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Original Article

ORIGINAL ARTICLE

Statin use linked with a decrease in the conversion from high-grade prostatic intraepithelial neoplasia (HGPIN) to prostate cancer

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Abstract

The roles of obesity, metabolic dysregulation and systemic inflammation to advance prostate carcinogenesis are unclear. This study investigates metabolic and inflammatory factors in the transition from high-grade prostatic intraepithelial neoplasia (HGPIN) to prostate cancer (PC). We prospectively followed 160 men diagnosed with HGPIN at biopsy and therefore at high-risk and clinically monitored for PC. Analyses investigated body mass index (BMI), waist circumference, waist–hip ratio (WHR), height, fat mass, lean mass percent body fat, NSAIDs, statins, metformin, diabetes, hypertension, hypercholesterolemia representing metabolic dysregulation on the risk of a PC diagnosis during follow-up. Systemic inflammation was estimated through measurement of 13 plasma cytokine levels. Statin use was significantly linked with overall PC at follow-up [odds ratio (OR) = 0.45, (0.23, 0.91), $P = 0.03$], with a somewhat stronger link with high-grade [OR = 0.39, (0.15, 1.04), $P = 0.06$] PC compared with low-grade PC [OR = 0.50, (0.23, 1.12), $P = 0.09$]. Non-statin cholesterol-lowering medications, BMI, WHR, diabetes, hypertension and percent body fat were not significantly associated with PC. Although blood IL-12p70, IL-2 and IL-1β levels were significantly lower among statin users, inflammatory markers were not significantly linked with PC and did not explain the observed relationship between statins and lower PC risk. In summary, this prospective study of HGPIN patients at high risk for PC finds that statin use was significantly associated with reduced risk of PC detection at follow-up. Systemic markers of inflammation did not mediate this association, suggesting that statins affect PC progression through alternative pathways.

Introduction

Prostate cancer (PC) remains the leading cancer diagnosis and the second leading cause of cancer-related death among USA men (1). This is a slow-growing tumor with controversial treatment options that may decrease patient quality of life. As a consequence, active surveillance is increasingly considered as an option for the treatment of low-risk PC. In this context, PC should be a prime target for interventions that slow tumor development among men at high-risk for PC or with small localized lesions such that PC surgery or other treatments can be avoided.

Deciding how best to intervene, however, has been challenging. Most PC risk factors such as race (1), inherited genetic variants (2) or somatic tumor markers (e.g. TMRPSS-ERG) (3) are not modifiable or poorly understood and difficult to target. Drugs such as finasteride block testosterone metabolism and reduce PC risk, but there are concerns that these drugs increase advanced PC risk (4,5). Results from the Selenium and Vitamin E Cancer Prevention Trial did not support the administration of either supplement (6), and investigations of diet, lifestyle or physical activity are inconsistent and have not translated to an approach to reduce PC risk (7–10). Alternatively, obesity appears to play a role in the development of advanced PC (11–13) and prostate tissue inflammation (14), while PC risk or progression may be inhibited by NSAIDs (15), statins (16,17) or metformin (18–20). A chemoprevention strategy based on such agents may

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Abbreviations

BMI	body mass index
BPH	benign prostatic hyperplasia
CI	confidence interval
DRE	digital rectal exam
FM	fat mass
HGPIN	high-grade prostatic intraepithelial neoplasia
LM	lean mass
OR	odds ratio
PC	prostate cancer
PSA	prostate-specific antigen
WC	waist circumference
WHR	waist–hip ratio
% BF	percent body fat

be promising; however, the literature is inconsistent, and the mediating pathways of the obesity/metabolism and PC link remain unresolved (21).

High-grade prostatic intraepithelial neoplasia (HGPIN) is a premalignant biomarker of PC and shares several characteristics with PC including enlarged epithelial cells with hyperchromatic nuclei and cell proliferation extending into the lumen (22–24). HGPIN also expresses fatty acid synthetase, selective DNA hypermethylation and TMRPSS-ERG gene fusion products similar to many PC samples (25). In this study, we created a cohort of men with HGPIN and without concurrent PC and followed these patients through subsequent prostate biopsy protocols for conversion to PC. This approach maximizes the opportunity to identify those metabolic factors which could affect prostate carcinogenesis after initiation and delay progression to a potentially fatal or aggressive tumor. Given that inflammatory pathways are thought to be involved in the early phases of prostate carcinogenesis and the development of HGPIN (26), we also measured 13 cytokine levels in blood collected at baseline, and explored the potential links with PC and the mediating role of inflammation on any observed link between identified risk factors with PC. Our results may identify the role of metabolic regulation in advancing HGPIN to PC, and may provide a new direction to avoid PC treatment in high-risk men.

