

## Alpha-methylacyl-CoA-racemase expression in variants and unusual patterns of prostate carcinoma

### Expresia alpha-methylacyl-CoA-racemazei în variante și pattern-uri neobișnuite de carcinom prostatic

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#### Abstract

**Introduction:** Alpha-methylacyl-CoA-racemase (AMACR) is a tumor marker expressed in prostate cancer. Literature data concerning its expression in different variants and unusual patterns of prostate carcinoma are uneven and less numerous. The present study investigates the AMACR expression in some rare forms of prostate carcinoma. **Material and methods:** Distinct variants and unusual patterns of prostate carcinoma (in pure or mixed form) were selected: foamy gland (9 cases), ductal adenocarcinoma (7 cases), pseudohyperplastic (5 cases), atrophic (3 cases), urothelial (3 cases), mucinous (2 cases), signet ring cell carcinoma (1 case) together with 20 cases of conventional prostate carcinomas. IHC study was performed using the following antibodies: AMACR, high molecular weight cytokeratin HMWCK, p63, PSA, prostein, CK5/6, CK7 and CK20. **Results:** Ninety percent of conventional prostate carcinomas cases were positive for AMACR. 5 out of the 30 variants/unusual patterns of prostate carcinoma cases were devoid of immunoreactivity for AMACR, including 2 ductal adenocarcinomas, 2 urothelial and an atrophic carcinoma. Carcinomas/areas of foamy, pseudohyperplastic and mucinous type have shown a marked heterogeneous expression of AMACR, with negative areas and foci of variable intense positive staining. **Conclusions:** This study confirms the heterogeneity of AMACR expression in prostate carcinomas and the fact that this marker's expression is reduced/absent in some cases of conventional or variants/unusual patterns of prostate carcinomas. In difficult cases or small biopsy specimens, the diagnosis of cancer should be based on correlation of data offered by the usual stains with findings provided by immunostaining for basal cells and AMACR.

**Keywords:** AMACR, immunohistochemistry, prostate, variants/unusual patterns of carcinoma

#### Rezumat

**Introducere:** Alfa-metilacyl-CoA racemaza (AMACR) este un marker tumoral exprimat în cancerul de prostată. Datele de literatură referitoare la expresia lui în variante și pattern-uri neobișnuite de carcinom prostatic

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sunt neuniforme și puțin numeroase. Studiul de față investighează expresia AMACR în unele forme rare de carcinom prostatic. **Material și metodă:** Au fost selectate variante distincte și pattern-uri neobișnuite de carcinom prostatic (ca forme pure sau mixte), după cum urmează: adenocarcinom cu celule spumoase (9 cazuri), adenocarcinom ductal (7 cazuri), carcinom pseudohiperplazic (5 cazuri), carcinom atrofic (3 cazuri), carcinom urotelial (3 cazuri), carcinom mucinos (2 cazuri), carcinom cu celule în "inel cu pecete" (1 caz) și 20 cazuri de adenocarcinom convențional de prostată. Studiul imunohistochimic s-a realizat cu ajutorul următorilor anticorpi: AMACR, HMWCK, p63, PSA, prostein, CK5/6, CK7 and CK20. **Rezultate:** 90% din adenocarcinoamele convenționale de prostată au fost pozitive pentru AMACR. 5 din cele 30 de cazuri de variante/pattern-uri neobișnuite de carcinom prostatic au fost lipsite de imunoreactivitate pentru AMACR: 2 adenocarcinoame ductale, 2 carcinoame uroteliale și un carcinom atrofic. Carcinoamele/ariile de carcinom cu celule spumoase, pseudohiperplazic și mucinos au demonstrat expresie heterogenă a AMACR cu arii negative și porțiuni cu reactive pozitivă de intensitate variabilă. **Concluzii:** Studiul de față confirmă heterogenitatea de expresie a AMACR în carcinoamele de prostată și faptul că expresia markerului este redusă/absentă în unele carcinoame convenționale și în variante/pattern-uri neobișnuite de carcinom prostatic. În cazurile dificile, datorită naturii leziunii sau a cantității limitate de tumoră în materialul biopsic, diagnosticul de cancer reprezintă rezultatul coroborării informațiilor oferite de colorațiile uzuale cu cele furnizate de imunoreacțiile de identificare a celulelor bazale și a AMACR.

**Cuvinte-cheie:** AMACR, imunohistochimie, prostata, variante/pattern-uri neobișnuite de carcinom

## Introduction

Along with a broader range of serum Prostate Specific Antigen (PSA) determination as an effort for prostate cancer early diagnosis, the more frequent problem confronting pathologists are minimally represented cases, also difficult to diagnose on routine stains, cancer foci seen in prostate needle-biopsy (NB) (1). Development of immunohistochemistry (IHC) applied in the prostate's pathology led to the discovery, in a first stage, of basal cells' markers as negative markers (2, 3), meaning that prostate adenocarcinomatous glands lack basal cells. Later on, alpha – methylacyl – CoA – racemase (AMACR), known as P504S (4, 5) was discovered and studied as a positive tumor marker consistently expressed in prostate cancer: AMACR has an important discriminating value for evaluation of prostate lesions (6-10). A few recent papers show some variability of the AMACR expression within the prostate carcinomas, which helps in the correct interpretation of problematic lesions (11, 12). Literature data concerning expression of AMACR in different variants and unusual patterns of prostate carcinoma are less numerous and inconsistent (8, 13-15). This paper aims to analyze, in terms of contribution to the histological diagnosis of difficult prostate lesions, the AMACR expression in variants and unusual patterns of prostate carcinoma.

