

**APPLICATION OF ALPHA-METHYLACYL COENZYME A
RACEMASE IMMUNOHISTOCHEMISTRY:
A HELP OR HINDRANCE IN THE DIAGNOSIS OF PROSTATE CANCER?**

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ABSTRACT

Since the discovery of alpha-methylacyl CoA racemase (AMACR) in prostate cancer in 2000, there have been a number of publications studying the diagnostic value of this enzyme. Although the role of this enzyme is still unknown, the application of AMACR immunohistochemistry in pathology practice has been increased sharply in the last few years. We are going to review the recent studies of the AMACR expression in prostate cancer and several benign and malignant entities by us and other investigators. Then we will discuss clinical application, limitation and pitfall of using this marker in details. This discussion is by no means a guidance for pathology practice, but it may provide some reference when interpreting AMACR immunostaining.

INTRODUCTION

Widespread use of PSA has resulted not only in increased number of prostate needle core biopsies performed each year, but also an increasing number of small foci of uncertain diagnoses of limited biopsy material (1-3). Although histological features of prostatic adenocarcinoma such as growth pattern, nuclear atypia, absence of basal cells, and presence of characteristic extra cellular material are important (4-5), when used alone, they are not entirely sensitive or specific to establish a definitive diagnosis of prostate cancer. Immunohistochemical stains for high-molecular weight keratins such as 34beta12 and more recently p63 have been used to identify basal cells which are typically present in benign glands but absent in prostatic adenocarcinoma (6-). Unfortunately, negative staining for basal cells in a few suspicious glands is not a definitive proof of malignancy, as benign conditions can have a patchy or discontinuous distribution of basal cells (8). Rarely, prostatic adenocarcinoma may also contain cells positive for basal cell markers. Therefore, a sensitive yet specific “positive” marker of prostatic adenocarcinoma would be very useful in raising the confidence level in diagnosis on limited specimens for prostate needle cores.

DISCOVERY OF AMACR

Alpha-methylacyl coenzyme A racemase (AMACR) is an enzyme first purified and characterized by investigators studying lipid metabolism. It was characterized in human tissues in 1995 (9). Later, Ferdinandusse et al demonstrated that AMACR, a protein with 382 amino acid residues, played a role in the beta-oxidation of branched chain fatty acids and fatty acid derivatives (10). In 2000, Xu et al (11), using cDNA subtraction in conjunction with high-throughput cDNA microarray screening, identified 3 three genes: P503S, P504S and P510S that showed differential expression in malignant and benign prostate glands. P504S, one of the gene product named with the cDNA clone number, was clearly identified as human alpha-methylacyl coenzyme a racemase (AMACR) (11). AMACR mRNA was overexpressed in the majority of prostate adenocarcinomas compared to low to undetectable levels in benign prostatic glands by Northern blots and real-time PCR analysis. Furthermore, they generated rabbit monoclonal antibodies specific for P503S and P504S (AMACR), respectively. It was demonstrated that AMACR (P504S) immunoreactivity in prostatic adenocarcinoma but not in benign prostatic glands, while P503S immunoreactivity was present in both malignant and benign glands in 5 prostate cases on paraffin-embedded human prostate tissues (11).

AMACR IN PROSTATE CANCER

In 2001, Jiang et al (12) examined at AMACR expression in 137 prostate cancer cases and 70 benign cases by immunohistochemistry using the same rabbit monoclonal antibody to AMACR (P504S). All 137 cases of carcinoma reportedly showed strong cytoplasmic expression regardless of Gleason grades. In addition, 88% of benign tissue samples were completely negative for AMACR, with the other 12% were only weakly and focally positive. In addition, benign prostatic lesions, such as, atrophy, and basal cell hyperplasia were completely negative. The authors therefore concluded that AMACR may be a useful adjunct in the diagnosis of prostate cancer on paraffin tissue (12).

In 2002, four additional studies supported the notion that AMACR may be a useful marker for prostate cancer. Rubin et al (13) found significant over-expression of AMACR in prostate cancer in three of four independent cDNA microarray analyses. By immunohistochemical analysis using a polyclonal AMACR antibody on tissue microarrays and needle core biopsy specimens, they showed that AMACR had 97% sensitivity and 100% specificity in the detection of prostate cancer (13). Similarly, Luo et al (14) showed 95% of prostate cancer cases were positive for AMACR compared to less than 4% of normal tissue and supported the findings that AMACR as a new positive marker that complements basal cell markers to enhance prostate cancer diagnosis. Jiang et al (15) investigated 73 small foci (<1mm) of prostate cancer on the prostate needle core biopsies and found 69 of 73 (95%) foci to be positive for AMACR while all 69 benign biopsies were all negative. This is of particular importance because the diagnosis of limited prostate cancer on needle biopsy is a major challenge for pathologists. Furthermore Beach et al (16) reported 153 of 186 (82%) prostate cancer specimens were positive for AMACR, while 21% of benign foci showed focal, faint, and noncircumferential staining, and concluded circumferential and diffuse luminal positivity was specific for prostate cancer.

