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Regular aspirin use and gene expression profiles in prostate cancer patients

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Abstract

Purpose—Pharmacoepidemiology studies suggest prognostic benefits of aspirin in prostate cancer. We hypothesized that aspirin induces transcriptional changes in tumors or normal prostate tissue.

Methods—We analyzed the prostatic transcriptome from men diagnosed with prostate cancer during follow up of the Physicians' Health Study 1 (PHS, $n = 149$), initially a randomized-controlled trial of aspirin. Aspirin target genes were identified through systematic literature review and a drug target database. We compared target gene expression according to regular aspirin use at cancer diagnosis and used whole-transcriptome Gene Set Enrichment Analysis to identify gene sets associated with aspirin use. Results were validated in the Health-Professionals Follow-up Study (HPFS, $n = 254$) and in Connectivity Map.

Results—Of 12 target genes identified from prior studies and 540 genes from the drug target database, none were associated with aspirin use. 21 gene sets were enriched in tumor tissue of aspirin users, 18 of which were clustered around ribosome function and translation. These gene sets were associated with exposure to cyclooxygenase inhibitors in Connectivity Map. Their association with cancer prognosis was U-shaped in both cohorts. No gene sets were enriched in normal tissue. In HPFS, neither the target genes nor the gene sets were associated with aspirin use.

Conclusions—Regular aspirin use may affect ribosome function in prostate tumors. Other putative target genes had similar expression in tumors from aspirin users and nonusers. If results

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are corroborated by experimental studies, a potential benefit of aspirin may be limited to a subset of prostate cancer patients.

Keywords

aspirin; transcriptome; ribosome; prognosis; prostate cancer

Introduction

Numerous pharmacoepidemiology studies have examined associations between regular aspirin use and prostate cancer prognosis [1–8]. Some studies report potential benefits of aspirin use on prostate cancer outcomes, while others report null associations. Drawing from RCTs primarily designed for non-cancer endpoints, a pooled patient-level analysis reported a hazard ratio of 0.52 (95% CI, 0.20 to 1.34) for aspirin and prostate cancer mortality after at least five years of follow-up [9].

Cellular and molecular effects of aspirin on carcinogenesis are likely multifold and are best studied in the context of colorectal carcinoma [10, 11]. In general, aspirin's active metabolite salicylate inhibits prostaglandin-endoperoxide synthases 1 and 2 (*PTGS1* and *PTGS2*, also COX1 and COX2). This post-transcriptional regulation via acetylation cannot be observed directly on a gene expression level. Several downstream effects and independent mechanisms are reflected by transcription changes, and aspirin also downregulates *PTGS2* expression [12, 13]. However, the molecular targets of aspirin in prostate cancer are unclear.

We hypothesized that aspirin effects reported from preclinical cancer research are mirrored by gene expression changes in prostate tissue after regular aspirin use in patients. We further hypothesized that long-term aspirin use may also cause alterations in the intratumoral gene expression of additional genes not identified in *in vitro* studies. We systematically reviewed the preclinical literature and used a drug target database to identify aspirin target genes in prostate cancer. In a prospective cohort that started as a large-scale RCT of preventative aspirin use, we assessed differences in these target genes according to aspirin use. We also used a discovery approach to identify other previously unexplored gene sets, and we validated these results in a second prospective cohort study and a database of drug effects on gene expression in cell lines [14, 15].

Methods

Study populations

The discovery cohort included a subset of participants in the Physicians' Health Study 1 (PHS) who reported a prostate cancer diagnosis over follow-up. PHS began in 1981 as an RCT of aspirin for prevention of cardiovascular disease and cancer among 22,071 healthy U.S. male physicians aged 40 to 84 years at baseline, and continued as a prospective cohort study [16]. The validation cohort was a subset of prostate cancer patients from the Health Professionals Follow-up Study (HPFS), a prospective cohort study of initially 51,529 U.S. male health professionals aged 40 to 75 years at baseline in 1986 [17].