## Material and methods

Fifty cases of prostate carcinoma were selected from archival material. 20 of them corresponded to conventional carcinomas with Gleason score between 4 and 10 and 30 prostate carcinomas representing pure or mixed variants and unusual patterns of prostate carcinoma, corresponding to: foamy gland carcinoma - 9 cases, ductal adenocarcinoma - 7 cases, pseudohyperplastic carcinoma - 5 cases; atrophic carcinoma - 3 cases, secondary urothelial carcinomas of the prostate - 3 cases, mucinous (colloid) carcinoma - 2 cases; signet ring cell carcinoma - 1 case.

Investigated prostate tumors were diagnosed in transurethral resection specimens of the prostate – TURP (28 cases), radical prostatectomy – RP (6 cases), needle-biopsy – NB (15 cases), cystoprostatectomy (1 case). Primary prostate tumors were graded using the Gleason grading system.

The diagnosis validation of carcinoma and the glandular or urothelial nature of the prostate tumors with more particular aspects was performed using antibodies anti - HMWCK, p63, PSA, prostein, CK5/6, CK7 and CK20 (Table 1). For the IHC study, formalin-fixed and paraffin-embedded tissue sections cut at 4 to 5 μm were placed on special (Super Frost Ultra Plus) slides for greater adhesion.

**Table 1. The antibodies' panel used for the IHC study**

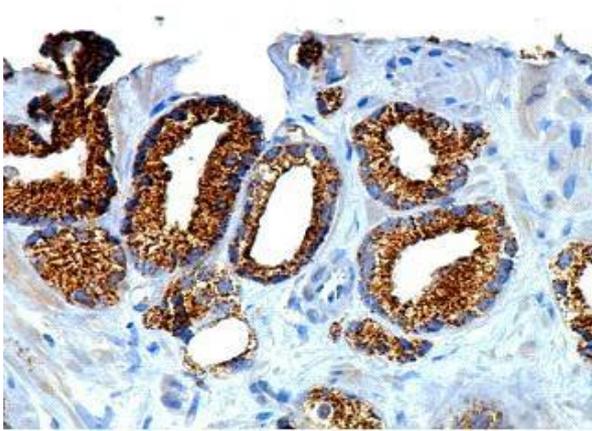
Antibody	Clone	Producer	Pretreatment	Dilution
Anti -AMACR	13H4	Dako	HIER - Dako Target Retrieval pH 9	RTU 1:200
Anti-HMWCK	34βE12	Dako	HIER - Dako Target Retrieval pH 6	RTU
Anti-p63	4A4	Dako	HIER - Dako Target Retrieval pH 9	RTU 1:100
Anti-PSA	Polyclonal	Dako	-	RTU
Anti - prostein	10E3	Dako	HIER - Dako Target Retrieval pH 6	RTU
Anti-CK5/6	D5/16B4	Dako	HIER- Dako Target Retrieval high pH	1:100
Anti-CK 7	OV-TL 12/30	Dako	K proteinase digestion / Dako Target Retrieval pH 6	RTU 1:75
Anti-CK20	K <sub>20</sub> .8	Dako	K proteinase digestion/ Dako Target Retrieval pH 9	RTU 1:50

With the exception of PSA, which did not require pretreatment, and CK7 and CK20, which were pretreated by enzymatic digestion in a first stage, for the rest of the antibodies antigen retrieval was achieved by boiling the slides in Target retrieval solution pH 6 or pH 9 for 20 min (*Table 1*). Considering the better results obtained with heat pretreatment by boiling for some cases we used this method to unmask the antigens also for CK7 and CK20.

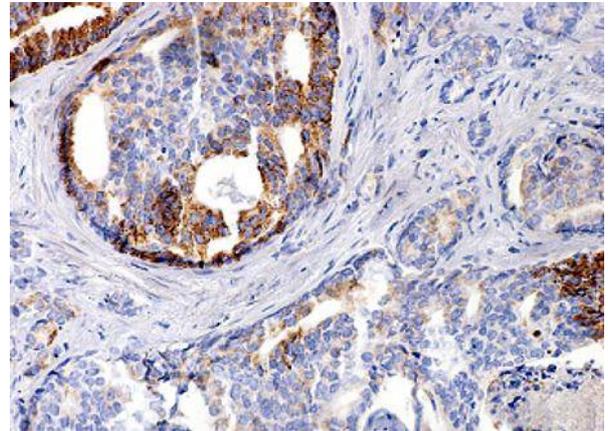
AMACR expression study was done on a single block from each case, using a rabbit monoclonal antibody to AMACR (Dako, Denmark) prediluted and concentrated. For concentrated form of antibody, the optimal concentration was determined by successive attempts, dilution of 1/200 being established as optimal in our laboratory conditions. Briefly, deparaffinized slides were hydrated and then placed in Target retrieval solution pH 9 and boiled for 20 min. Endogenous peroxidase activity was quenched by incubation with Dako peroxidase block for 5 min at room temperature. Slides were then washed and incubated with primary rabbit monoclonal antibody to AMACR for 30 min at room temperature. Secondary antirabbit/antimouse antibody-coated polymer peroxidase complex was applied for 30 min at room tempera-