Materials and methods

Study population

The Nashville Men's Health Study (NMHS) employs a multiclinic, rapid-recruitment protocol targeting men seeking a diagnostic prostate biopsy from a large urology group practice in Nashville, TN. Research recruiters approached a candidate prior to prostate biopsy. Eligibility criteria include (i) having a diagnostic prostate biopsy, (ii) age 40 years or older, (iii) English speaking, (iv) no exogenous androgen supplementation and (v) able to provide consent. If eligible, informed consent was obtained through a Vanderbilt IRB approved protocol prior to initiating data collection and biospecimen collection protocols.

It is standard of care for pathologists to report HGPIN at biopsy, such that the urologist may continue clinical follow-up. HGPIN was diagnosed using published criteria (22,23), and slides with suspected HGPIN were routinely sent to an external pathology lab for confirmation. We identified 313 patients diagnosed with HGPIN and without PC at initial biopsy. From medical chart review and patient contact, 160 had a second prostate biopsy after recruitment and were thus eligible to be diagnosed with PC. Review of medical charts and social security registries identified 38 deaths prior to a follow-up biopsy. There were no significant differences between participants with versus without a follow-up biopsy regarding baseline prostate-specific antigen (PSA) levels, prostate volume, body mass index (BMI), waist circumference (WC), waist–hip ratio (WHR), prior diabetes diagnosis, aspirin use, statin use or treatment for benign prostatic hyperplasia (BPH). However, HGPIN patients without a follow-up biopsy were recruited in the later years of recruitment (2012 or later: 5.1% versus 9.8%, $P = 0.02$), were significantly younger ($P < 0.001$) and more likely to be taking medication for hypertension (26.7% versus 32.1%, $P = 0.02$). However, hypertension medication use and year of recruitment were no longer associated with obtaining a follow-up biopsy after controlling for age.

Data collection

At recruitment, participants completed a baseline questionnaire to elicit detailed information on demographics, race/ethnicity, occupation, income, health history and lifetime weight. The trained recruiter measured weight (kg), height (within 0.1 cm) and circumference of the waist and hip using an anthropometric tape measure (Gullick II). Body composition [i.e. percent body fat (%BF), lean mass, fat mass] by bioelectrical impedance analysis was initiated approximately 5 years after recruitment was initiated (BIA; Tanita Corporation, Arlington Heights, IL). Measurement of lower urinary tract symptom severity was measured by the International Prostate Symptom Score (IPSS) (27). Current use of NSAIDs, hypertensive medication, cholesterol-lowering medications, diabetes medications and BPH medications were extracted from surgical medical records for the surgical biopsy procedure. The presence of hypertension, diabetes, hypercholesterolemia or BPH was determined by any reference to the condition in the medical record or taking any medication for treatment. Prebiopsy blood for serum and plasma (EDTA) was collected at recruitment and prior to the biopsy and prior to any drug administration for the biopsy procedure. Blood was immediately refrigerated after collection, then processed for serum or plasma, aliquoted and stored at –80°C on the same day of collection.

Inflammatory markers

Plasma cytokines under evaluation included GM-CSF, INF-γ, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-13, IL-12p70, IL-1β and TNF-α. Assays were conducted at the Vanderbilt University Medical Center Hormone Assay and Analytical Services Core using a high-sensitivity magnetic bead-based multianalyte panel from Millipore (Billerica, MA). All assays were conducted in duplicate and the average participant assay concentration as pg/ml. Coefficients of variation ranged from 4.7 to 6.8% across all analytes.

