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α-Methylacyl-CoA racemase expression and lethal prostate cancer in the Physicians' Health Study and Health Professionals Follow-up Study

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Abstract

Background—α-Methylacyl-CoA racemase (AMACR) is an enzyme that serves as a diagnostic biomarker of prostate cancer in clinical practice. Recent studies suggest that low AMACR expression is associated with biochemical recurrence and the development of fatal disease.

Methods—We conducted a prospective cohort study among 920 men aged 47–84 years, who were diagnosed with prostate cancer in the Physicians' Health Study and the Health Professionals Follow-up Study cohorts, and whose resected tissue specimens were available for immunohistochemical analysis. We used Cox proportional hazards regression to evaluate the association of AMACR expression with lethal prostate cancer over a 20-year follow-up period.

Results—In total, 68 men died from prostate cancer, and an additional 18 developed bony metastases during follow-up. We found that lower AMACR intensity was associated with higher prostate-specific antigen levels (p=0.003) and more advanced clinical stage (p=0.06) at diagnosis, and a non-significant trend for higher risk of lethal outcomes. The hazard ratio comparing the lowest to the highest quartile of AMACR expression intensity was 1.53 ((95% CI: 0.86, 2.73), p-for-trend across quartiles=0.07); this trend was further attenuated after adjustment for age, Gleason score, stage and cohort with a hazard ratio of 1.24 (95% CI 0.69, 2.22), p-for-trend=0.23.

Conclusions—Low AMACR expression in primary tumor specimens was not independently associated with the development of metastatic and lethal prostate cancer after treatment over a 20-year follow-up period, after adjustment for important clinical covariates at diagnosis.

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Introduction

α-Methylacyl-CoA racemase (AMACR) is a catalyst in the peroxisomal beta-oxidation of branched chain fatty acids found in dietary sources, such as meat and dairy products, and in the metabolism of drug molecules, such as ibuprofen (1). Protein expression of AMACR is elevated in localized prostate cancer as compared to benign prostatic epithelium (2, 3, 4), and indeed it is one of the most over-expressed genes in prostate cancer (5). AMACR protein expression is routinely used in surgical pathology to aid with difficult diagnoses. Polymorphisms leading to single amino acid substitutions in the *AMACR* gene have been linked to prostate cancer risk (6, 7).

AMACR expression is actually down-regulated in hormone-refractory metastatic tissue relative to the primary tumor (8) and we previously reported an association between low AMACR expression at diagnosis and an increased risk of biochemical recurrence and fatal prostate cancer (9). While it is unclear whether increased expression of AMACR is mechanistically involved in tumor initiation or progression, or is an epiphenomenon (8), experimental data suggest that AMACR is functionally important for prostate cancer cell growth in vitro (10). Given these associations and importantly, the current widespread use of AMACR immunohistochemistry in the setting of diagnostic pathology, we examined the potential role of AMACR expression as a prognostic biomarker in two, large prospective US cohorts.

Methods

Study population

We followed men diagnosed with prostate cancer who were participants in two studies, the Physicians' Health Study (PHS I and II), randomized trials of aspirin and nutritional supplements for the primary prevention of cancer and cardiovascular disease among US male physicians (11) and the Health Professionals Follow-up Study (HPFS), a prospective cohort of US male health professionals (12). PHS I began in 1982 among 22,071 physicians aged 40–84 years and PHS II began in 1997 among 14,641 physicians aged 50 years and older. HPFS began in 1986 when 51,529 health professionals aged 40–75 years completed a questionnaire on diet, lifestyle factors and medical diagnoses. None of the participants had a cancer diagnosis at baseline.

Case identification and follow-up

For both cohorts, a prostate cancer diagnosis was based initially on self report on mailed questionnaires and was confirmed through a review of medical records and pathology reports. Data were abstracted from medical charts to determine clinical data, including Gleason score, tumor-node-metastasis (TNM) stage and prostate specific antigen (PSA) levels at diagnosis.

The prostate cancer cases were followed for development of distant metastases, prostate cancer-specific and all cause mortality, or end of follow-up through either December 2008 or March 2009 (for the HPFS and PHS cohorts respectively). Development of bony metastases was ascertained through mailed questionnaires and confirmed by treating physicians. Deaths were identified through the National Death Index, postal system and next of kin. A prostate cancer death was based on evidence of extensive metastatic disease, and all causes of death were determined by an Endpoint Committee of physicians through medical record review. Follow-up data for mortality are >99% complete in the PHS and >98% in the HPFS.