ture. Slides were incubated with substrate/chromogen for 5-10 min at room temperature and then counterstained with hematoxylin. An AMACR-positive prostatic adenocarcinoma was used as a positive control and primary antibody was omitted from negative controls.

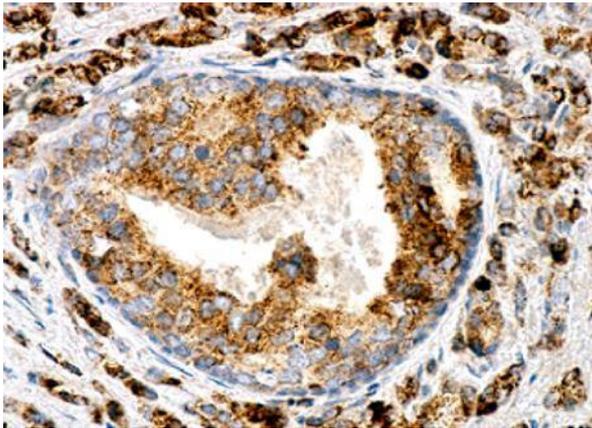
In the evaluation of the AMACR immunostaining, the location (cytoplasmic, luminal/subluminal) and the intensity of stain for AMACR were considered. AMACR protein expression was scored as *negative*, *weak stain* (faint cytoplasmic stain or granular apical staining which cannot be seen at low power magnification  $\leq 100\times$ ), *moderate* (diffuse granular moderate cytoplasmic stain) and *strong* (diffuse intense cytoplasmic stain), as previously described (11, 16, 17); only staining that was moderate or strong, significantly stronger than that of benign glands, and which can be easily observed at low power magnification ( $\leq 100\times$ ) was considered positive. We appreciated the AMACR reactivity as diffuse when  $> 75\%$  of tumor cells were intensely marked and heterogeneous when 1-75% of tumor cells were intensely and/or moderate labeled (11). For urothelial carcinoma only the tumors with nonfocal reactivity, present in over 10% of tumor cells, were considered positive.



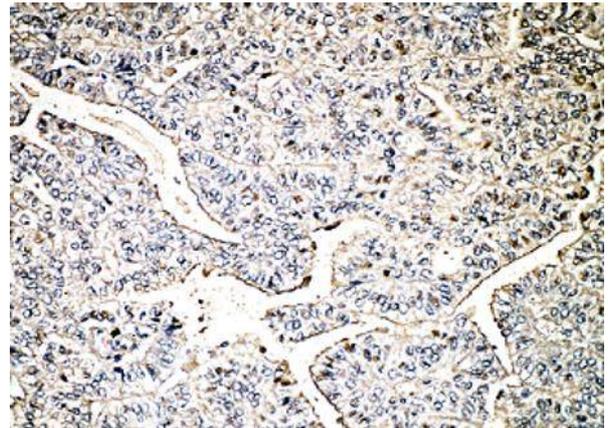
**Figure 1. Microacinary conventional prostate adenocarcinoma with granular intense circumferential stain.** Anti- Alpha-methylacyl-CoA racemase, EnVision System, Diaminobenzidine, hematoxylin counterstain x400.



**Figure 2. Heterogeneous staining for Alpha-methylacyl-CoA racemase in a prostate carcinoma with cribriform growth pattern.** Anti - Alpha-methylacyl-C6.0oA racemase, EnVision System, Diaminobenzidine, hematoxylin counterstain x200.



**Figure 3. Moderate to intense, circumferential staining into a High-Grade Prostatic Intraepithelial Neoplasia focus associated with carcinoma.** Anti - Alpha-methylacyl-CoA racemase, EnVision System, Diaminobenzidine, hematoxylin counterstain x400.

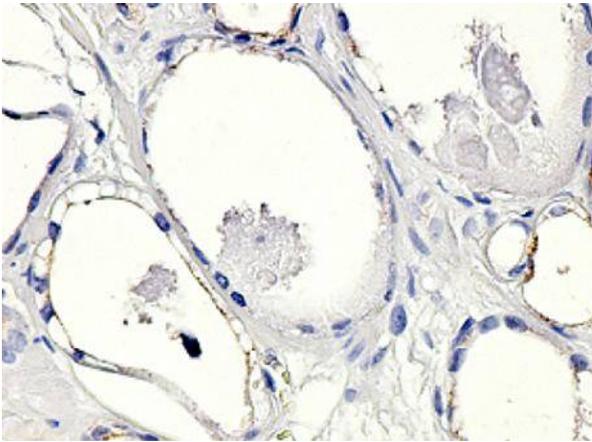


**Figure 4. Alpha-methylacyl-CoA racemase - negative ductal adenocarcinoma of the prostate.** Anti - Alpha-methylacyl-CoA racemase, EnVision System, Diaminobenzidine, hematoxylin counterstain x200.

