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Gene expression profiling of prostate tissue identifies chromatin regulation as a potential link between obesity and lethal prostate cancer

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Abstract

Background—Obese men are at higher risk of advanced prostate cancer and cancer-specific mortality though the biology underlying this association remains unclear. We examined gene expression profiles of prostate tissue to identify biological processes differentially expressed by obesity status and lethal prostate cancer.

Methods—Gene expression profiling was performed on tumor (N=402) and adjacent normal (N=200) prostate tissue from participants of two prospective cohorts, diagnosed with prostate cancer from 1982–2005. BMI was calculated from questionnaire immediately preceding cancer diagnosis. Men were followed for metastases or prostate cancer-specific death (lethal disease) through 2011. We identified Gene Ontology biological processes differentially expressed by BMI using Gene Set Enrichment Analysis. Pathway scores were computed by averaging signal intensities of member genes. Odds ratios for lethal cancer were estimated using logistic regression.

Results—Of 402 men, 48% were healthy weight, 31% were overweight, and 21% were very overweight/obese. Fifteen gene sets were enriched in tumor tissue, but not normal tissue, of very overweight/obese vs. healthy weight men; five of which were related to chromatin modification and remodeling (false discovery rate < 0.25). Patients with high tumor expression of chromatin-related genes had worse clinical characteristics (Gleason grade >7, 41% versus 17%, p-value = 3×10^{-4}) and increased risk of lethal disease independent of grade and stage (odds ratio = 5.26, 95% confidence interval = 2.37 to 12.25).

Conclusions—This study improves our understanding of the biology of aggressive prostate cancer and identifies a potential mechanistic link between obesity and prostate cancer death that warrants further study.

Keywords

Body mass index; chromatin modification; chromatin remodeling; gene expression; obesity; prostate cancer; prostate cancer-specific mortality

Introduction

Identification of risk factors that drive prostate cancer progression has been a challenge. Obesity is a modifiable risk factor linked to advanced disease and worse cancer-specific outcomes among prostate cancer patients.[1, 2] Given high rates of obesity, an understanding of the relationship between excess body weight and worse prostate cancer outcomes has important clinical and public health implications. While several mechanisms have been proposed,[3, 4] what drives the association between obesity and aggressive prostate cancer remains poorly understood.

In this study, we sought to explore the link between excess body weight and lethal prostate cancer using whole transcriptome gene expression profiles of prostate tissue. We assessed

differences in gene expression in tumor and adjacent normal tissue according to prediagnosis body mass index (BMI) and examined the role of these genes in prostate cancer-specific mortality.

Materials and Methods

Study population

This study was nested among prostate cancer patients in the prospective Physicians' Health Study (PHS) and Health Professionals Follow-up Study (HPFS). PHS I and II began in 1982 and 1997 respectively as randomized primary prevention trials of aspirin and supplements among 29,067 U.S. physicians.[5, 6] HPFS is an ongoing cohort study of 51,529 U.S. health professionals followed since 1986.[7] Both cohorts completed annual or biennial questionnaires on lifestyle and health. Incident prostate cancer was confirmed by review of medical records and pathology reports. The studies were approved by institutional review boards at the Harvard T.H. Chan School of Public Health and Partners Health Care. Written informed consent was obtained from study participants.

Following confirmation of diagnosis, archival formalin-fixed paraffin-embedded (FFPE) prostate tissue specimens from radical prostatectomy (RP) or transurethral resection of the prostate (TURP) were retrieved from treating hospitals. Gene expression profiling was performed on a subset of the cases with available tissue using an extreme case sampling design. In total, 402 patients diagnosed between 1982 and 2005 were included, comprising 113 lethal cases (metastatic disease or prostate cancer death) and 289 indolent cases (survived ≥8 years after diagnosis without evidence of metastases). For 200 of these men we also profiled adjacent normal tissue.

