# USE OF IMMUNOHISTOCHEMISTRY AS AN ADJUNCT IN THE DIAGNOSIS OF LIMITED ADENOCARCINOMA OF THE PROSTATE CANCER

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- The use of immunohistochemistry for basal cell markers and AMACR for the diagnosis of limited adenocarcinoma of the prostate should be used as an adjunct to the H&E diagnosis as there is false positive and negative staining with these markers.
- Examples of labeling of prostate adenocarcinoma with basal cell markers include: aberrant scattered HMWCK staining of cancer cells; rarely the retention of a basal cell layer in carcinoma; and diffuse expression of p63 in some prostate cancers.
- Basal cell markers are useful in distinguishing mimickers, such as adenosis, atrophy, radiation changes, and sclerosing adenosis, from prostatic adenocarcinoma recognizing that in some cases the staining pattern between mimickers and prostate carcinoma overlap.
- Basal cell stains are helpful but are associated with pitfalls in the diagnosis of HGPIN and its distinction from carcinoma, PIN-like ductal adenocarcinoma, and intraductal carcinoma.
- Although the vast majority of prostate adenocarcinomas label with antisera to PSA, there are some that are negative where the P501S (prostein) and PSMA (prostate specific membrane antigen) may be positive.
- Other examples of useful immunohistochemistry include stains for CD68 to distinguish prostatic xanthoma and nonspecific granulomatous prostatitis from high grade prostate cancer.

## Use of Basal Cell Markers and AMACR to Diagnose Limited Carcinoma

The most commonly used antibody to label basal cells in benign mimickers of prostate cancer, is high molecular weight cytokeratin (34ßE12, cytokeratin 5/6). High molecular weight cytokeratin immunoreactivity in benign glands is localized to the cytoplasm of basal cells and is negative in prostate cancer. More recently, antibodies to p63 have been shown to label the nuclei of basal cells in benign prostatic lesions.

Several studies comparing high molecular weight cytokeratin and p63 have showed p63 to be slightly superior. One study demonstrated that ck5/6 was superior to 34ßE12, although only a minority of pathologists use ck5/6. The use of a double cocktail combining HMWCK and p63 can increase the sensitivity of basal cell detection with a decrease in staining variability.

The use of high molecular weight cytokeratin or p63 in a focus with only a few atypical glands is not as diagnostic, since benign glands may not show uniform positivity with these markers. Negative staining for basal cell markers is most diagnostic when more than a few glands are present for evaluation and the morphologic features are very suspicious for carcinoma. Rather than used to establish a diagnosis of cancer, we use these antibodies to help verify a suspicious focus as cancer. If we favor, although are not

sure, that a focus is benign and the basal cell stains are negative, we will diagnose it as atypical rather than as cancer. In a small focus of atypical glands on prostate biopsy, negative staining for high molecular weight cytokeratin should not necessarily lead to a definitive malignant diagnosis in all cases, as almost half these biopsies on follow-up sampling are benign. If we are confident the focus is benign and stains performed at an outside institution are negative in a small focus of glands, we will still diagnose the focus as benign since certain mimickers of prostate cancer may not react with these antibodies

Alpha-methylacyl-CoA-racemase (AMACR), an enzyme involved in the beta-oxidation of branched-chain fatty acids, is significantly up-regulated in prostate cancer. Antibodies have been developed against its gene product, P504S protein. By immunohistochemistry, the majority of prostate cancers are positive for AMACR, the sensitivity varying amongst studies from 82%-100%. Often the staining is fine dot-like and luminal. Although the data is somewhat conflicting, some studies have shown relative decrease AMACR immunoreactivity in foamy gland, atrophic, and pseudohyperplastic prostate cancers. AMACR staining of PIN and mimickers of prostate cancer is discussed in chapters 5 and 7, respectively. As negative staining for basal cell markers especially in a small focus of atypical glands is not necessarily diagnostic of prostate cancer, positive staining for AMACR can increase the level of confidence in establishing a definitive malignant diagnosis.

Different cocktails have been investigated combining antibodies for AMACR and basal cell specific markers. One combination is with antibodies to p63 which label basal cell nuclei of benign glands and AMACR which stains cytoplasm of cancer. Although these authors have reported that this cocktail is essentially equal to each antibody used separately, in our experience a problem with this cocktail is that in some cases stains for p63 show some background staining of the cytoplasm in benign glands, which can be confused with AMACR immunoreactivity. With small foci of atypical glands, the lesion may not survive sectioning to do separate stains for basal cell markers and AMACR on different slides. A triple stain cocktail using a brown chromogen for both high molecular weight cytokeratin and p63 and a red chromogen for AMACR optimizes the preservation of tissue for immunohistochemistry and has been shown to be better than basal cell markers by themselves.