Statistical analysis

Analyses investigated obesity-related risk factors for conversion from HGPIN to PC during follow-up. Follow-up review identified 78 HGPIN patients who converted to PC at follow-up biopsy. Primary obesity exposures included BMI, WC, WHR, % body fat (%BF), total fat mass (kg) and total lean mass (LM, kg). Medications were grouped for analysis as statins, non-statins, aspirin, other NSAIDs, metformin and any antihypertensive medications. High-grade PC is defined as a Gleason sum score of 7 or more. Initial analyses compared PC outcomes to non-outcomes using chi-square or Wilcoxon tests. Since time to PC diagnoses was a function of clinical scheduling, we used multivariable logistic regression to calculate odds ratio (OR) and 95% confidence intervals (CIs) summarizing adjusted associations between obesity/medication variables and conversion to PC during follow-up. Standard models adjust for age, PSA and prostate volume as prior studies indicate a plausible link between obesity-related indices with PSA or prostate volume (28). Additional models were developed during the analysis as indicated in the Results section. Year of recruitment was not associated with PC and thus not included in the final model. Cytokines were analyzed as a continuous variable after natural log transformation and also as a categorical variable (low versus high) using the median value of each cytokine distribution. Mediation analysis was performed within the logistic regression framework using the PROCESS macro within SAS (29). Bootstrap sampling with 1000 iterations was used to generate CIs for indirect effects.

Results

Analysis includes 160 patients found with HGPIN at baseline biopsy and who also had at least one follow-up biopsy. Median age was 65 years and ranged from 41 to 85 years (Table 1). Approximately, 90% were recruited between 2003 and 2011, and the majority (78%) had one follow-up biopsy. The median PSA level was 5.6 ng/ml, and many had hypertension (53%), BPH (34%) or hypercholesterolemia (41%).

Table 1. Baseline study population characteristics

Factor	N	Min	25th	Median	75th	Max
Age (years)	160	41	59	65	69	85
PSA (ng/ml)	158	0.71	4.3	5.6	7.4	21
Prostate volume (ml)	154	12.0	32.0	45.0	63.0	567
BMI	160	20.1	25.8	27.9	31.3	47.5
Waist (cm)	160	78.7	97.8	104.1	111.8	148.6
WHR	160	0.85	0.99	1.01	1.06	1.19
Height (cm)	160	157.5	171.5	175.3	179.7	191.8
Fat mass (kg)	89	8.6	19.8	23.9	32.0	81.9
Lean mass (kg)	89	45.9	56.7	61.7	66.8	76.1
% Body fat	89	15.7%	24.1%	28.5%	32.7%	60.1%
					n	%
Comorbidity	Diabetes		Yes		16	10
			No		144	90
	Hypertension		Yes		84	53
			No		76	47
	Hypercholesterolemia		Yes		66	41
			No		94	59
	BPH		Yes		55	34
			No		105	66
Medication	Any statin		Yes		62	39
			No		98	61
	Any non-statin		Yes		14	8
			No		147	92
	Aspirin		Yes		68	43
			No		92	57
	Metformin		Yes		8	5
			No		152	95
# Biopsies	1				125	78
	2				29	18
	3–4				6	4
Year recruited	2003–2005				54	34
	2006–2008				43	27
	2009–2011				47	29
	2012–2016				16	10

n may be <160 due to missing data.
Body composition by bioelectric impedance analysis initiated in 2007.

A BMI >30 was marginally linked with high-grade PC (OR = 2.12, *P* = 0.09, age adjusted), but otherwise, body composition was not linked with either low-grade or high-grade PC (Table 2). PC at follow-up was not significantly associated with hypertension, BPH, diabetes, aspirin use or metformin use (Table 3). Additional control for PSA levels and prostate volume did not substantially alter these results.

In contrast, HGPIN patients with hypercholesterolemia were significantly less likely to be diagnosed with high-grade PC at follow-up [OR = 0.37 (0.14, 0.98), *P* = 0.04; Table 3]. Similarly, statin use was significantly linked with overall PC follow-up [OR = 0.45, (0.23, 0.91), 0.03], with consistent protective associations extending to low-grade PC and high-grade PC. Additional control for metformin and aspirin use in the model did not alter the association between statins and PC at follow-up [OR = 0.45 (0.21, 0.97), *P* = 0.04]. Interestingly, there was no indication that non-statin cholesterol-lowering medications were associated with PC [OR = 0.95 (0.29, 3.15), *P* = 0.93], although non-statin cholesterol-lowering medications were less frequently used.

