Research Article

Cancer Epidemiology, Biomarkers & Prevention

Intratumoral Sterol-27-Hydroxylase (*CYP27A1*) Expression in Relation to Cholesterol Synthesis and Vitamin D Signaling and Its Association with Lethal Prostate Cancer

Nabeela A. Khan[1], Konrad H. Stopsack[1,2], Emma H. Allott[2,3], Travis Gerke[2,4], Edward L. Giovannucci[2,5,6], Lorelei A. Mucci[2,6], and Philip W. Kantoff[1]

Abstract

Background: Higher intratumoral cholesterol synthesis is associated with a worse prognosis in prostate cancer. The vitamin D–regulated enzyme sterol-27-hydroxylase (CYP27A1) converts cholesterol to 27-hydroxycholesterol, potentially lowering intracellular cholesterol levels. We hypothesized that low *CYP27A1* expression is associated with high cholesterol synthesis, low vitamin D signaling, and higher risk of lethal prostate cancer.

Methods: In 404 patients from the prospective prostate cancer cohorts within the Health Professionals Follow-up Study (HPFS) and the Physicians' Health Study (PHS), we assessed intratumoral *CYP27A1* expression and proxies of cholesterol synthesis using transcriptome profiling, prediagnostic plasma 25-hydroxyvitamin D [25(OH)D; $n = 132$], and intratumoral vitamin D receptor protein expression (VDR; $n = 300$). Patients were followed for metastases and prostate cancer mortality (lethal cancer; median follow-up, 15.3 years).

Results: *CYP27A1* expression was lower in tumors with higher Gleason grade and higher expression of cholesterol synthesis enzymes, including the second rate-limiting enzyme, *SQLE*. We did not detect consistent associations between *CYP27A1* and 25(OH)D, VDR, or *CYP24A1* mRNA expression. Lower *CYP27A1* was associated with higher risk of lethal cancer in both cohorts, independent of *SQLE* [adjusted OR for lowest vs. highest quartile of *CYP27A1*, 2.64; 95% confidence interval (CI), 1.24–5.62]. This association was attenuated when additionally adjusting for Gleason grade (OR, 1.76; 95% CI, 0.75–4.17).

Conclusions: Low *CYP27A1* expression was associated with higher cholesterol synthesis and a higher risk of lethal disease.

Impact: These observations further support the hypothesis that intratumoral cholesterol accumulation through higher synthesis and decreased catabolism is a feature of lethal prostate cancer.

Introduction

Prostate tissue has long been recognized to contain considerable amounts of cholesterol, particularly when undergoing carcinogenic transformation (1). More recently, several studies have suggested that higher serum cholesterol levels are associated with increased risk of advanced stage, higher grade, or fatal prostate cancer (2–4), while others reported null associations (5). Higher intratumoral synthesis of cholesterol, as assessed through expression of the second rate-limiting enzyme of cholesterol synthesis, squalene monooxygenase (*SQLE*), is associated with a higher risk of lethal prostate cancer (6).

A key metabolite of cholesterol is 27-hydroxycholesterol (Fig. 1). Intriguingly, high expression of the enzyme that synthesizes 27-hydroxycholesterol, 27-hydroxylase (*CYP27A1*), has been reported to be associated with higher tumor grade in breast cancer yet better prognosis (7, 8). *CYP27A1* also catalyzes the 25-hydroxylation step of vitamin D, which might have a protective effect in various cancers including prostate cancer (9, 10). In prostate cancer, a recent study reported *CYP27A1* expression to be strongly inversely related to Gleason grade (11), but the association with long-term clinical outcomes is unknown.

We hypothesized that low *CYP27A1* expression, potentially resulting in cholesterol accumulation, occurs in prostate cancers that have higher expression of the cholesterol synthesis pathway. We also hypothesized that low *CYP27A1* expression is associated with low vitamin D signaling. To test these hypotheses, we conducted a cross-sectional analysis of two large, well-characterized populations of patients with prostate cancer. In a

[1]Department of Medicine, Memorial Sloan Kettering Cancer Center, New York, New York. [2]Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, Massachusetts. [3]Department of Histopathology and Morbid Anatomy, Trinity Translational Medicine Institute, Trinity College Dublin, Dublin, Ireland. [4]Department of Cancer Epidemiology, H. Lee Moffitt Cancer Center, Tampa, Florida. [5]Department of Nutrition, Harvard T.H. Chan School of Public Health, Boston, Massachusetts. [6]Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital, Boston, Massachusetts.

N.A. Khan and K.H. Stopsack contributed equally to this article.

Prior presentation: Presented in part at the Annual Meeting of the American Association for Cancer Research, Chicago, IL, April 14–18, 2018.

Corresponding Authors: Konrad H. Stopsack, Memorial Sloan Kettering Cancer Center, New York, NY 10065. Phone: 212-639-5851; Fax: 929-321-5023; E-mail: stopsack@mskcc.org; and Philip Kantoff, kantoff@mskcc.org

