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Utility of PTEN and ERG Immunostaining for Distinguishing High Grade PIN and Intraductal Carcinoma of the Prostate on Needle Biopsy

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Abstract

Intraductal carcinoma of the prostate and high grade prostatic intraepithelial neoplasia (PIN) have markedly different implications for patient care, but can be difficult to distinguish in needle biopsies. In radical prostatectomies, we demonstrated that PTEN and ERG immunostaining may be helpful to resolve this differential diagnosis. Here, we tested whether these markers are diagnostically useful in the needle biopsy setting. Separate or combined immunostains were applied to biopsies containing morphologically-identified intraductal carcinoma, PIN or borderline intraductal proliferations more concerning than PIN, but falling short of morphologic criteria for intraductal carcinoma. Intraductal carcinoma occurring with concurrent invasive tumor showed the highest rate of PTEN loss, with 76% (38/50) lacking PTEN and 58% (29/50) expressing ERG. Of biopsies containing isolated intraductal carcinoma, 61% (20/33) showed PTEN loss and 30% (10/33) expressed ERG. Of the borderline intraductal proliferations, 52% (11/21) showed PTEN loss and 27% (4/15) expressed ERG. Of the borderline cases with PTEN loss, 64% (7/11) had carcinoma in a subsequent needle biopsy specimen, compared to 50% (5/10) of PTEN-intact cases. In contrast, none of the PIN cases showed PTEN loss or ERG expression (0/19). On needle biopsy, PTEN loss is common in morphologically identified intraductal carcinoma yet is very rare in high grade PIN. Borderline intraductal proliferations, especially those with PTEN loss, have a high rate of carcinoma on resampling. If confirmed in larger prospective studies, these results

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suggest that PTEN and ERG immunostaining may provide a useful ancillary assay to distinguish intraductal carcinoma from high grade PIN in this setting.

Keywords

Prostatic intraepithelial neoplasia; intraductal carcinoma; prostatic carcinoma; PTEN; ERG

Introduction

Intraductal carcinoma of the prostate and high grade prostatic intraepithelial neoplasia (PIN) comprise the two main intraepithelial neoplastic lesions occurring in the prostate (1). When diagnosed as isolated lesions on needle biopsies, these two entities have dramatically different implications for patient prognosis and care (2–4). PIN is widely believed to be a non-obligate precursor lesion of invasive cancer while intraductal carcinoma is a high-grade malignant lesion, likely representing retrograde intraductal/intra-acinar spread of high grade invasive cancer in most cases (2, 4–11). Accordingly, PIN is frequently an isolated finding, occurring in biopsies without invasive carcinoma and, if not present in at least 2–3 separate biopsy cores, is not associated with an increased risk of cancer diagnosis on subsequent biopsies done within the following year (3). In stark contrast, intraductal carcinoma is associated with underlying high grade invasive carcinoma in more than 90% of cases (2, 11). While many groups do not even recommend re-biopsy for isolated PIN occurring in a single needle core biopsy, most recommend definitive therapy (surgery or radiation) for intraductal carcinoma in a prostate needle core biopsy even without concurrent invasive carcinoma (2, 11). Further, in the presence of concurrent invasive carcinoma, accurate recognition of intraductal carcinoma is also critical as recent studies have established that the presence of this lesion is associated with adverse prognosis following surgery, radiation or neoadjuvant chemotherapy or hormonal therapy (12–16).

The distinction of PIN from intraductal carcinoma on needle core biopsy is currently based exclusively on morphological assessment. Criteria for diagnosis of intraductal carcinoma (and distinction from PIN) have been proposed by several groups (2, 6, 9), yet even with strict application of these criteria to needle biopsy specimens we and others have encountered a number of cases where the intraepithelial proliferation shows borderline features, indeterminate between PIN and intraductal carcinoma (10). Given the critical implications of the diagnosis for patient care, use of an ancillary molecular or immunohistochemical test would be helpful in this setting. Recently, using radical prostatectomy specimens, we reported that PTEN protein loss occurs in the majority of morphologically identified intraductal carcinoma cases and was never observed in isolated high grade PIN (17). A similar study of borderline intraductal proliferations in radical prostatectomies showed that isolated lesions were entirely negative for ERG, while cancer-associated lesions or morphologically identified intraductal carcinoma were highly enriched (75%) for ERG expression (18). Here, we examined whether immunostaining for PTEN, ERG and basal cell markers (p63 and high molecular weight keratin [HMWK]) would be useful to distinguish intraductal carcinoma and high grade PIN in the more clinically relevant needle biopsy setting.

Materials and methods

Patient and Tissue Selection

This study, including tissue collection and immunohistochemical staining, was approved by the authors' Institutional Review Board. Prostate needle biopsy specimens containing intraductal carcinoma with concurrent invasive tumor (n=50) were collected from the surgical pathology files of the Johns Hopkins Hospitals (JHH), the Cleveland Clinic, and Miraca Life Sciences. Needle biopsies containing isolated intraductal carcinoma (n=33) without concurrent carcinoma were identified from the consultation files of JHH. All intraductal carcinoma cases were identified applying previously published morphologic criteria (2), and defined as malignant epithelial cells filling large acini and prostatic ducts, with preservation of basal cells (confirmed by p63 and/or HMWK immunostaining) forming either: (1) solid or dense cribriform patterns; or (2) loose cribriform or micropapillary patterns with either marked nuclear atypia (nuclear size >6 times normal or larger) or non-focal comedonecrosis.