Furthermore, in a multi-institutional study, a total of 807 prostatic specimens from 6 US

medical centers were analyzed using conventional and quantitative immunostaining analyses and found that of the 454 cases of prostatic adenocarcinoma, 441 (97%) were positive for AMACR, while 254 of 277 cases of benign prostate were negative for AMACR. Moreover, by using quantitative automated imaging analyses, AMACR immunostaining intensity and percentage in prostate cancer were also significantly higher than those in benign prostatic tissues (17).

Kumar-Sinha et al (18) found evidence that AMACR enzymatic activity is consistently elevated in prostate cancer tissue specimens indicating AMACR in prostate cancer is enzymatically active. The first study examining the predictive capacity of AMACR in patient outcome came in 2005, when Rubin et al (19) reported that lower AMACR expression in cancer cells was associated with worse patient outcome, independent of Gleason score, PSA, and margin status.

In 2004, in an effort to evaluate diagnostic utility of AMACR immunostaining in establishing definitive diagnosis from suspicious prostate biopsies, three urological pathologists analyzed 93 cases of atypical small acinar proliferations (ASAP) with combination of histology, immunostains for AMACR and HMWCK. It was found that AMACR immunoreactivity contributed a resolution of 12% to 24% cases unanimously or by consensus (20). Therefore, AMACR can increase the level of confidence in establishing a definitive malignant or benign diagnosis in atypical cases. In another study, AMACR immunoreactivity contributed the conversion from atypical diagnosis to cancer in 50% (34/76) of atypical cases by a genitourinary pathology expert and 10% (34/307) of atypical cases diagnosed by contributing pathologists (21). Even for experts in prostate pathology, AMACR is helpful for definitive diagnoses in difficult cases

AMACR IN VARIANTS OF PROSTATE CANCER

In addition to typical prostatic adenocarcinoma, there are several morphologic variants of prostate cancer such as transition zone cancer, foamy gland cancer, hyperplastic cancer. Most of the earliest studies of AMACR expression in prostate cancer dealt mainly with conventional acinar prostatic adenocarcinoma, making little note of expression in various morphologic subtypes of prostate cancer. In 2003, Leav et al (22) demonstrated AMACR expression in 25 of 25 cases of prostatic carcinoma of the transition zone, but noticed staining was less intense in tumors of lower Gleason grade.

The previously mentioned study by Beach et al (16) stated that 5 of 6 carcinomas in prostatectomy specimens with pseudohyperplastic patterns did express AMACR. In 2003, Zhou et al (23) looked at thirty needle core biopsies containing the so-called foamy gland carcinoma, a deceptively benign-appearing variant of prostatic adenocarcinoma. They reported that 68% of these tumors were positive for AMACR using the monoclonal antibody P504S and 62% were positive using the polyclonal antibody specific for AMACR. They also examined 17 needle biopsies with pseudohyperplastic carcinomas and reported 77% and 70% positive in AMACR immunoreactivity, respectively (23). Similar findings have recently reported 72% of malignant foamy glands positive for AMACR in 23 prostatectomy specimens (24).

The atrophic variant of prostatic adenocarcinoma is a mimicker of benign atrophy, often difficult to diagnose on needle core biopsy. Farinola et al (25) looked at AMACR expression in 19 cases of atrophic carcinoma and 16 cases of benign atrophy on needle core biopsy and found expression in nearly 70% of atrophic cancers but none of the cases of benign atrophy. In summary, expression of AMACR may be helpful in diagnosing deceptively bland variants of prostate cancer (i.e. pseudohyperplastic, foamy gland, and atrophic variants) when positive. One must interpret a negative stain in these variants with caution, because approximately 30% could be negative.

AMACR IN PROSTATE AFTER RADIATION OR HORMONAL THERAPY

Radiation therapy, a common treatment modality for prostate cancer, can induce marked histological changes in both malignant and benign prostatic tissues such as nuclear enlargement and hyperchromasia. Benign prostatic glands after radiation may be difficult to distinguish from malignant ones based on histological features alone. An additional marker such as AMACR might be useful as a tool to aid in the diagnosis of malignancy in prostatic glands with post radiation atypia. Yang et al (26) examined AMACR expression in 40 irradiated prostate specimens (28 with carcinoma) and 40 nonirradiated specimens (20 with carcinoma) and found that all 48 malignant cases showed strong expression of AMACR while all 32 benign cases were negative. Amin et al (27) looked at 26 patients with post radiation prostate cancer and reported AMACR expression in 94% of cases. The authors also observed that decreased AMACR expression in tumors correlated with treatment effects (27).

Hormonal treatment modalities such as androgen antagonists are also commonly used against prostate cancer, especially for advanced stage disease. Rubin et al reported a significant decrease in AMACR expression in metastatic hormone-refractory prostate cancers compared with hormone naïve cancers (13). On the other hand, Luo et al (14) reported 13 of 14 cases of hormone refractory metastatic cancers were positive including 71% showing strong expression of AMACR. In the study by Beach et al (16), all eight hormonally treated cases were positive. The largest study examining AMACR expression in hormone treated tumors was done by Suzue et al in 2005 (28). They looked at 64 patients with residual or recurrent prostate cancer following hormonal therapy. They found that AMACR expression was reduced significantly in the majority of post hormonal residual carcinomas, whereas in hormone-refractory metastatic tumors, AMACR expression was retained (28). This finding also indicates that AMACR may be functionally related to the development and progression of prostate cancer rather than a by-stander.