Cancer Epidemiol Biomarkers Prev 2019;28:1052–8

doi: 10.1158/1055-9965.EPI-18-1083

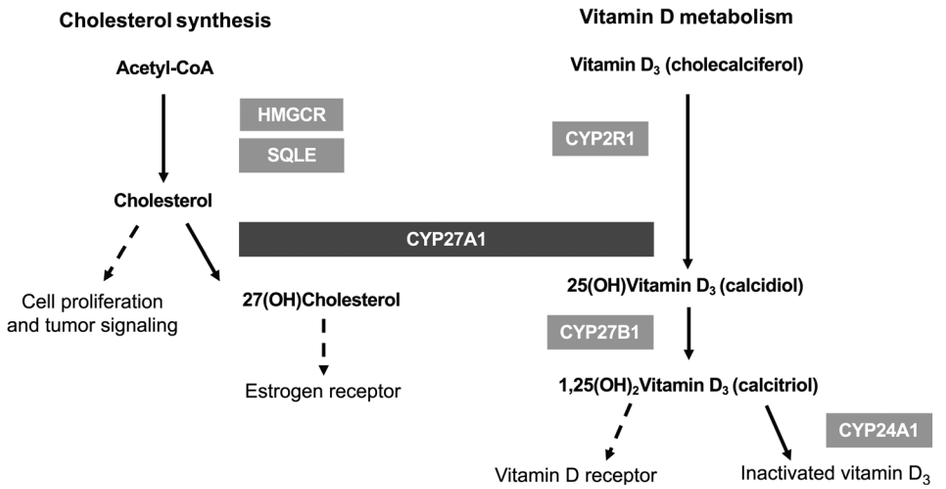

Figure 1.
CYP27A1 functions. CYP27A1 interacts with both cholesterol synthesis and vitamin D signaling pathways.

longitudinal design, we tested our hypothesis that low *CYP27A1* would be associated with a higher risk of lethal prostate cancer over long-term follow-up.

Methods

Study populations

We studied patients who were diagnosed with prostate cancer during follow-up of two prospective cohort studies, the Health Professionals Follow-up Study (HPFS) and the Physicians' Health Study (PHS).

The HPFS enrolled 51,529 male health professionals, aged 40 to 75 years, in 1986 (12). Participants have been followed through biannual questionnaires since. The PHS enrolled 29,071 male physicians, aged $\geq$40 years, in 1982, initially for randomized controlled trials of aspirin (13) and micronutrients (14). Blood samples were collected from cancer-free participants in 1982 (PHS) and in 1993–1995 (HPFS). Self-reported prostate cancer diagnoses in both cohorts were verified through review of medical records. Tissue from all patients included in this study also underwent centralized pathology review. Patients were followed prospectively for metastases and prostate cancer–specific death (lethal cancer). Adjudication of death causes was 98% complete in HPFS and 99% complete in PHS.

Within the prostate cancer biorepository from HPFS and PHS, we conducted a nested case–control study of whole-transcriptome profiling of the tumor tissue. It compared patients who developed lethal disease with those who remained metastasis-free for at least 8 years after cancer diagnosis (nonlethal disease) and oversampled patients with lethal outcome and those with available blood specimens from before cancer diagnosis (15).

Participants gave written informed consent by returning the baseline questionnaires. The research was approved by the institutional review boards at Harvard T.H. Chan School of Public Health and Partners Healthcare.

Tumor profiling and plasma levels

For all patients included in this study, we retrieved tumor specimens from cancer diagnosis from the treating hospital. Expert genitourinary pathologists performed centralized histologic rereview, including Gleason grading (16), and selected high-density tumor areas (>80% tumor cell density). Tumor tissue and, if available, adjacent noncancer prostate tissue, was measured on the Affymetrix GeneChip Human Gene 1.0 ST array (Gene Expression Omnibus: GSE62872), with postprocessing as described previously (17). Transcriptome profiling included mRNA expressions of *CYP27A1*, *SQLE*, and *CYP24A1*.

Plasma 25-hydroxy-vitamin D [25(OH)D] from blood samples before cancer diagnosis was measured as a part of a case–control study nested within HPFS. A radioimmunosorbent assay was used, as described previously (18).

To quantify vitamin D receptor (VDR) signaling (9), its expression in the cytoplasm and membrane was stained via IHC on tissue microarrays. Using a semiautomated quantitative image analysis system, the VDR score was generated as a combination of the relative area positively stained and the intensity of staining, as described previously (19). *TMPRSS2:ERG* status was determined using a genomically validated ERG IHC (20).

Statistical analysis

Our analysis plan had two main parts. First, we assessed cross-sectionally, how *CYP27A1* expression was associated with measures of vitamin D signaling and intratumoral cholesterol synthesis. Second, in a longitudinal analysis, we assessed the association between *CYP27A1* at cancer diagnosis and the risk of lethal disease over long-term follow-up. All tests were two-sided.

To assess the associations of 25(OH)D, VDR, *SQLE*, *CYP24A1*, ERG, and *CYP27A1*, we used linear regression. Values for 25(OH)D were adjusted for season and batch, as described previously (18); VDR scores were adjusted for differences in mean values between tissue microarrays (19). We modeled the predictor in categories and inspected plots to assess for potential nonlinear relationships, and we calculated tests for linear trend across quartiles by modeling the category medians (for 25(OH)D and VDR) or category indices (mRNA variables) as ordinal predictors. In a sensitivity analysis, we replaced *SQLE* as a proxy for cholesterol synthesis activity of the tumor by a summary score of all cholesterol synthesis genes (6). This summary score was the first principal component from principal components analysis of the cholesterol synthesis genes *CYP51A1*, *DHCR24*, *DHCR7*, *EBP*, *FDFT1*, *FDPS*, *GGPS1*, *HMGCR*, *HMGCS1*, *HSD17B7*, *IDI1*, *IDI2*, *LBR*, *LSS*, *MVD*, *MVK*, *NSDHL*, *PMVK*, *SC5DL*, *SQLE*, and *TM7SF2*. Higher levels indicated higher expression of the

cholesterol synthesis pathway, as 20 of the 21 cholesterol synthesis genes were positively loaded on this principal component.