Borderline intraductal proliferations more concerning than high grade PIN, but falling short of current criteria for intraductal carcinoma were collected from the consultation files of JHH from 2010 to early 2012 (n=60). Since 2010, we have diagnosed these cases descriptively as "atypical glands surrounded by basal cells where the differential diagnosis is between high grade prostatic intraepithelial neoplasia (PIN) and intraductal carcinoma of the prostate" and recommended follow-up biopsies in all cases. The morphologic characteristics of these cases are described in the Results section below. None of these lesions were associated with concurrent infiltrating prostatic adenocarcinoma or a previous known diagnosis of such. Information regarding clinical follow-up was obtained from medical records or from correspondence with outside physicians.

As a control group, we utilized needle biopsies containing high grade PIN sampled either with (n=7) or without (n=12) concurrent carcinoma in additional cores. These cases were identified from the surgical pathology files of JHH from 2010–2012. High grade PIN was defined as a tufted or micropapillary intraepithelial luminal proliferation, identifiable at low power, with nucleoli easily visualized at 20× magnification (1). No lesions with cribriform architecture were included in the high grade PIN group for this study. Of the PIN cases occurring with concurrent carcinoma, 57% (4/7) occurred with Gleason score 3+3 = 6 carcinoma and 43% occurred with Gleason 3+4 = 7 carcinoma.

Immunohistochemistry

Immunostaining for PTEN, ERG and basal cell markers was performed using two different strategies for cross validation purposes. On the first subset of cases (30/50 cases of intraductal carcinoma with concurrent invasive adenocarcinoma, 10/33 cases of isolated intraductal carcinoma, and 13/21 cases of borderline intraductal proliferations), we used a 3 color chromogenic quadruple immunostain for PTEN, ERG, p63 and HMWK (34βE12 or CK903) that has been described previously (17). In this assay, basal cells (p63 and HMWK) are labeled in red (alkaline phosphatase using Vector® Red as chromagen), PTEN is labeled in brown (horseradish peroxidase using 3,3' diaminobenzidine (DAB) as chromagen), and

ERG is labeled in purple (horseradish peroxidase using Vector® VIP purple as chromogen. In order to further validate the quadruple immunostain (and in part because the p63 antibody clone 4A4 used in the quadruple immunostain became commercially unavailable during the course of the study), we performed the PTEN, ERG and HMWK immunostains individually on adjacent tissue levels on the remainder of the cases, using the same antibody clones as in the quadruple stain, above and previously validated staining protocols (19, 20). Rates of PTEN/ERG staining were nearly identical for each class of lesions using the two immunostaining strategies, further validating the quadruple immunostain's equivalency to the individual stains.

Interpretation of immunohistochemistry

Cytoplasmic PTEN and nuclear ERG protein were visually scored using a previously validated dichotomous scoring system (19) by a urologic pathologist (TLL). All lesional glands were scored that met morphologic criteria for intraductal carcinoma, borderline intraductal proliferation or high grade PIN, based on side-by-side comparisons with a hematoxylin- and eosin-stained section. Lesions were scored only if the presence of basal cells could be documented by p63 and/or 34βE12 staining. As previously described (17, 19), cytoplasmic staining for PTEN was classified as negative if the intensity was markedly decreased or entirely negative across >90% of lesional epithelial cells within each gland when compared to the surrounding benign glands and/or stroma which provide an internal positive control. In a previous study, we found that using this scoring system, PTEN immunohistochemistry was 100% sensitive and 97.8% specific for *PTEN* genomic loss across a panel of 58 cell lines and between 75% and 86% sensitive for *PTEN* genomic loss in 119 genetically characterized prostate tumor tissues (19).

Staining for nuclear ERG was assessed in comparison to stromal endothelial cell staining, which provided an internal positive control for ERG in each section. Similarly, adjacent benign glands provided an internal negative control for ERG staining in all cases. Using cutoffs found to be nearly 90% specific for *ERG* gene rearrangement in a prior study (20), staining for ERG was considered positive if any lesional cells showed nuclear positivity, even those with somewhat weaker staining when compared to surrounding endothelial cells, and negative if no lesional cells were positive.

Statistical analysis

Fisher's exact tests were used to determine the correlation of PTEN and ERG protein expression with one another.

Results

PTEN and ERG expression in intraductal carcinoma and high grade prostatic intraepithelial neoplasia (PIN)

Intraductal carcinoma occurring with concurrent invasive tumor showed the highest rate of PTEN protein loss, with 76% (38/50) of cases lacking PTEN protein (Figure 1, Table 1). In total, 58% (29/50) of these cases expressed ERG. ERG expression was seen in 66% (25/38) of the PTEN loss cases, compared to only 33% (4/12) of the PTEN intact cases ($p=0.091$ by

Fisher's exact test; Table 2A). Overall, 70% (35/50) of cases had concurrent invasive carcinoma present on the same needle core as the intraductal tumor available for analysis. Of these cases, 97% (34/35) showed concordant PTEN and ERG staining between the intraductal and invasive carcinoma. The one discordant case showed PTEN loss in the intraductal component with intact PTEN in the invasive component in the background of negative ERG staining in both components.