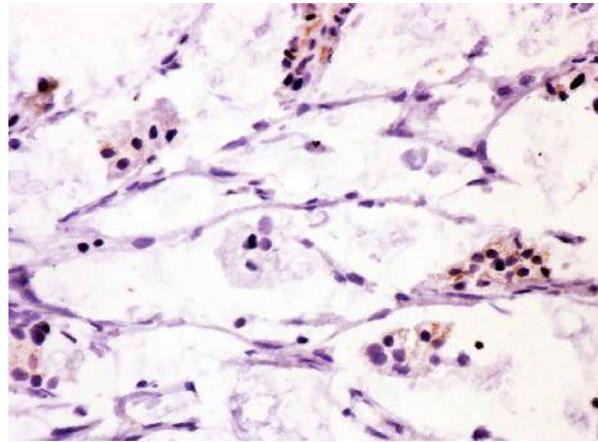
## Results

Eighteen out of the 20 conventional prostate carcinomas (90%) were positive for AMACR and only 2 cases (one diagnosed on TURP fragments, the other one on NB), with small number of malignant glands, were negative for AMACR. The cases of conventional carcinoma which were

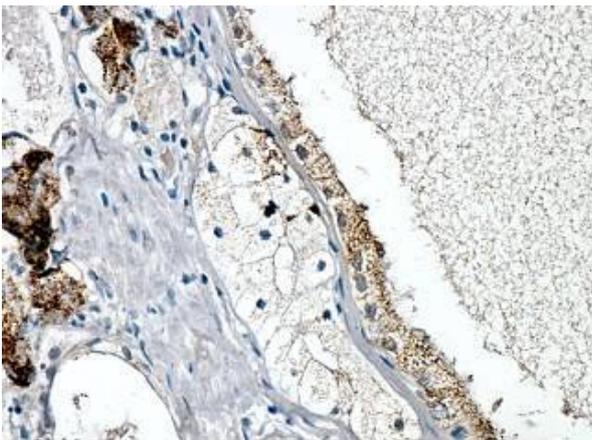
appreciated as AMACR-negative presented a complete lack of reactivity or had a weak positive stained glands, but not significantly stronger than the surrounding benign glands. Most conventional AMACR-positive prostate carcinomas showed an intense granular cytoplasmic stain (*Figure 1*) or a moderate, homogenous one, sometimes with luminal/subluminal enhancement in areas of glands for-



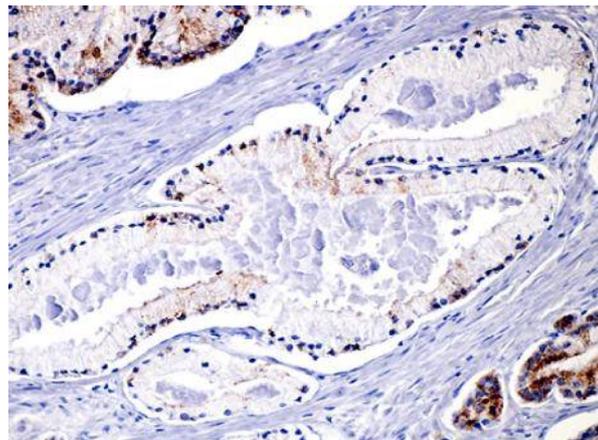
**Figure 5. Atrophic prostate adenocarcinoma without reactivity for Alpha-methylacyl-CoA racemase.** Anti - Alpha-methylacyl-CoA racemase, EnVision System, Diaminobenzidine, hematoxylin counterstain x400.



**Figure 6. Mucinous carcinoma with negative/weak positive areas of reactivity.** Anti- Alpha-methylacyl-CoA racemase, EnVision System, Diaminobenzidine, hematoxylin counterstain, x400.



**Figure 7. Heterogeneous Alpha-methylacyl-CoA racemase staining in foamy gland carcinoma with some tumoral glands devoided of reactivity.** Anti - Alpha-methylacyl-CoA racemase, EnVision System, Diaminobenzidine, hematoxylin counterstain x200.



**Figure 8. Heterogeneous expression with absent/weak reactivity in some glands of pseudohyperplastic adenocarcinoma.** Anti - Alpha-methylacyl-CoA racemase, EnVision System, Diaminobenzidine, hematoxylin counterstain, x200.

mation. In the case of prediluted antibody we have noticed a moderate staining of smooth and striated muscle cells, so we interpreted as positive only the intense cytoplasmic staining of tumor cells.