Gene expression profiling

To measure gene expression in archival FFPE tissue specimens, whole-transcriptome amplification using the WT-Ovation FFPE System V2 (NuGEN) was paired with microarray technologies using the GeneChip Human Gene 1.0 ST microarray (Affymetrix) as previously described.[8, 9] Expression profiles were processed by regressing out technical variables including mRNA concentration, block age, batch (96-well plate), percentage of probes above background, log-transformed average background signal, and median of the perfect match probes for each probe intensity of the raw data. The residuals were shifted to the original mean expression values and normalized using the robust multi-array average method.[10, 11] We mapped gene names to Affymetrix transcript cluster IDs using the NetAffx annotations as implemented in Bioconductor annotation package pd.hugene.1.0.st.v1, resulting in 20,254 unique gene names. Gene expression data are available through Gene Expression Omnibus (GSE79021).

Anthropometric data

BMI was calculated using height and weight reported on questionnaires immediately preceding cancer diagnosis. In HPFS, self-reported measurements of weight show high validity.[12] The mean prediagnosis BMI was 25.4 kg/m^2 (range 19.0–36.8 kg/m^2) and the mean time between BMI measurement and prostate cancer diagnosis was 1.3 years (range

0–11.3 years). Because the number of men in our study above the World Health Organization cut-off for obesity (BMI ≥30 kg/m^2) was low (N = 27), we divided BMI into the following categories for subsequent analyses: 18.5 to <25 (healthy weight), 25 to <27.5 (overweight), and ≥27.5 kg/m^2 (very overweight/obese), with a sensitivity analysis using BMI ≥30 for the top category.

Clinical and follow-up data

Information about age and date of diagnosis, prostate-specific antigen (PSA) level at diagnosis, and clinical and pathologic stage was abstracted from medical records and pathology reports. Study pathologists provided a standardized histopathologic review of each case including Gleason grading. Information on the development of metastatic disease was collected through follow-up questionnaires. Review of medical records and death certificates were used to determine date and cause of death. Lethal prostate cancer was defined as distant metastases or prostate cancer-specific death with follow-up through March 2011 (PHS) or December 2011 (HPFS).

Statistical analysis

Linear regression as implemented in the Bioconductor package *limma* was used to assess differential expression of individual genes by BMI.[13] Gene Set Enrichment Analysis (GSEA)[14] was performed to identify the association between BMI and expression of 589 Gene Ontology (GO) Biological Process gene sets from the Molecular Signature Database v4.0, using software from the Broad Institute (http://software.broadinstitute.org/gsea/index.jsp). Genes were ranked based on a signal-to-noise metric comparing very overweight/obese (BMI ≥27.5 kg/m^2) to healthy weight (BMI 18.5 to <25 kg/m^2) men. An Enrichment Score (ES) was calculated for each gene set based on a weighted Kolmogorov-Smirnoff statistic and the top ranked genes contributing to the ES were identified as the leading edge subset. A positive ES indicated gene set enrichment at the top of the ranked list (upregulated gene set); a negative ES indicated gene set enrichment at the bottom of the ranked list (downregulated gene set). Significance was estimated using 10,000 phenotype-based permutations. The normalized enrichment score (NES) and false discovery rate (FDR) were used to identify the top GO biological processes differentially expressed by prediagnosis BMI status. Gene sets with FDR < 0.25 were considered for subsequent analyses. The Enrichment Map Cytoscape Plugin[15] was used to visualize GSEA results as gene set networks.

To further explore the five chromatin-related gene sets identified by GSEA, we created a “metagene” score representing chromatin gene expression by averaging the normalized (mean centered, variance scaled) expression values of the leading edge genes from these gene sets.

We used t-tests to compare mean scores between tumor and adjacent normal tissue, and Pearson correlations to measure the relationship between the score and BMI. We used logistic regression adjusted for age and year at diagnosis to calculate odds ratios (ORs) and 95% confidence intervals (CIs) for the association between the “metagene” score and lethal prostate cancer. P-values were from the Wald test. We adjusted for Gleason grade and

clinical stage to test whether the score independently predicted lethal cancer. Finally, we used logistic regression to evaluate whether the "metagene" score mediated the association between BMI and lethal prostate cancer, adjusting for age, date at diagnosis, Gleason grade, and clinical stage. Simple mean imputation was used for individuals missing clinical stage (N = 7).

