Immunohistochemical expression of α-methylacyl coenzyme-A racemase in prostatic carcinoma: correlation with image morphometric parameters

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Background/aim
Prostate cancer is the second leading cause of death in men. Although prostate-specific antigen is the most commonly used biomarker for monitoring prostate cancer, it has poor specificity. This study aimed to evaluate the diagnostic utility of α-methylacyl coenzyme-A racemase (AMACR) as a predictive marker for prostate carcinoma. The expression of AMACR will be correlated with nuclear and glandular morphometric parameters with the aim to enhance the possibility of finding a sensitive immune marker for diagnosing prostatic carcinoma.
Patients and methods
Prostatic lesions including 30 benign prostatic hyperplasia, 25 prostatic intraepithelial neoplasia (PIN), and 50 prostatic carcinoma cases were included in this study. Immunohistochemical staining for AMACR was done in formalin-fixed, paraffin-embedded tissue sections. Nuclear and glandular morphometric parameters for all cases were evaluated using an image analysis system.
Results
All cases of benign prostatic hyperplasia as well as normal controls were negative for AMACR. In contrast, 28% of PIN cases and 94% of prostatic carcinoma cases were positively stained for AMACR. Its expression was associated with high-grade PIN and carcinoma. Glandular area and glandular area% were significantly different between the studied prostatic lesions, and they were significantly increased with grade in cases of carcinoma.
Conclusion
Expression of AMACR plays an important role in the diagnosis of prostatic lesions and may be used as a potentially important prostatic tumor marker. A combination of AMACR and morphometry is of great value in increasing the diagnostic accuracy of prostatic carcinoma and may have value for resolving suspicious cases.

Keywords:
α-methylacyl coenzyme-A racemase, immunohistochemistry, morphometric parameters, prostatic carcinoma

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Introduction

Benign prostatic hyperplasia (BPH) is the most common non-neoplastic urological condition in men. The incidence of BPH reaches 90% by 80 years of age [1]. Prostatic adenocarcinoma is also a frequent neoplasm and the second leading cause of cancer-related death in men [2,3]. The diagnosis of prostatic carcinoma is based on a combination of architectural, cytological, and ancillary features, and sometimes presents a diagnostic challenge for pathologists because of the presence of many benign mimics of malignancy [4,5]. Tissue diagnosis of prostate cancer can be difficult in needle biopsies or in small foci of cancer, and hence underdiagnosis might lead to delay in early management and adverse consequences for the patients [6,7].

On the other hand, prostatic intraepithelial neoplasia (PIN) is a condition that is usually diagnosed either during prostatic biopsy or during transuretheral resection of the prostate for treatment of BPH [8].

Although prostate-specific antigen (PSA) is the main marker for the diagnosis of prostatic carcinoma, it has been shown to be highly present in noncancerous tissues as well as in cancerous tissue. Thus, it has limited specificity to cancer [9]. Consequently, there should be increased efforts to identify a new diagnostic biomarker not only for prostatic cancer but also for biologically relevant diseases [5].

α-Methylacyl COA-racemase (AMACR) is one of the latest biomarkers to show overexpression in an early event

in prostatic carcinogenesis and has been reported to have sensitivity ranging from 82 to 100% [10,11]. AMACR is a well-characterized mitochondrial and peroxisomal enzyme that is overexpressed in prostate cancer, but its function has not been clarified yet. However, several reports showed a mechanistic relationship between AMACR expression and hormonal status. AMACR expression may be substantially diminished or entirely lost in prostate carcinoma after hormonal therapy [7,12,13]. In vitro, AMACR is an enzyme essential for the optimal growth of cells of prostatic carcinoma and it has the potential to be a complementary target together with androgen ablation in the treatment of prostatic carcinoma [3].

Quantitative measurements of microscopic features visualized using an automated image analyzer were used for diagnostic and prognostic purposes in surgical pathology. In many tumors, various morphometric parameters were used for accurate diagnosis and for exclusion of overlapping entities [14].