Statin use was not significantly associated with prostate volume, PSA levels, height, BMI, diabetes or BPH treatment (Supplemental Table 1, available at *Carcinogenesis* Online). However, statin use was significantly associated with WHR, year of recruitment and other medications. Addition of WHR [OR = 0.46 (0.23, 0.91), *P* = 0.03] or recruitment year [OR = 0.47 (0.23, 0.95), *P* = 0.04] to the logistic regression model did not alter the protective link between statins and PC. Statins also remained significantly associated with PC after controlling for use of non-statin medications to treat hypercholesterolemia [OR = 0.43 (0.21, 0.87), *P* = 0.02]. Similarly, control for metformin and aspirin use did not alter the association between statins and PC at follow-up [OR = 0.45 (0.21, 0.97), *P* = 0.04].

We investigated the association between PC, statins and a panel of 13 blood cytokine levels as an index of systemic inflammation (Table 4). Although blood cytokine levels were not significantly associated with overall PC, increased IL12p70 was marginally associated with a reduced risk of high-grade PC after adjustment of PSA and prostate volume [OR = 0.59 (0.32, 1.06), *P* = 0.08]. Blood IL-12p70, IL-2 and IL-1β levels were significantly lower among statin users (Table 5). However, our mediation analysis found no cytokine marker significantly mediated the relationship between statins and PC (Supplementary Table 2, available at *Carcinogenesis* Online).

Discussion

Cancer high-risk cohorts that are clinically monitored for progression, such as colon adenoma, atypical ductal hyperplasia

Table 2. Associations between obesity and diagnosis of PC at follow-up

Index	Unit	PC	Cases	OR[a]	95% CI	*P*	Cases	OR[b]	95% CI	*P*
BMI	kg/m^2	All PC	81	1.02	0.95, 1.09	0.61	79	1.02	0.95, 1.10	0.59
		High grade	33	1.04	0.96, 1.14	0.34	32	1.06	0.96, 1.16	0.23
		Low grade	48	1.01	0.93, 1.09	0.90	47	1.01	0.93, 1.09	0.90
	≥30 versus <30	All PC	81	1.16	0.59, 2.28	0.67	79	1.13	0.57, 2.25	0.72
		High grade	33	2.12	0.89, 5.05	0.09	32	2.08	0.84, 5.16	0.11
		Low grade	48	0.78	0.98, 1.08	0.54	47	0.76	0.33, 1.74	0.52
WC	cm	All PC	81	1.01	0.98, 1.04	0.44	79	1.01	0.98, 1.04	0.42
		High grade	33	1.02	0.98, 1.05	0.29	32	1.02	0.99, 1.06	0.20
		Low grade	48	1.01	0.98, 1.04	0.66	47	1.01	0.98, 1.04	0.64
	≥102 versus <102 cm	All PC	81	1.40	0.72, 2.69	0.32	79	1.21	0.62, 2.38	0.58
		High grade	33	2.19	0.85, 5.64	0.11	32	2.07	0.76, 5.59	0.15
		Low grade	48	1.12	0.53, 2.35	0.76	47	1.00	0.47, 2.15	0.99
WHR	0.1 unit	All PC	81	1.29	0.78, 2.13	0.32	79	1.37	0.82, 2.30	0.23
		High grade	33	1.29	0.65, 2.56	0.46	32	1.41	0.69, 2.88	0.34
		Low grade	48	1.29	0.74, 2.27	0.37	47	1.39	0.78, 2.48	0.27
	≥1.0 versus <1.0	All PC	81	1.31	0.69, 2.46	0.41	79	1.47	0.77, 2.3	0.25
		High grade	33	1.59	0.68, 3.75	0.29	32	1.85	0.74, 4.60	0.19
		Low grade	48	1.17	0.56, 2.42	0.67	47	1.39	0.66, 2.93	0.39
Height	cm	All PC	81	0.98	0.93, 1.03	0.36	79	0.97	0.92, 1.02	0.26
		High grade	33	0.99	0.92, 1.06	0.68	32	0.98	0.91, 1.05	0.59
		Low grade	48	0.97	0.91, 1.03	0.34	47	0.97	0.91, 1.03	0.30
	≥175 versus <175 cm	All PC	81	0.88	0.46, 1.67	0.69	79	0.84	0.43, 1.65	0.61
		High grade	33	1.03	0.43, 2.47	0.94	32	0.92	0.36, 2.35	0.86
		Low grade	48	0.83	0.40, 1.72	0.61	47	0.82	0.38, 1.78	0.62
FM	kg	All PC	44	0.98	0.94, 1.02	0.22	42	0.97	0.93, 101	0.16
		High grade	21	0.97	0.92, 1.02	0.27	20	0.97	0.92, 1.03	0.28
		Low grade	23	0.98	0.94, 1.03	0.42	22	0.98	0.93, 1.03	0.37
LM	kg	All PC	44	0.98	0.91, 1.05	0.57	42	0.98	0.91, 1.06	0.61
		High grade	21	1.01	0.92, 1.12	0.78	20	1.03	0.92, 1.14	0.61
		Low grade	23	0.95	0.87, 1.04	0.29	22	0.96	0.87, 1.06	0.39
% BF	%	All PC	44	0.97	0.91, 1.02	0.24	42	0.96	0.90, 1.02	0.17
		High grade	21	0.95	0.88, 1.02	0.18	20	0.95	0.88, 1.03	0.20
		Low grade	23	0.98	0.92, 1.05	0.64	22	0.98	0.91, 1.05	0.52