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## Use of Basal Cell Markers in Radiated Prostate

In addition to radical prostatectomy, external beam radiation and or interstitial radiotherapy (brachytherapy) are currently among the most common options available for the management of localized prostate cancer with a curative intent. Within the nonneoplastic prostatic glands, radiation results in glandular atrophy, squamous metaplasia, and cytologic atypia. Though one may find vascular radiation changes and stromal fibrosis, the stromal atypia characteristic of radiation in other organs is not usually seen. The degree of cytologic atypia in non neoplastic glands and degree of stromal fibrosis appear to be higher after brachytherapy compared to external beam radiation. Furthermore, the marked epithelial atypia tend to persist for a longer time (up to 6 years) following brachytherapy.

The distinction between irradiated nonneoplastic prostatic glands and carcinoma is best made on the architectural pattern of the glands. Within the radiated normal prostate, glands maintain their normal architectural configuration. In contrast to carcinoma, the nonneoplastic glands are separated by a modest amount of prostatic stroma. On higher magnification, there is piling up of the nuclei within irradiated normal prostate as well as an occasional recognizable basal cell layer. Multilayered cells in radiated benign glands frequently appear slightly spindled resembling urothelial metaplasia. The finding of scattered markedly atypical nuclei within well-formed acini is typical of radiated benign glands and rare in prostate carcinoma. Prostate carcinomas that are sufficiently differentiated to form glands rarely manifest the degree of atypia seen with radiation, and if present would be more uniformly present in all cells. Radiated nuclei showing atypia also have a degenerative, hyperchromatic smudgy appearance as opposed to malignant prostatic nuclei that usually contain prominent nucleoli, although occasional nucleoli can be seen in benign prostate glands with radiation affect. Irradiated nonneoplastic glands often are atrophic, in contrast to gland-forming prostatic adenocarcinomas that typically have abundant cytoplasm.

Radiated adenocarcinoma of the prostate may show either no recognizable difference from nonradiated cancer or the effects of radiation damage. In order to diagnose either pattern of cancer, the key feature is that architecturally the findings are inconsistent with benign glands. The presence of closely packed glands with a haphazard

infiltrative growth pattern is typical of adenocarcinoma without treatment affect and cannot be attributed to radiation change. Similarly, the presence of numerous infiltrating individual epithelial cells is diagnostic of carcinoma with treatment affect. Cancers not showing any treatment effect have typical prostate cancer nuclei with prominent nucleoli and glands with a modest amount of cytoplasm. Cancers with radiation effect typically demonstrate individual cells with abundant vacuolated cytoplasm or single cells with indistinct cytoplasm. Nuclei lack apparent nucleoli and are either large with bizarre shapes or pyknotic with smudged chromatin.

It has been demonstrated that high molecular weight cytokeratin immunohistochemistry can aid in the diagnosis of irradiated prostate by identifying basal cells within benign radiated glands. Expression of alpha-methylacyl-coenzyme A racemase (P504S) is usually maintained in radiated adenocarcinoma.

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## Immunohistochemistry for Selected Mimickers of Prostate Adenocarcinoma

### Adenosis

| *Adenosis* | *Cancer* |
|---|---|
| Lobular | Haphazard growth pattern |

| Small glands share features with admixed larger glands | Small glands differ from adjacent benign glands |
|---|---|
| Pale-clear cytoplasm | Occasionally amphophilic cytoplasm |
| Medium sized nucleoli | Occasionally large nucleoli |
| Blue mucinous secretions rare | Blue mucinous secretions common |
| Corpora amylacea common | Corpora amylacea rare |
| Basal cells present | Basal cells absent |

Features Shared in Adenosis and Cancer

- Crowded glands
- Crystalloids
- Medium sized nucleoli
- Scattered poorly formed glands and singles
- Minimal infiltration at periphery
- AMACR immunoreactivity

<u>Atrophy</u>

Post-atrophic hyperplasia (PAH) also often appears basophilic at low power. It consists of acini that are small and mostly round that are arranged in a lobular distribution. Often these acini appear to be surrounding a somewhat dilated "feeder" duct. Many of these lesions frequently resemble normal appearing resting breast lobules, and are referred to by some authors as lobular atrophy. The lesions appear hyperplastic since the close packing of multiple small acini suggests that there is an increase in their number compared to normal tissue. PAH glands have a much higher proliferation rate than nonatrophic benign glands. Although the glands may appear infiltrative, they appear invasive as a patch not as individual glands infiltrating in between larger benign glands. The basophilic appearance of glands of atrophy is due to their scant cytoplasm and crowded nuclei such that at low magnification one is merely seeing a nuclear outline of the gland.