AMACR IN PUTATIVE PRECURSOR LESIONS OF PROSTATE CANCER

Two prostatic lesions have been considered as potential premalignant lesions of the prostate. High grade prostatic intraepithelial neoplasia (HGPIN) is most likely a precursor of peripheral zone prostatic adenocarcinomas (29-30). Studies have shown that the risk of carcinoma on re-biopsy is increased when HGPIN is present (31-32). Several of the early studies of AMACR in prostate found increased expression in HGPIN, but rates varied from 13 to 72% (12-17). In 2004, Wu et al (33) analyzed AMACR

expression by immunohistochemistry in 3954 prostatic ducts and acini with HGPIN from 140 prostatectomy specimens. They found AMACR expression in 126 of 140 cases, but only 41 % of prostatic ducts or acini involved by PIN showed AMACR immunoreactivity. Significantly, 56% of HGPIN glands close to adenocarcinoma were positive compared to only 14% of HGPIN glands away from adenocarcinoma (33) which suggests a higher risk of finding cancer or developing cancer in areas adjacent to AMACR positive HGPIN.

Using gene expression profiles, Ashida et al showed that AMACR was one of 21 up-regulated genes seen in PIN lesions and considered it to be involved in the early stages of prostate carcinogenesis (34). Recently, Ananthranarayanan et al (35) looked at AMACR expression in 45 patients with isolated HGPIN in needle core biopsy, 12 radical prostatectomy specimens with prostatic carcinoma, and 6 cystoprostatectomies without prostatic carcinoma. They found that AMACR expression was increased in HGPIN lesions, but that proximity to carcinoma did not affect expression levels. This study was limited by the choice of small specimens from biopsy. Significantly, they also observed that AMACR expression was significantly increased in benign glands adjacent to adenocarcinoma and postulated a possible field effect in prostatic carcinogenesis (35).

Atypical adenomatous hyperplasia (AAH), also known as adenosis, is a lesion characterized by a well-circumscribed lobule of closely packed, crowded small glands without significant cytologic atypia, occurring mostly in the transition zone (30, 36). Seen in approximately 5-20% of transurethral and radical prostatectomy specimens, AAH may be difficult to distinguish from low-grade carcinoma because of their architectural similarities (30, 36, 37). Yang et al (38) examined AMACR in 40 cases of AAH and found focal expression in 10% of cases and diffuse expression in 7.5% of cases. Similarly, Gupta et al (39) observed AMACR expression in 31% of cases of AAH. These findings support the notion that a small subset of AAH may be a precursor of prostate cancer. They also indicate that AMACR expression in a lesion, in which AAH is a diagnostic consideration, must be interpreted with caution. Other features such as the presence of basal cells by high molecular weight keratin stains might also be useful in these circumstances.

AMACR IN BENIGN CONDITIONS

Many benign conditions such as small, crowded glands, atrophy, inflammatory atypia, and basal cell hyperplasia can mimic prostatic adenocarcinoma on needle core biopsies (40). Moreover, small foci of such conditions may be negative for high-molecular weight keratins (HMWK) (8). The initial studies examining AMACR expression in prostate noted an occasional small amount of expression in benign prostatic epithelium (12 to 21% of benign glands) using both polyclonal and monoclonal antibodies (12-17). The staining was reported as almost always fine granular, focal, weak, and noncircumferential (12-17). Benign prostatic hyperplasia (BPH) was often negative for AMACR (12, 16). In the study by Leav et al (22), the authors noted AMACR expression in eight BPH samples adjacent to adenocarcinoma but none of the other BPH cases. This could be a “field effect” phenomenon similar to that described above by Ananthranarayanan et al (35) in which benign glands adjacent to cancer seemed to show increased expression of

AMACR.

Recently, Herawi et al (42) examined AMACR expression in benign mimickers of prostate cancer seen on needle biopsies in consultation. They reported 15 of 19 (79%) cases of partial atrophy and 7 of 11 (64%) cases of crowded glands were positive for AMACR, although they did not mention extent or intensity of staining (42). We have also noticed increased AMACR expression in cases of partial atrophy (unpublished data), although the staining tends to be non-circumferential and less intense compared to carcinoma.

Nephrogenic adenoma (NA) is a benign lesion composed of small glandular structures that develops along the urothelium which is felt to be derived from shedding renal tubules (43). Although not commonly seen on prostate needle core biopsies, it may be mistaken for carcinoma when present. Skinnider et al (44) reported moderate to strong circumferential AMACR in 3 of 4 NAs of the prostatic urethra. Gupta et al (45) found AMACR expression in 28 of 38 (58%) NAs, ranging from patchy, focal staining to diffuse positivity. Some of these lesions were also negative for HMWK. Thus, nephrogenic adenomas are both morphologic as well as immunohistochemical mimickers of prostate carcinoma and must be kept in mind when examining small foci of suspicious glands on needle biopsy.