Within PHS and HPFS, we have created a tumor biorepository of primary prostate cancer tissue from patients predominantly from curative-intent prostatectomy (92% of patients) [18, 19]. The current study included all patients with transcriptome expression profiling from the primary tumor (149 from PHS, 254 from HPFS). Designed as “extreme case-control studies”, the subcohorts with transcriptome profiling oversampled patients who died from prostate cancer or developed metastases (lethal cancer) and those with available blood samples. Linked to the tumor repository are data sources including biennial questionnaires, through which patients reported diet, lifestyle factors, diagnoses, and medications including aspirin use, systematic medical record review, and detailed ascertainment of death causes (98% and 99% complete in HPFS and PHS, respectively).

Aspirin exposure

During the RCT phase of PHS (1982–1988), participants were assigned to one tablet of aspirin 325 mg every other day or matching placebo. Nonrandomized aspirin exposure of any dose after unblinding of the trial was assessed through annual questionnaires and was measured as tablets per week, irrespective of dose per tablet. In HPFS, nonrandomized aspirin exposure, quantified as tablets per week, was assessed through biennial questionnaires. Current regular aspirin use was defined as three or more days of use weekly before prostate cancer diagnosis in PHS and as two or more days of use weekly in HPFS, both based on the most recent or, if missing, second most recent questionnaire prior to prostate cancer diagnosis.

Tissue profiling

mRNA expression profiling from high-density tumor areas was performed using the GeneChip Human Gene 1.0 ST array (Affymetrix, Santa Clara, CA), resulting in 20,254 unique genes (Gene Expression Omnibus accession number GSE62872), as described previously [20]. Additionally, separate mRNA expression profiling of normal prostate tissue adjacent to the primary tumor was performed for a subset of the cases.

Retrieval of aspirin target genes

We searched PubMed for all articles describing gene expression changes in prostate cancer cell lines or tissue associated with aspirin use. The search term was “(aspirin OR “acetylsalicylic acid” OR salicylate) AND prostate AND (gene OR protein OR expression OR *RNA OR *histochemistry) AND (“1900/01/01”[Date - Entrez] : “2017/04/30”[Date - Entrez])”. Articles were screened stepwise for relevance based on title and abstract and then underwent full-text review. We then extracted all genes that were reported to be potentially differentially expressed in response to aspirin as putative aspirin targets in prostate cancer. We further explored the reference lists of included articles, including selected literature on aspirin effects in other cancers. In a separate effort to obtain a more comprehensive set of putative aspirin target genes, we searched the database DSigDB (<http://tanlab.ucdenver.edu/dsigdb>) for “aspirin” and “acetylsalicylic acid” and selected all genes from different sources [21].

Statistical analysis

Baseline characteristics between regular aspirin users and non-users were visually inspected. To test for differences in categorical characteristics, Fisher's exact test was used.

In the discovery set (PHS), we first tested whether the mRNA expression (continuous) of aspirin target genes in tumor tissue differed between regular aspirin users and non-users (binary). The two sets of target genes, one obtained from our systematic review and one from DSigDB, were tested separately. To account for multiple hypothesis tests in linear regression models, a false-discovery rate (FDR) q -value < 0.25 was considered significant.

In a second step, we performed a discovery approach using Gene Set Enrichment Analysis (GSEA) in order to identify predefined sets of functionally related genes that differ according to aspirin use in tumor tissue [22, 23]. From the Molecular Signature Database version 5.2 of the Broad Institute, a total of 3,609 Gene Ontology biological process gene sets with at least 15 genes but less than 500 genes were selected [24]. Using GSEA software, all genes were ranked based on a signal-to-noise ratio, contrasting aspirin use versus non-use. Based on 2,000 phenotype-based permutations, a normalized enrichment score was calculated using a weighted Kolmogorov-Smirnoff statistic. Gene sets with an FDR q -value < 0.25 were selected for further analysis. Enrichment Map was used to visualize findings [25]. Leading edge genes were selected if present in a majority of functionally related gene sets. A meta-gene score of leading edge genes was constructed by summing their normalized expression levels.

