Evaluation and Prognostic Significance of ACAT1 as a Marker of Prostate Cancer Progression

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INTRODUCTION. Prostate cancer is the second leading cause of cancer-related death among men in North America. While a majority of prostate cancer cases remain indolent, subsets of patients develop aggressive cancers, which may lead to death. The current methods of detection include digital rectal examination and the serum PSA test. However, due to lack of specificity, neither of these approaches is able to accurately discriminate between indolent and aggressive cancer, which is why there is a need for additional prognostic factors. Previously, we identified enzymes of the ketogenic pathway, particularly ACAT1, to be elevated in aggressive prostate cancer.

METHODS. In the current study, we assessed the diagnostic and prognostic potential of ACAT1 by analyzing its expression using immunohistochemistry on a tissue microarray consisting of 251 clinically localized prostate cancer patients who have undergone radical prostatectomy.

RESULTS. Using quantitative digital imaging software, we found that ACAT1 expression was significantly greater in cancerous cores compared to adjacent benign cores ($P < 0.0001$), in Gleason score (GS) $\geq$8 cancers versus GS$\leq$6 cancers ($P < 0.0001$), GS$\geq$8 cancers versus GS7 cancers ($P = 0.001$), as well as pT3/pT4 versus pT2 cancers ($P = 0.001$). In addition, ACAT1 predicted biochemical recurrence in univariate (HR, 1.81, CI = 1.13–2.9, $P = 0.0128$), and multivariate models (HR, 1.69, CI = 1.01–2.81, $P = 0.0431$) including pre-operative PSA level, Gleason score and pathological stage. In univariate time-to-recurrence analysis, ACAT1 expression predicted recurrence in ERG negative cases ($P = 0.0025$), whereas ERG positive cases did not display any differences.

DISCUSSION. Taken together, these findings indicate that ACAT1 expression could serve as a potential prognostic marker in prostate cancer, specifically in differentiating indolent and aggressive forms of cancer. *Prostate 74:372–380, 2014.* © 2013 Wiley Periodicals, Inc.

KEY WORDS: **ACAT1; prostate cancer; progression; ketogenesis**

Abbreviations: ACAT1, acetyl-coenzyme A acetyltransferase 1; HMGCS2, 3-hydroxy-3-methylglutaryl-CoA synthase 2; OXCT1, succinyl-CoA:3-ketoacid-coenzyme A transferase 1; BDH1, D-beta-hydroxybutyrate dehydrogenase; PSA, prostate specific antigen; TMA, tissue microarray; GS, Gleason score; BPH, benign prostate hyperplasia; HR, hazard ratio; ERG, Ets related gene; SD, standard deviation.

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INTRODUCTION

Prostate cancer is the most common solid organ tumor and the second leading cause of death due to cancer among men in North America [1]. Prostate-specific antigen (PSA) is a protein predominantly secreted by prostatic epithelial cells and is one of the best serum cancer biomarkers available [2,3]. Since the introduction of the PSA test in the late 1980's, prostate cancer diagnosis increased substantially, however, the reduction in mortality is not matched by the improved diagnosis. A major issue with PSA is its lack of specificity, as other non-malignant diseases of the prostate also display elevated serum PSA levels, including benign prostate hyperplasia (BPH) and prostatitis, which can lead to over-diagnosis [2]. In addition, PSA levels are a poor indicator of aggressiveness, leading to potential over-treatment of many patients.

It is well-established that the majority of prostate cancer cases are slow growing and often indolent; however, a subset of patients develop more aggressive cancers which are usually fatal [4,5]. Therefore, markers that are able to discriminate between the various forms of the disease are of utmost importance. One of the best prognostic indicators for prostate cancer is Gleason score (GS), which characterizes the glandular architecture of the prostate based on a score that represents the level of cancer "de-differentiation" [6,7]. The Gleason score is comprised of two numbers, each representing the most common Gleason patterns ranging from 1 to 5, where 1 represents a highly differentiated carcinoma and 5 represents an aggressive de-differentiated one. It is now accepted that the transition from pattern 3 to pattern 4 represents disease progression from low-grade to high-grade [8]. Gleason 7 cancers, which are comprised of pattern 3 and pattern 4, are considered an intermediate state, requiring definitive treatment such as prostatectomy or radiotherapy. In addition, a proportion of patients with Gleason score 6 prostate cancers are eligible for active surveillance instead of immediate curative therapy.

Many studies focusing on genomics, epigenomics, and proteomics have been conducted to identify biomarkers that complement PSA or correlate with disease aggressiveness [9–13]. However, these studies have come-up short on yielding useful biomarkers for diagnosis or prognosis. For example, recent interest on gene fusions, specifically the TMPRSS2-ERG fusion, which has been found in approximately 50% of prostate cancer cases, has been evaluated for its prognostic potential [14,15]. There have been many conflicting reports of TMPRSS2-ERG as a prognostic marker, as some studies correlated it with several clinicopathological characteristics such as disease stage and biochemical recurrence, whereas others found no association [16–18]. Since TMPRSS2-ERG fusion is a very early event in prostate carcinogenesis, ERG positive and negative prostate cancers may represent two disparate lineages of prostate cancer with their own biomarker repertoire.