There seems to be some differences in AMACR staining levels in benign prostatic conditions when comparing the monoclonal (P504S) or polyclonal antibodies specific for AMACR. Typically polyclonal AMACR antibodies demonstrated slightly higher background than monoclonal ones. Using the monoclonal antibody, Beach et al (16) reported that small benign glands including atrophy, basal cell hyperplasia, urothelial metaplasia, and most cases of adenosis were completely negative for AMACR. On the other hand, Rubin et al (13), using the polyclonal antibody, found increased expression in benign conditions such as post-atrophic hyperplasia. In 2003, Kunju et al (41) directly compared the two antibodies and found that 68% of benign glands showed weak expression of AMACR with the polyclonal antibody compared to only 7% using the monoclonal antibody. Sensitivity for prostatic adenocarcinoma was 100% using the polyclonal antibody compared to 94% for P504S. We have been using the rabbit monoclonal antibody, as it seems to have good specificity while not sacrificing much sensitivity for prostatic adenocarcinoma. Because of the wider application of AMACR antibodies, the staining conditions for AMACR immunohistochemistry are essential for interpretation. Overstaining can be a major problem since both benign and malignant glands would be all positive. Optimal staining condition for each AMACR antibody has to be tested out. When interpreting AMACR stains in prostates, one should always use the benign prostatic glands as a negative control, which should have very low AMACR staining, and compare to the lesions of interest.

AMACR IN OTHER NEOPLASMS

Since the original prostate cancer studies, several investigators have looked at AMACR expression in other tumors. Zhou et al (46), using the polyclonal antibody, found AMACR overexpression in colorectal, ovarian, breast, bladder, lung, and renal cell

carcinomas, as well as lymphomas and melanomas. Greatest overexpression was seen in colorectal carcinomas (92%) (46). Using the monoclonal antibody, Jiang et al (47) reported that 81% of hepatocellular carcinomas, 75% of renal cell carcinomas, 31% of urothelial carcinomas, and 27% of gastric carcinomas were positive for AMACR. They also reported that lung, breast, pancreas, bile duct, adrenal gland, salivary gland, ovary, thyroid, and endometrial cancers were negative or rarely positive, while AMACR expression was found in normal liver, kidney, and salivary gland tissue (47). Using a high-density tissue microarray, Witkiewicz et al (48) determined that AMACR was overexpressed in 42 of 160 invasive breast carcinomas, and was associated with a decrease in tumor differentiation.

In 2003, Jiang et al (49) examined AMACR expression 242 cases of colonic tumors including 176 carcinomas, 38 adenomas, and 28 hyperplastic polyps. Using immunohistochemistry, they determined AMACR was highly expressed in 75% of carcinomas and 79% of adenomas but only 4% of hyperplastic polyps. It was postulated that AMACR overexpression might be an early event in the adenoma-carcinoma sequence in colorectal tumor genesis (49). Recently, Chen et al (50) examined AMACR expression in 59 small intestinal adenocarcinomas and 66 colorectal adenocarcinomas and reported that 62% of colorectal tumors were positive compared to only 5% of small intestinal tumors. Therefore, AMACR expression level is important for differentiation of small bowel adenocarcinoma from colonic adenocarcinoma.

There are several studies in the literature regarding AMACR expression in renal tumors. Tretiakova et al reported expression in 41 of 41 papillary renal cell carcinomas (RCC) compared with 13 of 52 clear cell RCCs, 3 of 20 oncocytomas, 0 of 18 chromophobe RCCs and 0 of 15 sarcomatoid RCCs. In addition, they reported a 5.2-fold increase of mRNA levels in 7 of 8 papillary RCCs but no increase in 60 of 62 non-papillary renal tumors (51). Other papillary carcinomas including thyroid, breast, endometrium, ovary, and pancreas carcinomas were rarely positive (51). Lin et al (52), using AMACR immunohistochemistry, also observed 100% expression in 15 papillary RCCs but also reported expression in 69% of clear cell RCCs, 29% of chromophobe RCCs, and 25% of oncocytomas.

Suh et al (53) stained 17 cases of enteric-type primary adenocarcinomas of the bladder and observed 65% positivity, similar to the 70% expression rate they observed in colorectal carcinomas but much higher than the 14% expression rate they observed in conventional transitional cell carcinomas of the bladder. This data suggests that AMACR expression might be related to an “enteric phenotype” in certain tumors. Logani et al (54) reported P504S overexpression in 32% of metastatic colorectal carcinomas to the ovary compared with none of the 23 primary ovarian mucinous and endometrioid carcinomas. Perhaps AMACR expression in a mucinous ovarian neoplasm may suggest a metastasis rather than a primary ovarian neoplasm.

DOUBLE OR TRIPLE STAINS

Based on the above data, one can reasonably assume that positive staining for AMACR in small atypical glands with absence of basal cells can help establish a definitive

diagnosis of prostatic adenocarcinoma when HGPIN, AAH, and nephrogenic adenoma have been excluded. Therefore, the development of double or triple staining distinguishing prostate cancer from benign glands became attractive. In 2004, Jiang et al reported the double immunofluorescence with AMACR in red and HMWCK in green as well as double immunohistochemistry with AMACR in brown and HMWCK in blue. In 2004, Browne et al (55) further showed that AMACR, when used in combination with a basal cell stain such as HMWK or p63, can render a definitive diagnosis in up to 70% of cases that would otherwise have been called atypical and recommended both stains for such lesions. The authors also noted that a limitation of this approach at the time was the loss of tissue in these small lesions, suggesting that combining the two or three stains on a single slide would be more optimal (55).