R version 3.1.0 was used for all other analyses. All statistical tests were two-sided, with p-values < 0.05 considered statistically significant.

Results

Table 1 describes the clinical characteristics of the study population according to prediagnosis BMI. Among 402 men, 192 (47.8%) were healthy weight (BMI 18.5 to <25 kg/m^2), 126 (31.3%) were overweight (25 to <27.5 kg/m^2), and 84 (20.9%) were very overweight/obese (BMI ≥27.5 kg/m^2) prior to prostate cancer diagnosis. No statistically significant differences were observed for clinical characteristics across BMI categories. However, there was a suggestion of increased pathologic TNM stage with increasing BMI.

Gene sets enriched in prostate tissue of overweight and obese prostate cancer patients

We compared gene expression in the highest and lowest BMI categories. No individual genes were significantly differentially expressed by BMI in tumor or adjacent normal tissue after adjusting for multiple comparisons (data not shown). To test for sets of functionally related genes with subtle but coordinated changes in expression we applied GSEA.[14] GSEA identified fifteen gene sets upregulated and two gene sets downregulated in the tumor tissue of very overweight/obese vs. healthy weight patients with FDR < 0.25 (Figure 1, Tables S1, S2). Among these top results, there were several networks of overlapping gene sets involved in chromatin regulation, RNA processing, and cellular disassembly (Figure 2). These pathways were not differentially expressed in adjacent normal tissue, suggesting the results are tumor-specific (Tables S3, S4). To address differences in sample sizes for tumor and adjacent normal tissue, we repeated the GSEA on the subset of tumor samples that also had normal tissue data and found that 9 of 15 upregulated gene sets from the full analysis remained enriched at FDR < 0.25 (Tables S5 and S6). Finally, we ran a sensitivity analysis excluding 34 cases with gene expression assayed in TURP specimens. While the overall significance level decreased slightly due to a decrease in sample size, the top pathways remained largely unchanged (Tables S7-S12).

Characterization of chromatin gene set network

Five of the 15 gene sets enriched in tumor tissue of very overweight/obese patients included chromatin modification and remodeling genes involved in regulation of chromatin structure and function (Figure 2). All five of these chromatin-related gene sets were also ranked in the top ten in a sensitivity analysis using 30 kg/m^2 as the cutoff for the high BMI group (data not shown). Given the extensive interplay between epigenetics and metabolism and the critical role this interplay has in cancer, we chose to explore these findings further.[16] Similar analyses were done for the other gene sets identified but will not be the focus of this paper. These results can be found in the online supporting information (Tables S13–15).

To characterize the chromatin gene set network (Figure 2), we created a “metagene” score based on expression levels of the 35 genes that comprised the GSEA leading edge subset, which were all upregulated in the high BMI category (Table 2, see Table S16 for functional annotations). This “chromatin gene score” was greater in tumor tissue than in adjacent normal tissue (p-value = 2×10^{-4}). As expected, the “chromatin gene score” was positively associated with prediagnosis BMI in tumor tissue (p-value = 6×10^{-5}) but not in adjacent normal tissue (p-value = 0.46).

Table 3 illustrates the clinical characteristics of the cohort according to tumor “chromatin gene score”. The score was significantly positively associated with Gleason grade >7 (chi-square trend test p-value = 3×10^{-4}). It was positively, but not significantly, associated with pathologic stage T3/T4 disease (chi-square trend test p-value = 0.11).

Chromatin gene expression and lethal prostate cancer

The tumor “chromatin gene score” was positively associated with risk of lethal prostate cancer, with an OR of 6.78 (95% CI = 3.42–14.16) comparing extreme quartiles of the score. With adjustment for Gleason grade and clinical TNM stage, the OR for lethal prostate cancer was only slightly attenuated (OR = 5.26, 95% CI = 2.37–12.25) (Table 4). Adjustment for BMI did not alter these associations (results not shown). Results were similar in an analysis excluding TURP samples (Table S17).