To assess the association of *CYP27A1* expression (modeled in quartiles) and lethal disease, we used logistic regression to estimate ORs and 95% confidence intervals. Models were additionally adjusted for age (linear), year of diagnosis [categorical: pre-prostate–specific antigen (PSA) era, 1982–1988; peri-PSA era, 1989–1993; PSA era, 1994–2005), smoking status (binary: current smoker vs. never/prior smoking), family history of prostate cancer in father or brothers (binary: yes/no), body mass index (categorical: <25, 25–30, >30 kg/m^2), and hyperlipidemia (binary: any self-report of hyperlipidemia by the health professionals on questionnaires before cancer diagnosis vs. no such report). In separate models, we adjusted for Gleason grade (categorical: 5–6, 3+4, 4+3, 8, 9–10), statin use at cancer diagnosis (binary: yes/no), and *SQLE* expression (categorical: quartiles). In an exploratory analysis, we assessed the association of *CYP27A1* within low and high strata of cholesterol synthesis activity defined by *SQLE* and the cholesterol signature, and tested for statistical interaction using likelihood ratio tests. Given its strong association with lethal disease specifically in the highest quartile (21), *SQLE* in the fourth quartile was considered high; the upper half of the signature was considered high.

Results

Study populations and tumor characteristics at cancer diagnosis

Characteristics of 254 patients from HPFS and 150 patients from PHS at the time of prostate cancer diagnosis are shown in Table 1. Fifty-nine percent of patients had pathologically organ-confined cancers (T1/T2 N0 M0), and 59% were diagnosed in the PSA screening era. Ninety-two percent of tumor samples were from radical prostatectomy. For 202 patients, adjacent noncancerous prostate tissue was assessed. Plasma concentrations of 25(OH)D before cancer diagnosis were available for a subset of 132 patients from HPFS. VDR protein expression had been quantified for 300 patients.

Notably, *CYP27A1* expression was lower in higher grade, advanced stage, and ERG-positive cancers (Table 1). Compared with Gleason grade 5–6, tumors with Gleason grade 9–10 had on average 0.73 SD lower *CYP27A1* expression (95% CI, 0.38–1.08 SD; $P_{trend} < 0.001$). ERG-positive tumors had 0.27 SD lower *CYP27A1* expression (95% CI, 0.06–0.47) than ERG-negative tumors. *CYP27A1* expression was lower by 0.42 SD in tumors with advanced stage (based on combined clinical and pathologic stage) compared with localized tumors (95% CI, 0.06–0.79).

Cross-sectional analysis: vitamin D signaling, cholesterol synthesis, and *CYP27A1* expression

We assessed the association of circulating and intratumoral indicators of vitamin D signaling and *CYP27A1* mRNA expression. Circulating plasma 25(OH)D was not associated with *CYP27A1*; the difference in 25(OH)D expression between the lowest quartile of *CYP27A1* and the highest quartile was −0.8 ng/mL (95% CI, −5.2 to 3.5; $P_{trend} = 0.71$; Fig. 2A). *CYP27A1* expression was also not associated with VDR expression in the tumor; the difference in VDR expression score was 0.28 SD (95% CI, −0.06 to 0.61 SD) between the lowest and the highest quartile of *CYP27A1* ($P_{trend} = 0.09$; Fig. 2B). In contrast, we observed a weak positive association between *CYP27A1* and the expression of the VDR target gene *CYP24A1*, which had a 0.36 SD (95% CI, 0.09–0.63 SD) higher expression in the highest quartile of *CYP27A1* expression compared with the lowest quartile ($P_{trend} = 0.005$; Fig. 2C).

To assess the association between intratumoral cholesterol synthesis and *CYP27A1*, we used the second rate-limiting enzyme of cholesterol synthesis, *SQLE*, and a score summarizing the mRNA expression of all cholesterol synthesis enzymes as proxies. *CYP27A1* was lower in tumors with higher *SQLE* expression; the difference in *CYP27A1* between lowest and highest quartile of *SQLE* was −0.42 SD (95% CI, −0.69 to −0.14; $P_{trend} = 0.002$ across quartiles of *SQLE*; Fig. 2D). Similar results were observed when we used the summary score instead of *SQLE* as a proxy for cholesterol synthesis in the tumor, observing a difference in *CYP27A1* between lowest and highest quartile of the score of −0.49 SD (95% CI, −0.77 to −0.22; $P_{trend} = 0.001$).

In normal prostate tissue, we also did not observe associations between *CYP27A1* expression and plasma 25(OH)D and VDR expression. In contrast to tumor tissue, *CYP27A1* expression and *CYP24A1* expression were not associated in normal prostate

Table 1. Characteristics of patients with prostate cancer from the Health Professionals Follow-up Study (HPFS) and the Physicians' Health Study (PHS), by *CYP27A1* mRNA expression in tumor tissue

	Quartile of *CYP27A1* mRNA expression in tumor tissue			
	1st (lowest)	2nd	3rd	4th (highest)
N	101	101	101	101
Age at diagnosis, median (range)	67 (49–80)	66 (47–80)	66 (50–81)	65 (52–77)
Year of diagnosis, *n*				
Before 1993	31	47	43	37
After 1993	70	54	58	64
Gleason grade, *n*				
5–6	10	11	13	23
7 (3+4)	24	36	38	41
7 (4+3)	34	24	24	20
8	12	17	9	5
9–10	21	13	17	12
Stage, *n*				
T1/T2	50	56	65	68
T3	38	37	29	28
T4/N1/M1	13	8	7	5
PSA (ng/dL), *n*				
<4	6	9	9	9
4–10	50	41	49	58
>10	27	32	31	20
Missing	18	19	12	14
Current smoking at diagnosis, *n*	9	6	2	7
Body mass index (kg/m^2), *n*				
<25	54	53	45	41
25–30	35	43	54	52
>30	12	5	2	8
Hypercholesterolemia, *n*	32	29	30	24
Statin use at diagnosis, *n*	9	11	10	13
Plasma 25(OH)D (ng/mL), median (interquartile range)	25 (21–32)	24 (19–29)	27 (22–35)	26 (19–28)
TMPRSS2:ERG status, *n*[a]				
ERG-positive	53	47	47	35
ERG-negative	39	47	41	56