In the primary analysis, the meta-gene score (continuous) was compared between regular aspirin users and non-users at diagnosis (binary) using linear regression. In a sensitivity analysis, the association of cumulative duration of regular aspirin use from cohort enrollment to prostate cancer diagnosis (categorical; <1 year/none, 1–5 years, 5–10 years, >10 years) and the meta-gene score were analyzed. To test for linear trend across categories of cumulative duration of use, ordinal indices were modeled as a continuous predictor. Multivariable models were adjusted for age at cancer diagnosis (continuous), calendar year of cancer diagnosis (categorical, periods: randomized phase of PHS and pre-prostate specific antigen [PSA] era, 1982–1988; start of observational PHS follow up and peri-PSA era, 1989–1993; PSA era, 1994–2005), body mass index (BMI) at cancer diagnosis (ordinal: <25 , 25–30, >30 kg/m²), current smoking status at cancer diagnosis (binary), statin use at cancer diagnosis (binary), and Gleason grade (ordinal: 5–6, 4+3, 3+4, 8, 9–10). To test whether associations between aspirin use and meta-gene score differed according to diagnosis year, a multiplicative interaction term between aspirin use and diagnosis period (ordinal indicator) was added to univariable regression models.

In the validation set (HPFS), linear regression analyses for the association between current regular aspirin use at diagnosis and target genes were performed, as described above. We used the validation set to test the association between regular aspirin use and the meta-gene score identified by GSEA in PHS. Additionally, we assessed whether aspirin use was associated with gene expression changes in adjacent normal prostate tissue, including both target gene analyses and GSEA, similar to tumor tissue.

We used Connectivity Map of the Broad Institute's Drug Repurposing Hub (<https://clue.io>), data version 1.0.0.1 and software version 1.0.1.7, to further validate leading edge genes [14, 15]. This database compares the provided gene list to gene expression patterns observed when cancer cell lines, including the prostate cancer cell lines PC3 and VCAP, were challenged with different concentrations of 2,911 different compounds, including aspirin and other NSAIDs. We explored the Integrated Connectivity Viewer for connectivity scores for aspirin, other NSAIDs, and the compound class NSAIDs/cyclooxygenase inhibitors.

Finally, the associations of the meta-gene score and clinical/pathological covariates (coded as above) were assessed in univariable linear regression models with *p*-values derived from likelihood ratio tests. Using logistic regression, the risk of lethal cancer across tertiles of the meta-gene score was assessed, and multivariable models included covariates as above plus cancer stage (categorical: pT1/T2, pT3, pT4/N1/M1) and, in a separate model, plus current aspirin use at diagnosis (binary).

All statistical tests were two-tailed. Unless otherwise specified, the type 1 error level was 0.05. The research was approved by the institutional review board at Harvard T.H. Chan School of Public Health and Partners Healthcare.

Results

Characteristics of study populations

Among 149 patients from the PHS discovery cohort, 98 (66%) were current regular aspirin users at the time of prostate cancer diagnosis (Table 1). Among 254 HPFS patients, 111 (44%) were aspirin users. Of the current non-users in PHS, 27 (56%) had previously used aspirin regularly, while 50 of the non-users in HPFS (35%) were prior users. 66% of all men were diagnosed after widespread adoption of PSA screening, and 59% of cancers were organ-confined (T1/T2 N0 M0). Adjacent normal prostate tissue was profiled for 81 patients from PHS and for 120 patients from HPFS.

There were no major differences in baseline characteristics among aspirin users and non-users in PHS and HPFS except for a disproportionally greater number of aspirin users in PHS who were diagnosed with prostate cancer between 1988 (unblinding of PHS) and 1993 (*p* = 0.025).

Identification and validation of aspirin target genes

Of 108 retrieved articles, 71 and 20 were excluded after review of the title and the abstract, respectively. Full-text review of 17 articles identified 12 target genes from eight articles: *ABCB* [26], *AXIN2* [27], *BCL2* [28, 29], *BIRC5* [30], *CCND1* [27], *MMP9* [31], NF- κ B (modeled as *NFKB1* and *RELA*) [28, 29, 32], *PLAU* [31, 32], *PTGER3* [33], *SERPINE1* [31], and *TIMP1* [31]. Three target genes were added based on other literature [10, 11]; these include *PTGS2* [12, 13] and the WNT/ β -catenin pathway members *CTNNB1* and *MYC*, which have been implicated in aspirin effects in colorectal cancer [34].

Current aspirin use at cancer diagnosis was not significantly associated with differences in expression of any of the 15 putative target genes in PHS in either tumor or normal tissue (all

$q > 0.41$; Supplementary Table 1). To evaluate for a type 2 error, we repeated analyses in the HPFS cohort and observed similar null results (data not shown).