Recently, using an in-vitro quantitative proteomics approach, we identified enzymes of the ketogenic pathway to be elevated during prostate cancer progression [19]. After validation on a small clinical cohort, we found that ACAT1 in particular, a key enzyme within this pathway, was strongly associated with high-grade (GS$\geq$8) and castration-resistant metastatic prostate cancer. In this study, we assessed the diagnostic/prognostic potential of ACAT1 expression in relationship with ERG expression status using immunohistochemistry on a large cohort of prostate cancer patients, and analyzed the relationship between ACAT1 expression and clinicopathological features of prostate cancer.

MATERIALS AND METHODS

Patient Cohort and Pathology

A total of 251 patients who were diagnosed with localized prostate cancer and underwent radical prostatectomy between 1998 and 2001 at the University Health Network (UHN) in Toronto were included in this study. All samples along with their clinical and pathological follow-up data were obtained according to protocols approved by the Research Ethics Board at Mount Sinai Hospital, Toronto and UHN, Toronto.

The complete set of hematoxylin and eosin (H&E)-stained slides were reviewed by an expert pathologist (T. van der Kwast) and were assigned a Gleason score (WHO/ISUP 2005 criteria) [20], pathological stage (TNM), and surgical margin status.

Tissue Microarray Construction

Between 3 and 13 cores were taken from each of the 251 cases, to have representation of each primary, secondary, and occasionally, tertiary Gleason pattern present within the case. Matched benign tissue adjacent to the tumor was also taken from every patient. This resulted in a total of 1,438 cores within 7 tissue microarray (TMA) blocks. For each TMA, 5-μm serial sections were used for H&E staining, to verify the presence of tumor versus normal.

Immunohistochemical Staining

TMA 4 μm-slides were deparaffinized in xylene, dehydrated and blocked in hydrogen peroxide in

methanol for 10 min. Antigen retrieval was then performed using citrate buffer 10 mM, pH 6.0, in a microwave for 10 min. Slides were then blocked for 5 min in casein and incubated overnight with an ACAT1 polyclonal antibody (1:500 in PBS) (Sigma). Following 10 min of phosphate buffer saline (PBS) washing, slides were placed in secondary antibody for 30 min using the Polymer-HRP Immunohistochemistry kit (Biogenex, Fremont, CA) according to the manufacturer's instructions. After a 10 min wash in PBS, slides were developed with the addition of 3,3-diaminobenzidine (DAB) for 5 min. Slides were then counterstained with hematoxylin, dehydrated, and coverslipped.

Evaluation of Immunohistochemistry Staining

Immunostained slides were scanned and analyzed with the Aperio system at objective ×20. An in-house positive pixel count algorithm was developed to measure ACAT1 expression applied to individual cores using the TMA lab application. The positive pixel count algorithm provided information on intensity and percentage of positive pixels, which was used to calculate the H-score [21] using the following formula:

$$100 \times \frac{\%\text{ of pixels with weak intensity} \times 1 + \%\text{ of pixels with moderate intensity} \times 2 + \%\text{ of pixels with high intensity} \times 3}{\text{Total number of pixels}}$$

When assessing ACAT1 expression for each case (251 cases in total), the H-scores of all cancerous cores were averaged to determine an overall H-score for each case. In addition, each of the cores was independently reviewed by a pathologist to verify the positive pixel count results. When needed, cases were manually annotated to remove portions of the cores that had a different positivity level than the tissue of interest. Cores that presented with less than 15% total measured areas were excluded from analysis. Also, to ensure accuracy, 10% of all cores were individually assessed by a pathologist, strictly analysing the tissue of interest on each core.

Biochemical recurrence

Biochemical recurrence was defined as either if (a) two or more consecutive increases in serum PSA resulting in at least doubling of initial PSA, or (b) any increase in PSA resulting in values exceeding 0.2 ng/ml, or (c) there was at least a 10-fold increase in serum PSA between two consecutive samples. Patients not meeting any of these criteria were categorized as exhibiting "stable disease" [22].

Statistical analysis

Analysis of the relation between ACAT1 expression and Gleason score and pathological stage was done using two-tailed *t*-tests. Pearson correlation analysis was performed comparing ACAT1 expression, age, and preoperative PSA. Cox proportional hazards regression model was used to evaluate whether Gleason score, stage, ACAT1 staining, ERG staining, age, preoperative PSA or margin status had a relationship with biochemical recurrence. Each factor was coded as a binary variable, with the exception of Gleason score, which was categorized into the three groups: GS$\leq$6, GS7, and GS$\geq$8. Univariate disease-free survival was assessed using the Kaplan–Meier curve and log-rank tests. All tests were conducted with SPSS software.