The same year, Sanderson et al (56) examined 40 cases containing small foci of adenocarcinoma, HGPIN, atypical small acinar proliferations (ASAP) with and without PIN, and atypical favor benign glands using a p63/P504S cocktail. After the combined stain, one third of cases were re-classified to carcinoma (56). Molinie et al (57) observed an increase in both sensitivity and specificity for prostate adenocarcinoma when using a p63/P504S cocktail compared to using a basal cell marker alone and that the combined stain supports a diagnosis of cancer in 40% of cases previously considered as ASAPs. Another study in 2004 examined 101 small foci of prostate cancer, 104 foci of ASAP, 19 small foci of PIN, and 36 benign mimics of cancer and found that with the P504S/p63 cocktail, 89% of ambiguous lesions were reclassified more definitively versus 53% when using CK 5/6 alone (58).

In 2005, Hameed et al (59) reviewed 31 consecutive radical prostatectomy specimens and 150 prostate needle biopsies and selected sections showing foci of minimal prostatic adenocarcinoma, HGPIN, and common benign mimickers of prostate cancer. They reported that a cocktail containing p63 and AMACR was very useful in highlighting adenocarcinoma associated with HGPIN, flat and cribriform HGPIN, and distorted foci of minimal prostatic carcinoma and suggested the cocktail is essentially equivalent to using each antibody separately for immunohistochemical confirmation of cancer (59).

Jiang et al (60) recently assessed the usefulness of a 3-antibody cocktail combining AMACR, 34betaE12, and p63 (triple stain) with a double chromogen reaction. Examining 138 needle biopsies including 82 with small foci of cancer, they found that 95% of the malignant cases expressed AMACR and none expressed basal cell markers, while a positive AMACR and negative basal cell phenotype was 100% specific for cancer (60). Performing the triple stain seems to be a very sensitive and specific way to detect small foci of prostate cancer on needle core biopsies while utilizing as little tissue as possible, and we recommend its routine use on all cases of small atypical foci suspicious for cancer.

The major reason for PIN-4 triple stains (AMACR, P63 and HMWCK) becoming more popular is using only one slide and revealing the prostate cancer marker and basal cell markers on the same focus. However, it is important to know that condition of double or triple stain is more complicated than single stain and subject to tissue distortion understaining or overstaining.

LIMITATION AND PITFALLS

Although AMACR immunohistochemistry has shown promise to surgical pathologists when diagnosing small foci of prostate cancer, there are some diagnostic pitfalls to keep in mind. First, like many other immunohistochemical stains, AMACR staining has shown variability from laboratory to laboratory. In 2003, Magi-Galluzzi (61) et al found that although all 34 in-house cases of prostate cancer performed at an immunohistochemistry laboratory at a major teaching hospital were positive for AMACR, while only 80% of prostate cancer cases seen in consultation but performed from various immunohistochemistry laboratories were positive. Zhou et al (23) reported that close to 20% of their prostate cancer cases seen in consultation were negative for AMACR. These rates are significantly less than most initial reports on AMACR expression in prostate cancer (12-17). This finding may represent the selection of difficult cases for consultation but nevertheless indicated the increasing utility and difficulty in performing and interpretation of AMACR staining in different institutions.

As stated earlier, some histologic variants of prostate cancer show decreased expression of AMACR compared to conventional prostatic acinar adenocarcinoma. Approximately 30% of atrophic, foamy gland, and pseudohyperplastic variants of adenocarcinoma are reportedly negative. In addition, many hormonally treated residual prostatic adenocarcinomas show decreased AMACR expression (28). Therefore, a lesion with negative expression of both AMACR and basal cells should be interpreted with caution when suspecting such entities.

Conversely, some benign conditions have been shown to express AMACR on occasion, although the staining pattern is usually weaker and non-circumferential. These include adenosis, atrophy, and benign glands adjacent to cancer. One must also be aware that HGPIN and nephrogenic adenoma often strongly express AMCAR. Many tumors from other organ systems have been shown to express AMACR as well. Therefore AMACR may not be as useful as markers such as PSA to define a metastatic carcinoma from other organ.

Although AMACR immunochemistry has been used in clinical practice, the utility of AMACR in serum tests are only at experimental stages. A screening test in the clinical setting based on urinary AMACR may develop as a useful adjunct to serum PSA and digital rectal exam in the early detection of prostate cancer. Using Western blot analysis, Zielie (62) et al detected AMACR in the urine in all twelve patients with biopsy-proven prostate cancer, and showed AMACR detection was associated with cancer status by biopsy in 21 of 26 patients. Rogers et al (63), using quantitative reverse transcriptase-PCR to detect AMACR-to-PSA transcript ratios, also were able to predict prostate cancer status in patients. These initial studies hint at perhaps not only a promising future for AMACR immunohistochemistry, but also in other clinical applications.