AMACR staining was generally homogeneous and diffuse in the tumors with Gleason score 4-6, while the category of tumors with Gleason score 7-10 showed often an heteroge-

neous stain, with tumor areas intensely marked, co-existing with moderate, weak or even absent reactivity portions. Areas with cribriform architecture, with/without central necrosis, presented more often weak or absent reactivity (*Figure 2*). Foci of high-grade intraepithelial neoplasia (HG-PIN) associated to carcinomas showed intense to moderate AMACR reactivity (*Figure 3*), with a

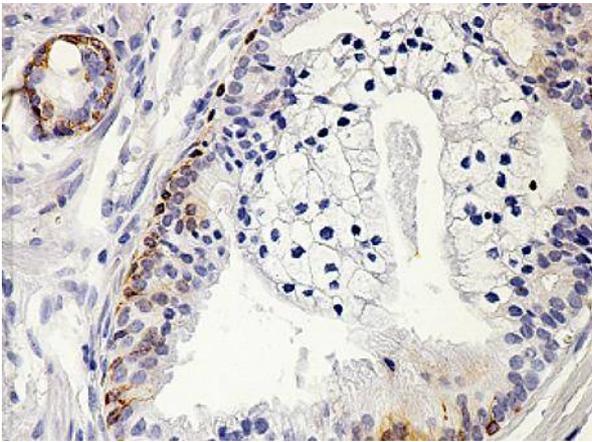
circumferential luminal to subluminal pattern or diffuse cytoplasmic staining.

From the 30 cases of variants/unusual patterns of prostate carcinoma, 5 cases were devoid of reactivity for AMACR: 2 ductal carcinomas (28.6%) (Figure 4), 2 urothelial carcinomas (66.7%) and an atrophic prostate carcinoma (33.3%) (Figure 5). The rest of 25 cases presented diffuse or heterogeneous positive staining for AMACR. Tumors/areas of mucinous (Figure 6), foamy (Figure 7) and pseudohyperplastic (Figure 8) type have shown a marked heterogeneous expression for AMACR, with negative foci and portions of positive staining with variable intensity. Interestingly, within a HGPIIN focus with a contingent of foamy cells these were devoid of AMACR immunoreactivity, while nonfoamy cells showed a moderate/intense granular positive staining, with luminal emphasis (Figures 9, 10). Signet ring cell carcinoma was characterized by a heterogeneous staining with areas of different intensity, with predominance of the moderate one.

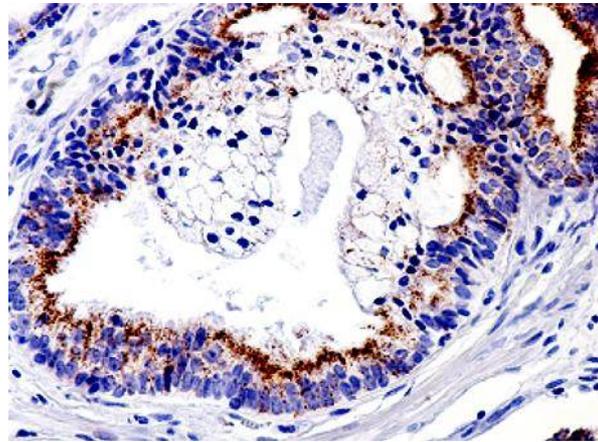
## Discussion

In prostate pathology diagnosis, IHC techniques are used in three main directions: a) the diagnosis of prostate carcinoma and its discrimination from some mimicking benign lesions b) the differential diagnosis of prostate carcinoma from extraprostatic tumor that secondary involves the gland c) and the identification of prostate cancer's metastases (18-21). The diagnosis of prostate cancer requires the absence of basal cells at the periphery of the glands. Evaluation of basal cells in the routine stains sometimes proves to be difficult, first because they can be confused with the stromal cells adjacent to the glandular basement membrane, with the endothelial cells of the vessels in the vicinity of malignant acini or with the tumor cells tangentially sectioned, and secondly because the basal cell layer is often discontinuous and/or difficult or impossible to identify on usual stain in some clear benign structures such as atrophic glands,

in lesions with architectural (adenosis) or cytological (PIN) atypia, as well as in the presence of crush or heat induced artifacts (20, 22-24). This is why the initial researches in the IHC domain, concerning prostate pathology, aimed to identify markers of basal cells. The highlighting of these cells at the periphery of glandular structures, based on IHC methods, excludes the cancer. Currently most used IHC markers for prostatic basal cells are high molecular weight cytokeratin (HMWCK), clone 34BE12, CK5/6 and p63 protein (25, 26, 27). The initial enthusiasm triggered by the discovery of these markers was slightly tempered by a number of proven limitations and shortcomings of IHC's methods for basal cell identification. The conclusion was that the absence of basal cells at the periphery of suspicious glandular structures, demonstrated by IHC techniques, biased the balance in favor of carcinoma, but did not allow its assertion (28). In these circumstances various papers have focused on the discovery of markers associated with prostate carcinoma, in other words, positive markers for adenocarcinoma of the prostate. Indeed, in 2000, the  $\alpha$  - Methylacyl Coenzyme A racemase (AMACR), an enzyme that plays an important role in the peroxisomal  $\beta$ -oxidation of dietary branched-chain fatty acids and C27-bile acid intermediates (4) was found. It was shown that AMACR gene and its product, P504S protein are overexpressed in prostate carcinoma cells compared with normal and benign prostate epithelium, thus AMACR could be considered a molecular marker of prostate cancer (29). Initial studies have reported a high specificity and sensitivity of this IHC marker for prostatic adenocarcinoma, with values close to 100% (5, 6, 30, 31). Further publications showed the existence of prostatic adenocarcinomas without immunoreactivity for AMACR (7, 8, 32) and also the marker's expression in some benign and premalignant prostatic or extraprostatic lesions (6, 31, 33-37). Our results, which show the presence of reactivity for AMACR in 90% of prostate conventional carcinoma cases, are consistent with