RESULTS

Associations Between ACATI Expression and Clinicopathological Variables

We assessed ACAT1 expression based on H-score intensity, in 251 prostate cancer specimens along with their adjacent benign matched tissues. In total, there were 1,541 cores, however, after excluding cores based on total area and improper tissue type, a total of 1,438 cores were used for the analysis (103 cores were excluded). Table I shows the clinicopathological characteristics of the patient cohort analyzed. Also, to ensure accuracy, 10% of all cores were individually assessed by a pathologist, strictly analysing the tissue of interest on each core. The correlation coefficient after independent assessment was 0.89, indicating strong positive correlation of the two independent analyses. Representative cores displaying ACAT1 expression are shown Figure 1A. Using an in-house quantitative algorithm, we assessed ACAT1 expression between benign and cancer cores. We observed significantly elevated ACAT1 expression in cancer cores (mean H-score = 40.3, SD = 27.3, n = 1174) compared to adjacent benign cores (mean H-score = 16.9, SD = 10.5, n = 264; $P < 0.0001$; Figure 1B).

Next, we assessed ACAT1 expression and its association with Gleason score (GS). After analysis, ACAT1 expression was observed to be higher in cases of high-grade (GS$\geq$8) prostate cancer (Fig. 1C). The distribution of ACAT1 expression according to GS was significantly different between GS$\leq$6 (mean H-score

TABLE I. Cohort Clinicopathological Characteristics

Clicinopathogical characteristics	
Number of patients	251
Gleason score	
4	5
5	36
6	87
7	103
8	13
9	5
10	2
Pathological stage	
pT2	163
pT3	83
pT4	5
Surgical margin status	
Positive	58
Negative	193
Average preoperative PSA (range)	8.6 (0.1–55.4)
Average follow-up time (range), y	4.43 (0.17–9.48)
Number of biochemical recurrences (%)	83 (33.7)
Median age (range), y	62 (32–75)

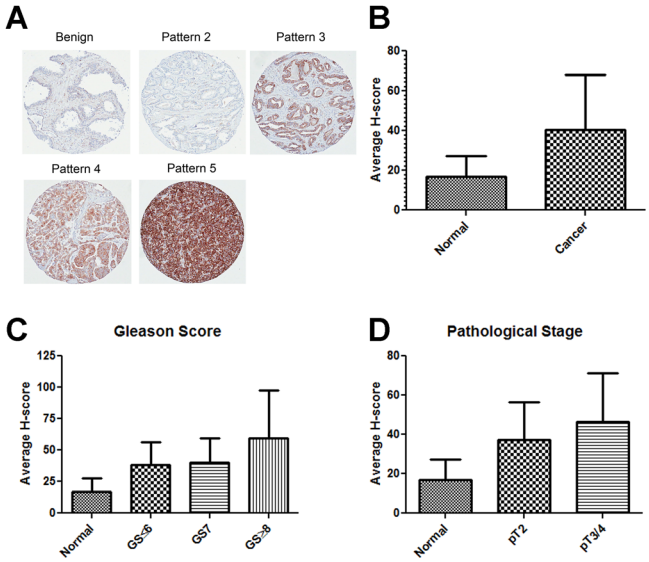

Fig. 1. ACAT1 staining versus clinicopathological parameters of prostate cancer. **A**: Representative prostate samples stained for ACAT1 expression at various Gleason patterns. **B**: Average H-score between normal and cancerous tissues. **C**: ACAT1 staining versus Gleason sore. **D**: ACAT1 staining versus pathological stage. Immunohistochemical staining was quantified using Aperio imaging Software. Error bars represent the standard deviation. For more details see text. Statistical comparisons between groups are shown in Table III.

$= 37.9$, SD $= 18.3$, n $= 128$), GS7 (mean H-score $= 40.2$, SD $= 19.3$, n $= 103$), and GS$\geq$8 (mean H-score $= 59.4$, SD $= 38.2$, n $= 20$) compared to benign tissues ($P < 0.0001$; Table II). In addition, there was a significant difference between GS$\leq$6 and GS$\geq$8 cases ($P < 0.0001$), as well as between GS7 and GS$\geq$8 cases ($P = 0.001$; Table III). However, there was no difference of ACAT1 expression between GS$\leq$6 and GS7 cases ($P = 0.372$). When we assessed GS7 (3 + 4) and GS7 (4 + 3) cases, we again, did not find a significant difference of ACAT1 expression.

We also assessed the relationship between ACAT1 expression and pathological stage (Figure 1D). Due to the small number of patients with pT4 stage tumors, we decided to combine pT3 and pT4 patterns to represent locally aggressive tumors. In organ confined pT2 tumors, ACAT1 displayed moderate expression patterns (mean H-score $= 37.3$, SD $= 19$, n $= 163$), whereas pT3/4 tumors displayed higher expression (mean H-score $= 46.6$, SD $= 24.7$, n $= 88$; Table II). There was a significant difference between pT2 and pT3/pT4 cases compared to benign controls ($P < 0.0001$; Table III). In addition, there was also a significant difference between organ confined pT2 tumors and the advanced pT3/pT4 tumors ($P = 0.001$).