Morphometric quantitative analysis of histologic images is an attempt to enhance the diagnostic pathology in three ways: by the application of individual criteria, thus contributing to their objective use; by increasing precision in the evaluation of quantitative criteria; and by evaluating features that are simply not appreciated by humans [15,16].

The aim of this study was to investigate the immunohistochemical expression of AMACR in benign and malignant prostatic lesions and to correlate its expression with morphometric parameters to evaluate their diagnostic utility as a sensitive immune marker in prostatic carcinoma.

Patients and methods

In this study, prostate needle biopsies or transurethral resection prostatic specimens were retrieved from different private laboratories as paraffin-embedded blocks. The Ethical Committee on human research at our Institute approved the protocol for the study. Two sections of 4 μm thickness were cut from each block. One section was stained with hematoxylin and eosin for histopathological evaluation, grading, and morphometric study. The other section was mounted on positively charged glass slides for immunohistochemical staining using anti-AMCR antibody.

The examined patients consisted of BPH (30 cases), PIN (25 cases), and prostatic carcinoma (50 cases). Ten biopsies containing normal prostatic tissue were used as controls.

Histological sections of prostate cancers were graded using the Gleason grading system, which evaluates tissue and cellular changes indicative of cancer. A Gleason score of 6 was designated low-grade cancer, 7 (3+4 or 4+3) as medium-grade cancer, and 8, 9, and 10 as high-grade cancers [17].

Immunohistochemical study

For immunostaining, the sections were deparaffinized and rehydrated through a graded series of alcohol. Endogenous peroxidase activity was blocked by freshly prepared 0.3% hydrogen peroxide in methanol for 20 min. Then microwave antigen retrieval was used, followed by incubation with AMACR antibody (p504s, clone no 13H4, 1 : 50 dilution). The Ultravision LP polymer system (Labvision, California, United States) and the chromogen diaminobenzidine were used to amplify and visualize the antigen–antibody complex. The expression of AMACR was evaluated in the entire section at a magnification of ×400. AMACR showed continuous diffuse or granular cytoplasmic staining of the glandular epithelium. The percentage positivity was graded from 0 to 3+ as follows: 0% cells, 0+ (negative); 1–10% cells, 1+; 11–50% cells, 2+; and more than 51% cells, 3+. Staining intensity was determined as mild, moderate, or strong [18].

Morphometric analysis

The morphometric analysis was performed at the Pathology Department, National Research Centre, using the Leica Qwin 500 Image Analyzer (LEICA Imaging Systems Ltd, Cambridge, UK), which consists of a Leica DM-LB microscope with a JVC color video camera attached to a computer system, Leica Q_500IW.

We place the slide to be examined on the stage of the microscope. The light source is set to the required level. Successful adjustment of illumination is checked for on the video monitor. The morphometric analysis was carried out on hematoxylin and eosin-stained slides to measure the glandular area at ×50 magnification and the nuclear area at magnification ×400. The areas to be measured are covered automatically by a green mask, which is called a binary image. The area of this binary image is then calculated, which reflects the area of object to be measured. The reading of each measurement appears in micrometers and finally the mean area in all fields examined is determined.

Statistical analysis

Data were analyzed using the statistical package SPSS version for windows (IBM, New York, United States). P values less than 0.05 were considered significant.

For comparison of the findings, Pearson's χ^2 and Spearman's correlation tests were performed.

Results

The present study was performed on a total of 105 cases. The prostatic specimens included 30 BPH, 25 PIN, and 50 prostatic carcinoma cases. Most cases of benign hyperplasia were associated with active and chronic prostatitis. The maximum numbers of cases were seen in the age group of 58–71 years. Cases of PIN were encountered in the age group of 63–73 years. They included 16 cases of low-grade PIN and nine cases of high-grade PIN. Prostatic adenocarcinoma accounted for almost 50% of all cases of the study. The majority of cases were encountered in the age group of 68–81 years. Prostatic carcinoma was graded according to Gleason grading system, which evaluates tissue and cellular changes indicative of cancer. A Gleason score of 6 was designated low-grade cancer, 7 (3+4 or 4+3) as medium-grade cancer, and 8, 9, and 10 as high-grade cancer.

