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TITLE: Molecular Epidemiology Investigation of Obesity and Lethal Prostate Cancer

PRINCIPAL INVESTIGATOR: Ericka Noonan

CONTRACTING ORGANIZATION:
Harvard T. H. Chan School of Public Health
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14. ABSTRACT
The major objective of the PCRP postdoctoral training award was to provide training and opportunities for the principal investigator (PI) to further her development as an independent prostate cancer researcher in the field of molecular epidemiology. During the award period the PI performed research incorporating tissue-level biomarker data into epidemiologic studies of prostate cancer progression to investigate the relationship between obesity, the tumor microenvironment, and lethal prostate cancer. Using whole transcriptome gene expression profiling data, she identified a chromatin gene signature that is enriched in the tumor tissue of overweight and obese prostate cancer patients. Furthermore, this chromatin signature was associated with poor prognosis. Upon further validation, these findings support an epigenetic link between obesity and prostate cancer survival. The support of the award has provided many opportunities to enhance the professional development of the PI. The coursework and research activities accomplished over the past year have strengthened her research skills. The PI has presented her research at a number of meetings and conferences throughout the year and has recently submitted a manuscript of the findings from this award. During this period she has developed new collaborations that have led to exciting new research opportunities.

15. SUBJECT TERMS
Lethal prostate cancer, obesity, tissue biomarkers, gene expression, growth factor signaling, inflammation, angiogenesis, molecular epidemiology

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INTRODUCTION

A significant challenge in prostate cancer research is the identification of factors that drive disease progression. Obesity is a particularly compelling risk factor for lethal disease due to its high prevalence in the United States and its potential as a modifiable risk factor. In the United States, one-third of men are obese and another one third are overweight[1]. While not related to overall prostate cancer risk, obesity is strongly linked with risk of advanced disease and worse cancer-specific outcomes[2-4]. However, what drives the association between obesity and lethal prostate cancer is not well understood. Obesity dysregulates multiple hormonal and metabolic pathways and is associated with higher levels of insulin and insulin-like growth factor-1 (IGF-1), lowers level of adiponectin, lower levels of testosterone, and higher levels of inflammatory cytokines, all of which may be factors in prostate progression through direct effects on the tumor microenvironment[5]. In this proposal we seek to develop a better understanding of the link between obesity and lethal disease, in order to improve our ability to develop successful interventions strategies and therapies for men diagnosed with prostate cancer.

KEYWORDS

Lethal prostate cancer, obesity, tissue biomarkers, gene expression, growth factor signaling, inflammation, angiogenesis, molecular epidemiology

ACCOMPLISHMENTS

What were the major goals of the project?

Mentored Training Plan

The goal of the mentored training plan was to provide training and opportunities for the principle investigator (PI) that will promote her career development as an independent prostate cancer researcher in the field of molecular epidemiology. The following tasks were proposed: 1) meet with mentor and collaborators to discuss research progress, 2) attend seminars and present research results at prostate cancer meetings at HSPH and the Dana Farber/Harvard Cancer Center (DF/HCC), 3) complete coursework in advanced biostatistics methods, epidemiology study design, biomarker and pathology techniques for epidemiology studies, and clinical research strategies, and 4) attend scientific conferences including the AACR Annual Meeting 2015.

Research Project

The objective of the research project was to quantify the link between the prostate tumor microenvironment and prostate cancer mortality, with a focus on obesity as a driver of lethal prostate cancer. The proposal focused on three key obesity-related biological processes including growth factor signaling, inflammation, and angiogenesis, and highlighted the integration of tissue biomarker data with anthropometric and cancer outcome data to elucidate the relationship between obesity and lethal disease. The research project aimed to 1) evaluate the association between obesity and markers of growth factor signaling, inflammation, and angiogenesis in the tumor microenvironment, 2) define the link between obesity, markers of growth factor signaling, inflammation, and angiogenesis in the tumor microenvironment and lethal disease, and 3) perform a discovery analysis on the association between obesity and lethal prostate cancer using gene expression data.

What was accomplished under these goals?

Mentored Training Plan

Task 1: Meet with mentor and collaborators

The PI consulted regularly with her primary mentor (Lorelei Mucci) as well as other key collaborators on the project (Stephen Finn, Svitlana Tyekucheva, Christopher Sweeney).

Task 2: Attend seminars and meetings

The PI regularly attended weekly Epidemiology Seminars offered by the Department of Epidemiology at HSPH and Bioinformatics/Omics Seminars offered by the Channing Division of Network Medicine at Brigham and Women's Hospital. In addition, she attended and presented research progress at monthly Patho-epidemiology Group meetings and Prostate Cancer Epidemiology Group meetings throughout the year. She was an active participant in a monthly prostate cancer journal club led by students and postdoctoral fellow at HSPH, which she led on two occasions. She participated in the monthly DF/HCC SPORE in Prostate Cancer meetings and

was selected for a travel award to present her research findings at the Annual Prostate Cancer SPORE Retreat in Fort Lauderdale, FL in March 2015 (see Appendix 1). In addition, as part of the Transdisciplinary Prostate Cancer Partnership (ToPCaP), the PI participated in conference calls and attended the Annual ToPCaP Retreat in Iceland in September 2014. Furthermore, she had the opportunity to present research findings at a number of meetings throughout the year including the DF/HCC Celebration of Junior Investigators in Cancer Science see (Appendix 2), the HSPH Program in Genetic Epidemiology and Statistical Genetics Seminar Series (see Appendix 3), and the Meeting on Lipid Metabolism and Metabolic Alterations in Prostate Cancer at Dana-Farber Cancer Institute (see Appendix 4). Details of the presentations are provided below (see Publications, conference papers, and presentations).

Task 3: Complete Coursework
Dr. Noonan completed the Harvard Catalyst Introduction to Translational Medicine course in fall 2014. In November 2014, she shadowed Dr. Sweeney, a medical oncologist at Dana-Farber Cancer Institute, to gain a clinical perspective of the important challenges in prostate cancer research. In addition, Dr. Noonan audited EPI203 Study Design in Epidemiologic Research in spring 2015.

Task 4: Attend scientific conferences
The PI attended the AACR Annual Meeting 2015 in Philadelphia, PA in April 2015. Her abstract was chosen for a talk in the Molecular and Genetic Epidemiology of Cancer 4: New Insights Minisymposium (see Appendix 5). She was awarded a Scholar-in-Training Award from the AACR Molecular Epidemiology Work Group to attend this meeting. As a result of this funding she was also able to attend and present a poster at the AACR Metabolism and Cancer Conference in Bellevue, WA in June 2015 (see Appendix 6). Details of the presentations are provided below (see Publications, conference papers, and presentations).

Research Project
Aim 1: Evaluate the association between obesity and markers of growth factor signaling, inflammation, and angiogenesis in the tumor microenvironment
We examined the association between obesity measures (body mass index (BMI) and waist circumference) at diagnosis and immunohistochemistry (IHC) markers of insulin/IGF-1 signaling (insulin receptor, IGF-1 receptor, PTEN, pAKT, pS6 and stathmin), histologic measures of acute and chronic inflammation, and histologic measures of microvessel density and morphology in prostate tumor tissue. No significant associations were identified for the insulin/IGF-1 signaling markers or for the microvessel density and morphology measures. We identified a positive association between BMI at diagnosis and severity of chronic inflammation in tumor tissue; however the statistical significance was borderline (Table 1, unpublished data).

Table 1. Association between obesity measures and presence of acute and chronic inflammation

		BMI (kg/m^2)		Waist Circumference (inches)	
	No.	Average	P-value	Average	P-value
Acute inflammation					
No	657	25.8	0.822	38.1	0.352
Yes	242	25.9		38.4	
Chronic inflammation					
No	125	25.4	0.045	37.6	0.143
Mild	448	25.8		38.2	
Moderate	253	26.0		38.0	
Severe	73	26.4		39.1	

P-values from t-test for acute inflammation and linear regression for chronic inflammation

Aim 2: Define the link between obesity, markers of growth factor signaling, inflammation, and angiogenesis in the tumor microenvironment and lethal disease
We evaluated the association between the tissue-level markers described in Aim 1 and lethal prostate cancer overall and by obesity status. Specifically, we were interested in whether any of these biological factors

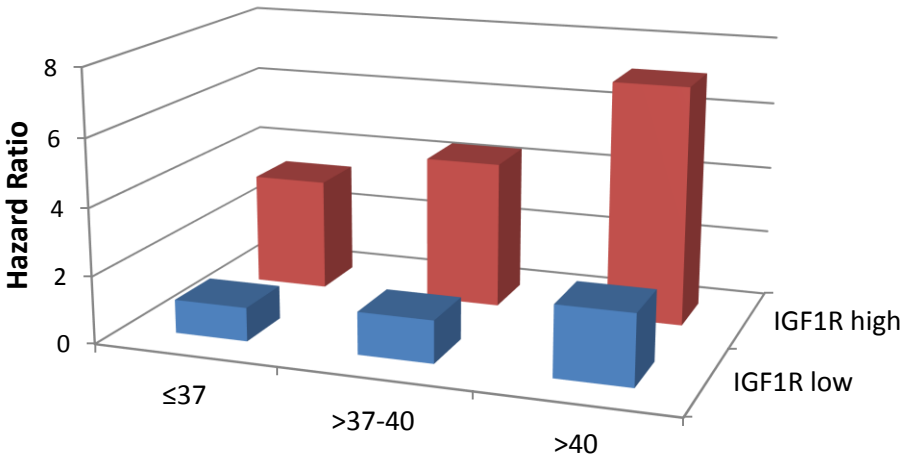
modified the association between obesity measures (BMI and waist circumference) at diagnosis and lethal prostate cancer (Aim 2a). Insulin/IGF-1 signaling: We did not identify any significant interactions for the insulin receptor, PTEN, pAKT, pS6, or stathmin. We did note, however, that the effect of waist circumference on prostate cancer survival after diagnosis is greater among men with tumors expressing IGF-1R compared to those not expressing the receptor (Table 2, unpublished data). The hazard ratio (95% CI) for lethal prostate cancer among men with low IGF-1R tumor expression was 0.90 (0.33, 2.45) per 8 inch increase in waist circumference compared to 3.37 (1.17, 9.7) among men with high IGF-1R tumor expression (interaction p-value = 0.072). These results were not observed when using BMI as the obesity measure. Figure 1 illustrates the hazard ratios for lethal prostate cancer according to cross-classified categories of waist circumference and IGF-1 receptor tumor status. Men with high IGF-1 receptor status and high waist circumference at diagnosis are at a seven fold greater risk of dying from prostate cancer compared to those with low receptor expression and healthy waist circumference. Inflammation: No significant interactions were observed between BMI or waist circumference and measures of acute and chronic inflammation. Angiogenesis: No significant interactions were observed between BMI or waist circumference and measures of microvessel density and morphology.

Table 2. Hazard ratios (95% Cis) for the association between obesity and lethal prostate cancer according to IGF-1 receptor tumor status

	IGF1R low [0,2]			IGF1R high [2.17,3]			
	#	N	HR (95% CI)	#	N	HR (95% CI)	P inter
Body mass index							
Continuous (per 5 kg/m^2)	33	486	1.27 (0.75, 2.16)	22	182	1.60 (0.82, 3.14)	0.533
Waist circumference							
Continuous (per 8 inches)	22	402	0.90 (0.33, 2.45)	20	143	3.37 (1.17, 9.7)	0.072

Cox proportional hazards regression model adjusted for age and year of diagnosis

Figure 1. Hazard ratios for lethal prostate cancer by cross-classified categories of waist circumference and IGF-1 receptor tumor status



Aim 3: Perform a discovery analysis on the association between obesity and lethal prostate cancer using gene expression data.

We evaluated whole transcriptome gene expression profiling data of prostate tumor and adjacent normal tissue to identify molecular alterations associated with a patient's prediagnosis body mass index (BMI). The results of this study are included in the manuscript "Gene expression profiling identifies chromatin regulation as a

molecular link between obesity and lethal prostate cancer" which has been submitted to *Science Translational Medicine* (under review, see Appendix 7). Briefly, Gene Set Enrichment Analysis identified fifteen gene sets upregulated in the tumor tissue of overweight/obese prostate cancer patients (BMI ≥ 27.5 kg/m^2; N=84) compared to healthy weight patients (BMI 18.5 to < 25 kg/m^2; N=192), five of which were related to chromatin modification and remodeling. Strikingly, these features were not found when comparing normal prostatic adjacent tissues of obese patients with healthy weight patients, suggesting that BMI might exert epigenetic modification only in cancer settings. Importantly, patients with high tumor expression of chromatin-related genes had worse clinical characteristics; 40.6% of men with high expression had Gleason grade >7 cancer compared to 16.8 with low expression (p-value = 3.21 x 10^{-4}). In addition, men with higher tumor expression of chromatin-related genes had a significantly increased risk of metastases or death from prostate cancer, independent of age and year at diagnosis, with an odds ratio of 6.78 (95% confidence interval = 3.42 to 14.16) for lethal outcome comparing extreme quartiles of expression. Of note, a number of the genes identified in this analysis were histone modifying enzymes, including acetyltransferases (KAT2A), deacetylases (HDAC 2,3,8 and SIRT1), methyltransferases (CARM1 and SUV39H2), and methylases (KDM4A). While these results warrant further study, they suggest that obesity may promote the metastatic potential of prostate cancer by influencing its histone profile.

What opportunities for training and professional development has the project provided?
This award has provided many opportunities to enhance the professional development of the PI. The coursework and research activities accomplished over the past year have strengthened Dr. Noonan's research skills related to the incorporation of tissue-level biomarker data into epidemiologic studies of prostate cancer progression. Furthermore, the PI has gained experience in preparing grants and manuscripts and has enhanced her communication skills through oral and poster presentations at numerous meetings and conferences. In addition, she has increased her professional network by forming new partnerships with basic science colleagues at the Dana-Farber Cancer Institute which has led to exciting new opportunities for reverse translation of the research findings from this project.

How were the results disseminated to communities of interest?
Results of the gene expression study were highlighted in a research news article on the Prostate Cancer Foundation website (pcf.org) on June 2, 2015.

What you plan to do during the next reporting period to accomplish the goals?

<u>Mentored Training Plan</u>
During the next reporting period, The PI will continue to meet with her mentor and collaborators for research and career guidance, attend seminars and meetings, and complete coursework that will strengthen her skill set. In addition, she will present research results at a scientific conference. The milestone for the mentored training part of this award (i.e. presentation of research results at a national meeting) has been achieved.

<u>Research Project</u>
Research-specific tasks that will be completed over the next reporting period include *Aim 3b: Quantify the extent to which the tissue-level markers from Aim 1 mediate the association between obesity and lethal disease using a mediation statistical analysis*. Dr. Noonan will attend the Methods for Mediation and Interaction course offered at HSPH in the spring to gain a better understanding of the methods required to complete this research aim. In addition, *Aim 3: Conduct Gene Set Enrichment Analysis using whole genome mRNA expression profiling data to find biological pathways enriched in the obesity-lethal prostate cancer relationship* will be expanded. Specifically, we plan to assess genes and predefined gene pathways for their ability to modify or mediate the association between obesity (e.g. BMI and waist circumference) and lethal prostate cancer to identify previously unknown biological mechanisms driving this relationship. In November, Dr. Noonan will attend the Harvard Catalyst Applications in Network Medicine: Gene Co-expression and Gene Regulatory Networks course to gain hands-on experience in analysis methods of gene co-expression networks and gene regulatory networks that can be incorporated into this research aim. In addition, we plan to follow-up on the chromatin gene expression results described previously (see Appendix 7). In collaboration with a group at Dana-Farber Cancer Institute, we have validated these findings in a high fat diet-induced obesity mouse model of prostate cancer. Going forward we plan to assess global histone modification patterns in human

prostate tissue to identify specific alterations linked to obesity. The milestones for the research part of this award include publication of 1-2 peer reviewed papers (18 months) and publication of 1-2 peer reviewed papers (24 months). We are on track for this goal with one manuscript currently submitted and another under preparation.