The DSigDB database listed a total of 562 genes as potentially influenced by aspirin, of which 22 were not included on our gene expression chip. Current aspirin use in PHS was not significantly associated with any of the remaining 540 genes (all $q > 0.68$ and > 0.93 in tumor and normal tissue, respectively; data not shown).

Derivation of the aspirin-related meta-gene score in PHS

We identified 21 Gene Ontology biological process gene sets significantly enriched in the tumor tissue of regular aspirin users compared to non-users at $q < 0.25$ using GSEA; 9 gene sets were significant at $q < 0.05$ (Supplementary Table 1). There were no significantly enriched gene sets in non-aspirin users. In normal prostate tissue from PHS participants, there was no significant enrichment at $q < 0.25$ according to aspirin use.

Of the enriched gene sets in tumor tissue, 18 were clustered around ribosome function, as demonstrated in the Enrichment Map (Figure 1). Given prior evidence suggesting a potential role of aspirin in acetylation of various metabolic pathways, including translation [11], we constructed a meta-gene score of leading edge genes related to ribosome function and translation. We selected the 12 genes that were shared among all leading edge genes of 15 of the 18 pathways (*RPL3*, *RPL5*, *RPL10*, *RPL11*, *RPL12*, *RPL23A*, *RPL24*, *RPS5*, *RPS14*, *RPS17*, *RPS19*, and *RPSA*). All were genes encoding ribosomal proteins. Results were similar when instead we selected 67 leading edge genes shared among at least 10 of the 18 pathways (data not shown).

As expected, the meta-gene score was 5.86 standard deviations (SD) higher in tumor tissue of aspirin users than in non-users in PHS (95% CI, 2.86 to 8.87; $p < 0.001$). However, in a sensitivity analysis, no association between years of cumulative aspirin use and the meta-gene score was identified (data not shown).

Validation of the aspirin-related meta-gene score in HPFS and Connectivity Map

In tumor tissue from patients in HPFS, the meta-gene score was not significantly different between aspirin users and non-users (mean difference, -1.76 ; 95% CI, -4.14 to 0.61 ; $p = 0.15$). Sensitivity analyses were inconsistent (Table 2).

In adjacent normal prostate tissue, all associations between measures of aspirin use and the meta-gene score were null, both in PHS and HPFS (Supplementary Table 3).

The association of aspirin use and the meta-gene score in tumor tissue was nearly unchanged after multivariable adjustment in PHS (difference, 5.48 SD; 95% CI, 2.39 to 8.57) and HPFS (-1.67 SD; 95% CI, -4.09 to 0.74). Similarly, multivariable adjustment did not alter the findings in normal tissue (data not shown). Further, the association of aspirin use and the meta-gene score did not differ significantly between different time periods of prostate cancer diagnosis, between localized or more advanced cancers, or between patients who died from their cancer or developed metastases (lethal cancer) and those who did not (Table 2).

In Connectivity Map, when the 12 leading edge genes that the meta-gene score represented were tested in all cell lines, aspirin had a nonspecific connectivity score of -43.8 (on a scale of -100 to 100 , with score values closer to -100 and 100 indicating higher concordance between observed and experimental gene expression pattern). The NSAID with the highest connectivity score was eprizole with a score of 97.7 . Among compound classes, cyclooxygenase inhibitors ranked third among 84 classes with a score of 88.7 (Supplementary Figure 1). This score was higher in VCAP cells (98.1) than in PC3 (79.1). Results were similar when the 67 leading edge genes were used instead, except for scores for cyclooxygenase inhibitors in VCAP (82.3) and PC3 (98.9).

Meta-gene score and cancer prognosis

The meta-gene score was inversely associated with age at cancer diagnosis in HPFS (-1.92 SD per decade; 95% CI, -0.09 to -3.74) but not in PHS (0.25 SD per decade; 95% CI, -2.06 to -2.56). It was not significantly associated with Gleason grade, cancer stage, calendar year of diagnosis, BMI, smoking status, or statin use in either cohort. Both low and high meta-gene scores, compared to values around the mean, were associated with lethal cancer in both cohorts (Table 3). This association persisted after adjustment for baseline characteristics and, unlike expected, after adjustment for aspirin use.