Of the needle biopsies containing isolated intraductal carcinoma, 61% (20/33) showed PTEN protein loss and 30% (10/33) expressed ERG. Of the cases with PTEN loss, 50% (10/20) expressed ERG protein while none of the PTEN intact cases expressed ERG (0/13, $p=0.0022$ by Fisher's exact test, Figure 1, Table 2B). In contrast, of the high grade PIN cases occurring with concurrent carcinoma in additional cores, 0% (0/7) showed PTEN loss or ERG expression. Similarly, of the isolated high grade PIN cases, 0% (0/12) showed PTEN loss or ERG protein expression (Figure 2, Table 1).

Clinical-pathologic features of borderline intraductal proliferations falling short of intraductal carcinoma

We identified 60 cases of borderline intraductal proliferations falling short of current criteria for intraductal carcinoma in our urologic consultation case files from 2010 to early 2012. We limited our search to this period because 2010 was when we first began to formally diagnose these lesions, and we wanted old enough cases to have at least 2 years of clinical follow-up. These cases were characterized by: 1) lumen-spanning proliferation with loose cribriform architecture beyond what would normally be seen in HGPIN, but lacking significant nuclear pleomorphism or necrosis to qualify for IDC-P (these cases are morphologically similar to those described in our previous radical prostatectomy study as "intraductal cribriform proliferations", [17]) (Figure 3A); and/or 2) atypical nuclei with significant pleomorphism but falling short of what is required for a diagnosis of IDC-P (<6 times larger than adjacent normal epithelial cells) (Figure 3C); and/or 3) dense cribriform or solid proliferation of atypical cells in incompletely represented large ducts on the edge of core biopsy specimens (Figure 3E). The majority of cases showed more than one of these features.

Of the 60 cases of borderline lesions, information about subsequent tissue sampling was available in 60% (36/60). Thirty-five of these patients underwent re-biopsy and one underwent an immediate radical prostatectomy despite the fact that he lacked a tissue diagnosis of carcinoma. The remainder of the patients (40%) failed to follow-up with their original urologist or elected for forgo an additional follow-up biopsy despite our recommendation. Of the 36 patients with additional tissue sampling after a diagnosis of this borderline lesion, the median age was 70 years old (range: 56 to 85). The number of cores involved by the borderline intraductal proliferation in each case ranged from 1 to 5 with a median of 1. There was a separate focus of atypical glands, suspicious for carcinoma, in 7 of 36 cases (19%). No concurrent invasive carcinoma was diagnosed in any case. The median interval to re-biopsy overall was 4 months, ranging between 0.6 and 3 years. On re-biopsy (or in one case, subsequent radical prostatectomy), 50% (18/36) of patients were diagnosed with prostatic carcinoma, with 83% (15/18) showing invasive tumor and 17% (3/18)

showing definitive IDC-P. For these patients with a subsequent diagnosis of carcinoma, the median interval to re-biopsy was 5.6 months. For patients with invasive tumor on re-biopsy, 53% (8/15) had a Gleason score of 6, 33% (5/15) had a Gleason score of 7, and 13% (2/15) had a Gleason score of 8. For two of the patients with Gleason score 6 carcinoma, the tumor was diagnosed on a second follow-up biopsy, following a re-diagnosis of borderline intraductal lesion on the first follow-up biopsy. These were the only two patients who had undergone two re-biopsies at the time of follow-up. Of the remaining patients, 44% (8/18) showed a borderline intraductal proliferation once again on re-biopsy, 17% (3/18) had a diagnosis of atypical glands, suspicious for prostatic carcinoma, 28% (5/18) showed HGPIN on re-biopsy, 11% (2/18) had a benign diagnosis on re-biopsy.

PTEN and ERG expression in borderline intraductal proliferations falling short of intraductal carcinoma

Of the 36 cases who underwent additional tissue sampling following the diagnosis of a borderline lesion, tissue was available for PTEN immunostaining in 58% (21/36) of cases and ERG immunostaining in 42% (15/36). Of these cases, 52% (11/21) showed PTEN protein loss and 27% (4/15) expressed ERG protein (Figure 3, Table 1). 50% (4/8) of the cases showing PTEN loss expressed ERG compared to 0% (0/7) of the PTEN protein intact cases (Table 2C, $p = 0.0769$ by Fisher's exact test). Of the cases with PTEN loss, 64% (7/11) had carcinoma sampled on a subsequent biopsy, including 29% (2/7) with Gleason score 6 tumors, 29% (2/7) with a Gleason score 7 tumor, 14% (1/7) with a Gleason score 8 tumor and 29% (2/7) with definitive intraductal carcinoma. The remaining 36% (4/11) of cases with PTEN loss had either PIN or a repeat diagnosis of borderline lesion on subsequent biopsy. Of the PTEN intact cases, 50% (5/10) had a subsequent diagnosis of carcinoma, including 80% (4/5) with Gleason score 6 tumors, 20% (1/5) with Gleason score 7 cancer.