NOTE: Within each quartile, absolute counts (out of 101 patients) closely approximate percentages.
[a]On the basis of IHC for ERG protein. Missing for 39 patients in total.

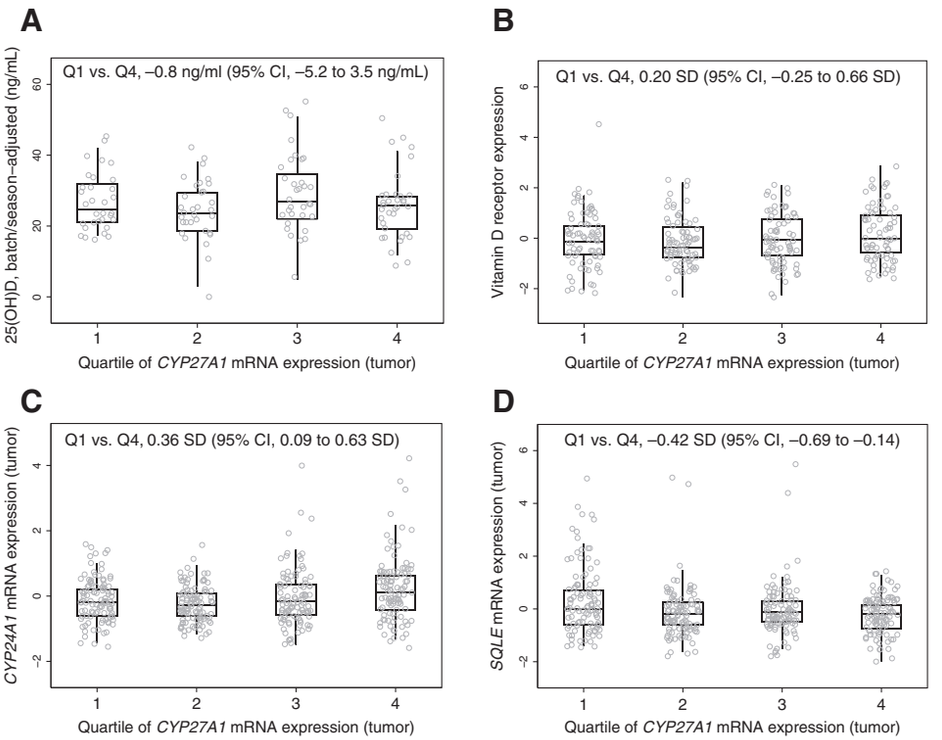

Figure 2.
CYP27A1 and biomarkers of cholesterol synthesis and vitamin D signaling. *CYP27A1* mRNA expression in tumor issue was associated with cholesterol synthesis enzyme expression, but less consistently with markers of vitamin D signaling. The diagrams show *CYP27A1* mRNA expression and plasma 25(OH)D (**A**), vitamin D receptor expression (**B**) in tumor tissue, *CYP24A1* mRNA (**C**), and *SQLE* mRNA (**D**). mRNA expressions and vitamin D receptor expression are dimensionless and expressed in SDs.

tissue (difference in *CYP24A1* expression between lowest and highest quartile of *CYP27A1*, 0.20 SD; 95% CI, −0.20 to 0.59; $P_{trend} = 0.33$), and there was no statistically significant difference in *CYP27A1* expression between the lowest and highest quartiles of *SQLE* (difference in *CYP27A1*, −0.15 SD; 95% CI, −0.54 to 0.25; $P_{trend} = 0.44$).

Longitudinal analysis: *CYP27A1* and lethal disease

Patients were followed a median of 15.3 years for the development of metastases or death from prostate cancer (lethal disease). Lower intratumoral *CYP27A1* mRNA expression was associated with a higher risk of lethal disease over long-term follow-up in both cohorts (Table 2). In HPFS, patients with