Pathology review and tissue microarray construction

For 920 cases, we retrieved archival formalin-fixed paraffin embedded tissue specimens from radical prostatectomy (PHS and HPFS) or transurethral resections of prostate (TURP, PHS only) for AMACR immunohistochemical staining.

Two study pathologists (MAR and SP) re-reviewed all specimens to provide a standardized Gleason scoring. Tissue microarrays (TMAs) were created using a manual tissue arrayer (Beecher Instruments, Silver Spring MD) as described previously (13). For each patient, a study pathologist circled the dominant nodule or the nodule with the highest Gleason score on a Hematoxylin and Eosin-stained slide, and three or more 0.6 mm tissue cores were used for the TMAs, with 3 to 14 cores per case.

Immunohistochemistry and scoring of the tissue microarrays

Immunohistochemistry for AMACR was performed on 5 micron TMA sections. We used a monoclonal antibody against AMACR (p504s, Zeta Co., Sierra Madra, CA). Slides were microwaved for 30 minutes in a pH 6.0 citrate buffer for antigen retrieval, and then incubated for 40 minutes with the primary p504s antibody. Secondary detection was with an anti-rabbit antibody for 30 minutes. Signal detection was by means of a streptavidin botin detection kit (Dako Developing System, Dako, Carpinteria, CA) for 5 minutes.

TMA slides were scored using a semi-automated quantitative image analysis system, ACIS II (Chromavision, San Juan Capistrano, CA), that gives highly reproducible results (9). The immuno-stained TMA slides were scanned into the ACIS II system, and a study pathologist (MB) electronically circled specifically the areas of prostate cancer within each TMA core on the digital image. The software assigned continuous intensity scores between 0–255 within circled areas of the brown chromagen staining. TMA construction and immunohistochemical evaluation were undertaken by laboratory personnel blinded to the clinical outcomes of the cases.

Statistical analysis

The intensity of staining was considered as a continuous variable and the mean intensity across multiple cores was standardized by TMA. We calculated TMA-specific z-scores to normalize intensity levels across TMAs. The standardized score was calculated using the following equation: $\text{intensity}_{z-score} = \text{intensity}_k - \text{mean}(\text{intensity}_k)/\text{SD}\,\text{intensity}_k$ where k represents the TMA and the mean z-score for each TMA is 0. The z-scores are then summed across the TMAs and categorized into quartiles based on the distribution in the study population. We compared the mean age at diagnosis (years), body mass index at baseline (kg/m^2) and prostate-specific antigen (PSA) levels at diagnosis (ng/mL), across quartiles of standardized AMACR intensity scores using analysis of variance and the F-test for trend, and Gleason score and TNM stage using the global chi-squared test. We used Cox proportional hazards regression to calculate the hazards ratio (HR) and the 95% confidence interval (CI) to assess the relation between AMACR expression and lethal prostate cancer (the development of bony metastases or prostate cancer death), defining the referent group as the highest levels of AMACR intensity based on quartiles. Survival was calculated as the time from a prostate cancer diagnosis to prostate cancer-specific death or development of bone metastases, censoring at date of death from other causes or at the end of follow-up (PHS – March 31, 2009 and in HPFS – December 31, 2008). Multivariate hazard ratios were adjusted for age at diagnosis (years), Gleason score (4–6, 7, 8–10), and TNM stage (T1/T2 vs. T3/T4 or N1 or M1). There was no significant heterogeneity ($p = 0.13$) in survival associated with AMACR expression between the PHS and HPFS, so we grouped the cohorts together to maximize power. We used the SAS program package, version 9.1 (SAS Institute, Cary NC) to perform statistical analysis with a significance level of 0.05. The Institutional

Review Board at Brigham and Women's Hospital and Partners Healthcare approved this study.

Results

The clinical characteristics for the PHS and HPFS are presented in Table I. During the 20-year follow-up period, 68 men died of prostate cancer and an additional 18 had bony metastases.

Men with lower AMACR intensity had higher Gleason scores (8–10), but the difference across quartiles was not significantly different (p=0.11, Table II). Men with lower AMACR expression (Q3, Q4) had a higher mean age (p=0.02) and higher PSA level at diagnosis (p=0.003). We also observed that men with lower AMACR intensity tended to have more advanced clinical stage at diagnosis (p=0.06) and increased prostate cancer mortality, (p=0.07) (Table II).

