BIOCARE MEDICAL

# PSA and NKX3.1:

## A Comparative IHC Study of Sensitivity and Specificity in Prostate Cancer

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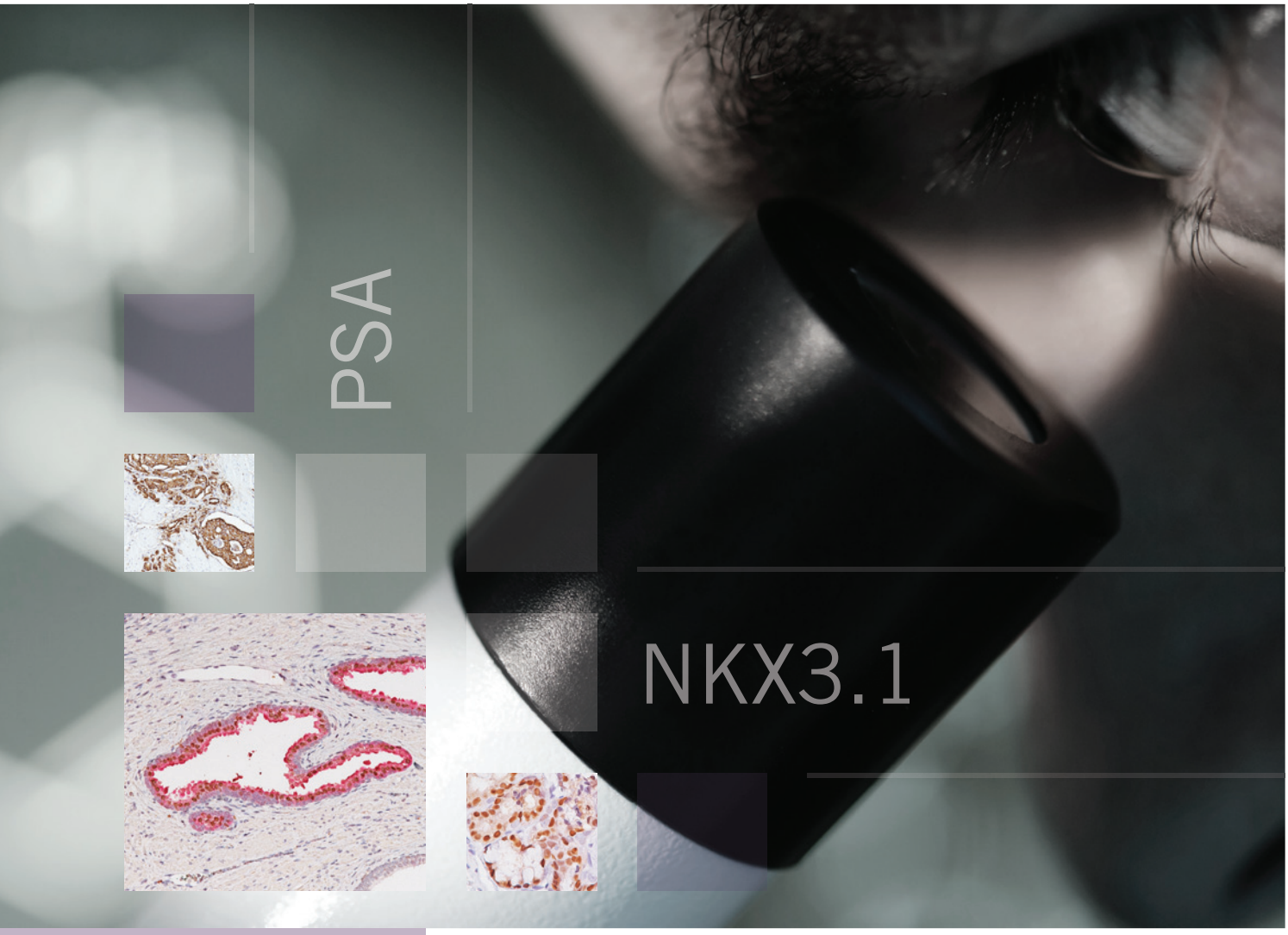




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# Background

Adenocarcinoma of the prostate can present as metastatic carcinoma, which is typically confirmed by immunohistochemistry with PSA antibodies. A new anti-PSA rabbit monoclonal (RM) antibody has been developed, which theoretically combines the advantages of high affinity, due to its rabbit origin, and high specificity, resulting from its monoclonal nature. Additionally, NKX3.1 protein has recently been shown to be a superior and sensitive marker in the majority of primary and metastatic prostatic adenocarcinomas. This study compared the staining sensitivity of a mouse monoclonal PSA (M) cocktail, the new PSA (RM), and NKX3.1 rabbit polyclonal (P). The PSA (RM) was also tested for specificity in over 600 cases of various normal and neoplastic tissues.