Table 2. *CYP27A1* mRNA expression in tumor tissue and lethal prostate cancer

	Quartile of *CYP27A1* mRNA expression in tumor tissue				
	1st (lowest)	2nd	3rd	4th (highest)	P_{trend}[a]
HPFS					
Cases: lethal, nonlethal, *n*	28, 34	20, 45	20, 44	15, 48	
OR (95% CI), unadjusted	2.64 (1.23–5.67)	1.42 (0.65–3.11)	1.45 (0.66–3.19)	1	0.018
PHS					
Cases: Lethal, nonlethal, *n*	8, 31	10, 26	10, 27	2, 36[b]	
OR (95% CI), unadjusted	4.65 (0.92–23.5)	6.92 (1.40–34.3)	6.67 (1.35–33.9)	1	0.12
Combined HPFS and PHS, OR (95% CI)					
Model 1: Unadjusted	2.74 (1.41–5.30)	2.09 (1.06–4.10)	2.09 (1.06–4.10)	1	0.005
Model 2: Adjusted[c]	3.04 (1.46–6.33)	2.17 (1.04–4.53)	2.40 (1.15–5.00)	1	0.007
Model 3: Model 2 + *SQLE*[d]	2.64 (1.24–5.62)	2.17 (1.03–4.58)	2.30 (1.08–4.88)	1	0.022
Model 4: Model 2 + cholesterol score[d]	2.86 (1.35–6.05)	2.02 (0.95–4.29)	2.40 (1.14–5.05)	1	0.015
Model 5: Model 3 + statin use at diagnosis	2.62 (1.23–5.57)	2.16 (1.02–4.57)	2.28 (1.07–4.85)	1	0.023
Model 6: Model 3 + Gleason	1.76 (0.75–4.17)	1.84 (0.77–4.41)	2.05 (0.87–4.86)	1	0.31
By cholesterol score,[e] OR (95% CI)					0.88[e]
Score < median	2.95 (1.12–7.81)	1.89 (0.67–5.41)	1.77 (0.65–4.79)	1	0.032
Score ≥ median	2.22 (0.89–5.57)	1.79 (0.72–4.48)	2.18 (0.85–5.60)	1	0.17

NOTE: Shown are case counts and ORs for lethal disease with 95% confidence intervals (CI). The fourth quartile (highest expression) served as the reference category.
[a]Test for linear trend across quartiles, modeled as ordinal indices.
[b]Because of the few events in the reference category for PHS ($n = 2$), the quartile-based ORs for PHS alone should be interpreted cautiously in light of probable sparse-data bias (36).
[c]Adjusted for age (linear), year of diagnosis (categorical), smoking status at cancer diagnosis (binary), body mass index (categorical), high serum cholesterol (binary).
[d]*SQLE* and summary score of expression levels for all cholesterol synthesis genes were modelled in quartiles.
[e]Analyses stratified by cholesterol synthesis score show unadjusted estimates within levels of the cholesterol summary score for HPFS and PHS combined. The *P* value is for multiplicative interaction between *CYP27A1* quartile indices (categorical) and cholesterol summary score (binary).

CYP27A1 mRNA expression in the lowest quartile had a 2.64-fold higher odds of lethal disease (95% CI, 1.23–5.67), compared with patients with *CYP27A1* in the highest quartile. In PHS, the OR was 4.65 (95% CI, 0.92–23.5). Combining both cohorts and adjusting for additional baseline characteristics, the OR was 3.04 (95% CI, 1.46–6.33; $P_{trend} = 0.007$ across quartiles of *CYP27A1*). The association of *CYP27A1* and lethal disease was attenuated somewhat when additionally adjusting for *SQLE* (OR for lowest vs. highest quartile of *CYP27A1*, 2.64; 95% CI, 1.24–5.62). Results were similar when adjusting for the summary score of cholesterol synthesis or when additionally adjusting for statin use at cancer diagnosis. With additional adjustment for Gleason grade, the association of *CYP27A1* and lethal disease was considerably attenuated and imprecisely estimated (OR, 1.76; 95% CI, 0.75–4.17).

As expected, *CYP27A1* expression in tumor-adjacent noncancerous prostate tissue was not associated with lethal disease (OR for lowest vs. highest quartile, 1.51; 95% CI, 0.66–3.44; $P_{trend} = 0.24$).

Finally, we assessed whether the association of intratumoral *CYP27A1* with lethal disease differed within levels of cholesterol synthesis. The association between *CYP27A1* and lethal disease did not differ when stratifying by the summary score of cholesterol synthesis (Table 2; $P_{interaction} = 0.88$), although it appeared to be slightly stronger in patients with low *SQLE* ($P_{interaction} = 0.12$).

Discussion

In this study, we assessed regulators of *CYP27A1*, which synthesizes 27-hydroxycholesterol from cholesterol, and associations of *CYP27A1* expression with long-term prognosis in patients with primary prostate cancer. We found *CYP27A1* expression to be low in tumors that had higher expression of cholesterol synthesis enzymes including *SQLE*. In contrast, we did not detect strong associations between several measures of vitamin D signaling and *CYP27A1*. Notably, low *CYP27A1* expression was associated with a higher risk of lethal disease, beyond the elevated risk associated with higher expression of the cholesterol synthesis pathway.

We observed a strong inverse relationship between *CYP27A1* expression and two different measures of intratumoral cholesterol synthesis, the expression of the second rate-limiting enzyme *SQLE* as well as a 21-gene signature of all enzymes in the cholesterol synthesis pathway. These observations suggest that in tumors with activated cholesterol synthesis, hydroxylation of cholesterol to 27-hydroxycholesterol is inhibited, perhaps giving these rapidly dividing cells a selective advantage when more cholesterol is available, for example, cell membrane formation. Concordantly, a preclinical study found the addition of 27-hydroxycholesterol to prostate cancer cell lines and xenografts attenuated their growth and decreased the expression of SREBP2, the main transcription factor regulating cholesterol synthesis (11). However, discordant experimental results, partially using the same cell line, have been reported as well (22).