[a]Adjusted for age.
[b]Adjusted for age, PSA and prostate volume.
High-grade PC: Gleason 7–10.
Common control group *n* = 73 HGPIN patients not diagnosed with PC at follow-up.

Table 3. Associations between comorbidity status and diagnosis of PC at follow-up

	PC	Cases	OR[a]	95% CI	*P*	Cases	OR[b]	95% CI	*P*
Hypertension	All	81	0.61	0.32, 1.17	0.14	79	0.72	0.37, 1.39	0.32
	High grade	33	0.77	0.33, 1.80	0.55	32	0.96	0.39, 2.35	0.93
	Low grade	48	0.54	0.26, 1.12	0.10	47	0.63	0.29, 1.34	0.22
Hypercholesterolemia	All	81	0.57	0.30, 1.10	0.09	79	0.52	0.26, 1.03	0.06
	High grade	33	0.43	0.18, 1.06	0.07	32	0.37	0.14, 0.98	0.04
	Low grade	48	0.71	0.34, 1.47	0.35	47	0.64	0.29, 1.39	0.26
Diabetes	All	81	0.94	0.33, 2.66	0.90	79	0.99	0.30, 3.16	0.98
	High grade	33	1.16	0.31, 4.30	0.82	32	1.18	0.29, 4.81	0.82
	Low grade	48	0.80	0.23, 2.82	0.72	47	0.74	0.17, 3.20	0.69
BPH	All	81	0.79	0.40, 1.53	0.48	79	0.87	0.43, 1.75	0.69
	High grade	33	1.13	0.48, 2.66	0.78	32	1.70	0.63, 4.62	0.30
	Low grade	48	0.60	0.29, 1.39	0.25	47	0.71	0.31, 1.60	0.40
Aspirin	All	81	0.73	0.38, 1.41	0.35	79	0.78	0.40, 1.53	0.47
	High grade	33	0.65	0.27, 1.55	0.33	32	0.60	0.24, 1.52	0.28
	Low grade	48	0.80	0.38, 1.69	0.56	47	0.87	0.40, 1.91	0.73
Statins	All	81	0.51	0.26, 0.98	0.04	79	0.45	0.23, 0.91	0.03
	High grade	33	0.45	0.19, 1.12	0.08	32	0.39	0.15, 1.04	0.06
	Low grade	48	0.56	0.27, 1.20	0.14	47	0.50	0.23, 1.12	0.09
Metformin	All	81	0.29	0.05, 1.50	0.14	79	0.32	0.06, 1.77	0.19
	High grade	33	0.31	0.03, 2.85	0.30	32	0.30	0.03, 2.96	0.30
	Low grade	48	0.26	0.03, 2.21	0.21	47	0.29	0.03, 2.64	0.27

[a]Adjusted for age.
[b]Adjusted for age, PSA and prostate volume.
High-grade PC: Gleason 7–10.