IMPACT

What was the impact on the development of the principal discipline(s) of the project?

Results from this study improved our understanding of the risk factors that promote prostate cancer progression and of the underlying biology that gives rise to more aggressive tumors. Specifically, in Aim 3 we identified a chromatin gene signature that is associated with poor prognosis. Future studies need to be done to validate these results; however, if confirmed, these findings have the potential to influence the clinical course of men diagnosed with prostate cancer through the identification of epigenetic biomarkers for high risk disease. Furthermore, epigenetic inhibitors that target HDACs have been tested in clinical trials and approved by the US Food and Drug Administration for use in treating specific cancers. Thus, understanding the specific role of obesity-related epigenetic events in prostate cancer progression could lead to new therapeutic targets to prevent or treat prostate cancer in both obese and non-obese men.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

CHANGES/PROBLEMS

Changes in approach and reasons for change

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them

Nothing to report.

Changes that had a significant impact on expenditures

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report.

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals.

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

PRODUCTS

Publications, conference papers, and presentations

Journal publications

Ebot EM, Gerke T, Labbé DP, Sinnott JA, Zadra G, Rider JR, Tyekucheva S, Wilson KM, Kelly RS, Shui IM, Loda M, Kantoff PW, Finn S, Vander Heiden MG, Brown M, Giovannucci EL, Mucci LA. Gene expression

profiling identifies chromatin regulation as a molecular link between obesity and lethal prostate cancer. [Submitted – see Appendix 7]

Labbé DP, Zadra G, **Ebot EM**, Mucci LA, Kantoff PW, Loda M, Brown M. Role of diet in prostate cancer: The epigenetic link. Oncogene. 2014 Dec 22; [Epub ahead of print – See Appendix 8]

Books or other non-periodical, one-time publications
Nothing to report.

Other publications, conference papers, and presentations
Using gene expression profiles of prostate cancer tissue to investigate the relationship between obesity and lethal prostate cancer, Meeting on Lipid Metabolism and Metabolic Alterations in Prostate Cancer, Dana-Farber Cancer Institute, Boston, MA, July 31, 2015 (oral presentation – See Appendix 4)

Identifying obesity-linked gene expression changes in prostate cancer, AACR Metabolism and Cancer Conference, Bellevue, WA, June 7-10, 2015 (poster presentation – See Appendix 6)

Identifying obesity-linked gene expression changes in prostate cancer, AACR Annual Meeting 2015, Philadelphia, PA, April 18-22, 2015 (oral presentation – See Appendix 6)

Obesity and chromatin remodeling – is there an epigenetic link between diet and prostate cancer, Eighth Annual Prostate Cancer SPORE Retreat, Fort Lauderdale, FL, March 15-17, 2015 (oral presentation – See Appendix 1)

Identifying obesity-linked gene expression changes in prostate cancer, Program in Genetic Epidemiology and Statistical Genetics Seminar Series, Boston, MA, February 13, 2015 (oral presentation, See Appendix 3)

Identifying obesity-linked gene expression changes in prostate cancer, DF/HCC Celebration of Junior Investigators in Cancer Science, Boston, MA, September 24, 2014 (oral presentation – See Appendix 2)

Website(s) or other Internet site(s)
Nothing to report.

Technologies or techniques
Nothing to report.

Inventions, patent applications, and/or licenses
Nothing to report.

Other Products
Nothing to report.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS
What individuals have worked on the project?
Name: Ericka Noonan
Project Role: PI/Postdoctoral Trainee
Researcher Identifier (e.g. ORCID ID): N/A
Nearest person month worked: 10
Contribution to Project: project management, statistical analysis, data interpretation, and manuscript preparation
Funding Support: N/A

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Past support (at time of application):

1) Sex-hormones and the TMPRSS2:ERG fusion in prostate cancer progression (NIH, PI Lorelei Mucci)
2) Training Program in Cancer Epidemiology (NIH, PI Meir Stampfer)

Current support (as of 8/31/15):
1) Molecular Epidemiology Investigation of Obesity and Lethal Prostate Cancer (DoD, PI Ericka Noonan)
2) Statistical methods for tumor expression data from archival tissues in clinical and epidemiologic research (NIH, PI Svitlana Tyekucheva)

What other organizations were involved as partners?
Organization name: Dana-Farber Cancer Institute
Location of Organization: Boston, MA
Partner's contribution to the project: Collaboration

SPECIAL REPORTING REQUIREMENTS
Nothing to report.

APPENDICES
1. Presentation: Annual Prostate Cancer SPORE Retreat
2. Presentation: DF/HCC Celebration of Junior Investigators in Cancer Science
3. Presentation: Program in Genetic Epidemiology and Statistical Genetics Seminar Series
4. Presentation: Meeting on Lipid Metabolism and Metabolic Alterations in Prostate Cancer
5. Presentation: AACR Annual Meeting 2015
6. Presentation: AACR Metabolism and Cancer Conference
7. Manuscript: Gene expression profiling identifies chromatin regulation as a molecular link between obesity and lethal prostate cancer
8. Manuscript: Role of diet in prostate cancer: The epigenetic link

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Obesity and chromatin remodeling- is there an epigenetic link between diet and prostate cancer

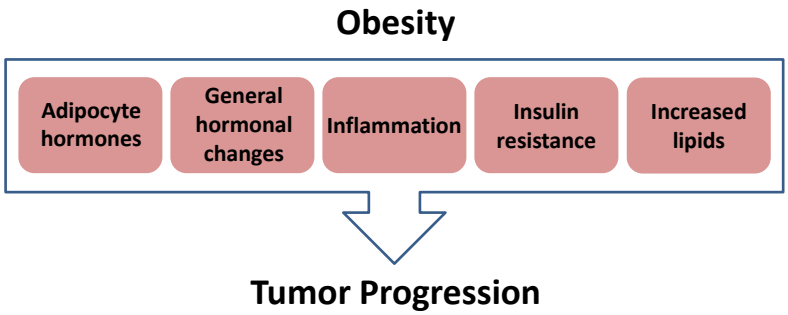
Ericka Ebot PhD
Postdoctoral Fellow, Epidemiology
Harvard T.H. Chan School of Public Health

David Labbé PhD
Postdoctoral Fellow, DFCI
Harvard Medical School

Eighth Annual Prostate Cancer Program Retreat
March 15, 2015

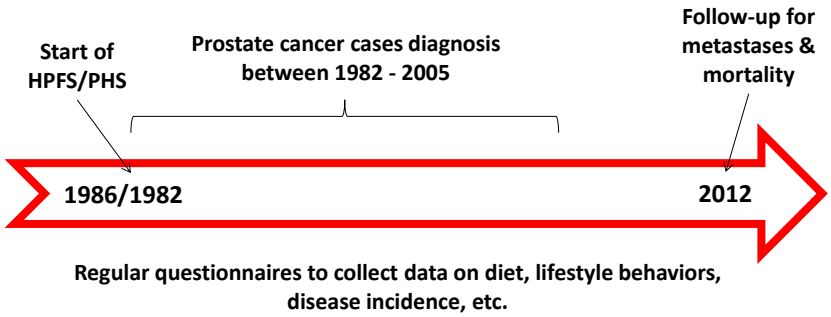
Background

- Obese men are at higher risk of developing advanced stage prostate cancer and have higher rates of cancer-specific mortality after diagnosis

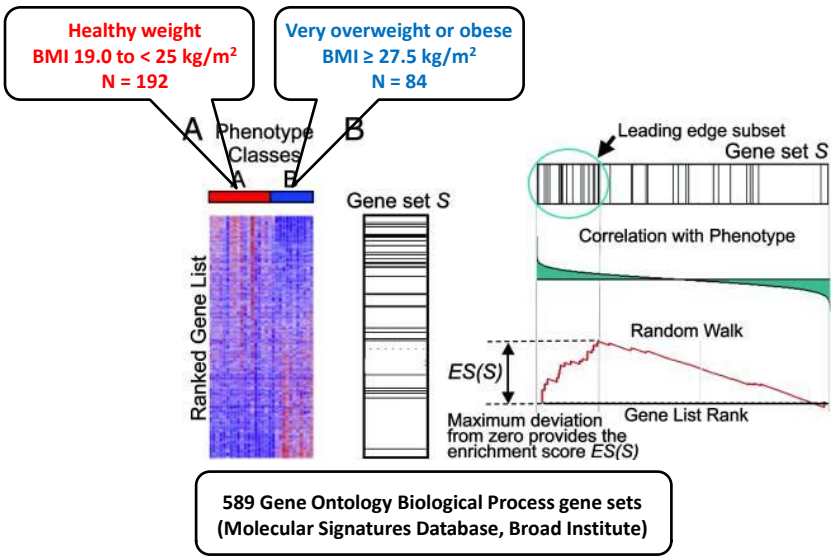


Aims

- Examine prostate-specific alterations associated with obesity using genome-wide mRNA expression profiles of tumor tissue
- Explore whether such alterations underlie the link between obesity and prostate cancer progression

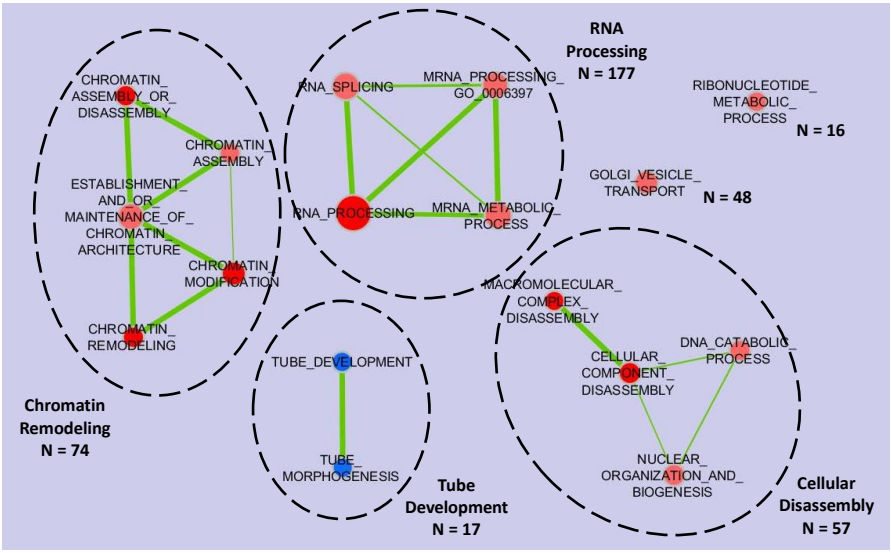


Gene Set Enrichment Analysis

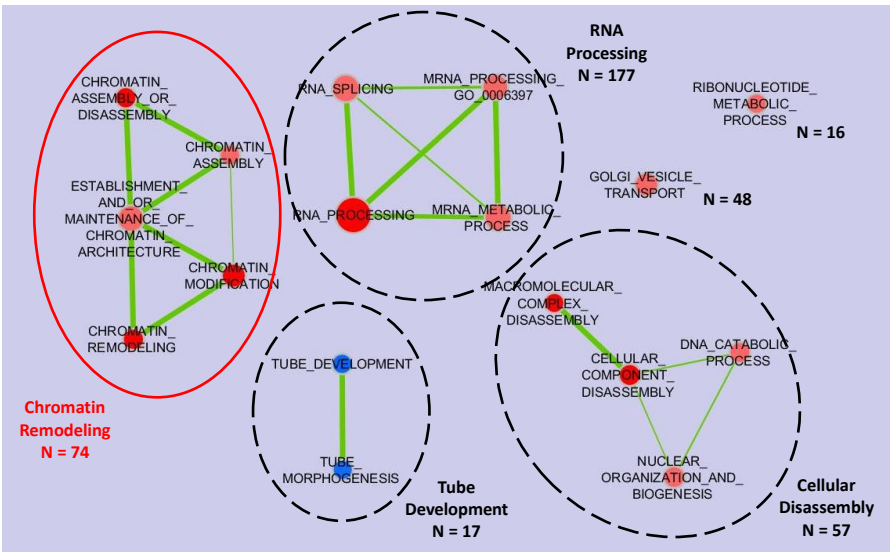


Subramanian et al. PNAS 2005

Enrichment Map of gene sets with FDR < 0.25



Enrichment Map of gene sets with FDR < 0.25



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

35 genes from leading edge subset

histone deacetylase activity
HDAC2, HDAC3, HDAC8, SIRT1

nucleosome remodeling
SWI/SNF: SMARCC2, SMARCA5, ARID1A, PBRM1, ACTL6A

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

score computed based on expression of chromatin-related genes

Chromatin score results

- Score associated with BMI (p-trend = 5.01e-6)
  - tumor specific (no association using expression values from adjacent normal tissue)

Chromatin score results

- Score associated with BMI (p-trend = 5.01e-6)
  - tumor specific (no association using expression values from adjacent normal tissue)
- Score associated with Gleason grade (p-trend =4.48e-6) and pathologic TNM stage (p-trend = 0.11)

Chromatin score results

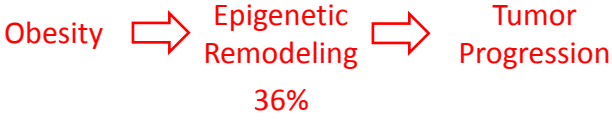
- Score associated with BMI (p-trend = 5.01e-6)
  - tumor specific (no association using expression values from adjacent normal tissue)
- Score associated with Gleason grade (p-trend =4.48e-6) and pathologic TNM stage (p-trend = 0.11)
- Score associated with lethal outcome (distant metastases or death from prostate cancer)

	# lethal	Odds ratio	P-value
Chromatin score			
quartile 1 (low expression)	15	ref	8.11E-05
quartile 2	23	2.03 (0.88, 4.81)	
quartile 3	25	2.04 (0.89, 4.79)	
quartile 4 (high expression)	50	5.01 (2.31, 11.38)	

Logistic regression model adjusted for age and date at diagnosis and Gleason grade

Chromatin score results

- Score associated with BMI (p-trend = 5.01e-6)
  - tumor specific (no association using expression values from adjacent normal tissue)
- Score associated with Gleason grade (p-trend =4.48e-6) and pathologic TNM stage (p-trend = 0.11)
- Score associated with lethal outcome (distant metastases or death from prostate cancer)