Next, we tested the association between ACAT1 expression and other variables including age, preoperative PSA, and surgical margin status. However, we did not find a significant correlation between ACAT1 expression and any of these variables (Table III).

Using previous ERG protein expression data conducted on the study cohort [23], we next assessed whether there was an association between ACAT1 and ERG staining. We did not find a statistically significant association, however, there was a trend for elevated ACAT1 expression in ERG positive cases ($P = 0.133$).

ACAT1 Expression and Biochemical Recurrence-Free Survival

We next evaluated whether there was a relationship between ACAT1 staining and other clinicopathological variables with biochemical recurrence. Before performing the analysis, we categorized all cases as either having low or high ACAT1 expression based on the median H-score of all cases. Any case below the median was considered low for ACAT1 expression, and any case higher than median H-score was considered high. Univariate Cox regression analysis showed that high ACAT1 expression (HR, 1.81, CI $= 1.13$–2.9, $P = 0.0128$), GS7 (HR, 1.97, CI $= 1.17$–3.31, $P = 0.0103$), GS$\geq$8 (HR, 3.99, CI $= 1.93$–8.28, $P = 0.0002$), pathological stage (pT3/4 tumors) (HR, 3.02, CI $= 1.91$–4.81, $P < 0.0001$), preoperative PSA (HR, 1.03, CI $= 1.01$–1.05, $P = 0.0061$) and positive surgical margin status

TABLE II. ACAT1 Expression Stratified by Clinical Characteristics*

Variable	Total	Mean ACAT1 H-score	Standard deviation
Benign cores	264	16.9	10.5
Cancerous cores	1,174	40.3	27.3
Stage			
pT2	163	37.3	19
pT3/pT4	88	46.6	24.7
Gleason score			
≤6	128	37.9	18.3
7	103	40.2	19.3
≥8	20	59.4	38.2
Surgical margin status			
Positive	58	39.9	22.4
Negative	193	42.7	18.8
ERG expression			
Positive	109	38.3	24
Negative	102	42.7	19

*For statistical comparisons see Table III.

(HR, 2.20, CI = 1.37–3.54, $P = 0.0011$), were all associated with shorter disease-free survival (Table IV). Univariate Kaplan–Meier/log-rank analysis revealed that high ACAT1 staining was associated with a significant decrease in biochemical recurrence-free survival (log rank $P = 0.0014$; Fig. 2A).

We also conducted multivariate Cox regression analysis, which revealed that ACAT1 expression (HR, 1.69, CI = 1.01–2.81, $P = 0.0431$), GS≥8 (HR, 2.69, CI = 1.2–6.04, $P = 0.017$), pathological stage (pT3/4 tumors) (HR, 3.42, CI = 2.02–5.78, $P < 0.0001$), preoperative PSA (HR, 1.02, CI = 1.0–1.06, $P = 0.0461$) and positive surgical margin status (HR, 2.31, CI = 1.37–3.88, $P = 0.0015$), were significant predictors of biochemical recurrence (Table IV). In addition, when combining ACAT1 expression and pathological stage, both these variables were able to further stratify the likelihood of biochemical recurrence. Among the subset of more advanced pT3/pT4 tumors, those with a high ACAT staining had a significantly increased likelihood of biochemical recurrence (log rank $P < 0.0001$; Fig. 2B). Furthermore, after performing a similar analysis with the GS≤6 group, any case that also displayed high ACAT1 expression had increased likelihood of biochemical recurrence compared to GS≤6 and low ACAT1 expression (log rank $P = 0.023$; Fig. 2C).

We next looked at whether biochemical recurrence could be further stratified based on ERG and ACAT1 expression. We observed that cases that were negative for ERG but displayed high ACAT1 expression, had a significantly increased likelihood of biochemical recurrence compared to ERG negative and low ACAT1 expression cases (log rank $P = 0.0025$; Fig. 2D). In contrast, ACAT1 lost its prognostic impact in the subset of ERG positive cases (Fig. 2D).

TABLE III. *P*-values for ACAT1 Expression by Clinical Characteristics

Variable	*P*-value
Normal vs. cancer	<0.0001
Normal vs. pT2	<0.0001
Normal vs. pT3/pT4	<0.0001
pT2 vs. pT3/pT4	0.001
pT3a vs. pT3b	0.707
Normal vs. ≤ 6	<0.0001
Normal vs. 7	<0.0001
Normal vs. ≥ 8	<0.0001
≤6 vs. 7	0.372
≤6 vs. ≥8	<0.0001
7 vs. ≥8	0.001
GS7 (3 + 4) vs. GS7 (4 + 3)	0.783
Age	0.43
Preoperative PSA	0.763
ERG postive cases	0.133
Surgical margin status	0.396