Discussion

Using two different approaches, one incorporating prior evidence and one being a discovery method, we aimed to understand how intratumoral gene expression in prostate cancers diagnosed in men who were regular aspirin users differs compared to those from men who did not use aspirin. In this study, a ribosome-related gene expression pattern with some biological plausibility emerged as being related to aspirin exposure in our discovery cohort. Connectivity Map data suggested that the observed gene expression changes could indeed be due to NSAID exposure, and the gene expression score was associated with cancer prognosis. However, these results were not supported by the validation cohort and sensitivity analyses.

In colorectal cancer, RCTs of aspirin have shown clinically meaningful decreases in cancer incidence and mortality [10]. In prostate cancer, at least three RCTs of aspirin treatment for cancer outcomes are underway (ClinicalTrials.gov, NCT00234299, NCT02420652, NCT02804815). However, aspirin has notable clinical toxicities, especially in older men [35]. If it can be shown that long-term aspirin use is indeed associated with gene expression changes in aspirin-target pathways in prostate cancer tissue, it would be better justified to prioritize aspirin further among several other medications considered by drug repurposing efforts for prostate cancer treatment [36]. Understanding the molecular tumor response to aspirin could also aid in identifying subgroups of patients who are more likely to benefit from aspirin treatment. Of note, a previous randomized-controlled trial (RCT) of the aspirin-related non-steroidal anti-inflammatory drug (NSAID) celecoxib as an addition to androgen-deprivation therapy was stopped early because of perceived futility [37].

Our systematic review of aspirin effects in cell culture and animal prostate cancer models identified twelve genes, for which mRNA or protein expression changes were reported when

aspirin was introduced in these model systems. This included genes involved in prostaglandin signaling (*PTGER3*), inflammatory response (NF- κ B), apoptosis (*BCL2*, *BIRC5*, *CCND1*), extracellular matrix remodeling (*MMP9*, *PLAU*, *SERPINE1*, *TIMP1*), the WNT/ β -catenin pathway (*AXIN2*), and the efflux pump P-glycoprotein (*ABCB1*). None of these putative target genes was differentially expressed according to aspirin use in our study. Aspirin use at cancer diagnosis was also not associated with differences in expression of key molecules of prostaglandin metabolism (e.g., *PTGS2*) and of other component of the WNT/ β -catenin signaling pathway. Further, we analyzed a set of 540 putative aspirin target genes from DSigDB, a database of drug–target interactions that is partly curated and partly populated through computational methods [21]. None of these genes emerged as being associated with aspirin use.

The time period of aspirin exposure merits consideration. Experimental studies focused on short-term aspirin administration for hours to days. In contrast, in our study, aspirin users were exposed to the medication for at least one year before cancer diagnosis. We do not know whether they continued aspirin up until the day of surgery. Higher periprocedural bleeding risks from aspirin were known during the study period [38, 39]. It is therefore likely that most patients discontinued aspirin for several days before undergoing prostate biopsy or prostatectomy. We would thus only be able to observe longer-lasting downstream effects of aspirin use on the intratumoral transcriptome. These considerations may also apply to trials of aspirin before prostatectomy or prostate biopsy (e.g., ClinicalTrials.gov, NCT00234299).

To identify gene expression patterns associated with long-term aspirin use that might not have emerged in short-term experimental aspirin treatment, we used Gene Set Enrichment Analysis. Ranking expression differences in biologically defined pathways according to aspirin exposure using permutation tests, we found functionally clustered sets of genes related to ribosome function and translation to be enriched among aspirin users in PHS. One may speculate that acetylation of RNA molecules, as reviewed by Alfonso and colleagues [11], could be a mechanistic basis. Targets related to ribosome function and translation did neither emerge in our prostate cancer-centered literature review nor in a screen for aspirin-acetylated proteins [40].