Chromatin score results

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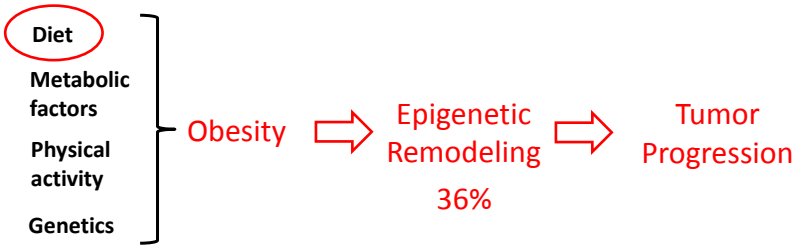
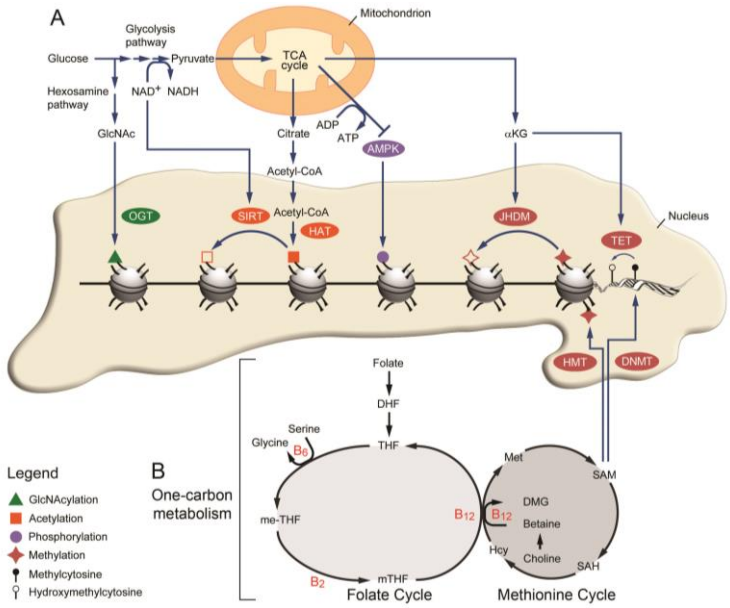
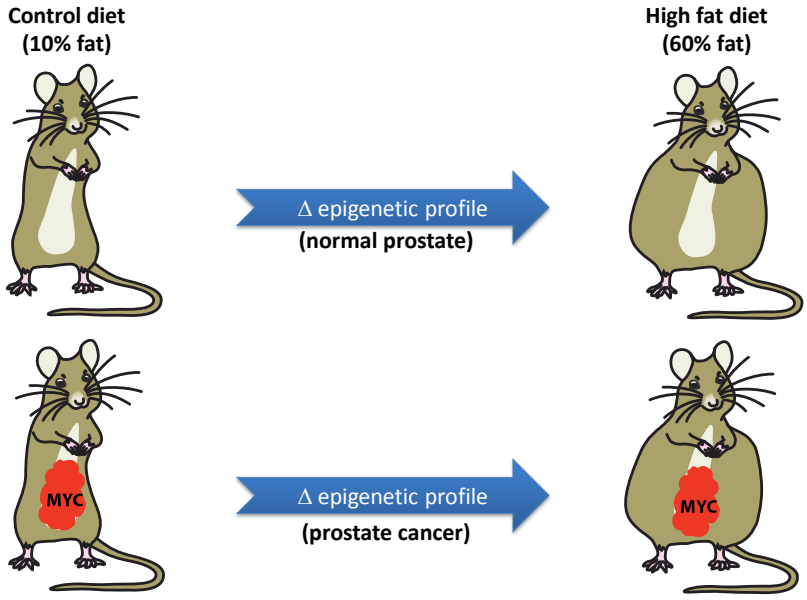


Epigenetic Remodeling Rely on Diet



Labbé DP.; Zadra G.; Ebot EM. *et al.*, *Oncogene* (2014) epub.

Project Design



Project Aims

- Determine the transcriptomic signature of diet in the prostate
  - RNA-seq
- Identify key dietary-related metabolomic changes
  - Metabolomic profiling (Metabolon)
- Map the prostatic epigenome
  - Histones PTMs (Mass Spec, Broad)
  - ATAC-seq
  - ChIP-seq
- Integrate transcriptomic and metabolomic with epigenomic data

Summary

- Genes involved in chromatin remodeling are enriched in the tumor tissue of overweight/obese men (not identified in analysis of adjacent normal tissue)
- Associated with worse clinical characteristics (Gleason grade) and poorer prostate cancer outcomes

Ongoing

- Validate if the chromatin remodeling signature identified in overweight/obese men is also observed by diet-induced obesity in the Hi-Myc mice
- Identify if diet-induced obesity lead to key alterations in the prostate epigenome through a global shift in the pool of metabolites available

Acknowledgements

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CIHR IRSC

NATIONAL CANCER INSTITUTE

DF/HCC prostate cancer SPORE

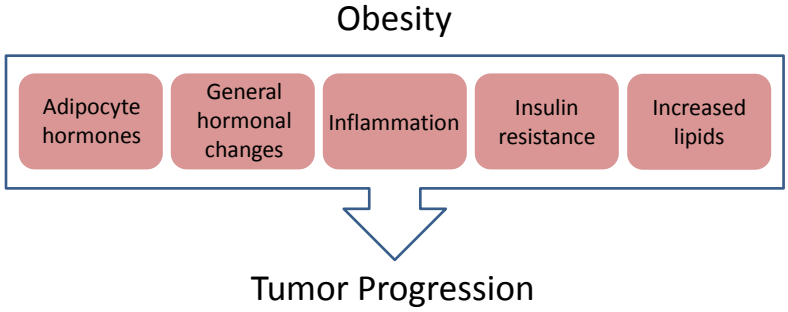
Identifying obesity-linked gene expression changes in prostate cancer

Ericka Ebot PhD MPH
Postdoctoral Fellow, Epidemiology
Harvard School of Public Health

DF/HCC Celebration of Junior Investigators in Cancer Science
September 24, 2014

Background

- Obese men are at higher risk of developing advanced stage prostate cancer and have higher rates of cancer-specific mortality after diagnosis



Aim

- Interrogate whole genome mRNA expression profiles from prostate tumor tissue to identify biological pathways differentially altered among patients who are overweight/obese and develop lethal prostate cancer

Methods

- **Study population:** Men with incident prostate cancer in the Health Professionals Follow-up and Physicians' Health Study who had available FFPE tissue
- **Obesity measures:** Self-reported body mass index (BMI) was taken from questionnaires closest to and before diagnosis
- **Clinical data:** Clinical information on age and date of diagnosis, PSA level at diagnosis, stage and Gleason grade were obtained from medical record review
- **Outcome data:** All men were followed for the development of lethal disease, defined by distant metastases or prostate cancer-specific death
- **Biomarker assessment:** Whole genome mRNA expression levels of tumor specimens were assayed using the Affymetrix 1.0 ST array

Clinical Characteristics	N=402
Lethal Outcome, N (%)	113 (28)
Follow-up, years, mean (SD)	13.2 (5.2)

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Follow-up, years, mean (SD)	13.2 (5.2)
Age at diagnosis, years, mean (SD)	65.7 (6.5)
Body mass index at diagnosis, N (%)	
Normal weight (18.5 – 25.0 kg/m^2)	192 (48)
Overweight (25.0 – 27.5 kg/m^2)	126 (31)
Overweight/Obese (≥ 27.5 kg/m^2)	84 (21)

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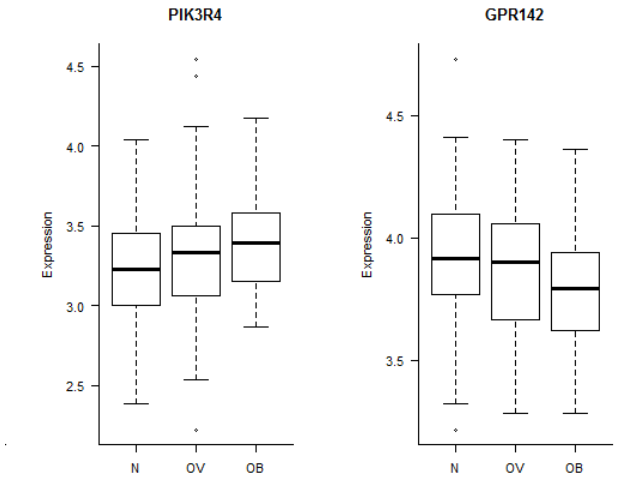
Gleason score, N (%)	
4-6	54 (14)
3+4	138 (35)
4+3	101 (26)
8-10	100 (25)
Pathological tumor stage, N (%)	
T2, Nx/N0	218 (59)
T3, Nx/N0	129 (35)
T4/N1/M1	20 (5)

Results: Top differentially expressed genes in tumors of overweight and obese men compared to normal weight men from trend test



Results: Top 10 Gene Ontology biological processes enriched in tumors of overweight/obese men compared to normal weight men determined by Gene Set Enrichment Analysis

Gene Set	No. of genes	P-value	FDR
CHROMATIN_REMODELING	25	0.002	0.23
CHROMATIN_MODIFICATION	52	0.006	0.29
ESTABLISHMENT_AND_OR_MAINTENANCE_OF_CHROMATIN_ARCHITECTURE	74	0.008	0.31
REGULATION_OF_GENE_EXPRESSION_EPIGENETIC	29	0.002	0.33
GENE_SILENCING	10	0.006	0.39
CHROMATIN_ASSEMBLY	16	0.008	0.43
CHROMATIN_ASSEMBLY_OR_DISASSEMBLY	26	0.002	0.58
RNA_PROCESSING	167	0.027	0.62
REGULATION_OF_NEURON_APOPTOSIS	12	0.022	0.63
ONE_CARBON_COMPOUND_METABOLIC_PROCESS	26	0.016	0.66

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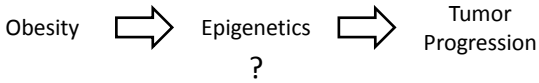

Summary and next steps

- GSEA identified chromatin remodeling as the top Gene Ontology biological process enriched in tumor tissue of overweight/obese men
  - Modification of histones, HDACs
- Further explore role of chromatin remodeling in the obesity-lethal prostate cancer relationship
  - Lethal-pathway analysis
    - Global Test P-value = 6.39E-8 (adjusted for age and year of diagnosis); 2.06E-6 (additionally adjusted for Gleason grade and stage)
  - Tumor features
    - Proliferation, apoptosis, inflammation, angiogenesis

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- Lorelei Mucci
- Prostate Cancer Patho-Epi Team
- DoD Prostate Cancer Research Program
- DF/HCC Prostate Cancer SPORE

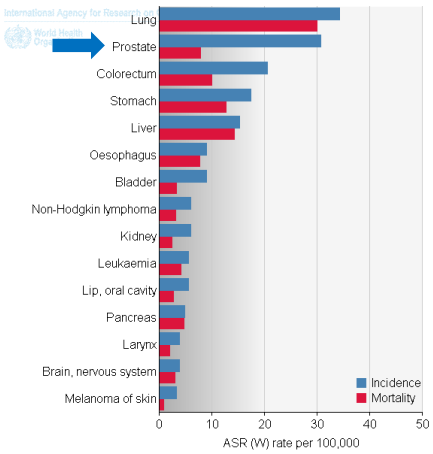
Identifying obesity-linked gene expression changes in prostate cancer

Ericka Ebot PhD MPH
Postdoctoral Fellow, Epidemiology
Harvard School of Public Health

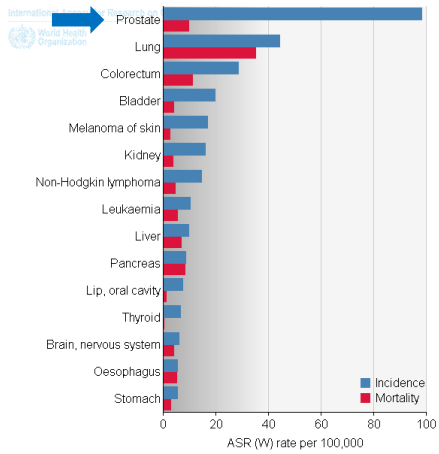
PGSG Seminar
Harvard School of Public Health
February 13, 2015

Prostate cancer incidence and mortality

World incidence and mortality in men



U.S. incidence and mortality in men

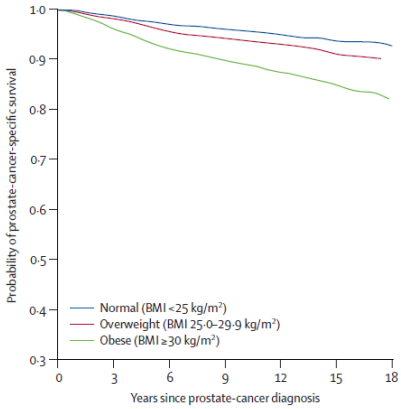


Lethal prostate cancer responsible for ~30,000 deaths/year in the U.S.

Globocan 2012 (IARC)

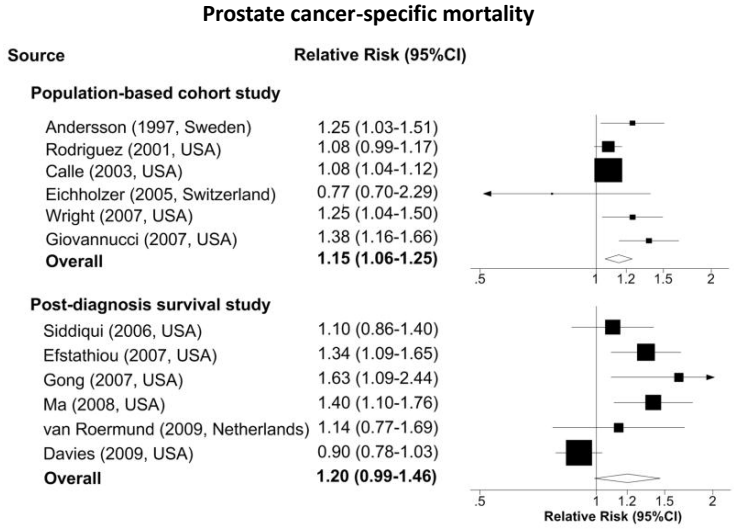
Obesity and aggressive prostate cancer

- 2,500 men with prostate cancer in Physicians' Health Study
- Obesity at baseline (1982)
- Followed for up to 28 years for prostate-cancer specific mortality
- Hazard ratio 1.47 (1.16-1.88) and 2.66 (1.62-4.39) for overweight men and obese men respectively



Ma et al. Lancet Oncology 2008

Obesity and aggressive prostate cancer

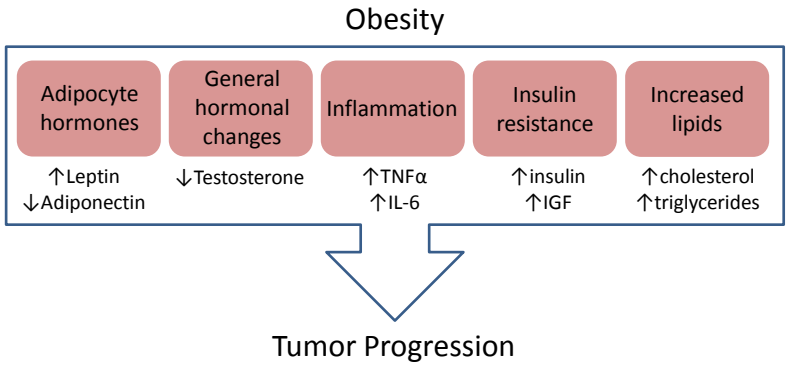


per 5 kg/m² increase in BMI

Cao et al. Cancer Prev Res 2011

Obesity and aggressive prostate cancer

- WICR/AICR 2014 Report on Diet, Nutrition, Physical Activity, and Prostate Cancer: *strong evidence* that being overweight or obese increases the risk of advanced prostate cancer and prostate cancer mortality (no conclusion could be drawn for total or non-advanced prostate cancer)