# Design

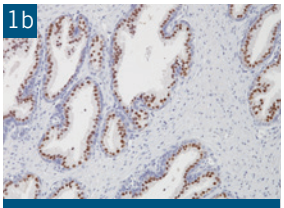
Formalin-fixed paraffin-embedded tissue microarrays (TMA) were deparaffinized in the usual manner, followed by antigen retrieval. PSA (M), PSA (RM) and NKX3.1 (P) (Biocare Medical) were optimized for staining prostate cancers, using an HRP micro-polymer detection system and visualization with DAB.

# Results

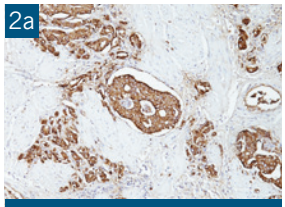
The PSA (RM) stained 163/167 (98%) cases of prostate cancer, including 94/94 cases with a Gleason score of 3 to 8, and 55/58 (95%) cases with a Gleason score of 9 or 10 (Table 1). All other cancers were negative and prostate was the only normal tissue stained by PSA (RM) (Table 2). PSA (RM) and PSA (M) antibodies stained 67 of 70 (95%) cases and were negative in the same cases, including Gleason scores 9 and 10 (Table 3). A comparison of PSA (M) and NKX3.1 on 71 cases of prostate adenocarcinoma (Grade II-IV) is summarized in Table 4.

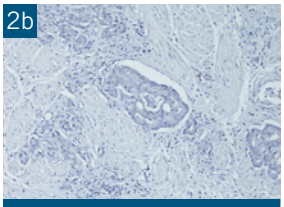
## Figure 1


1a PSA (+) in BPH prostate gland

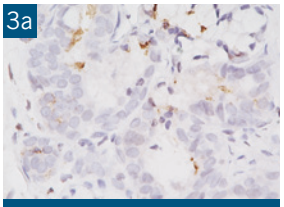

1b NKX3.1 (+) in BPH prostate gland

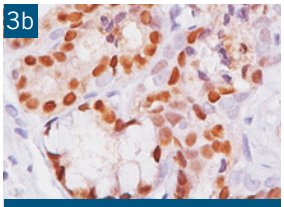
## Figure 2


2a PSA (+) in prostate cancer

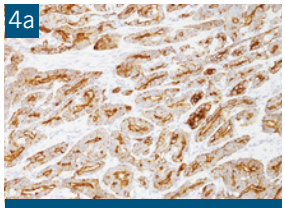

2b NKX3.1 (-) in prostate cancer

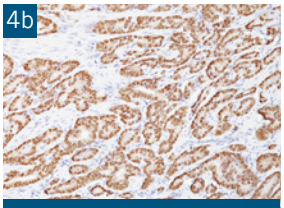
## Figure 3


3a PSA (-/focal) in prostate cancer

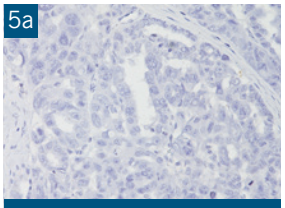

3b NKX3.1 positive in prostate cancer

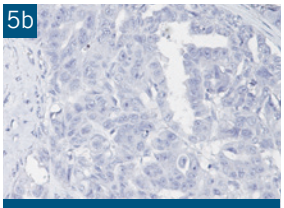
## Figure 4


4a PSA (+) in prostate cancer

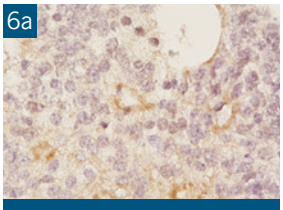

4b NKX3.1 (+) in prostate cancer

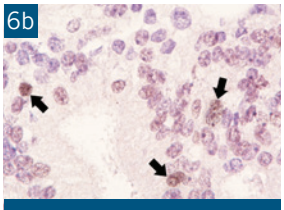
## Figure 5


5a PSA (-) in prostate cancer

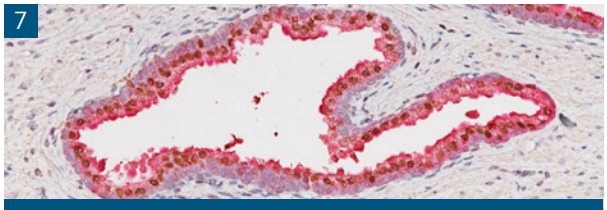

5b NKX3.1 (-) in prostate cancer

## Figure 6


6a PSA staining - difficult to interpret


6b NKX3.1 - readily distinguished

## Figure 7


7 PSA (red) + NKX3.1 (brown) Double Stain

## Table 1: Rabbit Monoclonal PSA

| Diagnosis | Cases | Positive | Negative | % + | % - |
|---|---|---|---|---|---|
| Prostate adenocarcinoma | 167 | 163 | 4 | 98% | 2% |
| Gleason Score 3-8 | 94 | 94 | 0 | 100% | 0% |
| Gleason score 9-10 | 58 | 55 | 3 | 95% | 5% |
| Stage III and IV | 44 | 43 | 1 | 98% | 0% |
| Hyperplasia | 20 | 20 | 0 | 100% | 0% |
| Chronic prostatis | 2 | 2 | 0 | 100% | 0% |
| Normal prostate | 10 | 10 | 0 | 100% | 0% |