Despite assessing multiple proxies of vitamin D signaling activity, including plasma 25(OH)D concentrations, VDR protein expression in tumor tissue, and mRNA expression of the VDR target gene *CYP24A1*, we did not find consistent evidence that showed *CYP27A1* to be strongly related to vitamin D signaling. However, these measures may not have fully captured an effect of exogenous vitamin D on *CYP27A1* expression, particularly if vitamin D is 25-hydroxylated directly within prostate cells without changing plasma 25(OH)D concentrations. This 25-hydroxylation step of vitamin D has indeed been observed in a nontumor prostate cell line, in which vitamin D also induced CYP27A1 expression (23). However, the 25-hydroxylase function of CYP27A1 may not be physiologically relevant in peripheral tissues as the prostate, but rather fulfilled by the microsomal 25-hydroxylase *CYP2R1* (24). Moreover, blood draws for 25(OH)D measurements preceded cancer diagnosis up to 9.8 years (median, 3.1 years). The correlation between two repeated 25(OH)D samples from HPFS participants over 3 years was relatively high ($r = 0.70$; ref. 25). Nevertheless, possible nondifferential misclassification of 25(OH)D at cancer diagnosis by using prediagnostic 25(OH)D may have added some degree of bias to the null. Ultimately, our observations lend no additional support to *CYP27A1* expression in prostate cancer tissue being tightly controlled by vitamin D signaling. We also assessed whether *TMPRSS2:ERG* status was associated with *CYP27A1* expression. Bidirectional influences between vitamin D signaling, including VDR and *CYP24A1*, and *TMPRSS2:ERG* have been reported in prostate cancer cell lines (26, 27), and we previously observed that ERG-positive tumors have higher VDR expression (19). In this study, we only observed a modest association between ERG status and *CYP27A1* expression, and *CYP24A1* expression did not differ by ERG status (data not shown).

In breast cancer, several studies found *CYP27A1* expression to be higher in high-grade compared with low-grade cancers (7, 8). In prostate cancer, a relatively strong, inverse relationship with Gleason grade has been reported previously (11) and was confirmed by our data. *CYP27A1* expression has also been reported to be lower in castration-resistant cancer tissue compared with tissue from castration-sensitive tumors (28). How *CYP27A1* expression would be associated with risk of clinically relevant outcomes, such as metastases or cancer-related death, was unknown. Our data indicated an approximately 2.6-fold higher odds of lethal cancer among the 25% patients with the lowest *CYP27A1* expression (first quartile), compared with the 25% with the highest expression (fourth quartile; Table 2). Given the tight association of *CYP27A1* and Gleason grade, it is unsurprising that these estimates were attenuated considerably when additionally adjusting for Gleason grade (OR, 1.76; 95% CI, 0.75–4.17). *CYP27A1* does not appear to be well suited as a prognostic marker. Our results are supported by a previous study that included a larger set of patients with prostate cancer from HPFS and found single-nucleotide polymorphisms within *CYP27A1* to be associated with the risk of lethal disease (18); however, we do not know if and how these single-nucleotide polymorphisms influence *CYP27A1* mRNA expression. While statistical power for interaction testing was low, we did not find that the association of *CYP27A1* expression and lethal disease differed across levels of cholesterol synthesis enzyme expression (Table 2).

Our results may be informative for mechanistic studies in both prostate and breast cancer. In preclinical breast cancer models, added 27-hydroxycholesterol stimulated tumor growth, acting partially as an endogenous selective estrogen receptor modulator (SERM; refs. 7, 29, 30). Upregulated cholesterol synthesis and production of 27-hydroxycholesterol in estrogen receptor–positive breast cancer under antiestrogen therapy has been suggested as a mechanism of therapy resistance (31, 32). Consequently, it has been suggested that SERM effects of 27-hydroxycholesterol are responsible for the association of lower *CYP27A1* expression with worse prognosis in breast cancer (33).

This association was most pronounced in premenopausal patients with estrogen receptor–positive tumors (8). In our study of prostate cancer, we also found a moderately strong association of lower *CYP27A1* expression with higher risk of lethal disease. Besides cholesterol accumulation as one potential mechanism, additional SERM-related mechanisms could be contributing. Estrogen receptor beta is expressed in at least a subset of prostate tumors (34), and future studies might need to consider 27-hydroxycholesterol or *CYP27A1* when studying estrogen receptor expression in prostate cancer.

It should be noted that we measured mRNA levels of *CYP27A1* and not protein expression. We are unaware of a study directly comparing mRNA and protein levels for CYP27A1 within the same patients. In a small number of breast tissue samples, CYP27A1 protein appeared to show changes in the opposite direction than *CYP27A1* mRNA (8); it is unclear how *CYP27A1* mRNA and CYP27A1 protein were associated on a individual-patient level. In a similarly designed study of prostate cancer tissue, CYP27A1 protein expression was lost in the tumor epithelium in contrast to normal glands, consistent with observations on the mRNA level (11). In this study, for which the Gleason grade distribution was unknown, only about a quarter of tumors were found to express CYP27A1 protein (11). If one assumed that CYP27A1 protein expression was lost in three quarters of tumors even in our study population, this could explain why risk estimates are relatively similar across the lowest three quartiles of *CYP27A1* mRNA expression. An additional limitation of our study is that only a relatively small subset of patients had prediagnostic plasma samples, which may have contributed to the null results for plasma 25(OH)D.

In summary, we found low intratumoral *CYP27A1* mRNA expression to be associated with higher markers of intratumoral cholesterol synthesis, higher Gleason grade, and a higher risk of lethal disease over long-term follow-up. We did not find strong and consistent associations of *CYP27A1* and circulating 25(OH)D or with two measures of intratumoral vitamin D signaling. Future studies should ideally attempt to directly measure intratumoral or circulating 27-hydroxycholesterol. Interestingly, serum 27-hydroxycholesterol concentrations were decreased by atorvastatin treatment and by vitamin D supplementation in two small-scale clinical trials among patients with breast cancer (8, 35). It remains to be seen how such interventions might affect intratumoral cholesterol and 27-hydroxycholesterol levels as well as clinical outcomes for patients with prostate cancer.