BMI, chromatin gene expression, and lethal prostate cancer

To explore whether chromatin modification and remodeling mediates the relationship between excess body weight and lethal prostate cancer, we assessed the association between BMI and lethal cancer with and without adjustment for tumor “chromatin gene score”. Per 5-unit increase in prediagnosis BMI, the OR for lethal prostate cancer was 1.70 (95% CI = 1.16–2.53). Adjustment for chromatin score reduced this OR to 1.41 (95% CI = 0.94–2.12). Adjustment for Gleason grade and clinical TNM stage did not affect these results (results not shown).

Discussion

There is compelling evidence linking obesity to aggressive prostate cancer, but the biology underlying this relationship is unclear. We found several networks of gene sets involved in chromatin regulation, RNA processing, and cellular disassembly enriched in the tumor tissue of overweight and obese prostate cancer patients compared to those of healthy weight. Focusing on chromatin-related gene sets, we found that tumors with high expression of these genes had higher Gleason grades and were at increased risk of lethal prostate cancer, independent of grade. This suggests that obesity may promote tumor progression in part by influencing the epigenetic state of prostate cancer.

Epigenetic alterations are a common feature of cancer and are emerging as important drivers of tumor progression.[17] In prostate cancer, DNA methylation has been linked to metastatic disease.[18] In addition, extensive remodeling of the histone code occurs in prostate cancer and, in cooperation with DNA methylation, results in transcription of key oncogenes, microRNAs, and cancer biomarkers.[19] The current analysis identified genes encoding

chromatin remodeling factors and histone modification enzymes, including histone deacetylases (HDACs). These mechanisms work together to regulate gene transcription as well as other cellular processes including DNA replication and DNA damage repair.[20] HDAC overexpression in prostate cancer specimens has been linked to adverse tissue features and worse outcomes.[21] Furthermore, global histone modification patterns have been correlated with recurrence.[22]

Epigenetic regulation mediates the reversible effects of environmental exposures and lifestyle factors on carcinogenesis and tumor progression.[23] Observational and experimental studies have begun to provide evidence for epigenetic alterations related to obesity; however, most human studies in this area were conducted in blood or adipose rather than tumor tissue and have focused on DNA methylation.[24] Our findings suggest that obesity impacts epigenetic regulation in prostate tumor tissue through chromatin-related processes.

Interestingly, our analysis of normal tissue found no association between BMI and chromatin-related gene expression, suggesting that characteristics specific to tumor tissue may render susceptibility to the effects of excess body weight. Along these lines, our group previously demonstrated that obesity is linked to worse prognosis among men with tumors harboring the *TMPRSS2:ERG* gene fusion,[25] supporting the idea that obesity interacts specifically with certain molecular features of prostate cancer to drive tumor progression. Further investigation is needed to determine what role such tissue factors play in the epigenomic rewiring observed in overweight and obese patients.

Few studies have the ability to evaluate prostate cancer gene expression signatures of patients with high BMI and to relate such signatures to disease outcomes. One study of 12 patients evaluated gene expression profiles of prostate tumor and matching normal tissue according to BMI at treatment and found an association of BMI with altered expression of lipid metabolism and cholesterol homeostasis genes.[26] A second study focused on gene expression in periprostatic adipose tissue by BMI among 18 prostatectomy patients.[27] These authors found altered expression of genes involved in adipogenic/antilipolytic, proliferative/anti-apoptotic, and mild immunoinflammatory processes in obese subjects. Most recently, a gene expression study assessing metabolic pathway genes in relation to BMI and prostate cancer outcomes among patients who had undergone prostatectomy identified aberrant metabolic gene expression associated with prostate cancer metastases, but no relation was found with BMI.[28]

Strengths of our study include its prospective design, well-characterized data on clinical and pathologic measures, including re-review of Gleason score, and long-term follow-up allowing for the study of lethal prostate cancer as the outcome. The cohort is almost exclusively white men, and our conclusions may not apply to men of other ethnic groups. A potential limitation of the study is the use of BMI as an imperfect measure of obesity; however, BMI is the most widely used method for assessing adiposity in epidemiologic studies, and its correlation with obesity-related biomarkers is comparable to more direct measures of body fatness.[29]

We cannot completely rule out that obesity affects prostate cancer outcomes at least in part through its effect on detection and treatment, rather than through true biological differences in tumors themselves.[30] To address PSA detection bias, Ma *et al.* tested the association between BMI and prostate cancer mortality in the PHS cohort separately by pre-PSA and PSA screening eras and noted that the association remained largely unchanged.[31] While obese patients may receive different treatments than non-obese patients,[30] our study includes primarily men who underwent prostatectomy as curative treatment, which limits the possible impacts of treatment differences that are observed in the overall patient population.