TABLE IV. Associations Between ACAT1 Expression and Biochemical Recurrence

Variable	Hazard ratio	95% CI	*P*-value
Univariate analysis			
High ACAT1	1.81	1.13–2.9	0.0128
Gleason grade 7	1.97	1.17–3.31	0.0103
Gleason grade ≥ 8	3.99	1.93–8.28	0.0002
Tumor stage (pT3/4)	3.02	1.91–4.81	<0.0001
ERG positivity	0.87	0.56–1.38	0.5714
Positive surgical margin	2.20	1.37–3.54	0.0011
Age	1.02	0.99–1.06	0.1604
Preoperative PSA	1.03	1.01–1.05	0.0061
Multivariate analysis			
High ACAT1	1.69	1.01–2.81	0.0431
Gleason grade 7	1.17	0.67–2.05	0.5643
Gleason grade ≥ 8	2.6	1.2–6.04	0.0167
Tumor stage (pT3/4)	3.42	2.02–5.78	<.0001
ERG positivity	0.65	0.38–1.11	0.114
Positive surgical margin	2.31	1.37–3.88	0.0015
Age	1.00	0.96–1.04	0.9327
Preoperative PSA	1.02	1.0–1.06	0.0461

CI, confidence interval.

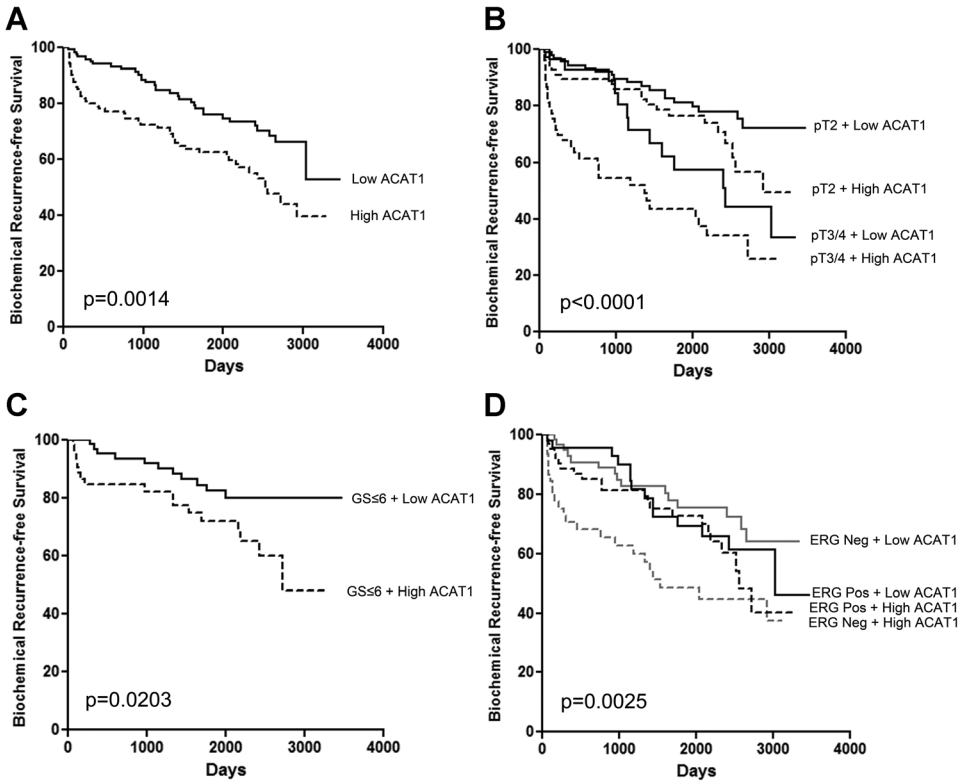

Fig. 2. Prognostic potential of ACATI staining as a marker of biochemical recurrence. **A**: Biochemical recurrence-free survival versus high ACATI and low ACATI cases. **B**: Univariate Kaplan–Meier/log-rank analysis of biochemical recurrence-free survival versus ACATI staining and pathological stage. **C**: Univariate Kaplan–Meier/log-rank analysis of biochemical recurrence-free survival vs. ACATI staining and GS $\leq$ 6 cases. **D**: Univariate Kaplan–Meier/log rank analysis of biochemical recurrence-free survival versus ACATI staining and ERG expression. For statistical comparisons seeTable IV.

DISCUSSION

Previously, we identified enzymes of the ketogenic pathway to be associated with prostate cancer progression. Of these enzymes, ACAT1 displayed the most interesting expression patterns, as it was found to be highly elevated in primary high grade and in castration-resistant metastatic prostate cancer specimens [19]. . In the current study, we validated the expression of ACAT1 by immunohistochemistry on a separate cohort of hormone-naïve prostate cancers and correlated the ACAT1 findings with various clinicopathological parameters and clinical outcomes, to evaluate its potential as a prognostic biomarker.