Discussion

Currently, the diagnosis of intraductal carcinoma remains a morphologic one, thus sensitive and specific criteria to accurately distinguish this lesion from common high grade PIN are essential. As originally defined by McNeal, intraductal carcinoma was characterized by ducts or acini lined by basal cells with an epithelial layer showing cytologic features of moderate- to high-grade dysplasia with the additional requirement that luminal extensions of the epithelial lining completely bridged the luminal diameter either as trabeculae or cell masses (6). Cohen et al proposed five major and several minor criteria that built on the original McNeal classification (9). In contrast to McNeal's criteria, Cohen's criteria included the expansile nature of the lesion, with involved glands more than twice the diameter of normal surrounding peripheral zone glands. Minor criteria included glands that branch at right angles, have smooth contours, and include a dual cell population with more atypical cells at the periphery and maturation towards the center of the lumen. Subsequent criteria put forth by the Epstein group (and utilized in this study) were the most stringent, requiring that the intraluminal proliferation either show a dense cribriform or solid architecture, or if not, have marked cytologic atypia defined on the basis of nuclear size, or comedonecrosis (2). Application of these criteria to define isolated intraductal carcinoma in prostate needle

biopsies was more than 90% sensitive for detection of underlying invasive carcinoma in subsequent radical prostatectomy specimens (11).

Given the vastly different clinical implications of the diagnosis of intraductal carcinoma versus the diagnosis of PIN, most morphologic criteria for intraductal carcinoma have emphasized specificity over sensitivity. High grade PIN most commonly has a tufted or micropapillary architecture with moderate, but not marked, cytologic atypia and nucleoli easily visible at 20× magnification (1). In contrast to intraductal carcinoma, solid architecture and comedonecrosis are never seen in PIN, however the classification of more loosely cribriform or lumen-spanning intraductal proliferations has been controversial (2, 4, 6, 7, 9–11) Should all lumen-spanning intraductal lesions be considered intraductal carcinoma? Does cribriform PIN exist? Previous studies of so-called “atypical cribriform lesions” using radical prostatectomy specimens have found that the vast majority occur within close proximity to invasive, frequently high grade, carcinoma (10). While a minority occur in isolation from invasive carcinoma and fail to satisfy the criteria for IDC-P, these lesions are relatively rare. Interestingly, while ERG was rearranged in approximately three-quarters of atypical cribriform lesions occurring in close proximity to invasive tumors, ERG rearrangement was not seen in isolated atypical cribriform lesions, suggesting that they may be molecularly distinct and most similar to PIN (18). Thus, the authors concluded that while rare true cases of cribriform PIN may exist, these cases are quite uncommon and when sampled on needle biopsy, all cribriform intraductal proliferations falling short of intraductal carcinoma should at least undergo a re-biopsy to exclude unsampled carcinoma.

These data and others in radical prostatectomy specimens strongly suggested that ancillary molecular tests may have significant utility for resolving the differential diagnosis of these difficult cribriform lesions (18, 21, 22). Because ERG FISH is expensive and time-consuming to perform, and ERG rearrangement can be seen in a subset of conventional PIN cases, in previous work, we focused on the utility of combined PTEN and ERG immunohistochemistry to distinguish PIN from intraductal carcinoma in radical prostatectomy specimens (17). In our previous study, we showed that PTEN loss by immunohistochemistry (which is highly concordant with the presence of an underlying PTEN deletion) occurs in over 80% of intraductal carcinoma (defined by Epstein criteria) and was not seen in morphologically typical high grade PIN lesions from grade- and stage-matched specimens. The common occurrence of PTEN loss in intraductal carcinoma not only provides a potential marker for this lesion, but also suggests a molecular mechanism for the aggressive behavior of tumors associated with intraductal carcinoma. Interestingly, in our previous study, we also examined loose, lumen spanning cribriform intraepithelial proliferations that fell short of intraductal carcinoma criteria but were adjacent to invasive carcinoma. All of these lesions had loss of PTEN, strongly suggesting that we may be under-recognizing some cases of intraductal carcinoma using current criteria. In this study, ERG was positive in a subset of intraductal carcinomas and frequently concordant with PTEN loss.

Because all previous molecular studies of intraductal lesions have been performed in radical prostatectomy specimens, where the distinction between intraductal carcinoma and PIN is often straightforward, in the current study we examined these markers in the more clinically

relevant setting of prostate needle biopsies. In line with our results from the radical prostatectomy study, we found that over three-quarters of morphologically identified intraductal carcinomas occurring with concurrent invasive adenocarcinoma show PTEN protein loss. In isolated intraductal carcinoma sampled without concurrent invasive tumor on needle biopsy, the rate of PTEN loss is similar at 60%. It should be noted that in our previous studies using this same method of PTEN detection by IHC, frequencies of PTEN loss approaching 75% were not seen even in high grade primary and hormone naïve metastatic prostate carcinomas (19, 23–25). This provides further support for the somewhat unique biological nature of intraductal carcinoma and associated invasive lesions. Strikingly, PIN sampled on needle biopsy with or without concurrent invasive carcinoma did not show PTEN loss in the present study, a finding consistent with our earlier study of PIN in radical prostatectomy specimens (17). ERG was expressed in 47% (39/83) of intraductal carcinomas overall on biopsy, and its expression was more commonly seen in cases with PTEN loss (60% or 35/58) than those without PTEN loss (16% or 4/25; $p=0.0002$ by Fisher's exact test), as has been previously reported (26–29). Interestingly, we did not see ERG expression in the 19 cases of isolated PIN examined in this biopsy study. Prior studies have shown ERG expression in up to 20% of PIN cases, however it is more commonly seen in PIN adjacent to invasive cancer (30, 31) or in isolated PIN diagnosed on needle biopsies from patients with a subsequent diagnosis of invasive cancer (32).