This analysis provides a comprehensive look at BMI-associated gene expression alterations in prostate tumor tissue. The findings improve our understanding of the biology of aggressive prostate cancer and provide additional support for a causal relationship between excess body weight and prostate cancer survival. Many new epigenetic targets are emerging for the treatment of cancer. If confirmed, this study could provide insight into novel therapeutic targets that could augment lifestyle changes for men diagnosed with the disease.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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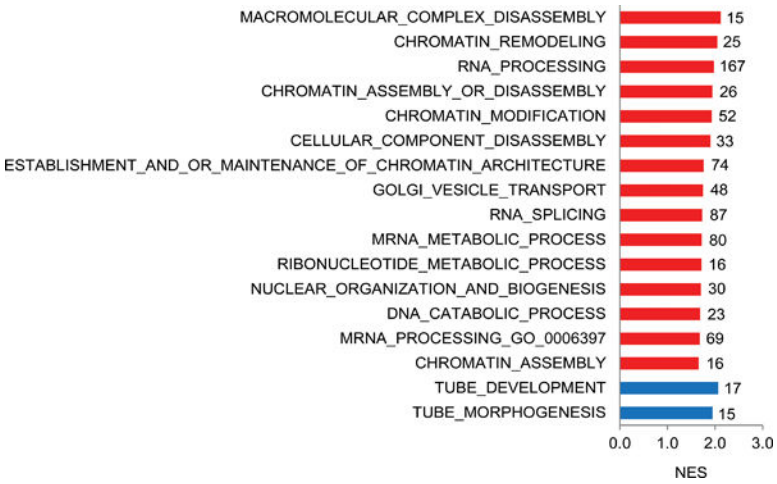

Figure 1.

Gene Ontology Biological Process gene sets enriched in tumor tissue of overweight/obese patients compared to healthy weight patients. Gene sets identified by Gene Set Enrichment Analysis with a false discovery rate less than 0.25 are shown. Gene Ontology terms are ordered according to the normalized enrichment signal. Numbers next to each bar represent the number of genes from the data set present in the particular biological process. Red bars represent upregulated gene sets and blue bars represent downregulated gene sets. NES = normalized enrichment score.

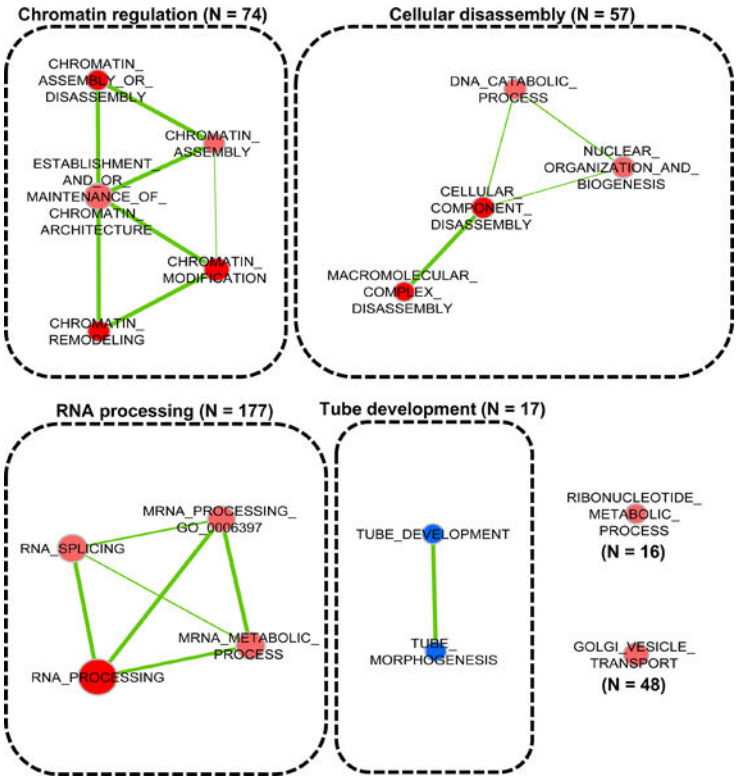

Figure 2.