## Table 2: Rabbit Monoclonal PSA

| Tissue Types | Cases | Positive | Negative | % + | % - |
|---|---|---|---|---|---|
| 33 FDA Normal Tissue Types | 33 | *1 | 32 | 97% | 100% |
| Pancreatic cancers | 21 | 0 | 21 | 0% | 100% |
| Renal cell cancers | 36 | 0 | 36 | 0% | 100% |
| Colon cancers | 126 | 0 | 126 | 0% | 100% |
| Bladder cancers | 90 | 0 | 90 | 0% | 100% |
| Lung cancers | 100 | 0 | 100 | 0% | 100% |
| Liver cancers | 16 | 0 | 16 | 0% | 100% |
| Melanoma | 13 | 0 | 13 | 0% | 100% |
| Breast cancer | 20 | 0 | 20 | 0% | 100% |
| GIST | 4 | 0 | 4 | 0% | 100% |
| Leimyosarcoma | 4 | 0 | 4 | 0% | 100% |
| Leiomyoma | 4 | 0 | 4 | 0% | 100% |
| Rhabdomyosarcoma | 5 | 0 | 5 | 0% | 100% |
| Seminoma | 5 | 0 | 5 | 0% | 100% |
| Stomach cancers | 6 | 0 | 6 | 0% | 100% |
| Esophageal cancers | 6 | 0 | 6 | 0% | 100% |

*Normal Prostate

## Table 3: PSA (RM) and PSA (M) Staining of Prostate Adenocacinoma

| Type | Cases | Positive | Negative | % + | % - |
|---|---|---|---|---|---|
| Rabbit Monoclonal PSA | 70 | 67 | 3 | 96% | 4% |
| Mouse Monoclonal PSA | 70 | 67 | 3 | 96% | 4% |

## Table 4: PSA (M) and NKX3.1 Staining of Prostate Adenocarcinoma

| Tumor Grade | PSA (M) | NKX3.1 (P) |
|---|---|---|
| II | 21/22 | 21/22 |
| III | 26/27 | 26/27 |
| IV | 19/22 | 20/22 |

# Discussion

PSA (RM) clone [EP1588Y] was highly sensitive for prostate cancer (Table 1). All other cancers tested were 100% negative, therefore distinguishing PSA (RM) as a highly specific antibody for prostate cancer (Table 2). When compared to an established PSA mouse monoclonal cocktail, no difference in sensitivity was observed (Table 3). In certain cases, the rabbit monoclonal antibody provided sharper staining, and in some cases, staining intensity was higher; however, in this study there was no clear advantage of either antibody.

The PSA (M) was compared to a relatively new antibody, NKX3.1. In a study by Johns Hopkins University Medical Center, NKX3.1 was shown to be a highly sensitive and specific marker of metastatic prostatic adenocarcinoma.[1] In the comparison of PSA (M) vs. NKX3.1, we observed a slightly higher sensitivity of NKX3.1 in Grade IV tumors, consistent with the report from Johns Hopkins. We observed staining of both antibodies in benign prostate hyperplasia (Figure 1-2), and positive and negative staining in invasive prostate cancer (Figures 2-5). In several cases, it was difficult to determine positive and/or background staining with the PSA (M) antibody (Figure 6). In this way, the nuclear staining of NKX3.1 is a clear advantage. Finally, the overall staining percentage is improved when both antibodies are employed for tumor of unknown origin; and thus a double stain could be employed for effective diagnosis (Figure 7).

# Conclusion

The newly developed PSA (RM) was 100% specific and demonstrated equivalent staining to PSA (M), and in some cases provided sharper staining. The NKX3.1 (P) was slightly superior to PSA (M) in Grade IV tumors. The strong nuclear staining of NKX3.1 results in easier interpretation of low expression cases, compared to the cytoplasmic staining of PSA, which can be ambiguous in these cases. PSA (RM) and NKX3.1 may be suitable for differential diagnosis and evaluation of tumors of unknown origin, particularly when used as a Multiplex IHC stain.

# References

Reference: 1. Gurel B, *et. al*. Am. J. Surg. Pathol., (2010) 24 1097.

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