We then tested the ribosome/translation-related leading edge gene set in Connectivity Map, a database of medication-related gene expression signatures of cancer cell lines. There was poor overlap of our gene set and aspirin-induced gene expression pattern changes. In contrast, the gene expression pattern induced by cyclooxygenase inhibitors (i.e., NSAIDs) as a compound class showed relatively good similarity to our gene set. Of note, Connectivity Map is similar to previously described sources of aspirin-related gene expression patterns in that its data stems from only short-term stimulation of cells within a timeframe of hours [14]. In contrast, attempts at validating the gene set, summarized as a meta-gene score, in HPFS showed associations in the opposite direction that were statistically different from PHS. A sensitivity analysis in PHS and HPFS according to cumulative duration of aspirin use also did not corroborate causality of the association of aspirin exposure and the meta-gene score. Collectively, while these validation steps are not supportive, the results from

PHS and Connectivity Map do suggest a potential effect of aspirin on ribosome function and translation in prostate cancer tissue.

When we evaluated how the meta-gene score was associated with cancer prognosis, we observed a U-shaped association in both PHS and HPFS. Both low and high expression levels of the meta-gene score, compared to average values, were associated with worse cancer prognosis. Given the sample size requirements for interaction tests, we were unable to formally assess whether this risk differed according to aspirin use. Assuming that aspirin would indeed increase the meta-gene score, then only patients with initially low scores would derive benefit, while those with otherwise average or high scores might be harmed.

Several limitations of our study may have contributed to partial null results. First, we relied on mRNA expression data only; cellular effects of aspirin are likely manifold and incompletely captured by changes in transcription [11]. Second, if aspirin has transient effects on prostatic gene expression, sensitivity analyses according to duration of use on a scale of years are unlikely to yield supportive evidence. Our dose-response analysis had inconclusive results, given that the vast majority of aspirin users in PHS had an intake of three or more tablets per week, the dose studied during the randomized phase of the study. Aspirin exposure was considerably lower in HPFS; exact doses were unknown. Third, while PHS initially was an RCT of aspirin, only 17 patients (11%) included in this analysis were diagnosed with prostate cancer before the randomized phase ended in 1988. However, differences in the meta-gene score according to aspirin use were virtually unchanged when adjusting for patient and tumor characteristics. Fourth, aspirin use is associated with significantly lower risk of lethal prostate cancer in the PHS cohort [8]. Therefore, cancers in aspirin users in PHS may have occurred despite aspirin use and hence may be driven by mechanisms unresponsive to aspirin. Our nested extreme case-control studies were oversampled for lethal cancers, potentially further underrepresenting cancers in which aspirin inhibited cancer progression.

In summary, we identified ribosome and translation-related genes sets that were differently expressed in long-term aspirin users compared to non-users in our discovery cohort. While findings in the validation cohort and sensitivity analyses were not supportive, this gene expression pattern was similar to an NSAID-induced gene expression pattern in Connectivity Map and it was associated with cancer prognosis. These results would at least require experimental confirmation, *e.g.* in presurgical aspirin trials, before they can be applied in guiding larger aspirin treatment studies for prostate cancer. Preclinically discovered aspirin targets could not be validated when assessing the prostatic transcriptome of long-term aspirin users. More generally, our study underscores that discovery-based results always require validation in independent cohorts. Inverse relations between aspirin use and prostate cancer prognosis observed in prior studies, if causal, may also be due to effects of aspirin on other tissues than the primary tumor or on the development of micrometastases. Studies aiming at identifying the mechanistic base may need to go beyond the primary tumor.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1.

Enrichment Map of 21 gene sets according to the Gene Ontology (GO) biological process database that were enriched at a false-discovery rate (FDR) $q < 0.25$ when comparing tumor tissue from aspirin users to non-users in the Physicians' Health Study 1 (PHS). Nodes represent gene sets and their size the number of genes. Lines and their thickness indicate overlap between gene sets.

Table 1

Baseline characteristics at cancer diagnosis of men with prostate cancer and tumor transcriptome profiling in the Physicians' Health Study 1 (PHS) and the Health Professionals Follow-up Study (HPFS), according to current regular aspirin use at prostate cancer diagnosis.