**Figure 9. Focus of High-Grade Prostatic Intraepithelial Neoplasia with foamy cells contingent: discontinuous basal cell layer at the periphery.** Anti - High Molecular Weight Cytokeratin (34 $\beta$ E12), EnVision System, Diaminobenzidine, hematoxylin counterstain, x400.



**Figure 10. The focus of High-Grade Prostatic Intraepithelial Neoplasia from previous figure: the foamy cells contingent lacks the reactivity for Alpha-methylacyl-CoA racemase.** Anti - Alpha-methylacyl-CoA racemase, EnVision System, Diaminobenzidine, hematoxylin counterstain, x400.

the literature data, reporting sensitivity and specificity rates of 80% -100% and 79% -100%, respectively (5, 6, 16, 30, 32, 35, 36, 38). These results highlight the importance of AMACR as a marker which enhances, on one hand, the ability to diagnose minimal foci of carcinoma and, on the other hand, increases the confidence of the pathologist's diagnosis of malignancy if an unequivocal positive reaction happened, doubled by the absence of reactivity for basal cells markers. Most conventional AMACR-negative prostate carcinomas reported in the literature are small tumors, present in NB specimens (32). Our data, showing the absence of AMACR immunoreactivity in two cases of conventional prostatic carcinoma, with small number of malignant glands, confirm these observations.

The values of sensitivity rate could be influenced by the tissue fixation and processing, the antibody type (mono- vs. polyclonal) and its concentration, the unmasking procedures used and even by the interpretation variables (11, 12, 19, 39). Magi-Galluzzi et al. reported 100% sensitivity for their own cases and 80% for cases came from different institutions, differences explained

by different processing of tissue material (32). Monoclonal antibody has been shown to be slightly less sensitive than polyclonal one in terms of prostate carcinoma detection (40), but the latter marks a greater number of benign glands, including postatrophic hyperplasia foci and is also accompanied by a more evident background staining (5, 14, 39). In our study, the use of monoclonal antibody could explain the 90% sensitivity rate for conventional prostate adenocarcinoma.

The interpretation of IHC staining is somehow subjective and could be a cause of different rates of sensitivity and even specificity for AMACR. Most authors consider that the specific stain for carcinoma should be easily detectable at low magnification ( $\leq 100\times$ ), circumferential, granular, luminal (apical) to subluminal or diffuse cytoplasmic staining and they grade the staining intensity into three (13, 17, 36, 41) or two tiered systems (42): strong, moderate and weak, respectively, strong and weak. Other authors quantify the IHC staining according to the percentage of positive cells (8) or using a score that combines the intensity of staining (3- strong, 2 - moderate, 1 - weak, 0 - negative) with the

percentage of stained cells (30). The overall assessment is that staining of the malignant glands must be significantly stronger than that of the adjacent benign glands (13, 41). Most authors consider as positive only the intense and moderate staining (12), but there are studies that find positive also the weak staining (8, 36), which could explain the high percentage of positive cases with normal glands or benign lesions. The light background staining, together with the fine granular staining of the epithelial and stromal cells, which can not be detected at low magnification ( $\leq 100\times$ ), are considered negative (15, 17). The reaction shouldn't be considered when the background staining is intense. Some authors report the weak/moderate stain of the suspected glands as positive only if the neighboring benign glands are completely negative, and the moderate staining is considered negative if normal glands are stained in the same way (13, 32). Therefore, when evaluating the expression of AMACR in prostate lesions, benign prostate glands should be used as negative control (39).

Considering all these interpretation problems, difficult prostate lesions should be evaluated considering the morphology on routine stained slides in conjunction with the AMACR and basal cells stainings (18). This can be done on serial paraffin block sections (33) or on the same section, using antibodies cocktails which combine AMACR with one or two basal cell markers, with single or double chromogen (27, 42-44). The advantages of the method that simultaneously reveals two basal cell markers (p63 and HMWCK) and AMACR are the increased sensitivity for the basal cells with circumferential staining of non-malignant glands, the easy detection of the atypical acini and minimal loss of tissue (17, 27), but there are, however, some disadvantages related to the difficulty of interpretation because of the understaining or overstaining (39).