In summary, multiple studies have shown that AMACR immunohistochemistry of is only a sensitive and specific marker for prostate cancer, but also practical for pathologic utility. However, we need to be aware that occasionally benign lesions may show positive AMACR staining and prostatic adenocarcinoma can be negative for AMACR.

Therefore, it is a good practice to use a combination of AMACR and basal cell markers such as 34bE12 and p63. Neoplasms from other organs may also express AMACR when a specimen outside the prostate is evaluated. Like any other good markers, application of AMACR should be used as an adjunct test. The diagnosis of prostate cancer should be primarily established on the morphological basis.

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REFERENCES

1. Catalona WJ, Richie JP, Ahmann FR, et al. Comparison of digital rectal exam and serum prostate specific antigen in the early detection of prostate cancer: results of a multicenter clinical trial of 6,630 men. *J Urol.* 1994;151:1283-1290.
2. DiGiuseppe JA, Sauvageot J, Epstein JI. Increasing incidence of minimal residual cancer in radical prostatectomy specimens. *Am J Surg Pathol.* 1997;21:174-178.
3. Nadler RB, Humphrey PA, Smith DS, et al. Effect of inflammation and benign prostatic hyperplasia on elevated serum prostate specific antigen levels. *J Urol.* 1995;154:407-413.
4. Epstein JI. Diagnostic criteria of limited adenocarcinoma of the prostate on needle biopsy. *Hum Pathol.* 1995;26:223-229.
5. Epstein JI, Yang XJ. *Prostate Biopsy Interpretation.* Third Edition. Lippincott, Williams and Wilkins, Philadelphia, PA, 2002.
6. Brawer MK, Peehl DM, Stamey TA, et al. Keratin immunoreactivity in the benign and neoplastic human prostate. *Cancer research.* 1985;45:3663-3667.
7. Signoretti S, Waltregny D, Dilks J, et al. p63 is a prostate basal cell marker and is required for prostate development. *Am J Pathol.* 2000;157:1769-1775.
8. Wojno KJ, Epstein LI. The utility of basal cell-specific anticytokeratin antibody (34 beta E12) in the diagnosis of prostate cancer: a review of 228 cases. *Am J Surg Pathol.* 1995;19:251-260.
9. Schmitz W, Albers C, Fingerhut R, Conzellan E. Purification and characterization of an alpha-methylacyl-CoA racemase from human liver. *Eur J Biochem.* 1995 Aug 1;231(3):815-22.
10. Ferdinandusse S, Denis S, Ijst L, et al. Subcellular localization and physiological role of alpha-methylacyl-CoA racemase. *J Lipid Res.* 2000;41:1890-1896.
11. Xu J, Stolk JA, Zhang X, et al. Identification of differentially expressed genes in human prostate cancer using subtraction and microarray. *CancerRes.* 2000;60:1677-1682.
12. Jiang Z, Woda BA, Rock KL, et al. P504S: a new molecular marker for the detection of prostate carcinoma. *Am J Surg Pathol.* 2001;25:1397-1404.
13. Rubin MA, Zhou M, Dhanasekaran SM, et al. alpha-Methylacyl coenzyme A racemase as a tissue biomarker for prostate cancer. *JAMA.* 2002;287:1662-1670.
14. Luo J, Zha S, Gage WR, et al. Alpha-methylacyl-CoA racemase: a new