	PHS		HPFS	
	Aspirin users	Nonusers	Aspirin users	Nonusers
<i>n</i>	98	51	111	143
Age, median (range)	66 (51–80)	65 (52–81)	66 (49–80)	66 (47–77)
Diagnosis year				
1982–1988 ^a	7 (7)	10 (20)	8 (7)	5 (4)
1989–1993	40 (41)	12 (24)	32 (29)	44 (31)
1994–2005 ^b	51 (52)	29 (57)	71 (64)	94 (66)
Stage, <i>n</i> (%)				
pT1/T2	60 (61)	29 (57)	65 (59)	85 (59)
pT3	31 (32)	17 (33)	38 (34)	45 (31)
pT4/N1/M1	7 (7)	5 (10)	8 (7)	13 (9)
Gleason grade, <i>n</i> (%)				
5–6	18 (18)	15 (29)	10 (9)	14 (10)
7 (3+4)	33 (34)	15 (29)	35 (32)	56 (40)
7 (4+3)	19 (19)	9 (18)	34 (31)	40 (28)
8	15 (15)	6 (12)	11 (10)	10 (7)
9–10	13 (13)	6 (12)	21 (19)	23 (16)
PSA, <i>n</i> (%) ^c				
<4	9 (10)	6 (14)	10 (11)	8 (7)
4–10	53 (62)	25 (60)	55 (60)	64 (53)
10	24 (28)	11 (26)	27 (29)	48 (40)
Missing	12	9	19	23
Tissue source, <i>n</i> (%)				
Prostatectomy	88 (90)	44 (86)	101 (91)	135 (94)
TURP ^d	10 (10)	7 (14)	10 (9)	8 (6)
BMI, mean (SD) ^e	25.3 (2.9)	24.8 (2.8)	25.8 (3.0)	25.2 (3.0)
Current smoking, <i>n</i> (%)	4 (4)	2 (4)	11 (10)	13 (9)
Current statin use, <i>n</i> (%)	6 (6)	4 (8)	16 (14)	17 (12)
Cumulative aspirin use, <i>n</i> (%)				
<1 year (none)	0 (0)	21 (41)	0 (0)	93 (65)
1–4 years	18 (18)	10 (20)	45 (41)	40 (28)

	PHS		HPFS	
	Aspirin users	Nonusers	Aspirin users	Nonusers
5–10 years	27 (28)	11 (22)	46 (42)	6 (4)
>10 years	53 (54)	9 (18)	20 (18)	4 (3)
Frequency of aspirin use, <i>n</i> (%)				
1–2 tablets/week	0 (0)	5 (10)	Not available ^f	
3 tablets/week	78 (80)	0 (0)		
4 tablets/week	20 (20)	0 (0)		
Past aspirin user, <i>n</i> (%)	Not applicable	27 (56)	Not applicable	50 (35)
Lethal cancer, <i>n</i> (%)	17 (17)	12 (24)	37 (33)	46 (32)

^a Randomized-controlled phase of PHS. Enrollment in HPFS started in 1986.

^b After widespread adoption of prostate-specific antigen screening.

^c Serum prostate specific antigen, in ng/ml.

^d Transurethral resection of the prostate. Includes one PHS patient with lymph node sample.

^e Body mass index, in kg/m², with standard deviation.

^f Aspirin use was reported by HPFS participants as <2 tablets per week vs. 2 or more tablets per week.

Table 2

Differences in 12-gene meta-gene score expression () in tumor tissue according to measures of aspirin exposure, in standard deviations of mRNA expression, with 95% confidence intervals (95% CI).

	PHS		HPFS	
	95% CI	<i>p</i>	95% CI	<i>p</i>
Use at diagnosis				
Non-use	0.0 (Reference)		0.0 (Reference)	
Current use	5.9 2.9 to 8.9	<0.001	-1.8 -4.1 to 0.6	0.15
Use at diagnosis				
Never	0.0 (Reference)		0.0 (Reference)	
Past	3.3 -1.8 to 8.4	0.21	0.3 -3.0 to 3.6	0.85
Current	7.7 3.5 to 11.9	0.001	-1.7 -4.3 to 1.0	0.22
Frequency of use				
Non-use	0.0 (Reference)		Not available ^b	
1–3 tablets/week	6.0 2.8 to 9.2			
4 tablets/week	6.7 2.0 to 11.4	0.75 ^c		
Current use (vs. non-use) by stage		0.22 ^d		0.75 ^d
Localized cancer (T1/T2)	4.3 0.5 to 8.0		-0.6 -3.5 to 2.4	
Advanced cancer (T3 or higher)	8.1 3.0 to 13.2		-3.5 -7.5 to 0.4	
Current use (vs. non-use) by outcome		0.76 ^d		0.21 ^d
Non-lethal cancer	5.6 2.2 to 8.9		-0.7 -3.3 to 1.9	
Lethal cancer	6.7 -0.8 to 14.2		-3.9 -9.0 to 1.1	
Current use (vs. non-use) by year of cancer diagnosis		0.20 ^d		0.39 ^d
1982–1988 ^e	8.4 1.1 to 15.8		0.3 -14.8 to 15.4	
1989–1993	8.4 1.4 to 15.4		-4.3 -9.0 to 0.5	
1994–2005 ^f	4.0 0.3 to 7.7		-0.7 -3.5 to 2.1	