Taken together, our data suggest that PTEN immunohistochemistry, either alone or in combination with ERG, may be useful as an ancillary test to distinguish intraductal carcinoma from PIN on prostate needle biopsy. In order to begin to formally test this hypothesis, we also studied the outcomes of difficult-to-classify borderline intraductal lesions sampled without concurrent carcinoma on needle biopsy. In this category, we included the controversial loose cribriform lesions described above, in addition to intraepithelial lesions with substantial cytologic atypia (but insufficient for a diagnosis of intraductal carcinoma) and lesions only partially represented at the edge of a biopsy core. As a group, these borderline intraductal lesions are analogous to those classified as atypical glands suspicious for carcinoma (ATYP) or atypical small acinar proliferations (ASAP) in that they do not appear to represent an entity in and of themselves, yet their presence in a needle biopsy signifies an increased risk for carcinoma on subsequent biopsies (1, 3, 33). In the current series, the risk of carcinoma diagnosis on subsequent biopsy was 50%, slightly higher than that seen following the diagnosis of atypical glands suspicious for carcinoma (3). Importantly, however, and in contrast to atypical glands suspicious for carcinoma, almost half of borderline intraductal cases with a subsequent diagnosis of invasive carcinoma showed Gleason score of 7 or higher, suggesting that many of these tumors are clinically significant and that a prompt diagnosis is required.

These data strongly suggest that current morphologic criteria for intraductal carcinoma on prostate needle biopsy, while quite specific, may not be optimally sensitive. Thus, we took the first steps to retrospectively examine the utility of PTEN and ERG immunohistochemistry to predict outcomes in these borderline intraductal lesions. We found that borderline lesions with PTEN loss on needle biopsy had a 64% risk of definitive carcinoma (intraductal or invasive) on subsequent biopsy, a slightly higher risk than seen in the overall population of borderline lesions, and a rate substantially higher than that seen

following a diagnosis of a small focus of atypical glands suspicious for carcinoma (ATYP or ASAP) (1, 3 33). Although this rate of carcinoma on re-biopsy was somewhat higher than that seen in the PTEN intact lesions (64% vs 50%) or for borderline lesions overall (50%), the current study of borderline lesions has a number of limitations that suggest it is not yet ready for routine clinical use in this context. Perhaps most importantly, it is limited by its modest sample size as clinical follow-up with available additional tissue for immunostaining was difficult to obtain in our consultation-enriched study population. Additionally, our study is limited by the fact that, due to current standards of care, all of the patients were followed up with a needle biopsy, which has limited sensitivity for detection of cancer compared to more thorough examination of a radical prostatectomy specimen. Thus, even if 100% of patients with borderline lesions showing PTEN loss had underlying carcinoma, we would not expect to detect all of these in a single follow-up needle biopsy. The increasing use of MRI-guided biopsy is rapidly improving the pervasive issue of tumor-undersampling with TRUS biopsies. Thus, it is our hope that larger future studies may improve upon our current data and are certainly required before PTEN loss may be used (in combination with morphologic evaluation) to recommend definitive therapy in a borderline intraductal lesion.

Despite these limitations, this study represents the first to use validated molecular markers as an ancillary test to help classify difficult intraepithelial lesions in the prostate with clinical follow-up. Given the clinical significance of distinguishing intraductal carcinoma from high grade PIN, ancillary molecular tests to help resolve ambiguous cases would be quite valuable to the practicing pathologist. While these stains (as with all immunohistochemistry) must always be interpreted in the context of morphology, they may be especially helpful adjunct markers for pathologists who do not see large volumes of urologic material and are less comfortable with the diagnosis of intraductal carcinoma on morphologic grounds alone. Importantly, PTEN loss is only seen in 60–70% of classic intraductal carcinoma lesions using current morphologic criteria. This means that if PTEN is intact, this does not rule out a diagnosis of intraductal carcinoma, reducing the negative predictive value of the test, and reinforcing the requirement for morphologic evaluation. On the other hand, if PTEN is lost, the positive predictive value is reasonably high as PTEN loss is rarely if ever seen in morphologically identified PIN.

Further, PTEN loss in an intraepithelial lesion would not only potentially help distinguish it from PIN, but even in a morphologically-identifiable case of intraductal carcinoma, would strongly suggest the presence of a concurrent underlying invasive carcinoma with PTEN loss as these lesions are highly concordant for PTEN status (17). Since we have previously shown that PTEN loss in invasive tumors is strongly associated with higher stage and grade (19, 25), worse outcomes (19, 23, 25) and upgrading (34), this is potentially valuable information to have on a needle biopsy. Though admittedly a small sample size, these data are supported by the current study. More than 70% (5/7) of the patients with borderline intraductal proliferations showing PTEN loss and a subsequent diagnosis of carcinoma had Gleason 7 or higher tumors or intraductal carcinoma (almost invariably associated with Gleason 7 or higher invasive carcinoma [2, 11]). In contrast, only 20% (1/5) of the borderline intraductal proliferations with intact PTEN and subsequent carcinoma were diagnosed with Gleason 7 tumor and none with definitive intraductal carcinoma. Given that PTEN loss is only about 60–70% sensitive for the detection of intraductal carcinoma on

needle biopsy, borderline lesions with intact PTEN not meeting current morphologic criteria for intraductal carcinoma would still need to be followed up with additional biopsies. However, if supported by larger prospective studies, these preliminary results suggest that this simple immunohistochemical assay for PTEN may ultimately be useful to help select cases that would benefit from immediate definitive therapy.