Our results show that ACAT1 expression is elevated in high grade (GS$\geq$8) prostate cancers compared with low and intermediate grade GS$\leq$7 tumors. We also observed elevated ACAT1 expression in more advanced pT3/pT4 tumors compared to organ confined pT2 tumors. These results are of importance, as patients who present with GS$\geq$8, harbour tumors displaying properties that are usually associated with poor prognosis [23].

After performing Kaplan–Meier survival analysis, ACAT1 expression was an independent prognostic marker for biochemical recurrence. When we performed survival analysis looking at both ACAT1 staining and tumor stage, it was observed that patients who displayed high ACAT1 expression along with having a tumor stage of pT3/pT4, had significantly worse prognosis (increased likelihood of biochemical recurrence) than other groups. In addition, ACAT1 was also able to discriminate between pT2 tumors, as pT2 cases that displayed high ACAT1 expression had worse prognosis than pT2 cases with low ACAT1 staining. After performing a similar analysis with Gleason score, we found that in cases of low-grade cancer (GS$\leq$6), patients that also displayed high ACAT1 expression had a significantly higher risk of biochemical recurrence than those with low ACAT1 expression. These results suggest that ACAT1 staining could be of clinical value as a prognostic marker at the time of biopsy diagnosis, if these findings could be confirmed in prostate biopsies.

After performing similar Kaplan–Meier survival analysis comparing ERG and ACAT1 expression, we

found that in ERG negative cases, patients that had high ACAT1 expression had an increased likelihood of biochemical recurrence. Such a trend was not observed when we performed the analysis on ERG positive cases, indicating that ERG expression might distinguish two distinct subsets of prostate cancers with their own biomarker profile. Recent studies have demonstrated such an effect, as different biomarkers displayed differential expression profiles in ERG negative and positive cases [24,25].

One of the major caveats of immunohistochemistry analysis is the reproducibility due to variability and the semi-quantitative nature of the approach. To address these, we decided to use a software that detects positive ACAT1 staining using an in-house generated algorithm for positive pixel count. Such an automated approach reduces variability of the scoring system, as the algorithm will always detect the same amount of ACAT1 positivity after each run. In addition, each of the cores was independently reviewed by a pathologist to verify the positive pixel count results detected by the software, to ensure the analysis was as accurate as possible.

The role of ACAT1 with respect to prostate cancer pathophysiology has yet to be elucidated. A recent study showed that ACAT1 was involved in androgen-mediated cholesterol metabolism in prostate cancer cell lines [26]. In another study, ACAT1 was found to be elevated in androgen-independent xenografts, further demonstrating its importance during prostate cancer progression [27]. Both these studies considered ACAT1 and its role with respect to cholesterol biogenesis. Interestingly, an alternative hypothesis as to why prostate cancer cells may over-express ACAT1 is to accelerate biosynthesis of cholesterol precursor molecules, as cholesterol has been shown to be involved in intratumoral de novo androgen biosynthesis [28]. Essentially, prostate cancer cells may be utilizing an alternate pathway to produce endogenous androgens to activate the AR signalling cascade, during times of androgen deprivation.

The ketogenic pathway is an alternate energy producing pathway that results in the formation of high energy ketone bodies such as beta-hydroxybutyrate [29]. Two recent studies looked at the importance of the ketogenic pathway with respect to tumor growth and progression in a human breast cancer cell line model [30,31]. In these studies, it was found that ACAT1, along with other ketogenic pathway enzymes, behaved functionally as a metabolic oncogene, as breast cancer cells over-expressing these enzymes had increased tumor growth and metastatic potential [30,31]. Interestingly, in these studies, it was observed that both BDH1 and HMGCS1/2 were rate-limiting with respect to ketone body formation, whereas ACAT1, HMGCS1/2 and OXCT1 were integral for ketone body re-utilization [30,31]. In order to better understand the mechanism of action of ACAT1 in prostate cancer progression, further studies, using RNA interference technology in prostate cancer cell lines and animal models, need to be conducted. Such functional studies can provide insight into whether ACAT1 has a direct mechanistic role on prostate cancer progression, which in turn can be utilized as a potential area of therapeutic intervention. ACAT1 inhibitors have been previously investigated for various other diseases including atherosclerosis [32,33], and may present and interesting avenue of therapeutic intervention for aggressive prostate cancers if this protein is indeed is implicated in prostate cancer pathobiology. In addition, assessing the expression of ACAT1 on other diverse clinical samples, such as hormone-naïve and hormone-refractory specimens, will provide better overall interpretation of the importance of this protein with respect to the development and progression of aggressive prostate cancer.

In conclusion, we assessed ACAT1 expression, using an automated scoring system, in a large series of prostate cancer cases and analyzed its relationship with the most relevant clinicopathological parameters. Overall, we have shown that ACAT1 expression is significantly elevated in (1) prostate cancer versus benign prostatic glandular tissue, (2) high-grade versus low/intermediate grade prostate cancer, and (3) advanced pT3/pT4 versus organ-confined pT2 tumors. In addition, ACAT1 expression is also an independent indicator of reduced biochemical recurrence-free survival. Going forward, further studies need to be conducted to assess the clinical utility of ACAT1 and its functional role during prostate cancer progression.