^a
^d *p* for linear trend.

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- ^b Aspirin use was reported by HPFS participants as <2 tablets per week vs. 2 or more tablets per week.
- ^c *p* for contrast to 1–3 tablets/week.
- ^d *p* for interaction.
- ^e Randomized-controlled phase of PHS. Enrollment in HPFS started in 1986.
- ^f After widespread adoption of prostate-specific antigen screening.

Table 3

Odds ratios for lethal prostate cancer (with 95% confidence intervals) associated with expression of the 12-gene meta-gene score in tumor tissue.

A. Unadjusted	PHS	HPFS
Linear, per 1 SD	0.98 (0.94 to 1.03)	1.01 (0.99 to 1.04)
<i>p</i>	0.50	0.33
Tertile 1	4.93 (1.50 to 16.2)	2.07 (1.03 to 4.15)
Tertile 2	1 (reference)	1 (reference)
Tertile 3	2.95 (0.86 to 10.1)	3.15 (1.59 to 6.24)
<i>p</i> _{trend} (linear)	0.27	0.19
<i>p</i> _{trend} (quadratic)	0.019	0.003
B. Adjusted for age and year of diagnosis^a		
Linear, per 1 SD	0.98 (0.94 to 1.03)	1.02 (0.99 to 1.05)
<i>p</i>	0.47	0.14
Tertile 1	6.79 (1.67 to 27.7)	1.82 (0.87 to 3.81)
Tertile 2	1 (reference)	1 (reference)
Tertile 3	4.27 (1.03 to 17.7)	3.14 (1.52 to 6.49)
<i>p</i> _{trend} (linear)	0.37	0.12
<i>p</i> _{trend} (quadratic)	0.012	0.008
C. Adjusted as in B plus baseline covariates^b		
Linear, per 1 SD	1.01 (0.95 to 1.07)	1.03 (0.99 to 1.07)
<i>p</i>	0.73	0.17
Tertile 1	5.02 (0.76 to 32.9)	1.76 (0.71 to 4.38)
Tertile 2	1 (reference)	1 (reference)
Tertile 3	6.42 (0.93 to 44.5)	3.51 (1.47 to 8.36)
<i>p</i> _{trend} (linear)	0.73	0.11
<i>p</i> _{trend} (quadratic)	0.055	0.022
D. Adjusted as in C plus aspirin use at diagnosis		
Linear, per 1 SD	1.25 (0.58 to 2.71)	1.03 (0.99 to 1.07)
<i>p</i>	0.57	0.17
Tertile 1	4.4 (0.65 to 29.6)	1.75 (0.70 to 4.33)
Tertile 2	1 (reference)	1 (reference)
Tertile 3	7.3 (1.00 to 53.1)	3.49 (1.47 to 8.31)
<i>p</i> _{trend} (linear)	0.51	0.011
<i>p</i> _{trend} (quadratic)	0.059	0.023

^a Adjusted for age at cancer diagnosis (continuous), year of cancer diagnosis (categorical, periods: randomized phase of PHS and pre-prostate specific antigen [PSA] era, 1982–1988; begin of observational PHS follow up and peri-PSA era, 1989–1993; PSA era, 1994–2005)

^b Additionally adjusted for body mass index at cancer diagnosis (ordinal: <25, 25–30, >30 kg/m²), current smoking status at cancer diagnosis (binary), statin use at cancer diagnosis (binary), Gleason grade (ordinal: 5–6, 4+3, 3+4, 8, 9–10), and cancer stage (categorical: pT1/T2, pT3, pT4/N1/M1).

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