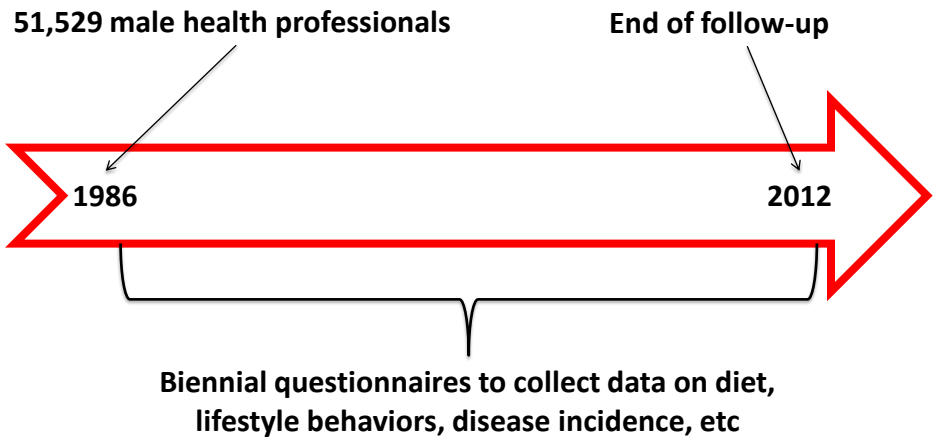


Aim

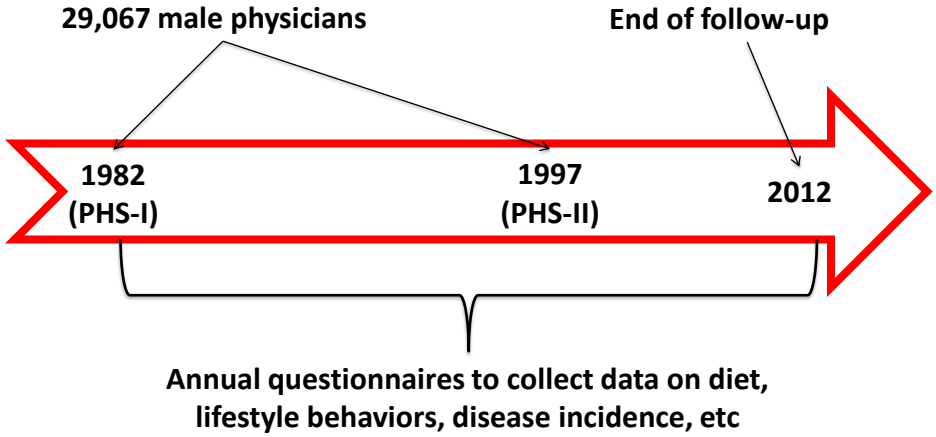
- Examine tissue-level alterations associated with obesity using whole transcriptome expression profiles of tumor and adjacent normal tissue from prostate cancer patients
- Explore whether such alterations underlie the link between obesity and prostate cancer progression

  - ➢ Better understand the underlying biology of aggressive prostate cancer
  - ➢ Identify subgroups of men with prostate cancer who are most likely to benefit from secondary prevention strategies including weight loss and other targeted therapies

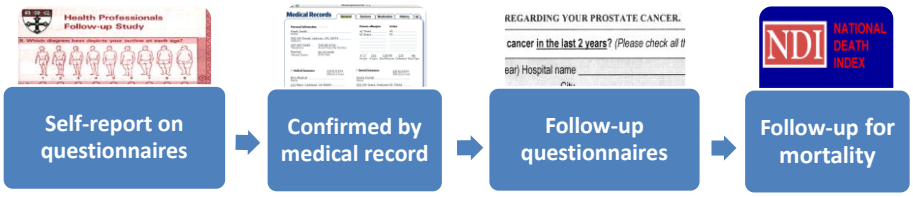
Health Professionals Follow-up Study



Physicians' Health Study



Prostate Cancer Ascertainment: PHS and HPFS



Study population

- **Harvard prostate tumor cohort:** prostate cancer cases (HPFS and PHS) with tissue specimens from radical prostatectomy (93%) or transurethral resection of the prostate (TURP, 7%)
  - Standardized histopathologic review of Gleason grade for each case by study pathologist
- **Gene expression profiling performed on tumor tissue of 402 patients in the prostate tumor cohort**
  - Subset also have expression profiling data for adjacent normal tissue
  - Cases diagnosed between 1982 and 2005
  - Extreme case design
    - 113 lethal cases: developed metastatic disease or died from prostate cancer
    - 289 indolent cases: survived at least 8 years after prostate cancer diagnosis without any evidence of metastases

Exposure and outcome data

- **Anthropometric data:** Self-reported body mass index (BMI) was taken from questionnaires closest to and before diagnosis (average = 1.3 years)
- **Clinical data:** Clinical information on age and date of diagnosis, PSA level at diagnosis, and stage were obtained from medical record review
- **Outcome data:** All men were followed for the development of lethal disease, defined by distant metastases or prostate cancer-specific death
- **Biomarker assessment:** Gene expression levels of tissue specimens were assayed using the Affymetrix Human Gene 1.0 ST array
  - 20,254 unique gene symbols after mapping transcript cluster IDs to gene names

Clinical characteristics of study population

	All men (N=402)
Age at diagnosis, years, mean (SD)	65.7 (6.5)
Year of diagnosis, N (%)	
before 1990 (pre-psa era)	45 (11.2)
1990-1993 (peri-psa era)	112 (27.9)
after 1993 (psa era)	245 (60.9)
PSA at diagnosis, ng/ml, median (q1, q3)	7.3 (5.3, 11.6)
Pathologic TNM stage, N (%)	
T2 N0 M0	218 (59.4)
T3 N0 M0	129 (35.1)
T4/N1/M1	20 (5.4)
Clinical TNM stage, N (%)	
T1/T2 N0 M0	349 (88.4)
T3 N0 M0	27 (6.8)
T4/N1/M1	19 (4.8)
Gleason grade, N (%)	
<7	57 (14.2)
3+4	138 (34.3)
4+3	102 (25.4)
>7	105 (26.1)
Tissue type, N (%)	
RP	368 (91.5)
TURP	34 (8.5)
Cohort, N (%)	
HPFS	254 (63.2)
PHS	148 (36.8)

organ-confined ↓ advanced

well differentiated ↓ poorly differentiated

Note: 63 missing PSA at diagnosis, 35 missing pathologic stage, 7 missing clinical stage

Clinical characteristics by prediagnosis BMI

	Prediagnosis BMI		
	19.0 - 25.0 kg/m^2 (N=192)	25.0 - 27.5 kg/m^2 (N=126)	27.5 - 36.8 kg/m^2 (N=84)
Age at diagnosis, years, mean (SD)	65.8 (6.5)	66.3 (6.5)	64.5 (6.2)
Year of diagnosis, N (%)			
before 1990 (pre-PSA era)	27 (14.1)	10 (7.9)	8 (9.5)
1990-1993 (peri-PSA era)	54 (28.1)	36 (28.6)	22 (26.2)
after 1993 (PSA era)	111 (57.8)	80 (63.5)	54 (64.3)
PSA at diagnosis, ng/ml, median (q1, q3)	7.9 (5.6, 12.0)	6.2 (4.8, 11.5)	7.7 (5.5, 10.7)
Pathologic TNM stage, N (%)			
T2 N0 M0	111 (62.7)	67 (58.8)	40 (52.6)
T3 N0 M0	54 (30.5)	43 (37.7)	32 (42.1)
T4/N1/M1	12 (6.8)	4 (3.5)	4 (5.3)
Clinical TNM stage, N (%)			
T1/T2 N0 M0	168 (88.9)	111 (91.0)	70 (83.3)
T3 N0 M0	13 (6.9)	6 (4.9)	8 (9.5)
T4/N1/M1	8 (4.2)	5 (4.1)	6 (7.1)
Gleason grade, N (%)			
<7	29 (15.1)	17 (13.5)	11 (13.1)
3+4	67 (34.9)	45 (35.7)	26 (31.0)
4+3	45 (23.4)	33 (26.2)	24 (28.6)
>7	51 (26.6)	31 (24.6)	23 (27.4)
Tissue type, N (%)			
RP	117 (92.2)	115 (91.3)	76 (90.5)
TURP	15 (7.8)	11 (8.7)	8 (9.5)
Cohort, N (%)			
HPFS	124 (64.6)	77 (61.1)	53 (63.1)
PHS	68 (35.4)	49 (38.9)	31 (36.9)

P-trend 0.14

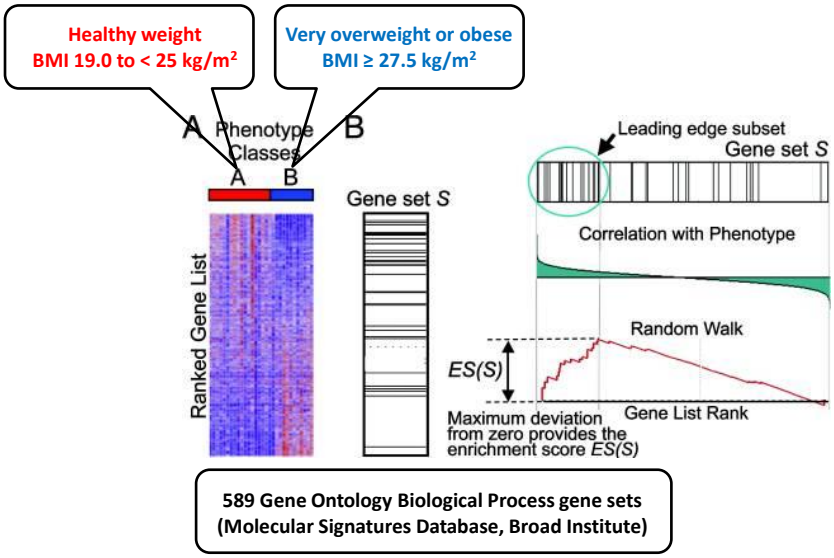
P-trend 0.40

Note: 63 missing PSA at diagnosis, 35 missing pathologic stage, 7 missing clinical stage

Analysis (1)

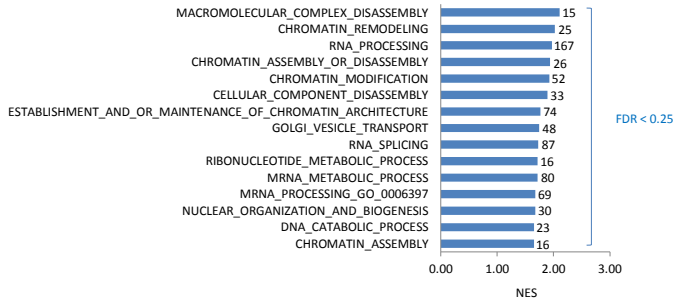
- **Gene Set Enrichment Analysis (GSEA):** identify functionally related sets of genes that are over- or under-represented in a given phenotype (Subramanian et al. PNAS 2005)
- **Enrichment Map:** organize results of GSEA as a network to identify redundancy in gene sets (Merico et al. PLoS One 2010)
- **GeneMANIA:** create protein interaction networks from list of genes using publicly available protein-protein interaction databases to aid in the interpretation of top scoring genes identified by GSEA (Warde-Farley et al. Nucleic Acids Res 2010)

Gene Set Enrichment Analysis

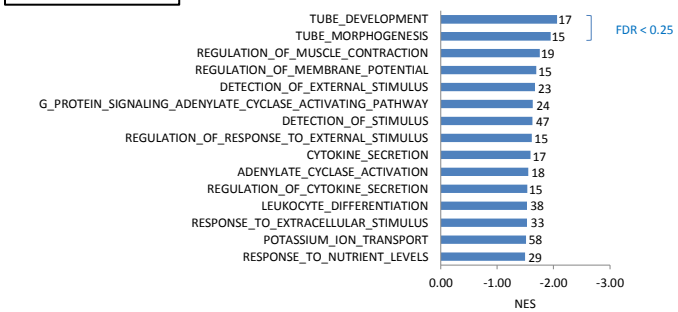


Subramanian et al. PNAS 2005

Upregulated

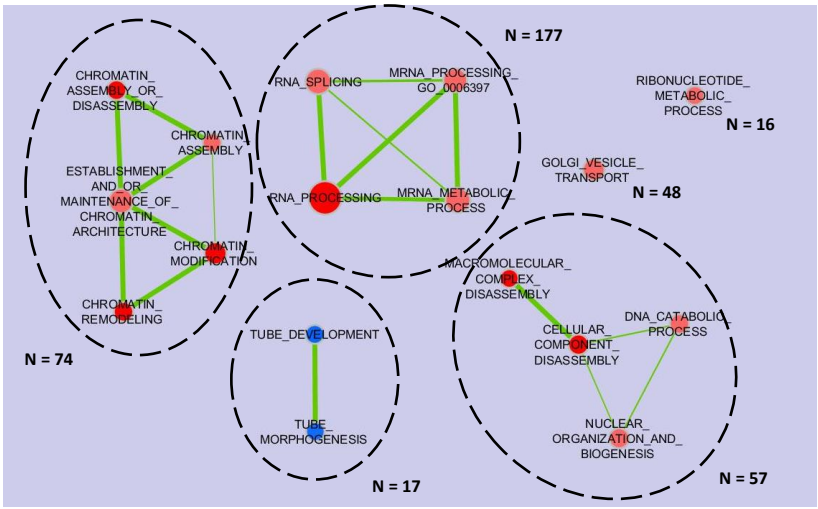


Downregulated



Top biological processes enriched in the tumor tissue of men with high BMI

Enrichment Map of gene sets with FDR < 0.25



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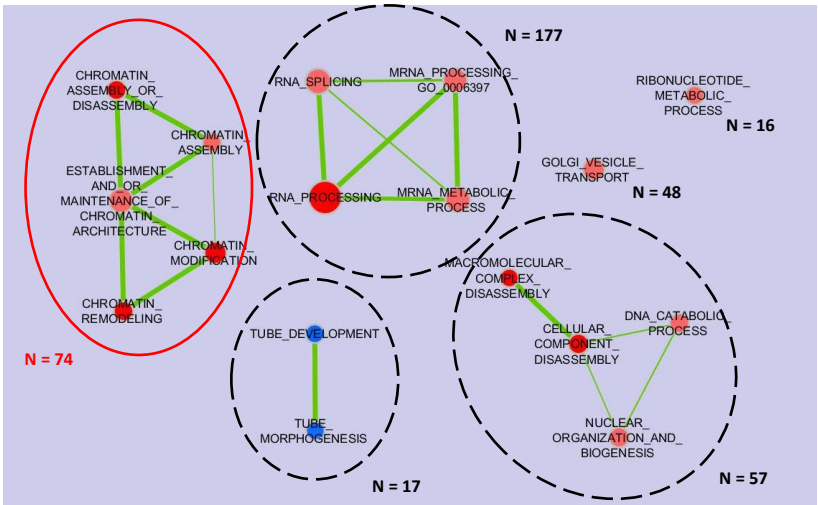

Top scoring chromatin-related genes from GSEA leading edge

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

Top scoring chromatin-related genes from GSEA leading edge

p-value < 0.05

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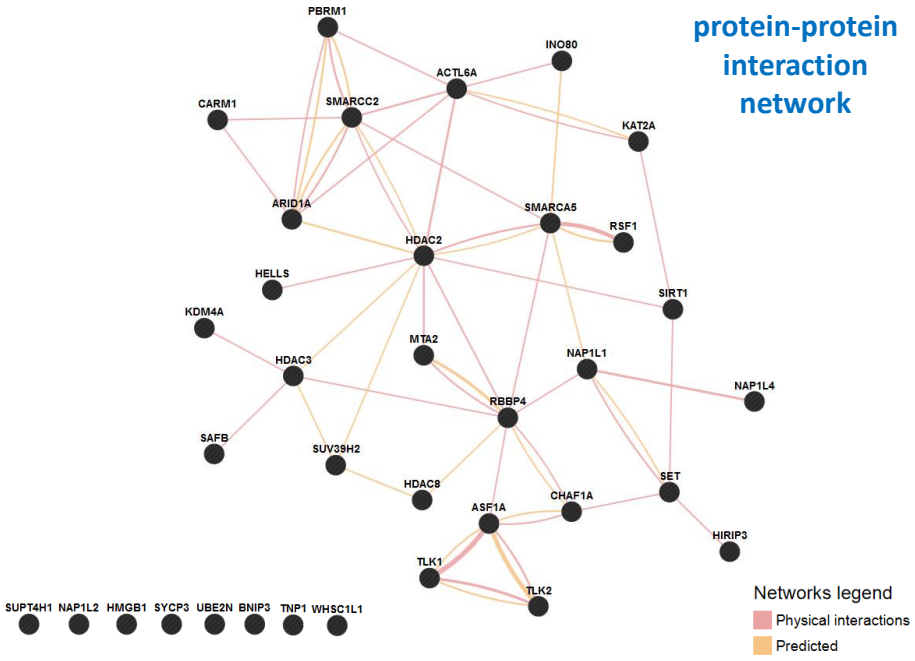
GeneMANIA protein-protein interaction network



