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INVITED COMMENTARY

Upcycling HOXB13: enhancing prostate cancer detection with a novel antibody†

Anke Augspach[1] and Mark A Rubin[1,2]*

[1] *Department for BioMedical Research, University of Bern, Bern, Switzerland*
[2] *Bern Center for Precision Medicine, University of Bern and Inselspital, Bern, Switzerland*

**Correspondence to: MA Rubin, University of Bern, Murtenstrasse 28, room 500, 3008 Bern, Switzerland. E-mail: mark.rubin@unibe.ch*

†Invited Commentary for Patel RA, Sayar E, *et al*. Characterization of HOXB13 expression patterns in localized and metastatic castration-resistant prostate cancer. *J Pathol* 2024; **262:** 105–120.

Abstract

Prostate cancer is one of the most prevalent and, upon metastasis, deadliest cancers in men. Timely identification is essential for effective treatment. Furthermore, accurate determination of prostatic origin is crucial for personalized therapy once the cancer has spread. However, current prostate cancer screening methods are lacking. A recent article in *The Journal of Pathology* addresses this issue by utilizing an improved antibody to reevaluate HOXB13 as a lineage marker for prostate cancer. The study's findings support the concept that, despite decreased expression in advanced prostate cancer, HOXB13 remains highly suitable for determining prostatic origin due to its androgen receptor independence, high specificity, and sensitivity.

Keywords: prostate cancer; HOXB13; lineage marker; neuroendocrine prostate cancer; precision oncology; androgen receptor

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In a recent publication in *The Journal of Pathology*, Patel, Sayar *et al* [1] reintroduced the transcription factor HOXB13 as a potential diagnostic biomarker for prostate cancer (PCa), specifically for advanced metastatic castration-resistant PCa (mCRPC). Although prior studies suggested using HOXB13 in this setting, the authors propose using a new antibody that enhances HOXB13 detection, enabling them to comprehensively assess HOXB13 sensitivity and specificity across a wide range of clinically annotated prostate samples.

Challenge

PCa is the second most common cancer in men worldwide and the second leading cause of cancer-related deaths among men. The 5-year relative survival rate for localized or regional PCa is nearly 100%, but this figure drops to 32% for mCRPC. This underscores the critical importance of early detection for patients. mCRPC is treated with androgen receptor signaling inhibitors (ARSi), such as enzalutamide or abiraterone, to reduce AR activity. However, resistance to ARSi inevitably develops and can lead to progression to a highly lethal form of AR-negative mCRPC. AR negative mCPRC consists of several subtypes, including several forms, including neuroendocrine prostate cancer (NEPC), a highly lethal subtype. With the growing number of men receiving ARSi and developing potentially AR-negative mCRPC, there is a pressing need for robust methods to detect potentially aggressive PCa at its earliest stage.

Biomarkers

Significant efforts have been directed towards developing molecular biomarkers for PCa to improve its diagnosis, some of which have received FDA approval, such as prostate cancer antigen 3 (PCA3) [2]. However, these biomarkers also have notable drawbacks, including challenges in establishing a cut-off value, low abundance at early stages, inability to detect NEPC, and lack of prostate specificity. In short, a crucial research objective is identifying and validating new biomarkers for PCa with improved sensitivity for early-stage detection and confirmation of prostatic origin in AR-negative mCRPC.

HOXB13 state of the art

Over the last two decades several publications have presented HOXB13 as a biomarker that may fulfill these

criteria. Notably, HOXB13 is expressed primarily in the adult prostate. HOXB13, a member of the homeobox protein family, is a critical regulator of epithelial differentiation in the prostate gland, playing an essential role in its development and secretory functions. Furthermore, it maintains elevated levels in the normal adult prostate, vital for ensuring regular morphology and proper secretory function of prostate epithelium. Another reason to consider HOXB13 as a marker for PCa is its association with AR, despite the controversial nature of this interaction, which may have activating or repressing effects depending on gene- and context-specific factors. It is noteworthy that although HOXB13 influences and regulates AR signaling, its expression is not dependent on AR. This characteristic distinguishes HOXB13 from other lineage markers, such as prostate-specific antigen (PSA) or NKX3.1. These features make it particularly intriguing for AR-negative mCRPC (Figure 1) [3,4].