Immunohistochemical expression of α-methylacyl coenzyme-A racemase

All cases of BPH as well as normal controls were negative for AMACR. In contrast, 28% of PIN cases and 94% of prostatic carcinoma cases were positively stained for AMACR (Table 1 and Fig. 1).

AMACR expression was associated with high grade: 78% of high-grade PIN and all cases of high-grade carcinoma were positively stained for AMACR (Table 2). Cell percentage expression of AMACR was significantly correlated with histopathological grades, where 70% of high-grade carcinoma showed more than 50% cell expression and 75% of low-grade carcinoma showed less than 10% cell expression (Fig. 2). Also, in PIN cases, 85.7% of high-grade cases showed 10–50% cell expression and the remaining cases showed 0–10% cell expression for AMACR. In contrast, AMACR staining intensity showed nonsignificant correlation with histopathologic grades (Table 3).

Table 1 α-Methylacyl coenzyme-A racemase expression in prostatic lesions

	N	Negative [*n* (%)]	Positive [*n* (%)]
BPH	30	30 (100)	–
PIN	25	18 (72)	7 (28)
Carcinoma	50	3 (6)	47 (94)

BPH, benign prostatic hyperplasia; PIN, prostatic intraepithelial neoplasia.P<0.05.

Morphometric results

All specimens were evaluated using image analysis for morphometric parameters, including nuclear area, glandular area, and glandular–stromal percentage area (glandular area%) (Fig. 3). Glandular area and glandular area% were significantly different between the studied prostatic lesions (Table 4), whereas nuclear area showed nonsignificant difference.

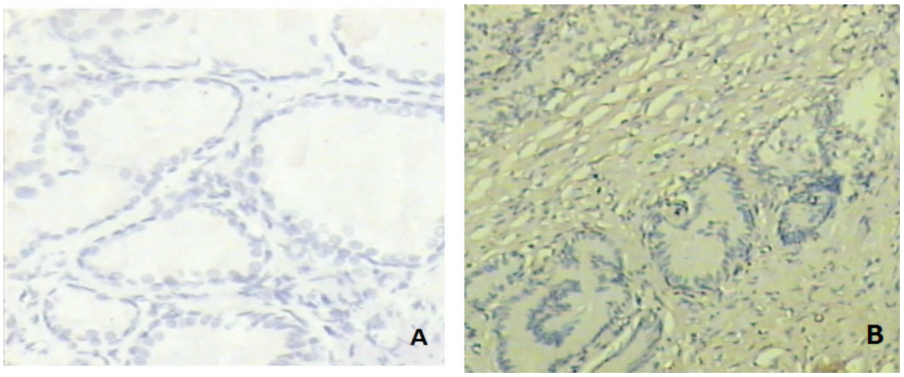
In addition, glandular area and glandular area% were significantly increased with grade in cases of carcinoma, whereas there was nonsignificant correlation with grade in PIN cases. Nuclear area showed nonsignificant correlation with grades (Table 5).

Table 2 Correlation of α-methylacyl coenzyme-A racemase expression with histopathologic grades in prostatic intraepithelial neoplasia and prostatic carcinoma

	Grades	*N*	Negative [*n* (%)]	Positive [*n* (%)]
PIN	Low	16	16 (100)	–
	High	9	2 (22)	7 (78)
	Total	25	18 (72)	7 (28)
Carcinoma	Grade I	10	2 (20)	8 (80)
	Grade II	30	1 (3.3)	29 (96.7)
	Grade III	10	0 (0)	10 (100)
	Total	50	3 (6)	47 (94)