- molecular marker for prostate cancer. *Cancer Res.* 2002;62:2220-2226.
15. Jiang Z, Wu CL, Woda BA, et al. P504S/alpha-methylacyl-CoA racemase: a useful marker for diagnosis of small foci of prostatic carcinoma on needle biopsy. *Am J Surg Pathol.* 2002;26:1169-1174.
 16. Beach R, Gown AM, De Peralta-Venturina MN, et al. P504S immunohistochemical detection in 405 prostatic specimens including 376 18 gauge needle biopsies. *Am J Surg Pathol.* 2002;26:1588-1596.
 17. Jiang Z, Wu CL, Woda BA, et al. Alpha-methylacyl-CoA racemase: a multi-institutional study of a new prostate cancer marker. *Histopathology.* 2004;45:218-25.
 18. Kumar-Sinha, C, Shah RB, Laxman B et al. Elevated alpha-methylacyl-CoA racemase enzymatic activity in prostate cancer. *Am J Pathol.* 2004;164:787-93.
 19. Rubin MA, Bismar TA, Andren O, et al. Decreased alpha-methylacyl CoA racemase expression in localized prostate cancer is associated with an increased rate of biochemical recurrence and cancer-specific death. *Cancer Epidemiol Biomarkers Prev.* 2005;14:1424-32.
 20. Jiang Z, Iczkowski KA, Woda BA, et al. P504S immunostaining boosts diagnostic resolution of "suspicious" foci in prostatic needle biopsy specimens. *Am J Clin Pathol.* 2004;121:99-107.
 21. Zhou M, Aydin H, Kanane H, Epstein JI. How often does alpha-methylacyl-CoA-racemase contribute to resolving an atypical diagnosis on prostate needle biopsy beyond that provided by basal cell markers? *Am J Surg Pathol.* 2004;28:239-43.
 22. Leav I, Mcneal JE, Ho SM, et al. Alpha-methylacyl-CoA racemase (P504S) expression in evolving carcinomas within benign prostatic hyperplasia and in cancers of the transition zone. *Hum Pathol.* 2003;34:228-233.
 23. Zhou M, Jiang Z, Epstein JI. Expression and diagnostic utility of alpha-methylacyl-CoA-racemase (P504S) in foamy gland and pseudohyperplastic prostate cancer. *Am J Surg Pathol.* 2003;27:772-778.
 24. Jiang Z, Woda BA, Wu CL et al. Discovery and Clinical Application of a Novel Prostate Cancer Marker: Alpha-Methylacyl CoA Racemase (P504S). *Am J Clin Pathol.* 2004;122:275-289.
 25. Farinola MA, Epstein JI. Utility of immunohistochemistry for alpha-methylacyl-CoA racemase in distinguishing atrophic prostate cancer from benign atrophy. *Hum Pathol.* 2004;35:1272-8.
 26. Yang XJ, Laven B, Tretiakova M, et al. Detection of alpha-methylacyl-coenzyme A racemase in postradiation prostatic adenocarcinoma. *Urology.* 2003 Aug;62(2):282-6.
 27. Amin M, Beach R, Gown AM, et al. Use of a novel immunohistochemical (IHC) panel (P504S, p63 and 34betaE12) in the diagnosis of post-radiation therapy (PRT) prostate cancer (PCa) [abstract]. *Mod Pathol.* 2003;16:139A.
 28. Suzue K, Montag AG, Tretiakova MG, et al. Altered expression of alpha-methylacyl-coenzyme A racemase in prostatic adenocarcinoma following hormone therapy. *Am J Clin Pathol.* 2005 Apr;123(4):553-61.
 29. Bostwick DG. Premalignant lesions of the prostate. *Semin Diag Pathol.* 1988;5:240-253.
 30. Bostwick DG. Prospective origins of prostate carcinoma: prostatic intraepithelial neoplasia and atypical adenomatous hyperplasia. *Cancer.* 1996;77:330-336.
 31. Weinstein MH, Epstein JI. Significance of high-grade prostatic intraepithelial neoplasia on needle biopsy. *Hum Pathol.* 1993;24:624-629.
 32. Kronz JD, Allan CH, Shaikh AA, et al. Predicting cancer following a diagnosis

of high-grade prostatic intraepithelial neoplasia on needle biopsy: data on men with more than one follow-up biopsy. *Am J Surg Pathol.* 2001;25:1079-1085.

33. Wu CL, Yang XJ, Tretiakova M, et al. Analysis of alpha-methylacyl-CoA racemase (P504S) expression in high-grade prostatic intraepithelial neoplasia. *Hum Pathol.* 2004;35:1008-13.
34. Ashida S, Nakagawa H, Katagiri T, et al. Molecular features of the transition from prostatic intraepithelial neoplasia (PIN) to prostate cancer: genome-wide gene-expression profiles of prostate cancers and PINs. *Cancer Res.* 2004;64:5963-72.
35. Ananthranarayanan V, Deaton RJ, Yang XJ et al. Alpha-methylacyl-CoA racemase (AMACR) expression in normal prostatic glands and high-grade prostatic intraepithelial neoplasia (HGPIN): association with diagnosis of prostate cancer. *Prostate.* 2005;63:341-6.
36. Bostwick DG, Srigley J, Grignon D, et al. Atypical adenomatous hyperplasia of the prostate: morphologic criteria for its distinction from well-differentiated carcinoma. *Hum Pathol.* 1993;24:819-832.
37. Kovi J. Microscopic differential diagnosis of small acinar adenocarcinoma of prostate. *Pathol Annu.* 1985;20:157-196.
38. Yang XJ, Wu CL, Woda BA, et al. Expression of alpha-Methylacyl-CoA racemase (P504S) in atypical adenomatous hyperplasia of the prostate. *Am J Surg Pathol.* 2002 Jul;26(7):921-5.
39. Gupta N, De Peralta-Venturina MN, Gown AM et al. P504S antibody expression in putative precursor lesion of prostate carcinoma (PCa)-high grade prostatic intraepithelial neoplasia (HGPIN) and atypical adenomatous hyperplasia (AAH) [abstract]. *Mod Pathol.* 2003;16:152.
40. Gaudin PB, Reuter VA. Benign mimics of prostatic adenocarcinoma on needle biopsy. *Anat Pathol.* 1997;2:111-134
41. Kunju LP, Rubin MA, Shen R, et al. Comparison of monoclonal antibody (P504S) and polyclonal antibody to alpha-methylacyl-CoA racemase in benign, atypical and malignant prostate tissues [abstract]. *Mod Pathol.* 2003;16:158A.
42. Herawi M, Parwani AV, Irie J, Epstein JI. Small glandular proliferations on needle biopsies: most common benign mimickers of prostatic adenocarcinoma sent in for expert second opinion. *Am J Surg Pathol.* 2005;29:874-80.
43. Mazal PR, Schaufler R, Altenhuber-Muller R et al. Derivation of nephrogenic adenomas from renal tubular cells in kidney-transplant recipients. *N Engl J Med.* 2002;347:653-659.
44. Gupta A, Wang HL, Policarpio-Nicholas ML, et al. Expression of alpha-methylacyl-coenzyme A racemase in nephrogenic adenoma. *Am J Surg Pathol.* 2004;28:1224-9.
45. Skinnider BF, Oliva E, Young RH, Amin MB. Expression of alpha-methylacyl-CoA racemase (P504S) in nephrogenic adenoma: a significant immunohistochemical pitfall compounding the differential diagnosis with prostatic adenocarcinoma. *Am J Surg Pathol.* 2004;28:701-5.
46. Zhou M, Chinnaiyan AM, Kleer CG et al. Alpha-Methylacyl-CoA racemase: a novel tumor marker over-expressed in several human cancers and their precursor lesions. *Am J Surg Pathol.* 2002;26:926-31.
47. Jiang Z, Fanger GR, Woda BA, et al. Expression of alpha-methylacyl-CoA racemase (P504s) in various malignant neoplasms and normal tissues: a study of 761 cases. *Hum Pathol.* 2003;34:792-6.