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References

1. Epstein JI, Netto GN. Biopsy interpretation of the prostate. 2nd ed.. Philadelphia, PA: Lippincott, Williams & Wilkins; 2008.
2. Guo CC, Epstein JI. Intraductal carcinoma of the prostate on needle biopsy: Histologic features and clinical significance. *Mod Pathol*. 2006; 19:1528–1535. [PubMed: 16980940]
3. Epstein JI, Herawi M. Prostate needle biopsies containing prostatic intraepithelial neoplasia or atypical foci suspicious for carcinoma: Implications for patient care. *J Urol*. 2006; 175:820–834. [PubMed: 16469560]
4. Shah RB, Zhou M. Atypical cribriform lesions of the prostate: clinical significance, differential diagnosis and current concept of intraductal carcinoma of the prostate. Review. *Adv Anat Pathol*. 2012; 19:270–279. [PubMed: 22692290]
5. Kovi J, Jackson MA, Heshmat MY. Ductal spread in prostatic carcinoma. *Cancer*. 1985; 56:1566–1573. [PubMed: 4027893]
6. McNeal JE, Yemoto CE. Spread of adenocarcinoma within prostatic ducts and acini. morphologic and clinical correlations. *Am J Surg Pathol*. 1996; 20:802–814. [PubMed: 8669528]
7. Rubin MA, de La Taille A, Bagiella E, Olsson CA, O'Toole KM. Cribriform carcinoma of the prostate and cribriform prostatic intraepithelial neoplasia: Incidence and clinical implications. *Am J Surg Pathol*. 1998; 22:840–848. [PubMed: 9669346]
8. Cohen RJ, McNeal JE, Baillie T. Patterns of differentiation and proliferation in intraductal carcinoma of the prostate: Significance for cancer progression. *Prostate*. 2000; 43:11–19. [PubMed: 10725861]
9. Cohen RJ, Wheeler TM, Bonkhoff H, Rubin MA. A proposal on the identification, histologic reporting, and implications of intraductal prostatic carcinoma. *Arch Pathol Lab Med*. 2007; 131:1103–1109. [PubMed: 17616999]
10. Shah RB, Magi-Galluzzi C, Han B, Zhou M. Atypical cribriform lesions of the prostate: Relationship to prostatic carcinoma and implication for diagnosis in prostate biopsies. *Am J Surg Pathol*. 2010; 34:470–477. [PubMed: 20182345]
11. Robinson BD, Epstein JI. Intraductal carcinoma of the prostate without invasive carcinoma on needle biopsy: Emphasis on radical prostatectomy findings. *J Urol*. 2010; 184:1328–1333. [PubMed: 20723921]
12. Cohen RJ, Chan WC, Edgar SG, Robinson E, Dodd N, Hoscek S, Mundy IP. Prediction of pathological stage and clinical outcome in prostate cancer: An improved pre-operative model incorporating biopsy-determined intraductal carcinoma. *Br J Urol*. 1998; 81:413–418. [PubMed: 9523662]
13. Watts K, Li J, Magi-Galluzzi C, Zhou M. Incidence and clinicopathological characteristics of intraductal carcinoma detected in prostate biopsies: A prospective cohort study. *Histopathology*. 2013; 63:574–579. [PubMed: 23931616]
14. Van der Kwast T, Al Daoud N, Collette L, Sykes J, Thoms J, Milosevic M, Bristow RG, Van Tienhoven G, Warde P, Mirimanoff RO, Bolla M. Biopsy diagnosis of intraductal carcinoma is