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REFERENCES

1. Gronberg H. Prostate cancer epidemiology. Lancet 2003;361:859–864.
2. Diamandis EP. Prostate-specific antigen: Its usefulness in clinical medicine. Trends Endocrinol Metab 1998;9:310–316.
3. Sardana G, Dowell B, Diamandis EP. Emerging biomarkers for the diagnosis and prognosis of prostate cancer. Clin Chem 2008;54:1951–1960.

4. Denmeade SR, Isaacs JT. A history of prostate cancer treatment. Nat Rev Cancer 2002;2:389–396.
5. Saraon P, Jarvi K, Diamandis EP. Molecular alterations during progression of prostate cancer to androgen independence. Clin Chem 2011;57:1366–1375.
6. Gleason DF. Classification of prostatic carcinomas. Cancer Chemother Rep 1966;50:125–128.
7. Gleason DF, Mellinger GT. Prediction of prognosis for prostatic adenocarcinoma by combined histological grading and clinical staging. J Urol 1974;111:58–64.
8. Lopez-Beltran A, Mikuz G, Luque RJ, Mazzucchelli R, Montironi R. Current practice of Gleason grading of prostate carcinoma. Virchows Arch 2006;448:111–118.
9. Grasso CS, Wu YM, Robinson DR, Cao X, Dhanasekaran SM, Khan AP, Quist MJ, Jing X, Lonigro RJ, Brenner JC, Asangani IA, Ateeq B, Chun SY, Siddiqui J, Sam L, Anstett M, Mehra R, Prensner JR, Palanisamy N, Ryslik GA, Vandin F, Raphael BJ, Kunju LP, Rhodes DR, Pienta KJ, Chinnaiyan AM, Tomlins SA. The mutational landscape of lethal castration-resistant prostate cancer. Nature 2012;487:239–243.
10. Khan AP, Poisson LM, Bhat VB, Fermin D, Zhao R, Kalyana-Sundaram S, Michailidis G, Nesvizhskii AI, Omenn GS, Chinnaiyan AM, Sreekumar A. Quantitative proteomic profiling of prostate cancer reveals a role for miR-128 in prostate cancer. Mol Cell Proteomics 2010;9:298–312.
11. Kron K, Pethe V, Briollais L, Sadikovic B, Ozcelik H, Sunderji A, Venkateswaran V, Pinthus J, Fleshner N, van der Kwast T, Bapat B. Discovery of novel hypermethylated genes in prostate cancer using genomic CpG island microarrays. PLoS ONE 2009;4:e4830.
12. Saraon P, Musrap N, Cretu D, Karagiannis GS, Batruch I, Smith C, Drabovich AP, Trudel D, van der Kwast T, Morrissey C, Jarvi KA, Diamandis EP. Proteomic profiling of androgen-independent prostate cancer cell lines reveals a role for protein S during the development of high grade and castrate-resistant prostate cancer. J Biol Chem 2012;287:34019–34031.
13. Taylor BS, Schultz N, Hieronymus H, Gopalan A, Xiao Y, Carver BS, Arora VK, Kaushik P, Cerami E, Reva B, Antipin Y, Mitsiades N, Landers T, Dolgalev I, Major JE, Wilson M, Socci ND, Lash AE, Heguy A, Eastham JA, Scher HI, Reuter VE, Scardino PT, Sander C, Sawyers CL, Gerald WL. Integrative genomic profiling of human prostate cancer. Cancer Cell 2010;18:11–22.
14. Perner S, Demichelis F, Beroukhim R, Schmidt FH, Mosquera JM, Setlur S, Tchinda J, Tomlins SA, Hofer MD, Pienta KG, Kuefer R, Vessella R, Sun XW, Meyerson M, Lee C, Sellers WR, Chinnaiyan AM, Rubin MA. TMPRSS2 ERG fusion-associated deletions provide insight into the heterogeneity of prostate cancer. Cancer Res 2006;66:8337–8341.
15. Tu JJ, Rohan S, Kao J, Kitabayashi N, Mathew S, Chen YT. Gene fusions between TMPRSS2 and ETS family genes in prostate cancer: Frequency and transcript variant analysis by RT-PCR and FISH on paraffin-embedded tissues. Mod Pathol 2007; 20:921–928.
16. FitzGerald LM, Agalliu I, Johnson K, Miller MA, Kwon EM, Hurtado-Coll A, Fazli L, Rajput AB, Gleave ME, Cox ME, Ostrander EA, Stanford JL, Huntsman DG. Association of TMPRSS2-ERG gene fusion with clinical characteristics and outcomes: Results from a population-based study of prostate cancer. BMC Cancer 2008;8:230.
17. Gopalan A, Leversha MA, Satagopan JM, Zhou Q, Al-Ahmadie HA, Fine SW, Eastham JA, Scardino PT, Scher HI, Tickoo SK, Reuter VE, Gerald WL. TMPRSS2-ERG gene fusion is not associated with outcome in patients treated by prostatectomy. Cancer Res 2009;69:1400–1406.