SWI/SNF superfamily-type complex

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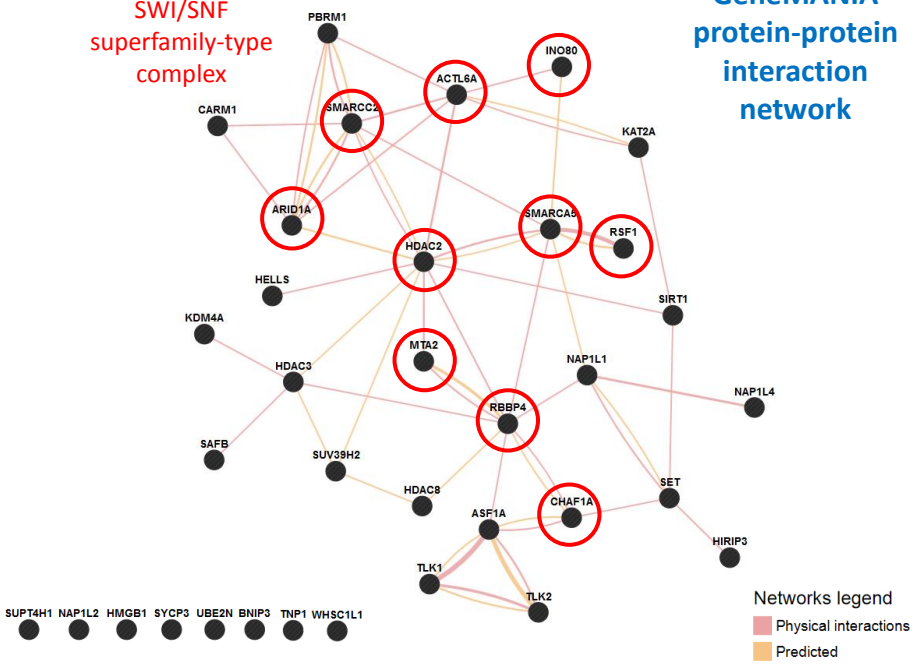

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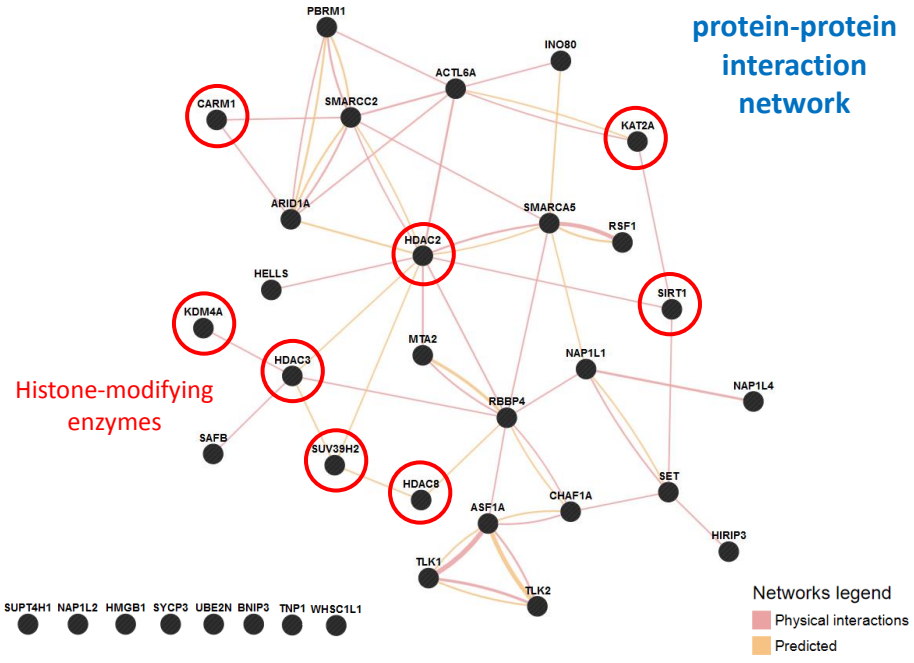

Histone-modifying enzymes

Analysis (2)

- Create chromatin 'metagene' score
  - Mean center and variance scale data
  - Average scaled expression of 35 top scoring chromatin-related genes
  - Range = -1.23 to 1.48
  - Continuous and categorical (quartiles)

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- Test associations between score and
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  - Clinic characteristics
  - Lethal prostate cancer outcome

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- Test associations between score and
  - Prediagnosis BMI ➡ P-value for trend = 5.01e-6
  - Clinic characteristics
  - Lethal prostate cancer outcome

Clinical characteristics by chromatin score

	Score (N = 402)				
	Q1	Q2	Q3	Q4	
Age at diagnosis, years, mean (SD)	66.7 (5.8)	71.0 (6.4)	64.5 (6.7)	66.5 (6.8)	
Year of diagnosis, N (%)					
before 1990 (pre-PSA era)	11 (10.9)	8 (8.0)	13 (13.0)	13 (12.9)	
1990-1993 (peri-PSA era)	29 (28.7)	26 (26.0)	26 (26.0)	31 (30.7)	
after 1993 (PSA era)	61 (60.4)	66 (66.0)	61 (61.0)	57 (56.4)	
PSA at diagnosis, ng/ml, median (q1, q3)	6.7 (5.2, 13.4)	7.2 (5.2, 10.2)	7.3 (5.4, 11.0)	8.1 (5.8, 11.5)	
Pathologic TNM stage, N (%)					
T2 N0 M0	57 (61.2)	60 (63.8)	58 (63.0)	43 (48.9)	
T3 N0 M0	32 (34.4)	29 (30.9)	28 (30.4)	40 (45.4)	**P-trend 0.11**
T4/N1/M1	4 (4.3)	5 (5.3)	6 (6.5)	5 (5.7)	
Clinical TNM stage, N (%)					
T1/T2 N0 M0	90 (90.0)	87 (87.0)	87 (89.7)	85 (86.7)	
T3 N0 M0	7 (7.0)	7 (7.0)	5 (5.2)	8 (8.2)	
T4/N1/M1	3 (3.0)	6 (6.0)	5 (5.2)	5 (5.1)	
Gleason grade, N (%)					
<7	20 (19.8)	13 (13.0)	15 (15.0)	9 (8.9)	
3+4	39 (38.6)	45 (45.0)	36 (36.0)	18 (17.8)	
4+3	25 (24.8)	18 (18.0)	26 (26.0)	33 (32.7)	**P-trend 4.48e-6**
>7	17 (16.8)	24 (24.0)	23 (23.0)	41 (40.6)	
Tissue type, N (%)					
RP	93 (92.1)	95 (95.0)	92 (92.0)	88 (87.1)	
TURP	8 (7.9)	5 (5.0)	8 (8.0)	13 (12.9)	
Cohort, N (%)					
HPFS	54 (53.5)	70 (70.0)	61 (61.0)	69 (68.3)	
PHS	47 (46.5)	30 (30.0)	39 (39.0)	32 (31.7)	

Note: 63 missing PSA at diagnosis, 35 missing pathologic stage, 7 missing clinical stage

Odds ratio and 95% CI for lethal prostate cancer

Chromatin score	Odds ratio[1]	P-value	Odds ratio[2]	P-value
Continuous, per 0.1 units	1.22 (1.14, 1.31)	4.13E-08	1.18 (1.09, 1.28)	9.18E-05
Categorical				
quartile 1	ref	1.03E-07	ref	8.97E-05
quartile 2	2.13 (1.02, 4.57)		1.93 (0.80, 4.77)	
quartile 3	2.25 (1.08, 4.82)		1.96 (0.82, 4.82)	
quartile 4	6.78 (3.42, 14.16)		5.14 (2.31, 12.06)	

[1]Logistic regression model adjusted for age and date of diagnosis
[2]Logistic regression model additionally adjusted for Gleason grade and clinical stage

Summary

- Genes involved in chromatin remodeling are enriched in the tumor tissue of overweight/obese men
  - Regulate transcription through nucleosome remodeling (SWI/SNF complex) and covalent histone modifications (histone deacetylases)
  - Form highly connected network of physical interactions
  - Tumor-specific (not identified in analysis of adjacent normal tissue)
- Associated with clinical characteristics, particularly Gleason grade
- Strongly associated with lethal prostate cancer outcome, even after adjusting for Gleason grade and stage

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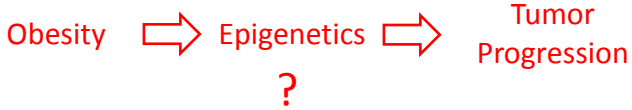
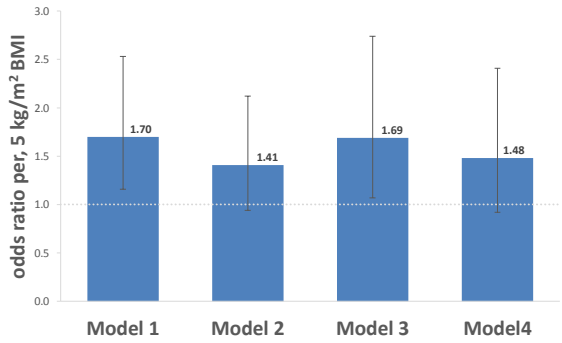


Analysis (3)

- Mediation analysis
  - Evaluate whether the score attenuates the association of BMI and lethal prostate cancer
  - Calculate the proportion of the effect of the exposure mediated by the intermediate on the odds ratio scale (Vanderwheele et al. Am J Epidemiol 2010)

Odds ratio and 95% CI for lethal prostate cancer



Model 1: base model (age and date of diagnosis)
Model 2: base model + score
Model 3: base model + Gleason grade
Model 4: base model + Gleason grade + score

~50% of the BMI effect on lethal outcome mediated by the score

Acknowledgments

- Lorelei Mucci
- Prostate Cancer Patho-Epi Team
- DoD Prostate Cancer Research Program
- DF/HCC Prostate Cancer SPORE

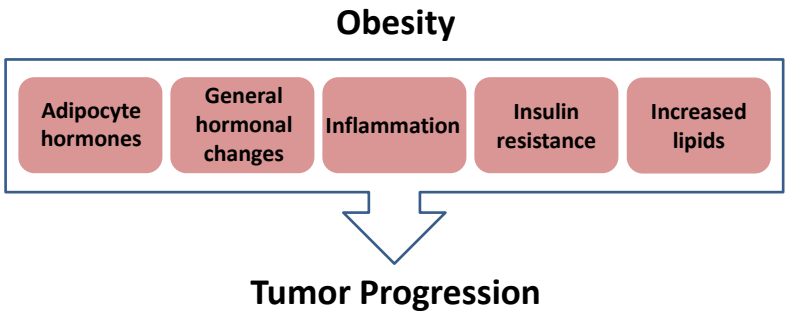
Using gene expression profiles of prostate cancer tissue to investigate the relationship between obesity and lethal prostate cancer

Ericka Ebot
Postdoctoral Fellow, Epidemiology
Harvard T.H. Chan School of Public Health

Lipid metabolism and metabolic alterations in prostate cancer
Dana-Farber Cancer Institute
July 31, 2015

Obesity and prostate cancer

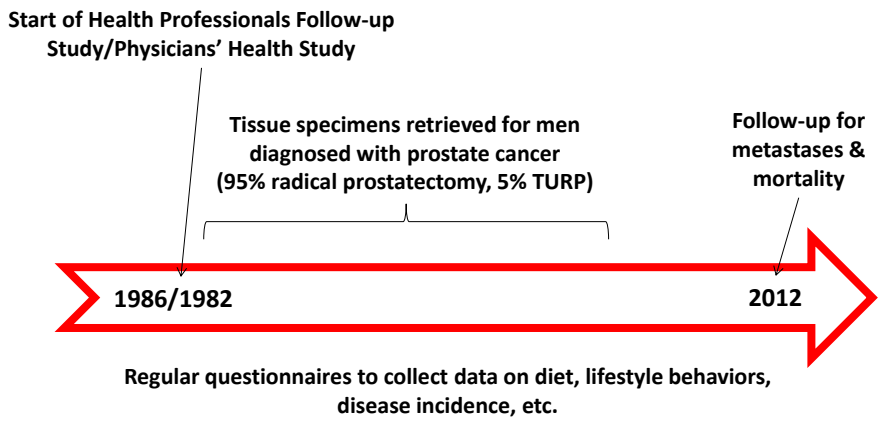
- Obese men are at higher risk of developing advanced stage prostate cancer and have higher rates of cancer-specific mortality after diagnosis



Aims

- Examine prostate-specific alterations associated with obesity using whole transcriptome gene expression profiles of tumor tissue
- Explore whether such alterations underlie the link between obesity and lethal disease

Prostate Tumor Tissue Cohort



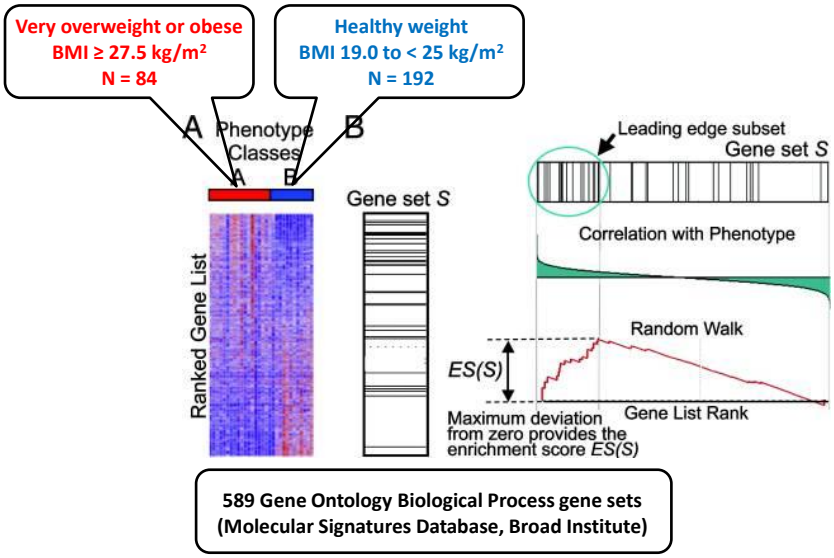
Methods

- **Study population:** 402 prostate cancer cases from the Prostate Tumor Tissue Cohort diagnosed between 1982 and 2005
  - 113 lethal (metastatic disease or prostate cancer-specific death)
  - 289 indolent (survived 8 years without lethal event)
- **Obesity measures:** Self-reported body mass index (BMI) was taken from questionnaires closest to and before diagnosis (average = 1.3 years)
- **Clinical data:** Clinical information was obtained from medical record review; Standardized histopathologic review of Gleason grade was performed for each case
- **Outcome data:** Prostate cancer cases were followed through questionnaires for details of clinical course; Deaths were ascertained by searches of the National Death Index
- **Biomarker assessment:** Whole transcriptome gene expression profiles of tumor tissue assayed using the Affymetrix GeneChip Human Gene 1.0 ST Array