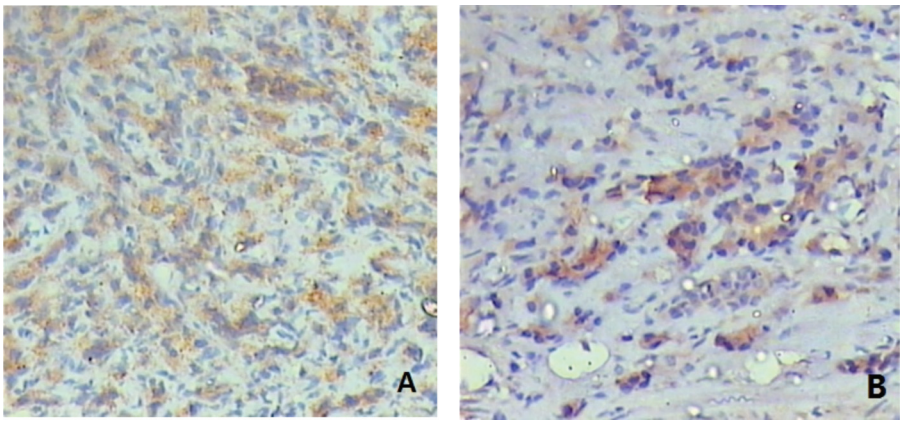
PIN, prostatic intraepithelial neoplasia.P<0.05.

Figure 1



Negative AMACR immunostaining in benign prostatic hyperplasia (a) and low-grade PIN (b) (immunohistochemistry, ×200). AMACR, α-methylacyl coenzyme-A racemase; PIN, prostatic intraepithelial neoplasia.

Figure 2



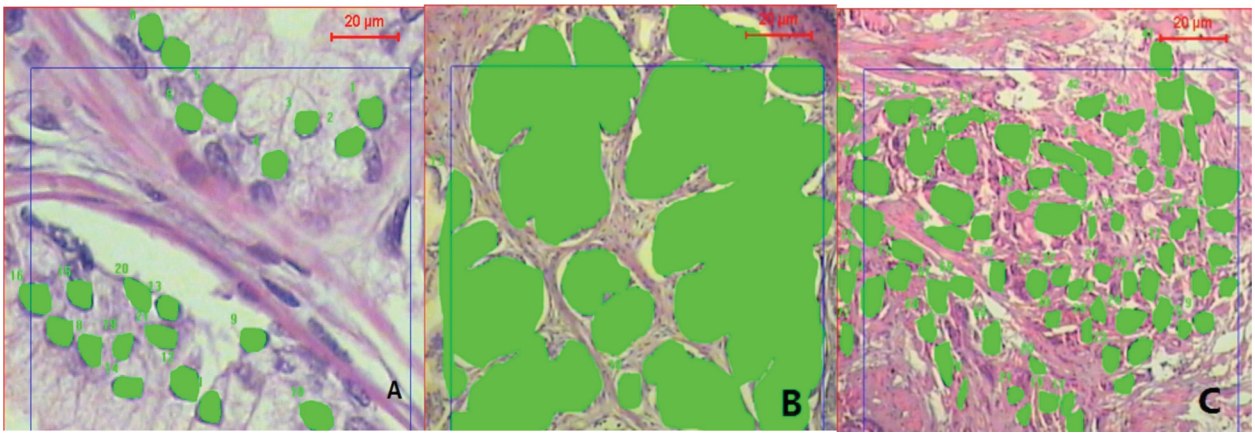
AMACR immunostaining in prostatic carcinoma showing (a) moderate cytoplasmic staining and high percentage of positively stained cells (score 3) and (b) strongly stained cytoplasm with low positively stained cell percentage (score 2) (Immunohistochemistry, ×200). AMACR, α-methylacyl coenzyme-A racemase.