Table 4. Blood cytokine levels and PC

	Outcome	Cases	OR[a]	95% CI	P	Cases	OR[b]	95% CI	P
GM-CSF	All PC	74	1.12	0.83, 1.53	0.46	72	1.00	1.00, 1.01	0.56
	High grade	29	0.90	0.58, 1.40	0.64	28	1.00	0.99, 1.01	0.81
	Low grade	45	1.28	0.89, 1.85	0.18	44	1.00	0.99, 1.01	0.47
IFNγ	All PC	74	1.00	0.74, 1.35	0.99	72	1.00	0.73, 1.37	0.98
	High grade	29	0.80	0.53, 1.20	0.28	28	0.85	0.55, 1.31	0.45
	Low grade	45	1.14	0.80, 1.62	0.47	44	1.16	0.80, 1.67	0.44
IL-2	All PC	74	0.90	0.59, 1.38	0.63	72	0.91	0.59, 1.41	0.66
	High grade	29	0.74	0.41, 1.33	0.32	28	0.84	0.45, 1.59	0.60
	Low grade	45	1.01	0.62, 1.64	0.97	44	1.04	0.63, 1.73	0.88
IL-4	All PC	74	1.10	0.87, 1.39	0.42	72	1.08	0.85, 1.38	0.53
	High grade	29	0.87	0.63, 1.20	0.40	28	0.85	0.60, 1.20	0.35
	Low grade	45	1.25	0.96, 1.64	0.08	44	1.26	0.94, 1.67	0.12
IL-5	All PC	74	1.04	0.79, 1.38	0.77	72	1.03	0.77, 1.38	0.83
	High grade	29	0.78	0.51, 1.18	0.24	28	0.76	0.49, 1.18	0.22
	Low grade	45	1.21	0.88, 1.65	0.23	44	1.20	0.87, 1.66	0.27
IL-6	All PC	74	0.97	0.73, 1.28	0.81	72	0.98	0.73, 1.31	0.88
	High grade	29	0.76	0.51, 1.14	0.19	28	0.84	0.55, 1.29	0.42
	Low grade	45	0.10	0.81, 1.50	0.55	44	1.11	0.81, 1.53	0.51
IL-7	All PC	74	0.95	0.68, 1.32	0.76	72	0.90	0.64, 1.26	0.54
	High grade	29	0.69	0.44, 1.09	0.11	28	0.70	0.44, 1.13	0.14
	Low grade	45	1.14	0.78, 1.67	0.50	44	1.10	0.75, 1.62	0.63
IL-8	All PC	74	1.15	0.78, 1.69	0.49	72	1.15	0.77, 1.73	0.49
	High grade	29	0.82	0.46, 1.45	0.49	28	0.80	0.44, 1.48	0.48
	Low grade	45	1.35	0.88, 2.07	0.17	44	1.38	0.88, 2.17	0.16
IL-10	All PC	74	0.93	0.63, 1.36	0.70	72	0.90	0.61, 1.33	0.60
	High grade	29	0.83	0.50, 1.39	0.48	28	0.84	0.49, 1.43	0.52
	Low grade	45	1.01	0.66, 1.53	0.98	44	0.99	0.65, 1.51	0.97
IL-13	All PC	74	1.05	0.88, 1.24	0.58	72	1.05	0.88,1.25	0.59
	High grade	29	0.89	0.71, 1.12	0.32	28	0.90	0.71, 1.14	0.39
	Low grade	45	1.17	0.95, 1.43	0.13	44	1.17	0.95, 1.44	0.15
IL-12p70	All PC	74	0.88	0.62, 1.25	0.49	72	0.86	0.60, 1.24	0.42
	High grade	29	0.58	0.34, 0.98	0.04	28	0.59	0.32, 1.06	0.08
	Low grade	45	1.09	0.73, 1.63	0.66	44	1.10	0.72, 1.67	0.66
IL-1β	All PC	74	1.05	0.69, 1.60	0.83	72	1.05	0.67, 1.63	0.84
	High grade	29	0.68	0.36, 1.29	0.24	28	0.71	0.36, 1.43	0.34
	Low grade	45	1.29	0.79, 2.09	0.31	44	1.35	0.80, 2.26	0.26
TNFα	All PC	74	1.02	0.74, 1.42	0.90	72	0.99	0.70, 1.40	0.95
	High grade	29	0.74	0.46, 1.17	0.19	28	0.71	0.43, 1.19	0.19
	Low grade	45	1.25	0.85, 1.83	0.26	44	1.26	0.83, 1.89	0.28

Cytokines were natural log transformed and evaluated as a continuous variable. Thus, each OR is the effect for a 1 log unit increase in cytokine. Adjusted for age.
[A]Adjusted for age.
[b]Adjusted for age, PSA and prostate volume.
High-grade PC: Gleason 7–10.