Clinical characteristics of prostate cancer cases in study (N=402)

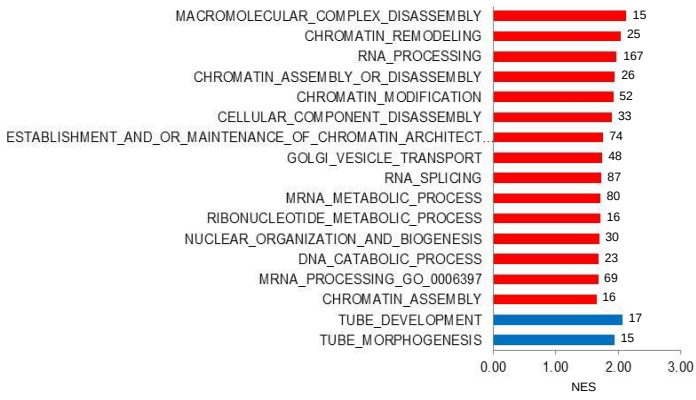
Age at diagnosis, years, mean	65.7
Year of diagnosis, %	
before 1990 (pre-PSA era)	11
1990-1993 (peri-PSA era)	28
after 1993 (PSA era)	61
PSA at diagnosis, ng/ml, median	7.3
Pathologic TNM stage, %	
T2 N0 M0	59
T3 N0 M0	35
T4/N1/M1	5
Gleason grade, %	
2-6	14
3+4	34
4+3	25
8-10	26
Tissue type, %	
Radical prostatectomy	92
TURP	9

Gene Set Enrichment Analysis



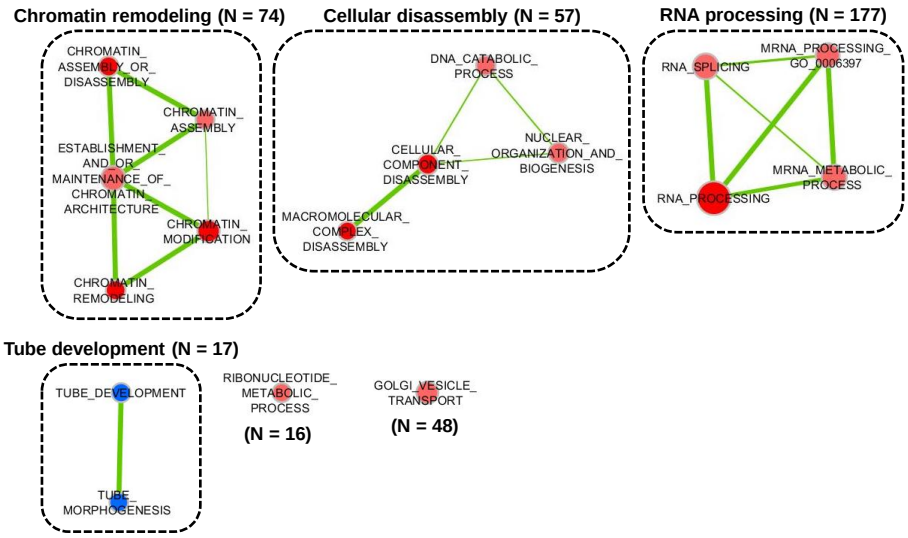
Subramanian et al. PNAS 2005

Top Gene Ontology biological process gene sets



FDR < 0.25

Enrichment Map of gene sets with FDR < 0.25



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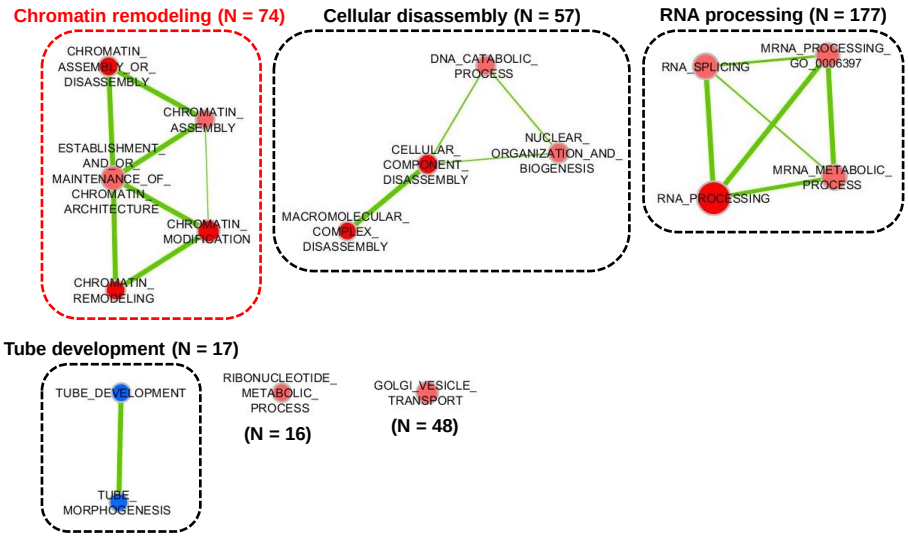

Gene symbol	Gene name
ACTL6A	actin-like 6A
ARID1A	AT rich interactive domain 1A (SWI-like)
ASF1A	ASF1 anti-silencing function 1 homolog A (S. cerevisiae)
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SAFB	scaffold attachment factor B
SET	SET nuclear oncogene
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SMARCA5	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily a, member 5
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SUPT4H1	suppressor of Ty 4 homolog 1 (S. cerevisiae)
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WHSC1L1	Wolf-Hirschhorn syndrome candidate 1-like 1

chromatin remodeling genes from leading edge subset

histone deacetylase activity
HDAC2, HDAC3, HDAC8, SIRT1

nucleosome remodeling
SWI/SNF: SMARCC2, SMARCA5, ARID1A, PBRM1, ACTL6A

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ACTL6A	actin-like 6A
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score computed based on expression of chromatin remodeling genes

Chromatin remodeling gene expression is associated with worse clinical characteristics

	Chromatin remodeling gene score		
	Quartile 1 (low expression)	Quartile 4 (high expression)	
Age at diagnosis, years, mean	66.7	66.5	
Year of diagnosis, %			
before 1990 (pre-PSA era)	11	13	
1990-1993 (peri-PSA era)	29	31	
after 1993 (PSA era)	60	56	
PSA at diagnosis, ng/ml, median	6.7	8.1	
Pathologic TNM stage, %			**P-value**
T2 N0 M0	61	49	**0.11**
T3 N0 M0	34	45	
T4/N1/M1	4	6	
Gleason grade, %			
2-6	20	9	
3+4	39	18	**P-value**
4+3	25	33	**3.2×10^{-4}**
8-10	17	41	
Tissue type, %			
Radical prostatectomy	92	87	
TURP	8	13	

Chromatin remodeling gene expression is associated with lethal outcome

	# lethal	Odds ratio[1]	P-value	Odds ratio[2]	P-value
Chromatin remodeling gene score					
Quartile 1 (low expression)	15	ref	1.03E-07	ref	8.11E-05
Quartile 2	23	2.13 (1.02, 4.57)		2.03 (0.88, 4.81)	
Quartile 3	25	2.25 (1.08, 4.82)		2.04 (0.89, 4.79)	
Quartile 4 (high expression)	50	6.78 (3.42, 14.16)		5.01 (2.31, 11.38)	

[1]Logistic regression model adjusted for age and year at diagnosis

[2]Logistic regression model additionally adjusted for Gleason grade

Summary

- Genes involved in chromatin remodeling are enriched in the tumor tissue of overweight/obese prostate cancer patients
  - Tumor-specific
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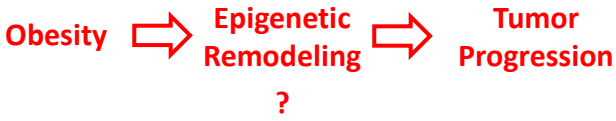
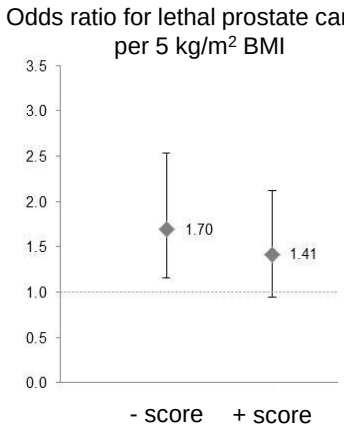


Chromatin remodeling gene expression mediates the relationship between BMI and lethal prostate cancer



36% of the association between BMI and lethal prostate cancer can be explained by the score

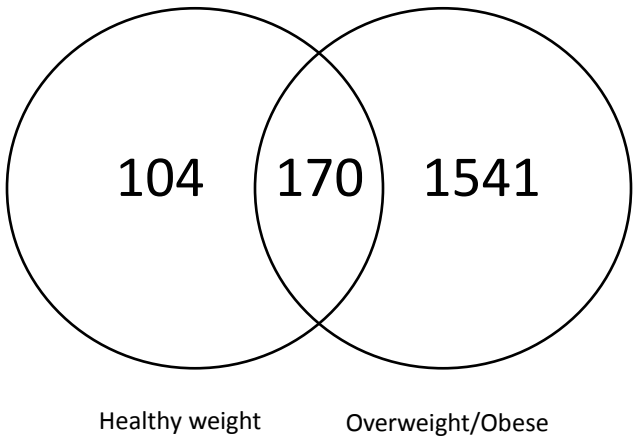
Conclusions

- These results provide support for a causal relationship between obesity and prostate cancer survival and identify a potential target for new treatment or secondary prevention strategies for prostate cancer patients
- Strengths:
  - First human study to look at gene expression alterations in prostate tissue by obesity status and relate such alterations to prostate cancer outcomes
  - Ability to integrate tissue-level biomarker data with exposure and clinical data and long-term follow-up for prostate cancer outcomes
- Limitations:
  - Detection and treatment bias among obese men
  - BMI as a measure of obesity/obesity-related metabolic disorders
  - Single BMI measurement prior to diagnosis

Future directions

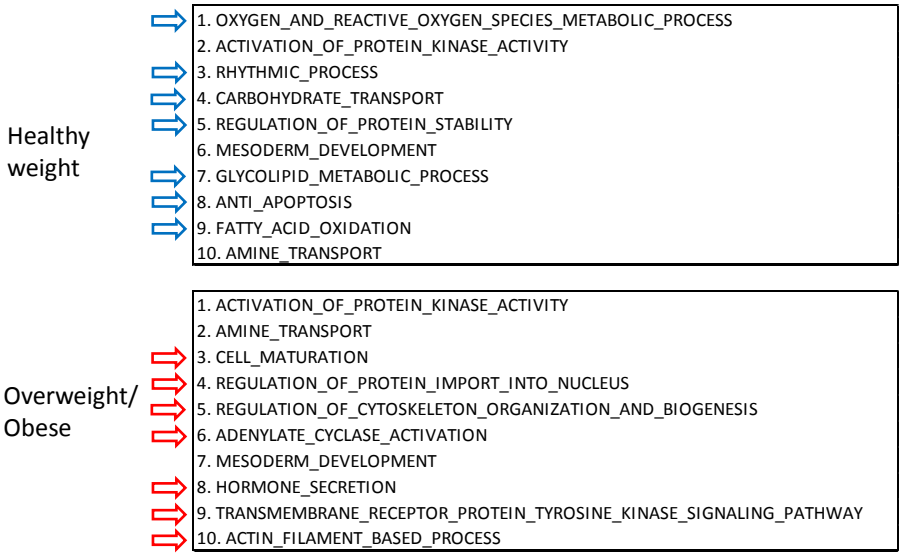
- Confirm chromatin remodeling results
  - Validate results in independent prostate cancer patient cohort
  - Measure chromatin modifications in prostate tissue
  - Test experimentally using diet-induced obesity mouse model of prostate cancer (David Labbé and Miles Brown, Dana-Farber Cancer Institute)
- Evaluate chromatin remodeling gene score in relation to other tissue biomarkers (e.g. ERG)
- Explore other pathways that modify the association between BMI and lethal prostate cancer

Number of differentially expressed genes in lethal tumors compared to indolent tumors by BMI status



p-value < 0.05

Top 10 biological processes enriched in lethal tumors compared to indolent tumors by BMI status



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Dana-Farber Cancer Institute

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Giorgia Zadra

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DoD Prostate Cancer Research Program

DF/HCC SPORE in Prostate Cancer

Identifying obesity-linked gene expression changes in prostate cancer

Ericka Ebot PhD MPH
Postdoctoral Fellow, Epidemiology
Harvard T.H. Chan School of Public Health

AACR Annual Meeting 2015
Molecular and Genetic Epidemiology of Cancer 4: New Insights
April 21, 2015

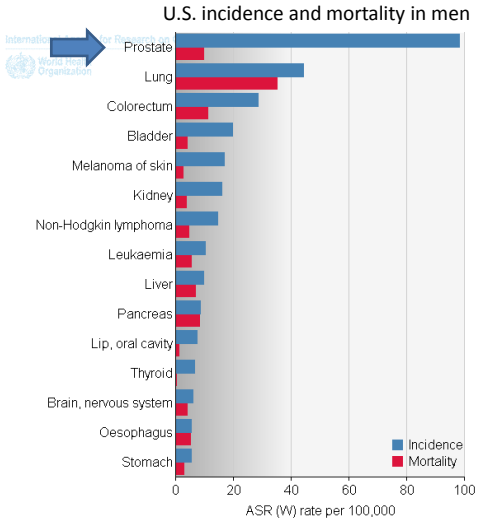
Disclosure Information

AACR Annual Meeting 2015
Ericka Ebot

I have no financial relationships to disclose.

I will not discuss off label use and/or investigational use in my presentation.