When there are concerns as to whether a focus represents PAH or adenocarcinoma, immunohistochemistry with antibodies to high molecular weight cytokeratin or p63 can be performed to resolve the issue, as PAH uniformly labels with basal cell markers. As opposed to partial atrophy (see below), PAH uncommonly expresses racemase.

Partial atrophy, another variant of atrophy, is the most common mimicker of prostate. Partial atrophy may still retain the lobular pattern of PAH, or have more of a disorganized diffuse appearance. Partial atrophy lacks the basophilic appearance of fully developed atrophy (simple atrophy, PAH) as the nuclei are more spaced apart. The presence of crowded glands with pale cytoplasm may lead to an overdiagnosis of low-grade adenocarcinoma. At higher power, however, the glands have benign features characterized by undulating luminal surfaces with papillary infolding. Most carcinomas have more straight, even luminal borders. In addition, the glands are partially atrophic with nuclei in areas reaching the full height of the cytoplasm. The nuclear features in partial atrophy tend to be relatively benign without prominent nucleoli, although nuclei may appear slightly enlarged with small nucleoli. As with adenosis, partial atrophy typically has a patchy basal cell layer and may express racemase.

Sclerosing Adenosis

Adenocarcinomas of the prostate composed of an admixture of glands, poorly formed glandular structures, and single cells would be assigned a high Gleason score (7 or 8). Prostatic adenocarcinomas with these scores are only rarely seen as limited foci within a TURP. The finding of only one or several small foci of a cellular lesion suspicious for high-grade carcinoma should prompt a consideration of sclerosing adenosis. Furthermore, although sclerosing adenosis may be minimally infiltrative at its perimeter, the lesion is still relatively circumscribed in contrast to high-grade prostate adenocarcinoma.

The glandular structures in sclerosing adenosis resemble those seen in ordinary adenosis. They are composed of cells with pale to clear cytoplasm and relatively benign-appearing nuclei. In many of the glandular structures, a basal cell layer can be identified on H&E-stained sections. This contrasts to carcinoma, where basal cells are absent. Sclerosing adenosis contains a dense spindle cell component that is typically lacking in adenocarcinomas. Usually, adenocarcinomas of the prostate show no apparent stromal response or at most a hypocellular fibrotic reaction. A rather unique feature of sclerosing adenosis is the presence of a hyaline sheath-like structure around some of the glands. The glands in ordinary adenocarcinoma lack such a collarette and have a “naked” appearance as they infiltrate the stroma.

Sclerosing adenosis contains a basal cell layer around most of the glandular structures as well as among the individual cells and cords of cells. The basal cells within sclerosing adenosis, however, are distinctive in their immunophenotypical staining and differ from ordinary basal cells. Ordinary basal cells of the prostate show no myoepithelial cell differentiation. They lack staining for muscle specific actin and ultrastructurally do not show contractile elements. Within sclerosing adenosis, the basal cells show muscle specific actin positivity consistent with myoepithelial cell differentiation. The dense spindle cell component in sclerosing adenosis also shows partial staining with keratin and muscle-specific actin consistent with myoepithelial cell

differentiation. Ultrastructural examination of several of these cases has verified their myoepithelial differentiation. There is no known association between sclerosing adenosis and adenocarcinoma of the prostate.

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Uncommonly, one can see occasional cancer cells that are positive for antibodies to high molecular weight cytokeratin and less likely p63, yet as long as these cells are not in a basal cell distribution, these cells represent aberrant expression of the antigen in cancer.

Rare lesions with the appearance of prostate cancer show high molecular weight cytokeratin staining in a basal cell distribution either from retention of basal cells by early invasive cancer or from high grade PIN outpouching. The lack of adjacent PIN in some cases and the large ratio of small atypical glands to PIN glands argue against high grade PIN outpouching as the sole explanation. In cases with adjacent high grade PIN, a comparison of the proximity and number of the small, atypical, infiltrative appearing glands to high grade PIN is helpful. The diagnosis of prostate cancer in the face of positive high molecular weight cytokeratin basal cell staining should be made with extreme caution, only in the face of unequivocal cancer on the H&E stain.