Enrichment Map of Gene Ontology Biological Process gene sets enriched in tumor tissue of overweight/obese patients compared to healthy weight patients. Gene sets identified by Gene Set Enrichment Analysis with a false discovery rate less than 0.25 are shown with an overlap coefficient cut-off of 0.5. Each gene set is a node and links represent gene overlap between sets. The larger the node the more genes in the gene set. Thicker lines represent more gene overlap between sets. Upregulated gene sets are in red and downregulated gene sets are in blue. Darker nodes represent more significant nominal p-values. The total number of genes in each gene set network is indicated.

Table 1

Characteristics of 402 men diagnosed with prostate cancer from 1982 to 2005 in the Health Professionals Follow-up Study and the Physicians' Health Study according to prediagnosis body mass index

		Prediagnosis BMI			
Characteristic	**All men (N=402)**	**18.5 to <25.0 kg/m^2 (N=192)**	**25.0 to <27.5 kg/m^2 (N=126)**	**≥27.5 kg/m^2 (N=84)**	**P-value[d]**
Age at diagnosis, years, mean (SD)	65.7 (6.5)	65.8 (6.5)	66.3 (6.5)	64.5 (6.2)	0.16
Year of diagnosis, N (%)					
Before 1990 (pre-PSA era)	45 (11.2)	27 (14.1)	10 (7.9)	8 (9.5)	0.47
1990–1993 (peri-PSA era)	112 (27.9)	54 (28.1)	36 (28.6)	22 (26.2)	
After 1993 (PSA era)	245 (60.9)	111 (57.8)	80 (63.5)	54 (64.3)	
PSA at diagnosis, ng/ml, median (Q1, Q3)[a]	7.3 (5.3, 11.6)	7.9 (5.6, 12.0)	6.2 (4.8, 11.5)	7.7 (5.5, 10.7)	0.12
Pathologic TNM stage, N (%)[b]					
T2 N0 M0	218 (59.4)	111 (62.7)	67 (58.8)	40 (52.6)	0.33
T3 N0 M0	129 (35.1)	54 (30.5)	43 (37.7)	32 (42.1)	
T4/N1/M1	20 (5.4)	12 (6.8)	4 (3.5)	4 (5.3)	
Clinical TNM stage, N (%)[c]					
T1/T2 N0 M0	349 (88.4)	168 (88.9)	111 (91.0)	70 (83.3)	0.53
T3 N0 M0	27 (6.8)	13 (6.9)	6 (4.9)	8 (9.5)	
T4/N1/M1	19 (4.8)	8 (4.2)	5 (4.1)	6 (7.1)	
Gleason grade, N (%)					
<7	57 (14.2)	29 (15.1)	17 (13.5)	11 (13.1)	0.96
3+4	138 (34.3)	67 (34.9)	45 (35.7)	26 (31.0)	
4+3	102 (25.4)	45 (23.4)	33 (26.2)	24 (28.6)	
>7	105 (26.1)	51 (26.6)	31 (24.6)	23 (27.4)	
Tissue type, N (%)					
RP	368 (91.5)	177 (92.2)	115 (91.3)	76 (90.5)	0.89
TURP	34 (8.5)	15 (7.8)	11 (8.7)	8 (9.5)	
Cohort, N (%)					
HPFS	254 (63.2)	124 (64.6)	77 (61.1)	53 (63.1)	0.82

		Prediagnosis BMI			
Characteristic	**All men (N=402)**	**18.5 to <25.0 kg/m^2 (N=192)**	**25.0 to <27.5 kg/m^2 (N=126)**	**≥27.5 kg/m^2 (N=84)**	**P-value[d]**
PHS	148 (36.8)	68 (35.4)	49 (38.9)	31 (36.9)	

[a] 63 men missing PSA at diagnosis.