Table 5. Statins and blood cytokine levels adjusted for age and PC status

Cytokine[a] (pg/ml)	Statin use		P
	Yes (*n* = 59)	No (*n* = 91)	
GM-CSF	44.8	55.2	0.25
IFN-γ	9.3	10.4	0.55
IL-2	2.6	3.5	0.04
IL-4	34.5	37.9	0.70
IL-5	2.4	2.5	0.83
IL-6	1.0	1.4	0.14
IL-7	2.8	3.4	0.21
IL-8	6.4	7.3	0.37
IL-10	9.9	11.2	0.41
IL-13	5.1	7.4	0.25
IL-12p70	1.8	2.5	0.04
IL-1β	1.7	2.4	0.01
TNFα	3.6	4.3	0.30

[a]Cytokine levels natural log transformed prior to analysis, and geometric means adjusted for age and PC status are reported.

or HGPIN cohorts, provide an opportunity to investigate and target cancer prevention strategies while minimizing detection biases related to differential health care practices. The primary strength of this study is the development of a new cohort of 160 HGPIN patients from a single urology clinic, with prospective follow-up over an extended period of time. At follow-up biopsy, 78 of these patients were diagnosed with PC. Initial analyses of obesity, including BMI, WC and WHR, found no significant association with the clinical conversion from HGPIN to PC at follow-up. Similarly, comorbidities such as diabetes or hypertension, or medications including NSAIDs or metformin, were not associated with clinical progression from HGPIN to PC. Alternatively, men taking a statin were significantly less likely to be diagnosed with PC, including a lower risk of both low-grade PC and high-grade PC, and after adjusting for differences in age, PSA levels and prostate size. From a panel of 13 blood inflammatory markers, statin users had significantly lower blood IL-1β, IL-2 and IL12p70 levels, and IL12p70 was also marginally significantly associated with high-grade PC at follow-up. However, mediation analyses found no evidence that the protective association between statin use and PC was mediated by any marker of systemic inflammation.

Statins represent a class of drugs that target the rate-limiting enzyme in hepatic cholesterol synthesis, reducing the risk of vascular events with infrequent and often well-tolerated side effects. Statins also inhibit PC cell proliferation *in vitro* across cell lines with varying degrees of androgen sensitivity and metastatic potential (i.e. LNCaP, DU145, PC3), suggesting statin use may delay PC progression to a potentially fatal phenotype (30). Two large prospective studies published in 2006 and 2007 reported a lower risk of advanced (stage III or above), metastatic or fatal PC among men taking a cholesterol-lowering drug for 5 or more years (16,17). These investigators reasonably presumed that most cholesterol-lowering drugs were statins. Similar results were reported in studies using pharmaceutical registries to estimate statin use (31,32) and studies of improved prognosis with statins following diagnosis and treatment (33–38). Any impact of statins on localized PC, high-grade PC or low-grade PC in these prior studies appears to be minimal (16,17,39,40). Indeed, statin use was not associated with the risk of either low-grade or high-grade PC in the placebo arm of the PC prevention trial where participants (baseline age 55 years or older, PSA < 3.0 ng/ml, normal DRE) are monitored under consistent protocols (41), suggesting statins provide little protection among otherwise healthy low-risk men. This HGPIN cohort does not necessarily generalize to a healthy sample of men at risk for PC, or to PC survivors after surgery or other treatment, but instead represents a clinical cohort with pathology indications that these men are at high risk for PC progression. Our results suggest statins reduce PC risk among high-risk men after initiation perhaps by inhibiting processes beyond the hyperplasia or dysplasia phases of PC progression.