There are also uncommon prostate adenocarcinomas that diffusely express p63, yet not HMWCK. Many of these tumors have distinctive morphology composed of atrophic glands lined by hyperchromatic nuclei that are often slightly spindled and minimally multilayered.

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## Use of Basal Cell Markers in the Differential Diagnosis of HGPIN

### PINATYP

A common scenario where it is difficult to distinguish acinar adenocarcinoma from high grade PIN is when there are a few atypical glands immediately adjacent to high grade PIN. The differential diagnosis is whether these small glands represent tangential sectioning or outpouching off of the high grade PIN glands or a small focus of carcinoma adjacent to the high grade. We refer to these foci at PINATYP. A diagnosis of carcinoma can be rendered only if the small atypical glands are too numerous or too far away from the high grade PIN glands to represent outpouching or tangential sectioning from the PIN glands. In cases of PINATYP, the lack of basal cells in the small atypical glands can be construed as evidence that these glands represent infiltrating cancer only if there are more than a few such glands. As high grade PIN glands can have discontinuous basal cells, one can envision tangential sections off PIN glands in which all cells would appear negative for basal cell markers, such that a few negative small atypical glands adjacent to PIN is not diagnostic of cancer. Some cases may have the appearance of PINATYP yet

will be entirely negative for basal cell markers; these foci may be diagnostic of cancer if there are a sufficiently large number of glands that are not immunoreactive. One may also see classic high grade PIN where some of the glands show the expected patchy basal cell layer and other identical glands are negative for the basal cell markers; these cases we would still diagnose as high grade PIN. Racemase does not differentiate between high grade PIN and cancer, as both typically express this antigen.

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PIN-like Ductal Adenocarcinoma

A more recently described variant of ductal adenocarcinomas closely resembles high grade prostatic intraepithelial neoplasia (HGPIN) and is composed of simple glands with flat, tufting or micropapillary architecture. PIN-like ductal adenocarcinoma differs from HGPIN by the presence of cystically dilated glands, a greater predominance of flat architecture, and less frequently prominent nucleoli. Verification often requires the immunohistochemical documentation of the absence of basal cells in numerous atypical glands. Although usual ductal adenocarcinoma is considered comparable to Gleason score 8, PIN-like ductal adenocarcinoma was accompanied by Gleason score 6 acinar carcinoma and behaved similar to Gleason score 6 acinar cancer.

Tavora F, Epstein JI. High grade prostatic intraepithelial neoplasia-like ductal adenocarcinoma of the prostate: A clinicopathologic study of 28 cases. Am J Surg Pathol (July) 32: 1060,1067, 2008.

Intraductal Carcinoma

Intraductal carcinoma of the prostate (IDC-P) in radical prostatectomy specimens is described as an atypical glandular lesion that spans the entire lumen of prostatic ducts or acini while the normal architecture of ducts or acini is still maintained. Rarely, IDC-P may be identified on biopsy material in the absence of infiltrating carcinoma. Our definition of IDC-P on needle biopsy was derived to identify objective morphological criteria that either architecturally or cytologically clearly exceed those seen in high grade PIN. It is critical to distinguish between high grade PIN and IDC-P, as the former is typically not treated with definitive therapy and recent data has questioned whether high grade PIN on needle biopsy even requires immediate rebiopsy within the first year following its diagnosis. Both entities share cytological features such nuclear enlargement,

hyperchromasia, and enlarged nucleoli. Although dense cribriform (more solid than luminal areas) and solid patterns are not architectural patterns associated with high grade PIN, loose cribriform and micropapillary patterns overlap between the two entities. To establish the diagnosis of IDC-P in the latter two patterns, other cytological features such as markedly enlarged nuclei (6 times larger than those in adjacent non-neoplastic cells) and non-focal comedonecrosis are required. Whereas, it has been accepted that classic high grade PIN can contain a rare gland with focal necrosis, more extensive necrosis is not acceptable. IDC-P also tends to show more prominent nuclear pleomorphism, as opposed to typical high grade PIN with its uniformly enlarged nuclei. Cases which do not satisfy the strict criteria for IDC-P on needle biopsy yet appear more atypical either architecturally or cytologically than usual high grade PIN can be diagnosed as borderline between IDC-P and high grade PIN with a strong recommendation for repeat biopsy.