- prognostic in intermediate and high risk prostate cancer patients treated by radiotherapy. *Eur J Cancer*. 2012; 48:1318–1325. [PubMed: 22405699]
15. O'Brien C, True LD, Higano CS, Rademacher BL, Garzotto M, Beer TM. Histologic changes associated with neoadjuvant chemotherapy are predictive of nodal metastases in patients with high-risk prostate cancer. *Am J Clin Pathol*. 2010; 133:654–661. [PubMed: 20231619]
 16. Efstathiou E, Abrahams NA, Tibbs RF, Wang X, Pettaway CA, Pisters LL, Mathew PF, Do KA, Logothetis CJ, Troncso P. Morphologic characterization of preoperatively treated prostate cancer: Toward a post-therapy histologic classification. *Eur Urol*. 2010; 57:1030–1038. [PubMed: 19853370]
 17. Lotan TL, Gumuskaya B, Rahimi H, Hicks JL, Iwata T, Robinson BD, Epstein JI, De Marzo AM. Cytoplasmic PTEN protein loss distinguishes intraductal carcinoma of the prostate from high-grade prostatic intraepithelial neoplasia. *Mod Pathol*. 2013; 26:587–603. [PubMed: 23222491]
 18. Han B, Suleman K, Wang L, Siddiqui J, Sercia L, Magi-Galluzzi C, Palanisamy N, Chinnaiyan AM, Zhou M, Shah RB. ETS gene aberrations in atypical cribriform lesions of the prostate: Implications for the distinction between intraductal carcinoma of the prostate and cribriform high-grade prostatic intraepithelial neoplasia. *Am J Surg Pathol*. 2010; 34:478–485. [PubMed: 20220513]
 19. Lotan TL, Gurel B, Sutcliffe S, Esopi D, Liu W, Xu J, Hicks JL, Park BH, Humphreys E, Partin AW, Han M, Netto GJ, Isaacs WB, De Marzo AM. PTEN protein loss by immunostaining: Analytic validation and prognostic indicator for a high risk surgical cohort of prostate cancer patients. *Clin Cancer Res*. 2011; 17:6563–6573. [PubMed: 21878536]
 20. Chaux A, Albadine R, Toubaji A, Hicks J, Meeker A, Platz EA, De Marzo AM, Netto GJ. Immunohistochemistry for ERG expression as a surrogate for TMPRSS2-ERG fusion detection in prostatic adenocarcinomas. *Am J Surg Pathol*. 2011; 35:1014–1020. [PubMed: 21677539]
 21. Dawkins HJ, Sellner LN, Turbett GR, Thompson CA, Redmond SL, McNeal JE, Cohen RJ. Distinction between intraductal carcinoma of the prostate (IDC-P), high-grade dysplasia (PIN), and invasive prostatic adenocarcinoma, using molecular markers of cancer progression. *Prostate*. 2000; 44:265–270. [PubMed: 10951489]
 22. Bettendorf O, Schmidt H, Staebler A, Grobholz R, Heinecke A, Boecker W, Hertle L, Semjonow A. Chromosomal imbalances, loss of heterozygosity, and immunohistochemical expression of TP53, RB1, and PTEN in intraductal cancer, intraepithelial neoplasia, and invasive adenocarcinoma of the prostate. *Genes Chromosomes Cancer*. 2008; 47:565–572. [PubMed: 18383208]
 23. Antonarakis ES, Keizman D, Zhang Z, Gurel B, Lotan TL, Hicks JL, Fedor HL, Carducci MA, De Marzo AM, Eisenberger MA. An immunohistochemical signature comprising PTEN, MYC, and Ki67 predicts progression in prostate cancer patients receiving adjuvant docetaxel after prostatectomy. *Cancer*. 2012; 118:6063–6071. [PubMed: 22674438]
 24. Gumuskaya B, Gurel B, Fedor H, Tan HL, Weier CA, Hicks JL, Haffner MC, Lotan TL, De Marzo AM. Assessing the order of critical alterations in prostate cancer development and progression by IHC: Further evidence that PTEN loss occurs subsequent to ERG gene fusion. *Prostate Cancer Prostatic Dis*. 2013; 16:209–215. [PubMed: 23545904]
 25. Chaux A, Peskoe SB, Gonzalez-Roibon N, Schultz L, Albadine R, Hicks J, De Marzo AM, Platz EA, Netto GJ. Loss of PTEN expression is associated with increased risk of recurrence after prostatectomy for clinically localized prostate cancer. *Modern pathology*. 2012; 25:1543–1549. [PubMed: 22684219]
 26. Yoshimoto M, Joshua AM, Cunha IW, Coudry RA, Fonseca FP, Ludkovski O, Zielenska M, Soares FA, Squire JA. Absence of TMPRSS2:ERG fusions and PTEN losses in prostate cancer is associated with a favorable outcome. *Mod Pathol*. 2008; 21:1451–1460. [PubMed: 18500259]
 27. Carver BS, Tran J, Gopalan A, Chen Z, Shaikh S, Carracedo A, Alimonti A, Nardella C, Varmeh S, Scardino PT, Cordon-Cardo C, Gerald W, Pandolfi PP. Aberrant ERG expression cooperates with loss of PTEN to promote cancer progression in the prostate. *Nat Genet*. 2009; 41:619–624. [PubMed: 19396168]
 28. Han B, Mehra R, Lonigro RJ, Wang L, Suleman K, Menon A, Palanisamy N, Tomlins SA, Chinnaiyan AM, Shah RB. Fluorescence in situ hybridization study shows association of PTEN

- deletion with ERG rearrangement during prostate cancer progression. *Mod Pathol.* 2009; 22:1083–1093. [PubMed: 19407851]
29. King JC, Xu J, Wongvipat J, Hieronymus H, Carver BS, Leung DH, Taylor BS, Sander C, Cardiff RD, Couto SS, Gerald WL, Sawyers CL. Cooperativity of TMPRSS2-ERG with PI3-kinase pathway activation in prostate oncogenesis. *Nat Genet.* 2009; 41:524–526. [PubMed: 19396167]
30. Mosquera JM, Perner S, Genega EM, Sanda M, Hofer MD, Mertz KD, Paris PL, Simko J, Bismar TA, Ayala G, Shah RB, Loda M, Rubin MA. Characterization of TMPRSS2-ERG fusion high-grade prostatic intraepithelial neoplasia and potential clinical implications. *Clin Cancer Res.* 2008; 14:3380–3385. [PubMed: 18519767]
31. Zhang S, Pavlovitz B, Tull J, Wang Y, Deng FM, Fuller C. Detection of TMPRSS2 gene deletions and translocations in carcinoma, intraepithelial neoplasia, and normal epithelium of the prostate by direct fluorescence in situ hybridization. *Diagn Mol Pathol.* 2010; 19:151–156. [PubMed: 20736744]
32. Park K, Dalton JT, Narayanan R, Barbieri CE, Hancock ML, Bostwick DG, Steiner MS, Rubin MA. TMPRSS2:ERG gene fusion predicts subsequent detection of prostate cancer in patients with high-grade prostatic intraepithelial neoplasia. *J Clin Oncol.* 2014; 32:206–211. [PubMed: 24297949]
33. Epstein JI. Atypical small acinar proliferation of the prostate gland. *Am J Surg Pathol.* 1998; 22:1430–1431. [PubMed: 9808137]
34. Lotan TL, Carvalho FLF, Peskoe SB, Hicks JL, Good J, Fedor H, Humphreys E, Han M, Platz EA, Squire JA, De Marzo AM, Berman DM. PTEN Loss is Associated with Upgrading of Prostate Cancer from Biopsy to Radical Prostatectomy. *Modern Pathology.* 2014 Jul 4. [Epub ahead of print].