48. Witkiewicz AK, Varambally S, Shen R, et al. Alpha-methylacyl-CoA racemase protein expression is associated with the degree of differentiation in breast cancer using quantitative image analysis. *Cancer Epidemiol Biomarkers Prev.* 2005;14:1418-23.
49. Jiang Z, Fanger GR, Banner BF, et al. A dietary enzyme: alpha-methylacyl-CoA racemase/P504S is overexpressed in colon carcinoma. *Cancer Detect Prev.* 2003;27:422-6.
50. Chen ZM, Ritter JH, Wang HL. Differential Expression of alpha-Methylacyl Coenzyme A Racemase in Adenocarcinomas of the Small and Large Intestines. *Am J Surg Pathol.* 2005;29:890-6.
51. Tretiakova MS, Sahoo S, Takahashi M, et al. Expression of alpha-methylacyl-CoA racemase in papillary renal cell carcinoma. *Am J Surg Pathol.* 2004;28:69-76.
52. Lin F, Brown RE, Shen T, et al. Immunohistochemical detection of P504S in primary and metastatic renal cell carcinomas. *Appl Immunohistochem Mol Morphol.* 2004 Jun;12(2):153-9.
53. Suh N, Yang XJ, Tretiakova MS, et al. Value of CDX2, villin, and alpha-methylacyl coenzyme A racemase immunostains in the distinction between primary adenocarcinoma of the bladder and secondary colorectal adenocarcinoma. *Mod Pathol.* 2005 Apr 1.
54. Logani S, Oliva E, Arnell PM, et al. Use of novel immunohistochemical markers expressed in colonic adenocarcinoma to distinguish primary ovarian tumors from metastatic colorectal carcinoma. *Mod Pathol.* 2005 Jan;18(1):19-25.
55. Browne TJ, Hirsch MS, Brodsky G et al. Prospective evaluation of AMACR (P504S) and basal cell markers in the assessment of routine prostate needle biopsy specimens. *Hum Pathol.* 2004 Dec;35(12):1462-8.
56. Sanderson SO, Sebo TJ, Murphy LM, et al. An analysis of the p63/alpha-methylacyl coenzyme A racemase immunohistochemical cocktail stain in prostate needle biopsy specimens and tissue microarrays. *Am J Clin Pathol.* 2004 Feb;121(2):220-5.
57. Molinie V, Herve JM, Lebret T, et al. Value of the antibody cocktail anti p63 + anti p504s for the diagnosis of prostatic cancer. *Ann Pathol.* 2004;24:6-16.
58. Molinie V, Fromont G, Sibony M, et al. Diagnostic utility of a p63/alpha-methyl-CoA-racemase (p504s) cocktail in atypical foci in the prostate. *Mod Pathol.* 2004;17:1180-90.
59. Hameed O, Sublett J, Humphrey PA. Immunohistochemical stains for p63 and alpha-methylacyl-CoA racemase, versus a cocktail comprising both, in the diagnosis of prostatic carcinoma: a comparison of the immunohistochemical staining of 430 foci in radical prostatectomy and needle biopsy tissues. *Am J Surg Pathol.* 2005;29:579-87.
60. Jiang Z, Li C, Fischer A, et al. Using an AMACR (P504S)/34betaE12/p63 cocktail for the detection of small focal prostate carcinoma in needle biopsy specimens. *Am J Clin Pathol.* 2005;123:231-6.
61. Magi-Galluzzi C, Luo J, Isaacs WB, et al. Alpha-methylacyl-CoA racemase: a variably sensitive immunohistochemical marker for the diagnosis of small prostate cancer foci on needle biopsy. *Am J Surg Pathol.* 2003;27:1128-1133.
62. Zielie PJ, Mobley JA, Ebb RG, et al. A novel diagnostic test for prostate cancer emerges from the determination of alpha-methylacyl-coenzyme a racemase in prostatic secretions. *J Urol.* 2004;172:1130-3.
63. Rogers CG, Yan G, Zha S, et al. Prostate cancer detection on urinalysis for alpha methylacyl coenzyme a racemase protein. *J Urol.* 2004;172(4 Pt 1):1501-3.