Prostate cancer incidence and mortality

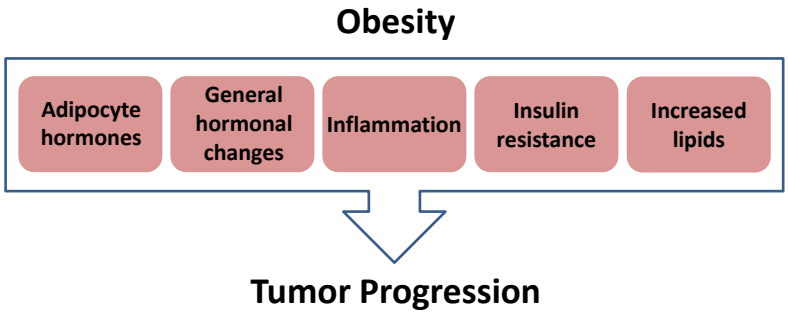


220,000 new cases and 30,000 deaths expected in the U.S. in 2015

Globocan 2012, IARC

Obesity and prostate cancer

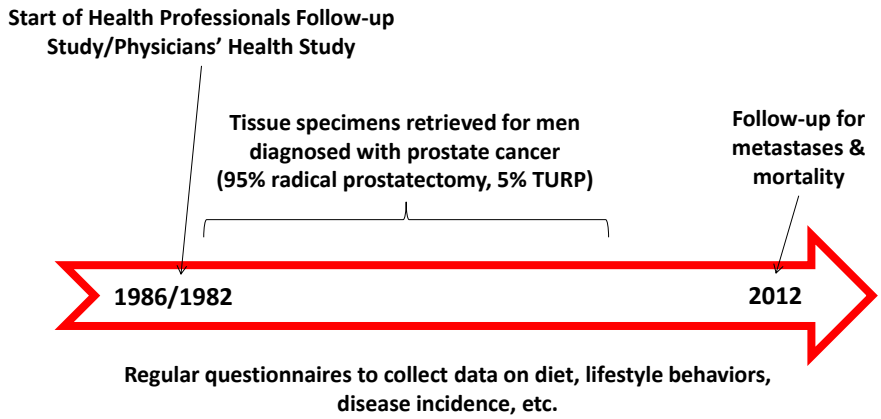
- Obese men are at higher risk of developing advanced stage prostate cancer and have higher rates of cancer-specific mortality after diagnosis



Aims

- Examine prostate-specific alterations associated with obesity using genome-wide mRNA expression profiles of tumor tissue
- Explore whether such alterations underlie the link between obesity and lethal prostate cancer
  - ➢ Better understand the underlying biology of aggressive prostate cancer
  - ➢ Identify subgroups of men with prostate cancer who are most likely to benefit from secondary prevention strategies including weight loss and other targeted therapies

Prostate Tumor Tissue Cohort



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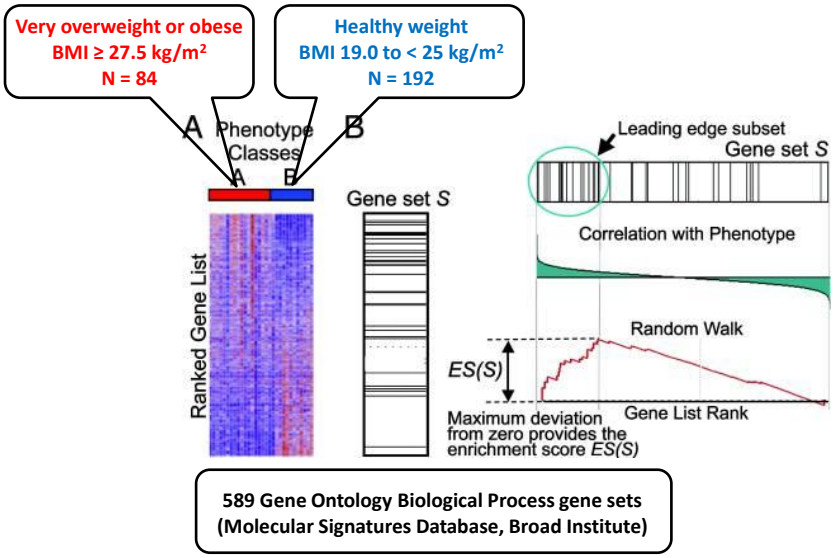
1990-1993 (peri-PSA era)	28
after 1993 (PSA era)	61
PSA at diagnosis, ng/ml, median	7.3
Pathologic TNM stage, %	
T2 N0 M0	59
T3 N0 M0	35
T4/N1/M1	5
Gleason grade, %	
2-6	14
3+4	34
4+3	25
8-10	26
Tissue type, %	
Radical prostatectomy	92
TURP	9

Gene Set Enrichment Analysis



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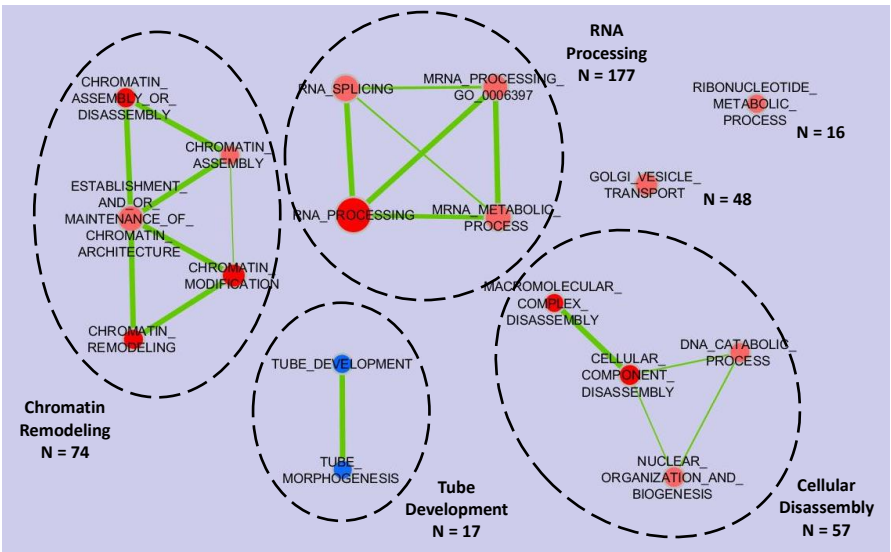
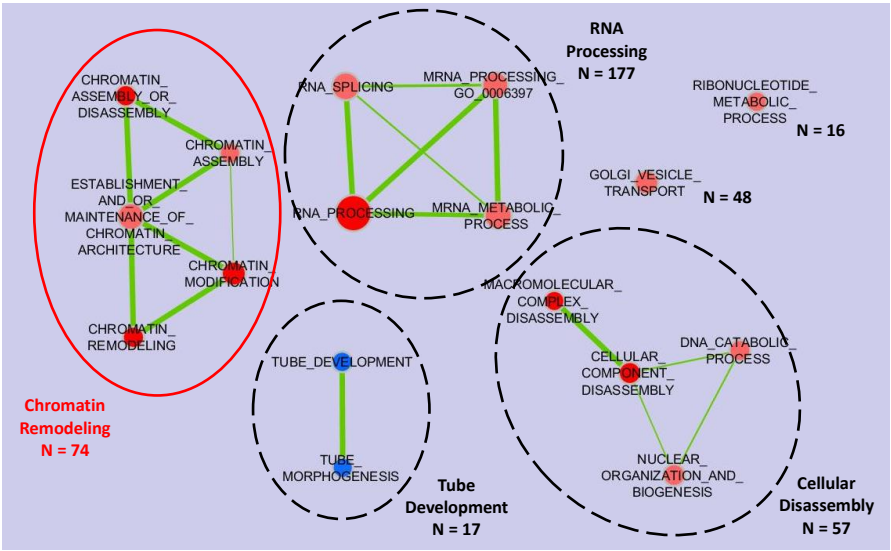
Enrichment Map of gene sets with FDR < 0.25



Enrichment Map of gene sets with FDR < 0.25



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

35 chromatin remodeling genes from leading edge subset

histone deacetylase activity
HDAC2, HDAC3, HDAC8, SIRT1

nucleosome remodeling
SWI/SNF: SMARCC2, SMARCA5, ARID1A, PBRM1, ACTL6A

Gene symbol	Gene name
INO80	INO80 homolog (S. cerevisiae)
SAFB	scaffold attachment factor B
SET	SET nuclear oncogene
HELLS	helicase, lymphoid-specific
HDAC2	histone deacetylase 2
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NAP1L2	nucleosome assembly protein 1-like 2
NAP1L4	nucleosome assembly protein 1-like 4
MTA2	metastasis associated 1 family, member 2
KAT2A	K(lysine) acetyltransferase 2A
RBBP4	retinoblastoma binding protein 4
HMGB1	high-mobility group box 1
HDAC3	histone deacetylase 3
PBRM1	polybromo 1
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TLK2	tousled-like kinase 2
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WHSC1L1	Wolf-Hirschhorn syndrome candidate 1-like 1
SMARCC2	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily c, member 2
HDAC8	histone deacetylase 8
HIRIP3	HIRA interacting protein 3
SYCP3	synaptonemal complex protein 3
ACTL6A	actin-like 6A
UBE2N	ubiquitin-conjugating enzyme E2N (UBC13 homolog, yeast)
RSF1	remodeling and spacing factor 1
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CHAF1A	chromatin assembly factor 1, subunit A (p150)
SIRT1	sirtuin 1
NAP1L1	nucleosome assembly protein 1-like 1
SUPT4H1	suppressor of Ty 4 homolog 1 (S. cerevisiae)

score computed based on expression of chromatin remodeling genes

Results: Tumor expression of chromatin remodeling genes is associated with Gleason grade

	Chromatin remodeling gene score		
	Quartile 1 (low expression)	Quartile 4 (high expression)	
Age at diagnosis, years, mean	66.7	66.5	
Year of diagnosis, %			
before 1990 (pre-PSA era)	11	13	
1990-1993 (peri-PSA era)	29	31	
after 1993 (PSA era)	60	56	
PSA at diagnosis, ng/ml, median	6.7	8.1	
Pathologic TNM stage, %			
T2 N0 M0	61	49	
T3 N0 M0	34	45	
T4/N1/M1	4	6	
Gleason grade, %			
2-6	20	9	**P-value 4.48e-6**
3+4	39	18	
4+3	25	33	
8-10	17	41	
Tissue type, %			
Radical prostatectomy	92	87	
TURP	8	13	

Results: Tumor expression of chromatin remodeling genes is associated with lethal outcome

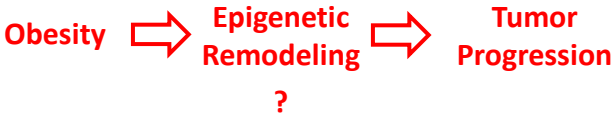
	# lethal	Odds ratio[1]	P-value	Odds ratio[2]	P-value
Chromatin remodeling gene score					
Quartile 1 (low expression)	15	ref	1.03E-07	ref	8.11E-05
Quartile 2	23	2.13 (1.02, 4.57)		2.03 (0.88, 4.81)	
Quartile 3	25	2.25 (1.08, 4.82)		2.04 (0.89, 4.79)	
Quartile 4 (high expression)	50	6.78 (3.42, 14.16)		5.01 (2.31, 11.38)	

[1]Logistic regression model adjusted for age and year at diagnosis

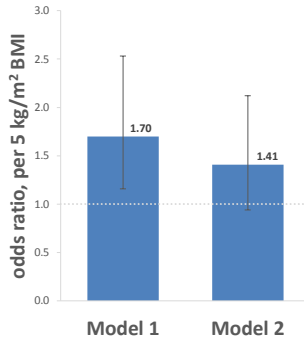
[2]Logistic regression model additionally adjusted for Gleason grade

Summary

- Genes involved in chromatin remodeling are enriched in the tumor tissue of overweight/obese men
  - Tumor-specific
- Associated with worse tumor characteristics (Gleason grade) and poorer prostate cancer outcomes

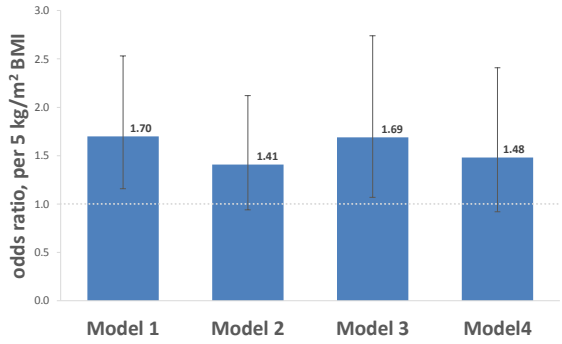


Results: Tumor expression of chromatin remodeling genes mediate the effect of BMI on lethal outcome



Model 1: base model (age and date of diagnosis)
Model 2: base model + gene score

Results: Tumor expression of chromatin remodeling genes mediate the effect of BMI on lethal outcome



Model 1: base model (age and date of diagnosis)
Model 2: base model + gene score
Model 3: base model + Gleason grade
Model 4: base model + Gleason grade + gene score

Conclusions and future directions

- This study provides evidence for involvement of chromatin remodeling in the obesity-lethal prostate cancer relationship
- Strengths:
  - First human study to look at gene expression alterations in prostate tissue by obesity status and relate such alterations to prostate cancer outcomes
  - Ability to integrate tissue-level biomarker data with rich exposure and clinical data and long-term follow-up for prostate cancer outcomes
- Limitations:
  - Detection and treatment bias among obese men
  - BMI as a measure of obesity/obesity-related metabolic disorders
  - Single BMI measurement prior to diagnosis

Conclusions and future directions

- Future work:
  - Validate gene expression results in independent cohort
  - Confirm epigenetic alterations in tumor tissue of obese patients
    - Histone acetylation, DNA methylation
  - Test experimentally using diet-induced obesity mouse model of prostate cancer (David Labbé and Miles Brown, Dana-Farber Cancer Institute)
  - Evaluate gene score in relation to other obesity biomarkers
  - Explore additional gene sets identified in Gene Set Enrichment Analysis

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Identifying obesity-linked gene expression alterations in prostate cancer

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[1]Harvard T.H. Chan School of Public Health; [2]Dana-Farber Cancer Institute; [3]Brigham and Women's Hospital; [4]Fred Hutchinson Cancer Research Center; [5]Trinity College Dublin Medical School; [6]Massachusetts Institute of Technology

BACKGROUND

- Obese men are at higher risk of developing advanced stage prostate cancer and have higher rates of cancer-specific mortality after diagnosis
  - Biological mechanism(s) are not well understood
- Using gene expression profiles of tissue from prostate cancer patients, we aimed to identify biological pathways that are differentially altered among overweight/obese compared to healthy weight men

METHODS

Study population
- Men diagnosed with prostate cancer in the Health Professionals Follow-up Study (HPFS) and Physicians' Health Study (PHS) for whom archival FFPE tissue was available
  - HPFS and PHS participants were followed with regular questionnaires to collect self-reported data on diet, lifestyle behaviors, medical history, and disease outcomes
  - Medical records and pathology reports were reviewed to confirm prostate cancer diagnosis and provide clinical data on stage and grade of cancer, PSA level at diagnosis, and initial treatments
  - Radical prostatectomy (92%) and TURP (8%) specimens were obtained from prostate cancer cases diagnosed between 1982-2005
  - Men were followed through 2012 for development of metastases and prostate cancer-specific death

Anthropometric data
- Self-reported body mass index was taken from the closest study questionnaire prior to prostate cancer diagnosis (average time = 1.3 years)

Outcome data
- Lethal prostate cancer defined as cancer that forms distant metastases or leads to cancer-specific death

Clinical data
- Study pathologists provided a standardized histopathologic review of each case including Gleason grading
- Tumor stage, PSA at diagnosis, age at diagnosis, and calendar year at diagnosis were obtained from medical record review

Gene expression data
- Whole genome mRNA expression profiling was performed on tumor (N=402) and adjacent normal (N=200) tissue using the Affymetrix GeneChip Human Gene 1.0 ST Array
  - 113 lethal and 289 indolent (survived at least 8 years without lethal event)

Statistical analysis
- Gene Set Enrichment Analysis (GSEA) was performed to identify differentially expressed Gene Ontology biological process gene sets between extreme BMI categories
- Chromatin remodeling gene score was computed for each tumor by averaging the normalized signal intensities of all member genes
- Odds ratios for lethal disease were estimated using logistic regression

ACKNOWLEDGMENTS

- This project was supported by the US Army Prostate Cancer Program, Prostate Cancer Foundation, National Cancer Institute, and DF/HCC SPORE in Prostate Cancer; EE supported by NRSA Training Program in Cancer Epidemiology (T32 CA-09001)

RESULTS

Table 1. Clinical characteristics by BMI status

	All men (N=402)	BMI 19.0 - 25.0 kg/m² (N=192)	25.0 - 27.5 kg/m² (N=126)	27.5 - 36.8 kg/m² (N=84)
Age at diagnosis, years, mean (SD)	65.7 (6.5)	65.8 (6.5)	66.3 (6.5)	64.5 (6.2)
Year of diagnosis, N (%)				
before 1990 (pre-PSA era)	45 (11.2)	27 (14.1)	10 (7.9)	8 (9.5)
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)	7.3 (5.3, 11.6)	7.9 (5.6, 12.0)	6.2 (4.8, 11.5)	7.7 (5.5, 10.7)
Pathologic TNM stage, N (%)				
T2 N0 M0	218 (59.4)	111 (62.7)	67 (58.8)	40 (52.6)
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 (%)				
T1/T2 N0 M0	349 (88.4)	168 (88.9)	111 (91.0)	70 (83.3)
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)
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)