A recurring concern in the investigations of statins and PC derives from whether clinical studies adequately control for detection biases induced by the effect of statins on increased PSA testing, lower PSA levels and a smaller prostate size (42–44). For example, increased PSA testing among statin users with health care access could lead to earlier PC detection, potentially decreasing the detection of advanced PC in the future. Most prior studies control for the number of PSA tests, but whether this is sufficient to separate PC screening and detection from PC pathophysiology is not always clear. Lower PSA levels among statin users could lead to the appearance of a protective association and perhaps, more so with localized PC with PSA levels near a clinical decision level. However, this assumes that the decrease in PSA from statin use is uniform across localized PC with low PSA levels compared with advanced PC, which can present with much higher PSA levels. This assumption may not be valid, however, as analyses of change in PSA levels with initiation of statins in a Veterans Administration clinical cohort found PSA declines were greater with statin use among men with higher prestatin PSA levels (43). PSA levels in this VA cohort of healthy men were low (median = 0.9 ng/ml), however, and the effect of statins on PSA change among men with elevated PSA levels (20 ng/ml or more) consistent with advanced or metastatic PC remains unclear. In this study, median PSA was approximately 5.6 ng/ml, and most participants were initially referred for biopsy in response to an elevated PSA level. Unlike PC patients under active surveillance or monitored for biochemical recurrence following surgery where PC progression is estimated by an increase in PSA change beyond a threshold, PSA levels play less of a role in PC detection following HGPIN diagnosis. In this study, the number of follow-up biopsies was not associated with baseline PSA levels ($P = 0.54$) or with follow-up PSA levels in the subset of participants with available data ($n = 68$, $P = 0.83$). Furthermore, all participants received at least one follow-up biopsy, and we also evaluated the impact of the number of follow-up biopsies, indicating our results are unlikely a consequence of any effect of statins on PC detection.

Inflammation is believed to play an important role in the early phases of prostate carcinogenesis (26). Statins alter the activity of Ras and Ras-like proteins, CEBPE/F, p21, p27 and elements of the MAPK and PI3K-ATK pathways (45), reducing NF-κB activation and a pro-inflammatory response (46). Prior studies have also reported an interaction between statins and anti-inflammatory NSAIDs such that the combined use further reduces PC risk beyond statin use alone (17,30,32). However, we found little evidence for interaction between statins and aspirin use on PC in this study (P-interaction = 0.49), and the statin–PC association was stronger without concurrent aspirin use [OR = 0.39 (0.14, 1.11) $P = 0.08$, age adjusted] than within aspirin users [OR = 0.67 (0.25, 1.79), $P = 0.42$, age adjusted]. We also assayed 13 cytokine levels in blood collected at baseline. Although blood levels of IL-1β, IL-2 and IL-12p70 were significantly lower among statin users, consistent with a lower level of systemic inflammation, no measured cytokine was found to mediate the significant protective link between statin use and PC. These results suggest that systemic inflammatory signaling may not be the pathway linking statins to PC in this high-risk cohort, but alternatively, the mechanism may involve altered lipid levels, steroid hormone metabolism or prostate cell regulatory pathways.

This study has several limitations. The clinical significance of HGPIN is controversial as rates of PC detection following HGPIN have varied over time and with differences in screening and detection protocols (23). We did not have data on the presence of multifocal HGPIN. The majority of HGPIN cases were non-Hispanic white, and thus, our results may not generalize to other race/ethnicity groups. Not all HGPIN cases received a follow-up biopsy, and thus, we cannot make a statement about undiagnosed PC in those with incomplete follow-up. However, we found that follow-up biopsy status was not significantly related to statins, aspirin, BMI or other factors under study, suggesting selection bias at follow-up among statin users is unlikely to explain the significant association between statin use and PC. We could not ascertain statin use during the follow-up period but rather assume that statin use is persistent during the study period as would be typical to control hyperlipidemia. The detection of PC via prostate biopsy has inherent limitations, and it is possible that a portion of baseline HGPIN patients had undetected PC. We repeated our analysis after removing PC cases diagnosed within 1 year of recruitment in an attempt to remove as many prevalent PCs at baseline as possible, and our results did not change.

In this prospective study of HGPIN patients at high risk for a future PC, we report that statin use significantly reduced the risk of PC detection at follow-up. Elevation of systemic markers of inflammation did not mediate this association, suggesting that statins affect PC progression through alternative pathways.

Supplementary material

Supplementary material can be found at *Carcinogenesis* online.

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