Figure 1. PTEN loss and ERG expression are common in morphologically diagnosed intraductal carcinoma of the prostate on needle biopsy

(A) Dense cribriform to solid architecture in isolated intraductal carcinoma case (arrows, 200× magnification). (B) Quadruple immunostain for PTEN (brown), ERG (purple) and basal cells (red) on case in (A) demonstrates PTEN loss in intraductal carcinoma (arrow) compared to nearby benign gland (arrowhead). ERG is expressed in nuclei of intraductal proliferation, although it is less intense than nearby endothelial cells (gray arrow). (C) Dense cribriform intraductal carcinoma with nearby invasive carcinoma (200× magnification). (D)

Quadruple immunostain for PTEN (brown), ERG (purple) and basal cells (red) on case in (C) demonstrates PTEN loss and ERG expression in intraductal carcinoma cells (inset) relative to entrapped benign cells (inset, arrowhead). The surrounding invasive carcinoma is concordant with the intraductal carcinoma for these markers. **(E)** Intraductal carcinoma with marked cytological atypia (200× magnification). Although this case does not show dense cribriform or solid intraductal proliferation, it qualifies as intraductal carcinoma due to the presence of atypical nuclei (arrow) greater than $6 \times$ the size of surrounding benign nuclei (arrowhead). **(F)** Quadruple immunostain for PTEN (brown), ERG (purple) and basal cells (red) on case in (E) demonstrates PTEN loss in intraductal carcinoma cells (arrow) relative to nearby benign glands (arrowhead). ERG is also expressed in this case.

Figure 2. PTEN loss and ERG expression are not seen in morphologically diagnosed high grade PIN on needle biopsy

(A) High grade PIN with tufted architecture (arrow, 200× magnification). Nuclear enlargement and nucleoli are apparent at 20× magnification (arrow) compared to surrounding benign glands (arrowhead). Nucleoli are easily visible (inset). (B) Quadruple immunostain for PTEN (brown), ERG (purple) and basal cells (red) on case in (A) demonstrates intact PTEN and absence of ERG staining. (C) High grade PIN with micropapillary architecture. This case contained concurrent invasive adenocarcinoma. (D)

Quadruple immunostain for PTEN (brown), ERG (purple) and basal cells (red) on case in (C) demonstrates intact PTEN and absence of ERG staining.

Figure 3. PTEN and ERG expression in borderline intraepithelial proliferations more concerning the PIN, but insufficient for a diagnosis of intraductal carcinoma using current morphologic criteria

(A) Borderline proliferation with loose cribriform architecture, unusual for PIN, but insufficient for diagnosis of intraductal carcinoma (100× magnification). (B) Quadruple immunostain for PTEN (brown), ERG (purple) and basal cells (red) on case in (A) demonstrates PTEN loss relative to adjacent benign cells (inset shows involved gland from different area of core; arrowhead demonstrates nearby benign gland) and diffuse expression of ERG. (C) Borderline proliferation with substantial cytologic atypia (arrow) but lacking

sufficient atypia to qualify as intraductal carcinoma (630× magnification). **(D)** Quadruple immunostain for PTEN (brown), ERG (purple) and basal cells (red) on case in (C) demonstrates pagetoid spread of PTEN-negative, ERG-positive cells (arrow). **(E)** Borderline proliferation (200× magnification) with dense cribriform architecture which is highly suspicious for intraductal carcinoma but insufficiently represented at the edge of the needle core. **(F)** Quadruple immunostain for PTEN (brown), ERG (purple) and basal cells (red) on case in (E) demonstrates retention of PTEN and lack of ERG expression in the proliferation.

Table 1

Rate of PTEN loss and ERG expression in spectrum of intraepithelial prostate proliferations

Intraepithelial lesion	PTEN loss	ERG expression
Intraductal carcinoma with concurrently sampled invasive carcinoma	76% (38/50)	58% (29/50)
Isolated intraductal carcinoma	61% (20/33)	30% (10/33)
Borderline intraductal proliferations	52% (11/21)	27% (4/15)
PIN with concurrently sampled invasive carcinoma	0% (0/7)	0% (0/7)
Isolated PIN	0% (0/12)	0% (0/12)

Table 2

A: PTEN and ERG status of intraductal carcinoma sampled with invasive carcinoma on needle biopsy (p=0.091 by Fisher's exact test)		
	ERG negative	ERG positive
PTEN intact	8	4
PTEN Loss	13	25

B: PTEN and ERG status of isolated intraductal carcinoma on needle biopsy (p=0.0022 by Fisher's exact test)		
	ERG negative	ERG positive
PTEN intact	13	0
PTEN Loss	10	10

C: PTEN and ERG status of borderline intraductal proliferations falling short of morphologic diagnosis of intraductal carcinoma (p=0.0769 by Fisher's exact test)		
	ERG negative	ERG positive
PTEN intact	7	0
PTEN Loss	4	4