[b] 35 men missing pathologic TNM stage.

[c] 7 men missing clinical TNM stage.

[d] P-values based on ANOVA for age at diagnosis; χ^2 test for year of diagnosis, Gleason grade, and cohort; Kruskal-Wallis test for PSA at diagnosis; Fisher exact test for pathologic TNM stage, clinical TNM stage, and tissue type.

SD = standard deviation; Q1 = lower quartile; Q3 = upper quartile.

Table 2

Chromatin-related leading-edge genes identified by Gene Set Enrichment Analysis

Gene symbol	Gene name
ACTL6A	actin-like 6A
ARID1A	AT rich interactive domain 1A (SWI-like)
ASF1A	ASF1 anti-silencing function 1 homolog A (S. cerevisiae)
BNIP3	BCL2/adenovirus E1B 19kDa interacting protein 3
CARM1	coactivator-associated arginine methyltransferase 1
CHAF1A	chromatin assembly factor 1, subunit A (p150)
HDAC2	histone deacetylase 2
HDAC3	histone deacetylase 3
HDAC8	histone deacetylase 8
HELLS	helicase, lymphoid-specific
HIRIP3	HIRA interacting protein 3
HMGB1	high-mobility group box 1
INO80	INO80 homolog (S. cerevisiae)
KAT2A	K(lysine) acetyltransferase 2A
KDM4A	lysine (K)-specific demethylase 4A
MTA2	metastasis associated 1 family, member 2
NAP1L1	nucleosome assembly protein 1-like 1
NAP1L2	nucleosome assembly protein 1-like 2
NAP1L4	nucleosome assembly protein 1-like 4
PBRM1	polybromo 1
RBBP4	retinoblastoma binding protein 4
RSF1	remodeling and spacing factor 1
SAFB	scaffold attachment factor B
SET	SET nuclear oncogene
SIRT1	sirtuin 1
SMARCA5	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 5
SMARCC2	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily c, member 2
SUPT4H1	suppressor of Ty 4 homolog 1 (S. cerevisiae)
SUV39H2	suppressor of variegation 3–9 homolog 2 (Drosophila)
SYCP3	synaptonemal complex protein 3
TLK1	tousled-like kinase 1
TLK2	tousled-like kinase 2
TNP1	transition protein 1 (during histone to protamine replacement)
UBE2N	ubiquitin-conjugating enzyme E2N (UBC13 homolog, yeast)
WHSC1L1	Wolf-Hirschhorn syndrome candidate 1-like 1

Table 3

Characteristics of 402 men diagnosed with prostate cancer from 1982 to 2005 in the Health Professionals Follow-up Study and the Physicians' Health Study according to chromatin gene score