Infiltrating cribriform acinar adenocarcinoma (Gleason pattern 4 or Gleason pattern 5 with comedonecrosis) closely mimics cribriform IDC-P. Most cases of IDC-P would be diagnosed as cribriform carcinoma if immunohistochemistry demonstrating basal cells had not been performed. In some cases, the contour and branching pattern of normal duct architecture distinguishes IDC-P from infiltrating carcinoma. Ultimately, the presence of a basal cell layer either identified on routine hematoxylin and eosin prepared slides or with immunohistochemistry rules out infiltrating acinar prostate adenocarcinoma. Despite the presence of comedonecrosis, Gleason pattern 5 adenocarcinoma is ruled out also by the identification of a basal cell layer. Although there are extremely rare cases of early small foci of non-cribriform carcinoma of the prostate with focal retention of basal cell layer, this has never been described in cribriform, solid, or micropapillary prostate acinar carcinoma.

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## Prostate Adenocarcinoma vs. Urothelial Carcinoma

Even in poorly differentiated prostatic carcinomas, there is typically relatively little pleomorphism or mitotic activity compared to poorly differentiated urothelial carcinoma. Poorly differentiated prostate cancers may have enlarged nuclei and prominent nucleoli, yet there is little variability in nuclear shape or size from one nucleus to another. High-grade urothelial carcinomas often reveal marked pleomorphism with tumor giant cells. A subtler finding is that the cytoplasm of prostatic adenocarcinoma is often very foamy and pale imparting a “soft” appearance. In contrast, urothelial carcinomas may demonstrate hard glassy eosinophilic cytoplasm or more prominent squamous differentiation. The findings of infiltrating cords of cells or focal cribriform glandular differentiation are other features more typical of prostatic adenocarcinoma than urothelial carcinoma. Urothelial cancer tends to grow in nests, even when poorly differentiated. Although the above distinction between urothelial carcinoma and prostatic

adenocarcinoma on H&E stained sections is valid for almost all cases, we have seen rare cases where prostate adenocarcinoma has had marked pleomorphism identical to urothelial carcinoma. Consequently, in a poorly differentiated tumor involving the bladder and prostate without any glandular differentiation typical of prostate adenocarcinoma, the case should be worked up immunohistochemically.

Approximately 95% of poorly differentiated prostatic adenocarcinomas show PSA and PSAP staining although it may be focal. While some studies claim superiority of PSA over PSAP in staining prostatic carcinoma, other articles have demonstrated poorly differentiated prostatic carcinomas that lacked PSA staining but still maintained their immunoreactivity with antibodies to PSAP. In our own hands, PSA has in general been more sensitive. Monoclonal antibodies to PSAP have lower sensitivities than their polyclonal counterparts. We have compared PSA staining in a group of poorly differentiated prostatic adenocarcinomas with "poor" PSA staining to newer prostate specific markers including prostate specific membrane antigen (PSMA), p501S (Prostein) and NKX 3.1. Completely negative staining was seen in 15% (PSA), 12% (PSMA), 17% (P501S) and 5% (NKX 3.1) of the cases. Five per cent of the cases were negative for all four markers combined. A similar 5% rate of "false negativity" is found when combining PSA and PSAP stains. Therefore, the lack of immunoreactivity to prostate specific markers in a poorly differentiated tumor within the prostate, especially if present in limited amount, does not exclude the diagnosis of a poorly differentiated prostatic adenocarcinoma.

In a poorly differentiated tumor occurring in the bladder and the prostate where the differential diagnosis is between high-grade prostatic adenocarcinoma and urothelial carcinoma, focal strong staining for either marker can be used reliably to make the diagnosis of prostatic adenocarcinoma, since PSAP and PSA false positivity have not been convincingly described in urothelial carcinomas.

In general, various cytokeratins (CK7, CK20, high molecular weight cytokeratin) show strong positivity in cases of urothelial carcinoma involving the prostate. Although CK7 and CK20 are more frequently seen in urothelial carcinoma as compared to adenocarcinoma of the prostate, they may also be positive in adenocarcinoma of the prostate, such that in our experience they are not that helpful in this differential diagnosis. We and others have found high molecular weight cytokeratin to be positive in more than 90% of urothelial carcinomas. In contrast, high molecular weight cytokeratin is only rarely (8%) expressed, and usually in a very small percentage of cells, in adenocarcinoma of the prostate. P63 is another useful marker in differentiating high grade urothelial from prostatic adenocarcinoma. Using tissue microarrays, we found p63 to have a greater specificity albeit lower sensitivity for urothelial carcinoma compared to high molecular weight cytokeratin (100% specificity and 83% sensitivity). Other markers that also appear highly specific but only of modest sensitivity for urothelial carcinoma include uroplakin and thrombomodulin (49%-69 % sensitivity).

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