The role of HOXB13 in PCa tumorigenesis and, thus, its value as a PCa marker have also been controversial. While numerous studies have shown that germline mutations in the *HOXB13* gene are associated with PCa, and microarray-based transcriptome analyses revealed the progressive upregulation of *HOXB13* in PCa [5], it remains unclear whether HOXB13 plays a tumor-suppressive or pro-oncogenic role in PCa. In addition, reported values for the sensitivity and specificity of this biomarker are inconsistent between published studies [6,7].

HOXB13: what's new?

Patel, Sayar *et al* [1] attribute the conflicting outcomes of prior HOXB13 studies in PCa to the reduced specificity of previously employed HOXB13 antibodies, which are mainly polyclonal and susceptible to batch variations, rendering them unsuitable for widespread use. Furthermore, most previously published antibodies have not undergone formal validation for application in formalin-fixed paraffin-embedded (FFPE) tissues, potentially contributing to the significant variation in HOXB13 expression reported in PCa. The authors present data on a new anti-HOXB13 monoclonal antibody, clone D7N8O from Cell Signaling Technologies (Danvers, MA, USA). The investigators verified the specificity of D7N8O through extensive *in vitro* studies. D7N8O was shown to be suitable for immunoblots and FFPE samples. Additionally, specificity was validated *in vivo* during mouse embryo development, as the results aligned with previously reported *Hoxb13* expression patterns. Although it is generally understood that HOXB13 is elevated in the prostate, the authors identified a gap in knowledge regarding the specific temporal fluctuations of HOXB13 during human and murine development. They used RT-PCR to track *Hoxb13* mRNA in mouse embryos over 4 months and observed that expression was crucial for establishing prostatic luminal cell identity and reached its peak in adult mouse prostate tissue. This dynamic pattern of Hoxb13 mRNA and protein, with a peak occurring in adult mouse prostate, was also validated in human tissue. The researchers determined that HOXB13

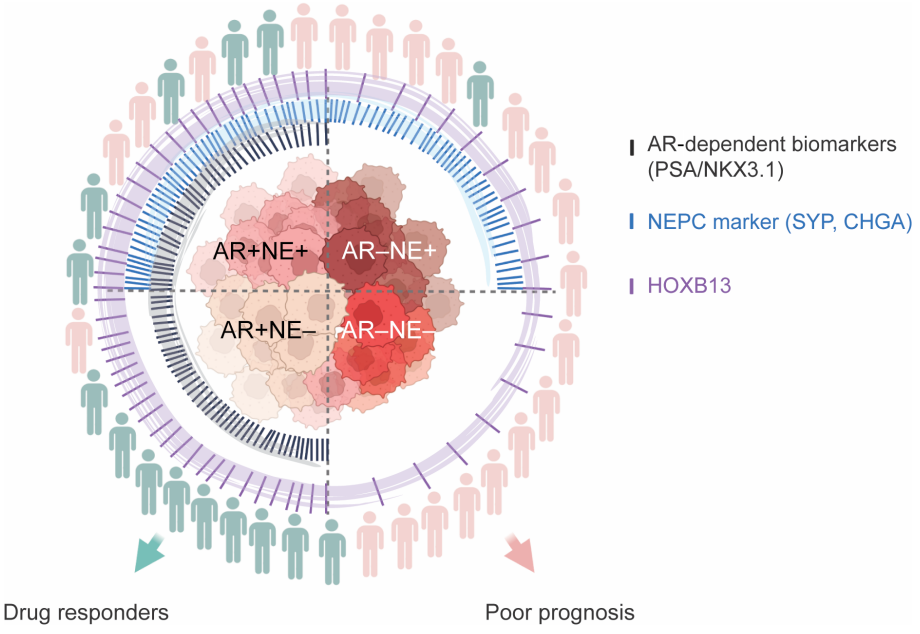

Figure 1. Advanced metastatic CRPC can be roughly categorized into four groups based on intact androgen receptor (AR+) signaling or the expression of neuroendocrine markers (NE+). Subtypes that are negative for AR tend to be associated with a poor prognosis due to limited treatment options and lack of response to androgen deprivation therapy and AR signaling inhibitor therapies. Current state-of-the-art PCa screening relies on PSA expression (gray), which is dependent on AR signaling, thereby missing the detection of AR-negative subtypes. While there are some lineage markers for NE subtypes (blue) in development, these markers may overlook cases that are negative for NE markers, thereby not covering the entire spectrum of PCa. In contrast, HOXB13 (purple) is expressed in all PCa subgroups.