18. Nam RK, Sugar L, Yang W, Srivastava S, Klotz LH, Yang LY, Stanimirovic A, Encioiu E, Neill M, Loblaw DA, Trachtenberg J, Narod SA, Seth A. Expression of the TMPRSS2 ERG fusion gene predicts cancer recurrence after surgery for localised prostate cancer. Br J Cancer 2007;97:1690–1695.
19. Saraon P, Cretu D, Musrap N, Karagiannis GS, Batruch I, Drabovich AP, van der Kwast T, Mizokami A, Morrissey C, Jarvi KA, Diamandis EP. Quantitative proteomics reveals that enzymes of the ketogenic pathway are associated with prostate cancer progression. Mol Cell Proteomics 2013;12(6):1589–1601.
20. Epstein JI, Allsbrook WC Jr, Amin MB, Egevad LL. The 2005 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of Prostatic Carcinoma. Am J Sur Path 2005;29:1228–1242.
21. Detre S, Saclani Jotti G, Dowsett M. A "quickscore" method for immunohistochemical semiquantitation: Validation for oestrogen receptor in breast carcinomas. J Clin Pathol 1995; 4: 876–878.
22. Yu H, Diamandis EP, Wong PY, Nam R, Trachtenberg J. Detection of prostate cancer relapse with prostate specific antigen monitoring at levels of 0.001 to 0.1 microG/L. J Urol 157:913–918.
23. Brimo F, Montironi R, Egevad L, Erbersdobler A, Lin DW, Nelson JB, Rubin MA, van der Kwast T, Amin M, Epstein JI. Contemporary grading for prostate cancer: Implications for patient care. Eur Urol 2013;63:892–901.
24. Kron K, Liu L, Trudel D, Pethe V, Trachtenberg J, Fleshner N, Bapat B, van der Kwast T. Correlation of ERG expression and DNA methylation biomarkers with adverse clinicopathologic features of prostate cancer. Clin Cancer Res 2012;18:2896–2904.
25. Karnes RJ, Cheville JC, Ida CM, Sebo TJ, Nair AA, Tang H, Munz JM, Kosari F, Vasmatzis G. The ability of biomarkers to predict systemic progression in men with high-risk prostate cancer treated surgically is dependent on ERG status. Cancer Res 2010;70:8994–9002.
26. Locke JA, Wasan KM, Nelson CC, Guns ES, Leon CG. Androgen-mediated cholesterol metabolism in LNCaP and PC-3 cell lines is regulated through two different isoforms of acyl-coenzyme A:Cholesterol Acyltransferase (ACAT). Prostate 2008; 68:20–33.
27. Leon CG, Locke JA, Adomat HH, Etinger SL, Twiddy AL, Neumann RD, Nelson CC, Guns ES, Wasan KM. Alterations in cholesterol regulation contribute to the production of intratumoral androgens during progression to castration-resistant prostate cancer in a mouse xenograft model. Prostate 2010;70: 390–400.
28. Mostaghel EA, Solomon KR, Pelton K, Freeman MR, Montgomery RB. Impact of circulating cholesterol levels on growth and intratumoral androgen concentration of prostate tumors. PloS ONE 2012;7:e30062.
29. Fukao T, Lopaschuk GD, Mitchell GA. Pathways and control of ketone body metabolism: On the fringe of lipid biochemistry. Prostaglandins Leukot Essent Fatty Acids 2004;70:243–251.
30. Martinez-Outschoorn UE, Lin Z, Whitaker-Menezes D, Howell A, Lisanti MP, Sotgia F. Ketone bodies and two-compartment tumor metabolism: Stromal ketone production fuels mitochondrial biogenesis in epithelial cancer cells. Cell Cycle 2012;11: 3956–3963.

31. Martinez-Outschoorn UE, Lin Z, Whitaker-Menezes D, Howell A, Sotgia F, Lisanti MP. Ketone body utilization drives tumor growth and metastasis. Cell Cycle 2012;11:3964–3971.

32. Leon C, Hill JS, Wasan KM. Potential role of acyl-coenzyme A: cholesterol transferase (ACAT) Inhibitors as hypolipidemic and antiatherosclerosis drugs. Pharm Res 2005;22:1578–1588.

33. Yoshinaka Y, Shibata H, Kobayashi H, Kuriyama H, Shibuya K, Tanabe S, Watanabe T, Miyazaki A. A selective ACAT-1 inhibitor, K-604, stimulates collagen production in cultured smooth muscle cells and alters plaque phenotype in apolipoprotein E-knockout mice. Atherosclerosis 2010;213: 85–91.