosal carcinoma	1	0
Infiltrating adenocarcinoma		
Moderately differentiated	7	0
Poorly differentiated	5	0
Signet cell carcinoma	8	0
Pancreas		
Infiltrating adenocarcinoma		
Moderately differentiated	10	0
Poorly differentiated	8	0
Thyroid		
Normal thyroid	5	0
Follicular adenoma	13	0

TMA's Stained	No. Cases	Positive
Papillary carcinoma	26	0
Follicular carcinoma	13	0
Misc. epithelial tumors stained using standard slides		
Adrenal cortical	10	0
Renal cell		
Papillary	6	0
Clear cell	5	0
Chromophobe	2	0
Hepatocellular carcinoma	13	0
Lung		
Squamous cell carcinoma	7	0
Adenocarcinoma	4	0
Bronchioloalveolar carcinoma	1	0
Adenosquamous carcinoma	1	0
Carcinoids		
Gastrointestinal	8	0
Lung	3	0
Liver	4	0
Testicular germ cell		
Mixed	2	0
Seminoma	2	0
Teratoma	1	0
Yolk Sac	1	0
Total Nonprostate cases	383	1

* Data are from Chung et al.¹¹ It is Included here as it is the same antibody using the same IHC staining protocol.

TABLE 3

The Average Percentage of Positively Stained Cells and the Calculated Staining Scores of NKX3.1, PSA and PSAP for Normal Prostate, Primary and Metastatic Prostate Carcinoma

	NKX3.1			PSA ^a			PSAP		
	% Positive (Range)	Staining Score(SD)		% Positive (Range)	Staining Score(SD)		% Positive (Range)	Staining Score(SD)	
Normal Prostate	92.0 (38.3–100)	218.3 (85.22)		965.0 (0–100)	241.7 (76.88)		97.6 (0–100)	287.8 (50.96)	
Primary Ca	84.7 (25–100)	179.1 (77.84)		87.3 (10–100)	180.7 (91.36)		98.6 (85–100)	249.2 (64.35)	
Lymph Node Met	74.2 (0–100)	155.4 (84.78)		80.1 (0–100)	1743.0 (99.14)		94.4 (0–100)	235.6 (78.24)	
Distant Site Met	54.0 (0–88.3)	111.4 (85.36)		30.8 (0–100)	50.8 (92.83)		74.2 (0–100)	162.0 (116.49)	
Average	80.5	175.9 (87.83)		83.1	186.0 (101.29)		95.0	248.5 (77.32)	

Ca indicates Carcinoma; Met-Metastases.

TABLE 4

Sensitivity of NKX3.1, PSA and PSAP for Metastatic Prostate Carcinoma

	NKX3.1	PSA	PSAP
Lymph Node	59/59 (100%)	58/59 (98.3%)	59/59 (100%)
Distant	9/10 (90%)	7/10 (70%)	9/10 (90%)
Overall	68/69 (98.6%)	65/69 (94.2%)	68/69 (98.6%)

Only cases in which the same cores were able to be evaluated for each of the 3 markers were used.