If the initial reports showed a positive intense, generally uniform, staining for AMACR in prostate carcinomas (8, 14), a series of more recent research signals the heterogeneity of AMACR ex-

pression in the prostate conventional carcinomas (11, 32). Our study confirms the variability of AMACR expression in prostate conventional carcinomas, with negative/weakly positive areas mainly associated to cribriform pattern, appearance previously reported by Herawi and Epstein (12). The data regarding the relationship between AMACR staining pattern and Gleason score are quite controversial. While some authors consider that there is no correlation between AMACR expression and Gleason score (9, 17), other researchers report a significant correlation between the heterogeneous AMACR expression and Gleason score  $\geq 7$  (11). This heterogeneous stain in high Gleason score tumors has not major implications because the diagnostic problems are caused by tumors with Gleason score below 6 which are generally diffuse and homogeneous stained as demonstrated by our study. The biological significance of heterogeneous AMACR expression is not known. According to some authors the AMACR heterogeneity is related to the type of the antibody used, rarely seen with the polyclonal antibody (40), while according to other authors this heterogeneity would indicate the existence of more dedifferentiated tumor areas and would have some prognostic significance in terms of tumor progression or metastasizing (11). It is hypothesized that the AMACR expression levels in benign and malignant glands may occur in response to the local changes in the concentration or metabolism of branched chain fatty acids; on the other hand, the increased expression of AMACR in benign glands would indicate preneoplastic changes at the biochemical level that might precede the morphological preneoplastic evidences (11).

Of variants and unusual patterns of prostate carcinoma, the atrophic carcinoma, the foamy gland and pseudohyperplastic are most difficult to diagnose, especially in quantitative limited tissue fragments, such as those of NB.

Atrophic carcinoma is a very rare pattern of prostate carcinoma, found mostly in RP and NB specimens, rarely in fragments of TURP. This tumor pattern can be confused with atrophy foci because the basal cells are sometimes diffi-

cult to be identified and the secretory cells occasionally have enlarged nuclei and prominent nucleoli (45). In extreme situations, IHC makes the difference and from this point of view the basal cell markers are useful by demonstrating these cells in the atrophy foci, but is worthy to keep in mind that basal cells may be present only focally or even absent at the periphery of the atrophic glands (21, 45). The absence of AMACR reactivity observed by us in one case of atrophic carcinoma (33.3%) confirms similar data from literature showing the absence of AMACR expression in 30% of atrophic carcinoma (46) and advocates for limited value of this marker for the differential diagnosis of atrophic prostate lesions, more so since there are reported cases of prostatic atrophy, especially foci of partial atrophy positive for AMACR (36, 45).

The foamy gland carcinoma is another variant of prostate cancer sometimes difficult to be diagnosed in NB fragments due to the apparent benign cytology (47). In difficult cases, the absence of basal cells and the positivity for AMACR are important criteria to conclude diagnosis, but the literature data show that within foamy gland carcinoma category, AMACR expression is significantly reduced compared with conventional carcinomas. Thus, Rubin et al. (9) showed that AMACR expression in the foamy gland variant was significantly higher compared with benign glands, but lower than that of localized prostate carcinomas. Our data on the marked variability of the AMACR expression in the "foamy gland" carcinoma, with some negative areas, are consistent with the observations of other authors showing that only 68% and 62% of foamy gland prostatic carcinomas express AMACR, depending on the type of antibody: monoclonal, respectively polyclonal (13). Based on these data, caution should be practiced in the interpretation of a limited focus of prostate glands with abundant vacuolated cytoplasm, with no reactivity for basal cell markers and AMACR.

Pseudohyperplastic carcinoma, a prostate carcinoma variant recently described, is often un-

derdiagnosed because tumor glands can be mistaken for hyperplastic glands. In particular the pseudohyperplastic carcinoma variant composed of large markedly dilated glands, arranged back to back, with straight luminal contour, lined by cells with abundant cytoplasm is difficult to recognize as malignant (47). The demonstration of carcinomatous nature of the glands requires IHC methods for basal cell, AMACR having a limited value if we take in account the fact that 9-30% of pseudohyperplastic carcinomas lack the reactivity for AMACR, dependent, according to some authors, to the type of used antibody (mono-vs. polyclonal) (8, 19). Our data show a heterogeneous staining, with negative areas and zones with different staining intensities within the same tumor, conforming to other authors' observations (13), aspect which, as with foamy gland and atrophic carcinoma, requires caution in assessing the malignant nature of the lesion based on AMACR expression.

Mucinous carcinoma is one of the less common variants of prostate carcinoma, characterized by the presence of a minimum 25% tumor contingent showing extracellular mucin lakes (23). The presence of a mucinous tumor in the NB material may induce question of a primary prostate tumor versus a secondary involvement of the gland by a colorectal, bladder or urethral adenocarcinoma. PSA is proving extremely useful in establishing the prostate or colorectal origin of the tumor, considering that intestinal tumors do not express PSA (48). The differentiation of mucinous bladder adenocarcinoma from mucinous prostate carcinoma is a real problem because PSA has also been reported in some bladder adenocarcinomas (49). AMACR can not be considered a useful marker to discriminate prostate mucinous tumors because, as our data show, it is present, although heterogeneously expressed, in mucinous prostate carcinoma, in 62% - 92% of colorectal carcinomas, with a weaker reactivity in the mucinous type (16, 50) and in a significant proportion of primary urinary bladder adenocarcinomas (51).