Disclosure of Potential Conflicts of Interest

P.W. Kantoff is a board member at Context Therapeutics; has ownership interest (including stock, patents, etc.) at Context Therapeutics, Tarveda, Placon, Seer, and DRGT; and is a consultant/advisory board member for Bavarian Nordic, DRGT, New England Research Institutes, Sanofi, Thermo Fisher, OncoCellMDX, Progenity, Seer, Tarveda, GE, Janssen, Merck, and Genentech/Roche. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: N.A. Khan, K.H. Stopsack, E.L. Giovannucci, L.A. Mucci, P.W. Kantoff
Development of methodology: N.A. Khan, P.W. Kantoff
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): E.L. Giovannucci, L.A. Mucci
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K.H. Stopsack, T. Gerke, P.W. Kantoff
Writing, review, and/or revision of the manuscript: N.A. Khan , K.H. Stopsack, E.H. Allott, T. Gerke, E.L. Giovannucci, L.A. Mucci, P.W. Kantoff
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): T. Gerke
Study supervision: P.W. Kantoff

Acknowledgments

We would like to thank the participants and staff of the Health Professionals Follow-up Study and the Physicians' Health Study for their valuable contributions. In particular, we would like to recognize the contributions of Liza Gazeeva, Siobhan Saint-Surin, Robert Sheahan, and Betsy Frost-Hawes. We would like to thank the following state cancer registries for their help: AL, AZ, AR, CA, CO, CT, DE, FL, GA, ID, IL, IN, IA, KY, LA, ME, MD, MA, MI, NE, NH, NJ, NY, NC, ND, OH, OK, OR, PA, RI, SC, TN, TX, VA, WA, WY. The authors assume full responsibility for analyses and interpretation of these data. The Health Professionals Follow-up Study is supported by the NIH (U01 CA167552). The Physicians' Health Study was supported by the NIH (CA097193, CA34944, CA40360, HL26490, HL34595). The Department of Defense supported K.H. Stopsack (W81XWH-18-1-0330) and P.W. Kantoff (W81XWH-14-1-0515). This research was funded in part by the Dana-Farber/Harvard Cancer Center Specialized Programs of Research Excellence program in Prostate Cancer 5P50 CA090381 and the NIH/NCI Cancer Center Support Grants P30 CA008748 and P30 CA06516. E.H. Allot was supported by an Irish Cancer Society John Fitzpatrick fellowship. K.H. Stopsack and L.A. Mucci are Prostate Cancer Foundation Young Investigators.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received October 3, 2018; revised January 9, 2019; accepted March 5, 2019; published first March 13, 2019.

References

1. Schaffner CP. Prostatic cholesterol metabolism: regulation and alteration. Prog Clin Biol Res 1981;75A:279–324.
2. Platz EA, Clinton SK, Giovannucci E. Association between plasma cholesterol and prostate cancer in the PSA era. Int J Cancer 2008; 123:1693–8.
3. Batty GD, Kivimaki M, Clarke R, Davey Smith G, Shipley MJ. Modifiable risk factors for prostate cancer mortality in London: forty years of follow-up in the Whitehall study. Cancer Causes Control 2011;22:311–8.
4. Mondul AM, Weinstein SJ, Virtamo J, Albanes D. Serum total and HDL cholesterol and risk of prostate cancer. Cancer Causes Control 2011;22: 1545–52.
5. Jacobs EJ, Stevens VL, Newton CC, Gapstur SM. Plasma total, LDL, and HDL cholesterol and risk of aggressive prostate cancer in the Cancer Prevention Study II Nutrition Cohort. Cancer Causes Control 2012;23:1289–96.
6. Stopsack KH, Gerke TA, Sinnott JA, Penney KL, Tyekucheva S, Sesso HD, et al. Cholesterol metabolism and prostate cancer lethality. Cancer Res 2016;76:4785–90.
7. Nelson ER, Wardell SE, Jasper JS, Park S, Suchindran S, Howe MK, et al. 27-Hydroxycholesterol links hypercholesterolemia and breast cancer pathophysiology. Science 2013;342:1094–8.
8. Kimbung S, Chang CY, Bendahl PO, Dubois L, Thompson JW, McDonnell DP, et al. Impact of 27-hydroxylase (CYP27A1) and 27-hydroxycholesterol in breast cancer. Endocr Relat Cancer 2017;24:339–49.
9. Feldman D, Krishnan AV, Swami S, Giovannucci E, Feldman BJ. The role of vitamin D in reducing cancer risk and progression. Nat Rev Cancer 2014;14: 342–57.
10. Shui I, Giovannucci E. Vitamin D status and cancer incidence and mortality. Adv Exp Med Biol 2014;810:33–51.
11. Alfaqih MA, Nelson ER, Liu W, Safi R, Jasper JS, Macias E, et al. CYP27A1 loss dysregulates cholesterol homeostasis in prostate cancer. Cancer Res 2017;77:1662–73.
12. Giovannucci E, Liu Y, Platz EA, Stampfer MJ, Willett WC. Risk factors for prostate cancer incidence and progression in the health professionals follow-up study. Int J Cancer 2007;121:1571–8.