Low AMACR intensity was associated with a nonsignificantly increased risk of lethal prostate cancer in crude analyses; men in the lowest quartile of AMACR intensity had a (nonsignificant) 53% increased risk of developing lethal disease when compared to the highest quartile of expression (95% CI: 0.86, 2.73), (p-for-trend = 0.07). These nonsignificant associations for both lethal and fatal disease were attenuated after adjusting for age, and further attenuated after adjusting for Gleason score, stage and cohort (p-for-trend = 0.23 & 0.27) (Table III).

Discussion

Several studies have described decreased AMACR expression in metastatic prostate cancer as compared with clinically localized cancer (3, 8), raising the possibility that decreased AMACR expression might be a marker of tumor progression. Consistent with this, we found that men with higher stage disease were more likely to exhibit lower AMACR expression in tumors. Although the differences were not significant, we also found a higher proportion of poorly differentiated tumors (Gleason 8–10) in men with lower levels of AMACR expression, consistent with the suggestion that AMACR may be a marker of tumor differentiation (8). However, AMACR expression in prostate tumor tissue was not a significant predictor of developing metastatic or fatal prostate cancer after adjusting for age, Gleason score and clinical stage at diagnosis.

One previous study reported significant inverse associations between prostate tumor AMACR expression and the risks of biochemical failure and fatal prostate cancer (9). In that study, we used similar semi-automated quantitative methods to assess tumor AMACR expression in a surgically treated cohort for its association with biochemical failure and in a watchful waiting cohort for its association with cancer-specific mortality. Important differences make direct comparisons between the two studies difficult. In particular, the watchful waiting cohort was from the pre-PSA screening era (vs. 50% PSA-detected disease in the current HPFS/PHS cohorts), had a higher mortality rate [19.2%] vs. HPFS/PHS cohorts [7.2%], and by definition did not undergo surgical therapy (vs. 95% of HPFS/PHS cohorts). The watchful cases were diagnosed through transurethral resection, perhaps representing transitional zone cases. In contrast to the surgical cases in both the present and previous study, we observed a direct relation of AMACR expression and stage in the watchful waiting cohort. Indeed, the observed association with lethal disease in the previous study emerged only after adjustment for stage. The surgically treated cohort in the earlier paper was followed only for biochemical failure. The different findings may be explained by

the known imperfect correlations between biochemical failure and the subsequent development of lethal prostate cancer.

In summary, our findings are consistent with prior data that downregulated AMACR expression is associated with poorer outcomes in prostate cancer. However the attenuated magnitude and lack of statistical significance after adjusting for clinical variables suggest that tumor AMACR expression at diagnosis is not a useful prognostic biomarker for lethal disease after treatment.

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Table I

Clinical characteristics of men diagnosed with prostate cancer in Physician's Health Study (PHS) and Health Professionals Follow-up Study (HPFS) cohorts, 1983–2009 and 1986–2008.

	PHS (N=303)	HPFS (N=617)	Total (n=920)
Case characteristics	**N (%), mean (SD) or median (Interquartile range (25%–75%) - IQR)**	**N (%), mean (SD) or median (IQR)**	**N (%), mean (SD) or median (IQR)**
AMACR mean intensity (IQR)	123.7 (112–135)	131.3 (121–143)	128.5 (116.7–140.7)
Age at diagnosis (yrs)	66.3 (6.3)	65.7 (6.3)	65.9 (6.3)
Follow-up time (yrs)	10.0 (4.2)	11.6 (3.9)	11.4 (4.0)
Body mass index (kg/m^2, baseline)	24.5 (2.5)	25.3 (3.2)	25.0 (3.0)
missing (%)	16 (5.3)	14 (2.3)	30 (3.3)
PSA at diagnosis (ng/mL)	6.7 (4.5–9.3)	7.2 (5.1–12.0)	7.1 (5.0–11.1)
missing (%) *	46 (15.2)	104 (16.9)	150 (16.3)
Gleason score			
4–6	185 (61.1)	137 (22.2)	322 (35.0)
7	73 (24.1)	378 (61.3)	451 (49.0)
8–10	30 (9.9)	100 (16.2)	130 (14.1)
missing (%)	15 (5.0)	2 (0.3)	17 (1.9)
Clinical stage			
T1, NX/NO	152 (50.2)	291 (47.2)	443 (48.2)
T2, NX/NO	127 (41.9)	241 (39.1)	368 (40.0)
T3/T4 or N1/M1	9 (3.0)	53 (8.6)	62 (6.7)
missing (%)	15 (5.0)	32 (5.2)	47 (5.1)

* 65% of men with missing PSA data (n=97/150) were diagnosed in the pre-PSA era (≤ 1992)