Table 3 Correlation of α-methylacyl coenzyme-A racemase positivity with prostatic intraepithelial neoplasia and prostate carcinoma

	Prostatic carcinoma [*n* (%)]			PIN [*n* (%)]		*P*-value
	Grade I	Grade II	Grade III	Low	High	
Intensity						
Weak	1 (12.5)	13 (44.8)	4 (40)	–	4 (57.1)	>0.05
Moderate	1 (12.5)	14 (48.3)	1 (10)	–	2 (28.6)	
Strong	6 (75)	2 (6.9)	5 (50)	–	1 (14.3)	
Total	8	29	10	–	7	
Percentage						
0–10	6 (75)	3 (10.3)	3 (30)	–	1 (14.3)	>0.05
10–50	2 (25)	11 (37.9)	–	–	6 (85.7)	
≥50	–	15 (51.8)	7 (70)	–	0	
Total	8	29	10	–	7	

PIN, prostatic intraepithelial neoplasia.

Figure 3



Binary image (green) using image analysis demonstrating (a) nuclear area in a case of benign prostatic hyperplasia (×400), and (b) glandular area in benign prostatic hyperplasia (×50), and (c) glandular area in prostatic carcinoma (×50).

Correlation between α-methylacyl coenzyme-A racemase expression and morphometric parameters

Glandular area and glandular area% but not nuclear area were significantly correlated with AMACR expression in carcinoma cases (Table 6). In contrast, all three morphometric parameters showed nonsignificant correlation with AMACR expression in different histologic grades (Table 7).

Discussion

PSA is a glycoprotein that is produced by prostatic glandular epithelial cells. Although PSA is still recognized as the best tumor marker available for the detection and treatment monitoring of prostate cancer, it has some limitations. This is because it is prostate gland specific but not prostate cancer specific [19]. This raises a need for implementation of other immune markers with well-established sensitivity and specificity for unambiguous diagnosis.

AMACR is an enzyme that is involved in peroxisomal oxidation of dietary branched-chain fatty acids and showed overexpression in early prostatic carcinogenesis [11]. Although several reports showed a relationship between AMACR expression and hormonal status, conflicting data exist on the effect of hormone therapy on AMACR expression. Suzue et al. [13] showed significant reduction in AMACR expression following hormone therapy, whereas Kuefer et al. [20] stated that there is no effect of hormone therapy on AMACR expression.

Our study aimed to investigate immunohistochemical expression of AMACR in benign and malignant prostatic lesions and to correlate its expression with morphometric parameters to evaluate their diagnostic utility as a sensitive immune marker for prostatic cancer.

In recent years, the prostatic biomarker AMACR has been used as adjuvant to morphology in diagnostically challenged cases, especially in the presence of a small focus of malignancy or due to the many benign mimickers of malignancy [4,10].

In the present study, taking into consideration the morphology in conjunction with immunostaining of AMACR, all cases of BPH showed negative staining with AMACR. This was in accordance with the study of Wu et al. [21], which demonstrated lack of AMACR expression in BPH. However, a previous study by Luo et al. [22] identified AMACR expression in 4% of benign prostatic glands. On the other hand, Jiang and colleagues [23,24] showed that AMACR protein assessed by western blot and by immunohistochemistry was negative in benign glands adjacent to malignant ones.

In our study, positive diffuse or granular cytoplasmic staining was detected in 94% of prostatic carcinoma. Similar to our finding, Ozgur et al. [3] evaluated AMACR expression in the majority of their cases. However, they have determined a nonsignificant relationship with tumor grade and AMACR expression.

In contrast to the results of Ozgur et al. [3], our findings revealed an increased proportion of positive cells through increasing grades of prostatic carcinoma, but no association between AMACR staining intensity and different grades. However, AMACR could be one of the immune markers that have a role in distinguishing between ordinary and aggressive cases of prostatic carcinoma and could have prognostic value [5].

On the other hand, the present study revealed positive staining of AMACR in 78% of high-grade PIN cases, whereas all cases of low-grade PIN were negative for AMACR. Similar to these findings, Rubin et al. [25] identified AMACR protein expression in high-grade PIN and noted that AMACR alone may not be a useful marker, especially in diagnostically challenging cases. However, they explained that this could be because of the small number of cases.