		Chromatin gene score				
Characteristic	**All men (N=402)**	**Quartile 1 (low) (N=101)**	**Quartile 2 (N=100)**	**Quartile 3 (N=100)**	**Quartile 4 (high) (N=101)**	**P-value[d]**
Age at diagnosis, years, mean (SD)	65.7 (6.5)	66.7 (5.8)	65.0 (6.4)	64.5 (6.7)	66.5 (6.8)	0.04
Year of diagnosis, N (%)						
Before 1990 (pre-PSA era)	45 (11.2)	11 (10.9)	8 (8.0)	13 (13.0)	13 (12.9)	0.83
1990–1993 (peri-PSA era)	112 (27.9)	29 (28.7)	26 (26.0)	26 (26.0)	31 (30.7)	
After 1993 (PSA era)	245 (60.9)	61 (60.4)	66 (66.0)	61 (61.0)	57 (56.4)	
PSA at diagnosis, ng/ml, median (Q1, Q3)[a]	7.3 (5.3, 11.6)	6.7 (5.2, 13.4)	7.2 (5.2, 10.2)	7.3 (5.4, 11.0)	8.1 (5.8, 11.5)	0.71
Pathologic TNM stage, N (%)[b]						
T2 N0 M0	218 (59.4)	57 (61.3)	60 (63.8)	58 (63.0)	43 (48.9)	0.38
T3 N0 M0	129 (35.1)	32 (34.4)	29 (30.9)	28 (30.4)	40 (45.5)	
T4/N1/M1	20 (5.4)	4 (4.3)	5 (5.3)	6 (6.5)	5 (5.7)	
Clinical TNM stage, N (%)[c]						
T1/T2 N0 M0	349 (88.4)	90 (90.0)	87 (87.0)	87 (89.7)	85 (86.7)	0.94
T3 N0 M0	27 (6.8)	7 (7.0)	7 (7.0)	5 (5.2)	8 (8.2)	
T4/N1/M1	19 (4.8)	3 (3.0)	6 (6.0)	5 (5.2)	5 (5.1)	
Gleason grade, N (%)						
<7	57 (14.2)	20 (19.8)	13 (13.0)	15 (15.0)	9 (8.9)	2×10^{-4}
3+4	138 (34.3)	39 (38.6)	45 (45.0)	36 (36.0)	18 (17.8)	
4+3	102 (25.4)	25 (24.8)	18 (18.0)	26 (26.0)	33 (32.7)	
>7	105 (26.1)	17 (16.8)	24 (24.0)	23 (23.0)	41 (40.6)	
Tissue type, N (%)						
RP	368 (91.5)	93 (92.1)	95 (95.0)	92 (92.0)	88 (87.1)	0.27
TURP	34 (8.5)	8 (7.9)	5 (5.0)	8 (8.0)	13 (12.9)	
Cohort, N (%)						
HPFS	254 (63.2)	54 (53.5)	70 (70.0)	61 (61.0)	69 (68.3)	0.06

		Chromatin gene score				
Characteristic	**All men (N=402)**	**Quartile 1 (low) (N=101)**	**Quartile 2 (N=100)**	**Quartile 3 (N=100)**	**Quartile 4 (high) (N=101)**	**P-value[d]**
PHS	148 (36.8)	47 (46.5)	30 (30.0)	39 (39.0)	32 (31.7)	

[a] 63 men missing PSA at diagnosis.

[b] 35 men missing pathologic TNM stage.

[c] 7 men missing clinical TNM stage.

[d] P-values based on ANOVA for age at diagnosis; χ^2 test for year of diagnosis, Gleason grade, and cohort; Kruskal-Wallis test for PSA at diagnosis; Fisher exact test for pathologic TNM stage, clinical TNM stage, and tissue type.

SD = standard deviation; Q1 = lower quartile; Q3 = upper quartile.

Table 4

Odds ratios and 95% confidence intervals for lethal prostate cancer according to chromatin gene score

Chromatin gene score	N lethal events	OR (95% CI)[b]	P-value[a]	OR (95% CI)[c]	P-value[a]
Continuous, per 0.1 units	113	1.22 (1.14, 1.31)	4×10^{-8}	1.18 (1.09, 1.28)	4×10^{-5}
Categorical					
Quartile 1 (low)	15	ref	1×10^{-7}	ref	5×10^{-5}
Quartile 2	23	2.13 (1.02, 4.57)		1.89 (0.78, 4.66)	
Quartile 3	25	2.25 (1.08, 4.82)		2.09 (0.88, 5.11)	
Quartile 4 (high)	50	6.78 (3.42, 14.16)		5.26 (2.37, 12.25)	

[a] Quartiles modeled as a continuous variable (quartile 1 = 0, quartile 2 = 1, quartile 3 = 2, quartile 4 = 3) to test for linear trend across categories.

[b] Adjusted for age and year at diagnosis (continuous).

[c] Additionally adjusted for Gleason grade (continuous: <7 = 0, 3+4 = 1, 4+3 = 2, >7 = 3) and clinical TNM stage (continuous: T1/T2 N0 M0 = 1, T3 N0 M0 = 2, T4/N1/M1 = 3).