Table II

Clinical characteristics by quartiles of mean AMACR intensity of men diagnosed with prostate cancer in PHS and HPFS, 1983–2009

	Quartiles of AMACR intensity (TMA-adjusted z-score)				
	Highest expression (Q1)	**Q2**	**Q3**	**Lowest expression (Q4)**	
Intensity z-score (min-max)	**(0.61, 2.94) n=230**	**(+0.06, 0.61) n=230**	**(−0.57, +0.05) n=230**	**(−4.46, −0.58) n=230**	
Characteristics					**p-value**
AMACR intensity	150.5 (0.90)	134.5 (0.48)	124.3 (0.43)	104 (0.84)	--
AMACR intensity z-score	1.20 (0.03)	0.32 (0.01)	−0.23 (0.01)	−1.29 (0.04)	--
Age at diagnosis (yrs)	65.0 (0.4)	65.7 (0.4)	66.1 (0.4)	66.8 (0.4)	0.02 **
Body mass index (baseline)	24.9 (0.2)	25.1 (0.2)	24.9 (0.2)	25.2 (0.2)	0.79 **
Follow-up time (yrs)	11.2 (0.3)	11.4 (0.3)	11.5 (0.3)	11.2 (0.3)	0.82 **
log(PSA at diagnosis) (ng/mL)	2.0 (0.1)	1.8 (0.1)	2.0 (0.1)	2.2 (0.1)	0.003 **
missing (%) §	33 (14.4)	31 (13.5)	36 (15.7)	50 (21.7)	
Gleason score					
4–6	79 (34.4)	87 (37.8)	77 (33.5)	79 (34.4)	0.11 †
7	124 (53.9)	110 (47.8)	114 (49.6)	103 (44.8)	
8–10	24 (10.4)	28 (12.2)	35 (15.2)	43 (18.7)	
missing (%)	3 (1.3)	5 (2.2)	4 (1.7)	5 (2.2)	
Clinical stage					
T1, NX/NO	109 (47.4)	118 (51.3)	112 (48.7)	104 (45.2)	0.06†
T2, NX/NO	99 (43.0)	90 (39.1)	95 (41.3)	84 (36.5)	
T3/T4 or N1/M1	10 (4.4)	12 (5.2)	10 (4.4)	30 (13.0)	
missing (%)	12 (5.2)	10 (4.4)	13 (5.7)	12 (5.2)	
Prostate cancer death					
Yes	16 (7.0)	11 (4.8)	16 (7.0)	25 (10.9)	0.07 ‡
No	214 (93.0)	219 (95.2)	214 (93.0)	205 (89.1)	

** F-test for analysis of variance, test for trend

† Global chi-squared test

‡chi-square test for trend across quartiles

Table III

Hazard ratios (HR's) of fatal and lethal (boney metastases or fatal) prostate cancer associated with AMACR intensity z-scores in PHS and HPFS, 1983–2009

Intensity z-score	Q1 (n=230) (0.61, 2.94)			Q2 (n=230) (+0.06, 0.61)		Q3 (n=230) (−0.57, +0.05)		Q4 (n=230) (−4.46, −0.58)		
Cox proportional hazards model	**HR**	**95% CI**		**HR**	**95% CI**	**HR**	**95% CI**	**HR**	**95% CI**	**p-for-trend**
Endpoint = prostate cancer death										
Crude HR	1.00	reference	--	0.72	(0.33, 1.57)	0.97	(0.47, 1.98)	1.60	(0.84, 3.06)	0.07
Age-adjusted HR	1.00	reference	--	0.69	(0.32, 1.51)	0.93	(0.45, 1.89)	1.47	(0.77, 2.81)	0.12
Multivariate-adjusted HR **	1.00	reference	--	0.69	(0.32, 1.51)	0.88	(0.43, 1.81)	1.28	(0.67, 2.45)	0.27
Endpoint = prostate cancer bony metastases or prostate cancer death										
Crude HR	1.00	reference	--	0.71	(0.36, 1.41)	1.00	(0.53, 1.88)	1.53	(0.86, 2.73)	0.07
Age-adjusted HR	1.00	reference	--	0.69	(0.35, 1.38)	0.97	(0.52, 1.81)	1.43	(0.80, 2.55)	0.11
Multivariate-adjusted HR **	1.00	reference	--	0.62	(0.31, 1.26)	0.89	(0.47, 1.66)	1.24	(0.69, 2.22)	0.23

** adjusted for age (yrs), gleason (4–6,7,8–10), stage (T1/T2 vs. T3/T4 or N1 or M1) and cohort (PHS v. HPFS)