In 1982, Diamond et al. [26] had introduced nuclear morphometry to aid in the prognostic evaluation of

Table 4 Morphometric parameters in prostate lesions *P*-value

	Normal	BPH	PIN	Carcinoma	
Glandular area	242	692	239	851	<0.05
Glandular area%	22	61	22	72.5	<0.05
Nuclear area	58	57	40.5	43.25	>0.05

BPH, benign prostatic hyperplasia; PIN, prostatic intraepithelial neoplasia.

Table 6 Correlation of morphometric parameters versus α-methylacyl coenzyme-A racemase expression in carcinoma

	AMACR expression		*P*-value
	Negative (*N*=3)	Positive (*N*=47)	
Glandular area	220	830	<0.05
Glandular area%	20	74	<0.05
Nuclear area	39	44	>0.05

AMACR, α-methylacyl coenzyme-A racemase.

Table 5 Correlation of morphometric parameters with histopathologic grades of prostate carcinoma and prostatic intraepithelial neoplasia

	Prostate carcinoma			*P*-value	PIN		*P*-value
	Grade I	Grade II	Grade III		Low	High	
Glandular area	464	736	1002	<0.05	430	450	>0.05
Glandular area%	26	35	67	<0.05	24	27	>0.05
Nuclear area	38	47	44	>0.05	39	36	>0.05

PIN, prostatic intraepithelial neoplasia.

Table 7 Correlation of morphometric parameters versus α-methylacyl coenzyme-A racemase expression with grades of carcinoma

	Grade I		Grade II		Grade III		*P*-value
	Negative (2)	Positive (8)	Negative (11)	Positive (29)	Negative (0)	Positive (10)	
Glandular area	395	460	712	740	998	1015	<0.05
Glandular area%	21	27	30	38	59	68	<0.05
Nuclear area	33	37	41	49	39	45	>0.05

prostate carcinoma. They observed that nuclear roundness but not area was useful in distinguishing long-term survivors among stage B patients from those who developed metastases. Many other studies evaluating nuclear area, ellipticity factor, nuclear perimeter and shortest and longest nuclear factors, showed that elliptical shape measurement to be the best in distinguishing patients with good or poor prognosis [27,28]. Veltri et al. [14] used quantitative nuclear structures to assess prostate histology and progression of prostatic carcinoma. Using computer-assisted digital image analysis, they demonstrated that, when prostate carcinoma is initiated and progressed, significant alterations in nuclear size, shape, and heterochromatin organization are found, and key nuclear structural and transcriptional proteins as well as multiple nuclear bodies can lead to malignant changes.

In our study, all specimens were evaluated using image analysis for morphometric parameters, including nuclear area and glandular–stromal area percentage. Glandular area and area% were significantly different between the groups, whereas nuclear area showed nonsignificant difference between the different lesions. In addition, glandular area and glandular area% were significantly increased with grade in carcinoma, whereas there was a nonsignificant correlation with grade in PIN. Nuclear area showed nonsignificant correlation with histopathological grades.

Pathologists play an important role in the diagnosis of prostatic lesions to distinguish biologically different types. Hence, the application of combined computer image analysis and different immune biomarkers to assess tissue-based morphological and molecular parameters as well as glandular architecture will be very helpful for the diagnosis of prostatic cancer before the treatment decision is taken and may have a role in the assessment of prognosis.

In our work, on studying the correlation between the AMACR expression and morphometric parameters in prostatic carcinoma, it was found that glandular area and glandular area%, not nuclear area, were significantly correlated with AMACR expression in carcinoma cases. In contrast, all three morphometric parameters showed nonsignificant correlation with AMACR expression in different histologic grades.

Conclusion

Expression of AMACR plays an important role in the diagnosis of prostatic lesions, and may be used as a potentially important prostatic tumor marker. A combination of AMACR and morphometry is of great value in increasing the diagnostic accuracy of prostatic carcinoma and may have value for resolving suspicious cases.

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Conflicts of interest

There are no conflicts of interest.

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