intensity, although consistently expressed across all samples (in contrast to previous studies with other antibodies), diminished with increasing Gleason risk score. Contrary to findings from other groups, there were no significant differences in survival rates among patients grouped by HOXB13 H-scores [8,9].

To investigate *in silico* RNA expression of *HOXB13* in mCRPC, a comprehensive cohort was established encompassing 121 LuCAP PDX models, 98 mCRPC patients from the University of Washington's rapid autopsy program (UW-Tan), and 266 mCRPC patients from the Stand Up to Cancer (SU2C) dataset. The cohorts were stratified based on AR and NE score. In line with previous findings, the researchers validated the presence of lower, yet detectable, levels of HOXB13 in AR-negative PCa and NEPC subgroups. This finding was reinforced by studying 52 patients from the University of Washington rapid autopsy program, which provides a unique opportunity to investigate extensively treated patients with prior androgen deprivation therapy and ARSi. However, analysis of follow-up data again demonstrated that although the HOXB13 level varied significantly across PCa subtypes, it did not have a significant prognostic value for overall survival.

Next, the authors decided to untangle the controversial AR/HOXB13 relationship. Consistent with prior studies, the authors discovered that, unlike PSA or NKX3.1, which AR regulates, HOXB13 levels are not influenced by AR. This indicates that HOXB13 expression is independent of AR and may be a suitable lineage marker despite the observed decrease in HOXB13 levels in AR-negative subtypes (Figure 1).

The authors also examined the molecular mechanisms underlying the decrease in HOXB13 expression in NEPC. At the genetic level, they determined that HOXB13 was enhanced in a PTEN−/− background. However, RB1/TP53 double knockout or RB1/TP53/PTEN triple knockout, known to replicate lineage plasticity to NEPC, exhibited significantly reduced levels of HOXB13, confirming its reduction in AR-negative settings. Consistent with these findings, the authors observed *HOXB13* hypermethylation in NEPC tumors, which aligns with a prior report suggesting a correlation between HOXB13 and CpG methylation patterns [3].

The authors recommend HOXB13 as a biomarker for determining prostatic origin. Despite previous analyses by other groups, the authors revisited this question with their new antibody. They compared HOXB13 to NKX3.1, a previously proposed prostatic origin marker. Their analysis, conducted in the largest cohort tested across different molecular PCa subtypes, revealed that HOXB13 outperformed NKX3.1 regarding sensitivity, particularly in AR-negative cases.

Conclusion

This carefully designed and rigorously validated study clarifies the controversial role of HOXB13 as a lineage marker in PCa. As such, this study claims that HOXB13 levels alone are insufficient to provide information about PCa prognosis. However, due to its high sensitivity and specificity, it may serve as an excellent marker for determining prostate origin, addressing a significant clinical need given the rise in mCRPC. While this is an intriguing concept, one potential drawback of this hypothesis, as acknowledged by the authors, is that HOXB13 is also implicated in various other types of tumors, including breast, ovarian, endometrial, renal, and malignant melanomas [10]. The major finding of this study is the association of decreased HOXB13 with higher-grade localized PCa and, notably, advanced tumors exhibiting a loss of AR or NEPC. While the new D78NO HOXB13 antibody used to illustrate this finding undoubtedly represents an excellent resource for research, its clinical utility is less clear due to the limited settings where it may be beneficial. Further research must be conducted to validate the clinical utility of the D78NO antibody across various cancer types and expand its applicability. Additionally, prospective studies assessing its performance in predicting treatment response or patient outcomes could provide valuable insights into its clinical relevance.

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Author contributions statement

AA and MAR planned the commentary. MAR provided context. AA performed a literature search for relevant articles and wrote the article. MAR edited and revised the manuscript.

Data availability statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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