13. Steering Committee of the Physicians' Health Study Research Group. Final report on the aspirin component of the ongoing Physicians' Health Study. N Engl J Med 1989;321:129–35.
14. Christen WG, Gaziano JM, Hennekens CH. Design of Physicians' Health Study II–a randomized trial of beta-carotene, vitamins E and C, and multivitamins, in prevention of cancer, cardiovascular disease, and eye disease, and review of results of completed trials. Ann Epidemiol 2000;10: 125–34.
15. Sinnott JA, Peisch SF, Tyekucheva S, Gerke T, Lis R, Rider JR, et al. Prognostic utility of a new mRNA expression signature of Gleason score. Clin Cancer Res 2017;23:81–7.
16. Epstein JI, Egevad L, Amin MB, Delahunt B, Srigley JR, Humphrey PA, et al. The 2014 International Society of Urological Pathology (ISUP) Consensus Conference on Gleason Grading of prostatic carcinoma: definition of grading patterns and proposal for a new grading system. Am J Surg Pathol 2016;40:244–52.
17. Penney KL, Pettersson A, Shui IM, Graff RE, Kraft P, Lis RT, et al. Association of prostate cancer risk variants with TMPRSS2:ERG status: evidence for distinct molecular subtypes. Cancer Epidemiol Biomarkers Prev 2016;25: 745–9.
18. Shui IM, Mucci LA, Kraft P, Tamimi RM, Lindstrom S, Penney KL, et al. Vitamin D-related genetic variation, plasma vitamin D, and risk of lethal prostate cancer: a prospective nested case-control study. J Natl Cancer Inst 2012;104:690–9.
19. Hendrickson WK, Flavin R, Kasperzyk JL, Fiorentino M, Fang F, Lis R, et al. Vitamin D receptor protein expression in tumor tissue and prostate cancer progression. J Clin Oncol 2011;29:2378–85.
20. Pettersson A, Graff RE, Bauer SR, Pitt MJ, Lis RT, Stack EC, et al. The TMPRSS2:ERG rearrangement, ERG expression, and prostate cancer outcomes: a cohort study and meta-analysis. Cancer Epidemiol Biomarkers Prev 2012;21:1497–509.
21. Stopsack KH, Gerke TA, Andren O, Andersson SO, Giovannucci EL, Mucci LA, et al. Cholesterol uptake and regulation in high-grade and lethal prostate cancers. Carcinogenesis 2017;38:806–11.
22. Raza S, Meyer M, Goodyear C, Hammer KDP, Guo B, Ghribi O. The cholesterol metabolite 27-hydroxycholesterol stimulates cell proliferation via ERbeta in prostate cancer cells. Cancer Cell Int 2017;17:52.
23. Tokar EJ, Webber MM. Chemoprevention of prostate cancer by cholecalciferol (vitamin D3): 25-hydroxylase (CYP27A1) in human prostate epithelial cells. Clin Exp Metastasis 2005;22:265–73.
24. Jones G, Prosser DE, Kaufmann M. Cytochrome P450-mediated metabolism of vitamin D. J Lipid Res 2014;55:13–31.
25. Platz EA, Leitzmann MF, Hollis BW, Willett WC, Giovannucci E. Plasma 1,25-dihydroxy- and 25-hydroxyvitamin D and subsequent risk of prostate cancer. Cancer Causes Control 2004;15:255–65.
26. Kim JS, Roberts JM, Bingman WE III, Shao L, Wang J, Ittmann MM, et al. The prostate cancer TMPRSS2:ERG fusion synergizes with the vitamin D receptor (VDR) to induce CYP24A1 expression-limiting VDR signaling. Endocrinology 2014;155:3262–73.
27. Washington MN, Weigel NL. 1{alpha},25-Dihydroxyvitamin D3 inhibits growth of VCaP prostate cancer cells despite inducing the growth-promoting TMPRSS2:ERG gene fusion. Endocrinology 2010;151:1409–17.
28. Tamura K, Furihata M, Tsunoda T, Ashida S, Takata R, Obara W, et al. Molecular features of hormone-refractory prostate cancer cells by genome-wide gene expression profiles. Cancer Res 2007;67:5117–25.
29. Warner M, Gustafsson JA. On estrogen, cholesterol metabolism, and breast cancer. N Engl J Med 2014;370:572–3.
30. Umetani M, Domoto H, Gormley AK, Yuhanna IS, Cummins CL, Javitt NB, et al. 27-Hydroxycholesterol is an endogenous SERM that inhibits the cardiovascular effects of estrogen. Nat Med 2007;13:1185–92.
31. Simigdala N, Gao Q, Pancholi S, Roberg-Larsen H, Zvelebil M, Ribas R, et al. Cholesterol biosynthesis pathway as a novel mechanism of resistance to estrogen deprivation in estrogen receptor-positive breast cancer. Breast Cancer Res 2016;18:58.
32. Nguyen VT, Barozzi I, Faronato M, Lombardo Y, Steel JH, Patel N, et al. Differential epigenetic reprogramming in response to specific endocrine therapies promotes cholesterol biosynthesis and cellular invasion. Nat Commun 2015;6:10044.
33. Nelson ER. The significance of cholesterol and its metabolite, 27-hydroxycholesterol in breast cancer. Mol Cell Endocrinol 2018;466: 73–80.
34. Nanni S, Benvenuti V, Grasselli A, Priolo C, Aiello A, Mattiussi S, et al. Endothelial NOS, estrogen receptor beta, and HIFs cooperate in the activation of a prognostic transcriptional pattern in aggressive human prostate cancer. J Clin Invest 2009;119:1093–108.
35. Going CC, Alexandrova L, Lau K, Yeh CY, Feldman D, Pitteri SJ. Vitamin D supplementation decreases serum 27-hydroxycholesterol in a pilot breast cancer trial. Breast Cancer Res Treat 2018;167:797–802.
36. Greenland S, Mansournia MA, Altman DG. Sparse data bias: a problem hiding in plain sight. BMJ 2016;352:i1981.