In the case of signet ring cells prostate carcinoma, another rare variant of prostate tumors, la-

beled as such when 25-50% of tumors cells present a signet ring appearance (52-54), it is necessary to establish the primary (prostate) or secondary (stomach, colon, bladder, urethra) nature of the tumor. More frequently than in the pure type, the signet ring tumor cells are focally present in some conventional high-grade prostate adenocarcinomas (55). Unfortunately, the positive staining for AMACR of a signet ring cell carcinoma is not an argument for the prostate origin of the tumor because it has also been reported in gastric and colonic signet ring cell carcinomas (50, 56). The immunoreactivity for AMACR has also a limited value in the discrimination of prostate carcinoma with a signet ring cell contingent from the nephrogenic adenoma with signet ring features, since its expression was detected in more than 50% of cases of nephrogenic adenoma (57). According to our data, which show the presence of moderate reactivity for AMACR in signet ring cells prostate carcinoma the marker's expression can be used to differentiate signet ring carcinomatous cells from vacuolated lymphocytes or stromal cells, which are AMACR-negative. AMACR cannot distinguish between signet ring cells carcinoma and the hormonally-induced vacuolated carcinomatous cells, as in both cases the staining can be positive.

Ductal prostatic adenocarcinoma representing less than 1% of prostate carcinomas (as the dominant pattern) can be present as an exophytic, papillary lesion in the prostatic urethra (58, 59). Besides the two classical subtypes of ductal adenocarcinoma, subtype A or primary ducts adenocarcinoma and subtype B or secondary ducts adenocarcinoma (59-61), in recent years a third subtype emerged as an entity: HG-PIN - like ductal adenocarcinoma, composed of individual glands lined by pseudostratified tall columnar cells (62). Literature data notify that AMACR is expressed in 77% of classical ductal adenocarcinomas (12), an aspect confirmed by our results which indicate a 28.6% rate of AMACR-negative ductal adenocarcinomas. The AMACR positivity percentage for the HG-PIN-like prostatic adenocarcinoma is variably re-

ported: 50% by Hameed and Humphrey (63) and 93% by Tavora and Epstein (62).

Since basal cells are identified in one third of ductal adenocarcinomas (12), at the periphery of cribriform and papillary HGPIN glands and in the foci of intraductal carcinoma of the prostate (23), and the expression of AMACR in these lesions is variable (12, 64), the discrimination of aforementioned entities can not rely on IHC methods, but only on information supplied by the usual stain, perhaps supplemented with clinical data (18).

The distinction between primary or secondary prostate urothelial carcinoma and poorly differentiated prostate carcinoma is sometimes difficult, so much more because there is the possibility of synchronous tumors (27). In equivocal cases, PSA and PSAP may be used to confirm the prostatic origin of the tumor, but with the caution that the two markers are negative in up to 27% and 19% of poorly differentiated prostate carcinoma respectively (65). AMACR has no discriminatory value because both tumors (glandular prostatic and urothelial) can express it in extremely varied percentages reported for urothelial carcinomas, depending on tumor location and stage: from 31-32% (66, 67) up to 83% cases (8) with an intermediate rate of 50% cases reported for the upper urinary tract urothelial carcinomas (68). In such situations CK7, CK20, 34 $\beta$ E12 and/or p63 may prove to be most valuable for the correct diagnosis (69).

## Conclusions

AMACR is a sensitive marker of conventional prostate adenocarcinoma, but only in terms of optimizing and standardizing of both, IHC technique and results report for each laboratory. This study confirms the heterogeneity of AMACR expression in prostate carcinomas. Thus, in a minority of cases of conventional or unusual patterns and variants of prostate carcinomas, the AMACR expression is reduced or absent. In the case of difficult and/or limited prostate lesions represented in the biopsy material, it is necessary to correlate

data provided by H&E stain with the determination of AMACR and basal cell markers.

When the pathologist faces prostate lesions that could be variants of prostate carcinoma such as atrophic, pseudohyperplastic or foamy glands and with negative basal cell stainings, the positivity for AMACR certainly increases the confidence in diagnosing a carcinoma. On the other hand, the heterogeneity of AMACR expression in these variants of prostate carcinoma confines the marker's value, so in the case of a tumor from this category, minimum represented in the biopsy material which is AMACR-negative or weakly positive, re-biopsy is required.

The significance of AMACR heterogeneous expression in prostate carcinomas, the involvement of this marker in prostate's oncogenesis and its possible prognostic significance still remain to be clarified.

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## Abbreviations

AMACR - Alpha-methylacyl-CoA racemase  
 CK - Cytokeratin  
 HMWCK - High Molecular Weight Cytokeratin  
 HIER - Heat Induced Epitope Retrieval  
 IHC - Immunohistochemistry  
 NB - Needle Biopsy  
 PSA - Prostate Specific Antigen  
 PSAP - Prostate Specific Acid Phosphatase  
 RP - Radical Prostatectomy  
 TURP - Transurethral Resection of the Prostate

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