63 missing PSA at diagnosis, 35 missing pathologic stage, 7 missing clinical stage

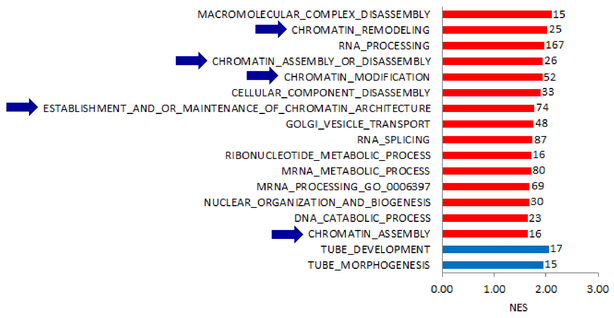

Figure 1. Top Gene Ontology biological process gene sets from GSEA (FDR < 0.25)

Table 2. Chromatin remodeling genes enriched in high vs. low BMI tumors

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

Table 3. Clinical characteristics by chromatin remodeling gene score

	All men (N=402)	Chromatin remodeling gene score Quartile 1 (N=101)	Quartile 2 (N=100)	Quartile 3 (N=100)	Quartile 4 (N=101)
Age at diagnosis, years, mean (SD)	65.7 (6.5)	66.7 (5.8)	71.0 (6.4)	64.5 (6.7)	66.5 (6.8)
Year of diagnosis, N (%)					
before 1990 (pre-PSA era)	45 (0.11)	11 (10.9)	8 (8.0)	13 (13.0)	13 (12.9)
1990-1993 (peri-PSA era)	112 (0.28)	29 (28.7)	26 (26.0)	26 (26.0)	31 (30.7)
after 1993 (PSA era)	245 (0.61)	61 (60.4)	66 (66.0)	61 (61.0)	57 (56.4)
PSA at diagnosis, ng/ml, median (q1, q3)	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)
Pathologic TNM stage, N (%)					
T2 N0 M0	218 (59.4)	57 (61.2)	60 (63.8)	58 (63.0)	43 (48.9)
T3 N0 M0	129 (35.1)	32 (34.4)	29 (30.9)	28 (30.4)	40 (45.4)
T4/N1/M1	20 (5.4)	4 (4.3)	5 (5.3)	6 (6.5)	5 (5.7)
Clinical TNM stage, N (%)					
T1/T2 N0 M0	349 (88.4)	90 (90.0)	87 (87.0)	87 (89.7)	85 (86.7)
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)
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)

63 missing PSA at diagnosis, 35 missing pathologic stage, 7 missing clinical stage

Table 4. Odds ratios for lethal prostate cancer according to chromatin remodeling gene score

Chromatin remodeling gene score	N lethal	Odds ratio[1]	P-value	Odds ratio[2]	P-value
Continuous, per 0.1 units	113	1.22 (1.14, 1.31)	4.13E-08	1.18 (1.09, 1.28)	4.31E-05
Categorical					
quartile 1 [-1.2,-0.2]	15	ref	1.03E-07	ref	8.11E-05
quartile 2 [-0.2, 0]	23	2.13 (1.02, 4.57)		2.03 (0.88, 4.81)	
quartile 3 [0, 0.2]	25	2.25 (1.08, 4.82)		2.04 (0.89, 4.79)	
quartile 4 [0.2, 1.5]	50	6.78 (3.42, 14.16)		5.01 (2.31, 11.38)	

[1]adjusted for age and year at diagnosis
[2]additionally adjusted for Gleason grade

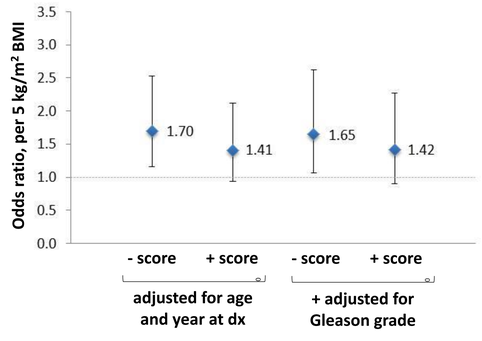

Figure 2. Odds ratios for lethal prostate cancer per 5kg/m² increase in BMI

CONCLUSIONS

- Genes involved in chromatin remodeling (including histone modification and nucleosome remodeling genes) were enriched in the tumor tissue of overweight/obese vs healthy weight men
  - Not enriched in analysis of adjacent normal tissue
- Chromatin remodeling pathway was positively associated with pathologic stage (p-value = 0.11) and Gleason grade (p-value = 4.48×10^{-6}).
- Chromatin remodeling pathway was associated with progression to lethal prostate cancer independent of Gleason grade
- These results support a role for chromatin remodeling as a mediator of the effect of obesity on prostate cancer progression

Title: Gene expression profiling identifies chromatin regulation as a molecular link between obesity and lethal prostate cancer

Authors: Ericka M. Ebot[1]*, Travis Gerke[1], David P. Labbé[2], Jennifer A. Sinnott[3], Giorgia Zadra[4], Jennifer R. Rider[5], Svitlana Tyekucheva[6], Kathryn M. Wilson[5], Rachel S. Kelly[1], Irene M. Shui[7], Massimo Loda[4], Philip W. Kantoff[8], Stephen Finn[9], Matthew G. Vander Heiden[8], Myles Brown[2], Edward L. Giovannucci[10], Lorelei A. Mucci[5]

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One Sentence Summary: Genes involved in chromatin regulation are upregulated in the tumor tissue of overweight and obese prostate cancer patients compared to healthy weight patients, and their expression is associated with worse clinical characteristics and poorer prostate cancer-specific survival.

Abstract: Obese men are at higher risk of developing advanced prostate cancer and have higher rates of cancer-specific mortality. However, the biological mechanisms explaining these associations are unknown. Using gene expression data, we aimed to identify molecular alterations in prostate tumor tissue associated with obesity. Gene Set Enrichment Analysis identified fifteen gene sets upregulated in the tumor tissue of obese prostate cancer patients (N=84) compared to healthy weight patients (N=192), five of which were related to chromatin modification and remodeling. These gene sets were not identified in an analysis of adjacent normal tissue. Patients with tumors with high expression of chromatin-related genes had worse clinical characteristics (Gleason grade >7, 41% versus 17%, p-trend = 3.21×10^{-4}) and poorer prostate cancer-specific survival independent of Gleason grade (lethal outcome, OR = 5.01,

95% CI = 2.31 to 11.38). Mediation analysis further supported a role for chromatin regulation in the obesity-lethal prostate cancer relationship. These findings identify a promising link between obesity and prostate cancer survival that if confirmed could lead to new treatment or prevention strategies for prostate cancer patients.

Introduction

A significant challenge in prostate cancer research is the identification of risk factors that drive disease progression. Obesity is a potential modifiable risk factor that has been linked to advanced disease and worse cancer-specific outcomes among prostate cancer patients (*1-3*). In the Health Professionals Follow-up Study (HPFS) and the Physicians' Health Study (PHS), our group demonstrated that men who are overweight or obese are at a higher risk of dying of prostate cancer (*4*) and have higher rates of prostate cancer-specific mortality after diagnosis (*5*). Obesity is a major health problem that affects more than 30% of adults in the United States (*6*). Therefore, an understanding of the relationship between excess body weight and worse prostate cancer outcomes has important public health implications. While several mechanisms have been proposed (*7, 8*), the drivers of the association between obesity and aggressive prostate cancer are not well understood.

In this study, we sought to develop a better understanding of the link between obesity and lethal prostate cancer using whole transcriptome gene expression profiles of prostate tissue from men diagnosed with the disease. We assessed gene expression alterations 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.

Results

Patient characteristics by prediagnosis BMI

Table 1 describes the clinical characteristics of the study population overall and according to categories of prediagnosis BMI. Among the 402 men included in the study, 192 (47.8%) were healthy weight (BMI 18.5 to <25.0 kg/m^2), 126 (31.3%) were overweight (BMI 25.0 to <27.5 kg/m^2), and 84 (20.9%) were very overweight or obese (BMI ≥27.5 kg/m^2) prior to prostate cancer diagnosis. No statistically significant differences were observed for any of the clinical

characteristics by prediagnosis BMI. However, an increase in pathologic TNM stage disease with increasing BMI was noted; 47.4% of men in the highest BMI category had T3 or T4 stage disease compared to 37.3% of men in the lowest BMI category (p-trend across BMI categories = 0.14).

Gene set enrichment analysis identifies biological processes enriched in tumor tissue of obese prostate cancer patients

We compared gene expression data from individuals in the highest BMI category (very overweight/obese; N=84) to those in the lowest BMI category (healthy weight; N=192) to identify molecular alterations in prostate tissue associated with obesity. After adjusting for multiple comparisons, no single gene was found to have significant differential expression between BMI categories in either the tumor or adjacent normal samples (data not shown). We applied Gene Set Enrichment Analysis (GSEA) (*9*) to detect coordinated changes in the expression of functionally related genes by prediagnosis BMI. We identified 15 gene sets upregulated and 2 gene sets downregulated in the tumor tissue of very overweight/obese prostate cancer patients compared to the healthy weight patients at a false discover rate (FDR) level of 0.25 (Figure 1 and Tables S1 and S2). Among these top results, we identified several networks of overlapping gene sets involved in chromatin regulation, RNA processing, and cellular disassembly (Figure 2). These pathways were not identified in a similar analysis using expression data from adjacent normal tissue suggesting the results are tumor-specific (Tables S3 and S4). To address differences in sample sizes for tumor and adjacent normal tissue, we performed GSEA on the subset of tumor samples that also have normal expression data and found that the majority (9 of 15) of up-regulated gene sets identified in the full analysis remained enriched at an FDR level of 0.25 (Tables S5 and S6).

Characterization of chromatin gene set network

Five of the 15 gene sets enriched in the tumor tissue of obese patients included chromatin modification and remodeling genes involved in the regulation of chromatin structure and function (Figure 2). Given the importance of epigenetics in cancer development and progression (*10*), we chose to further characterize this gene set network in our prostate cancer cohort. Specifically, we selected the 35 genes within the chromatin gene set network making up the GSEA leading edge subset (Table 2) and created a "metagene" score based on the expression levels of these genes. We found that the "chromatin gene score" was greater in tumor tissue compared to adjacent normal tissue (p-value = 2.3×10^{-4}). Also, as expected, the "chromatin gene score" was positively associated with prediagnosis BMI in tumor tissue (p-value = 5.7×10^{-5}) but not in adjacent normal tissue (p-value = 0.46).

Expression of chromatin modification and remodeling genes is associated with clinical characteristics

Table 3 illustrates the clinical characteristics of the cohort according to the "chromatin gene score" in tumor tissue. We found that the score was significantly associated with Gleason grade; 40.6% of men in the highest score quartile had a Gleason grade >7 compared to 16.8% in the lowest quartile (p-trend across quartiles = 3.21×10^{-4}). In addition, though not significant, we observed an increase in the percentage of pathologic T3 or T4 stage with increasing score, from 38.7% in the lowest quartile to 51.2% in the highest quartile (p-trend across quartiles = 0.11).

Expression of chromatin modification and remodeling genes is associated with lethal prostate cancer

Logistic regression analysis showed a positive association between the "chromatin gene score" in tumor tissue and lethal prostate cancer. Men with a higher score had a significantly increased risk of metastases or death from prostate cancer, independent of age and year at diagnosis, with an odds ratio (OR) of 6.78 (95% confidence interval (CI) = 3.42 to 14.16) for lethal outcome

comparing extreme quartiles of the score (Table 4). After further adjusting for Gleason grade the odds ratio for lethal prostate cancer was attenuated slightly but remained statistically significant (OR = 5.01, 95% CI = 2.31 to 11.38 comparing extreme score quartiles) (Table 4).

Expression of chromatin modification and remodeling genes mediates the relationship between BMI and lethal prostate cancer

To explore whether chromatin modification and remodeling mediates the relationship between obesity and lethal prostate cancer, we assessed the association between BMI and lethal outcome with and without adjustment for the "chromatin gene score". Among the men in this study, the odds ratio for lethal prostate cancer was 1.70 (95% CI = 1.16 to 2.53) per 5-unit increase in prediagnosis BMI, adjusted for age and year at diagnosis (Figure 3, Model 1). Addition of the score to the base model reduced the odds ratio for lethal prostate cancer to 1.41 (95% CI = 0.94 to 2.12) per 5-unit increase in prediagnosis BMI (Figure 3, Model 1). Further adjustment for Gleason grade did not have an effect on these results (Figure 3, Model 2). The association between the score and lethal prostate cancer remained unchanged with BMI added to the model. Moreover, in a mediation analysis (*11*), we determined that 36% of the association between BMI and lethal prostate cancer was explained by the score. No appreciable difference in the results were observed when accounting for Gleason grade (proportion mediated = 31%).

Discussion

There is compelling evidence linking obesity to aggressive prostate cancer, but the underlying causes of this relationship are unclear. In this study we used whole transcriptome gene expression profiling data to identify biological processes that may mediate the adverse effects of excess body weight on prostate tumor tissue. We found that expression of genes involved in chromatin regulation is enriched in the tumor tissue of overweight and obese prostate cancer patients compared to those of healthy weight. Tumors with high expression of the identified

genes had less favorable clinical characteristics, including Gleason grade. Moreover, greater tumor expression was associated with an increased risk of prostate-cancer specific mortality, independent of Gleason grade. While these results warrant further study, they suggest that obesity may promote tumor progression by influencing the epigenetic state of prostate cancers.

Epigenetic alterations, including DNA methylation and histone modifications, are a common feature of cancer and are emerging as important drivers of tumor progression. A dysregulated epigenome can prevent normal tissue differentiation and results in altered transcription of genes involved in cancer-related cellular processes, such as proliferation, apoptosis, inflammation, and invasion (*10*). Both global DNA hypomethylation and promoter-localized DNA hypermethylation occur in prostate cancer and have been linked to metastatic disease (*12*). In addition to DNA methylation, alterations in chromatin structure also represent an important component of transcription deregulation during tumor progression. Extensive remodeling of the histone code through gain and loss of histone marks occurs in prostate cancer and, in cooperation with DNA methylation, results in transcription of key oncogenes, microRNAs, and cancer biomarkers (*13*).

The current analysis identified genes encoding components of the mammalian SWI/SNF complex, an ATP-dependent nucleosome remodeling complex important for transcriptional regulation (*14*), and histone modification enzymes, including a number of histone deacetylases (HDACs) that contribute to inactive chromatin and gene silencing (*15*). These mechanisms work together to regulate gene transcription as well as other cellular processes including DNA replication and DNA damage repair (*16, 17*). Mutations and altered expression of genes encoding these regulators have been identified in prostate cancer (*10*). In particular, accumulating evidence supports a role for HDACs in prostate cancer. HDAC overexpression in prostate cancer specimens has been linked to adverse tissue features and worse prostate

cancer outcomes (*18-21*). Furthermore, global histone modification patterns were shown to correlate with risk of prostate cancer recurrence (*22-24*).

Epigenetic regulation mediates the reversible effects of environmental exposures and lifestyle factors on carcinogenesis and tumor progression (*25*). Observational and experimental studies have begun to provide evidence for epigenetic alterations related to obesity (*26*). However, most human studies in this area were conducted in blood or adipose rather than tumor tissue (*27*). A recent study looked at the association between obesity and gene methylation in breast cancer and found BMI-associated gene methylation alterations in estrogen receptor-positive breast tumors (*28*). To date, DNA methylation has been the most well studied epigenetic regulatory mechanism. Our novel findings suggest that obesity impacts epigenetic regulation in prostate tumor tissue through chromatin-related processes. Metabolism influences the availability of metabolites used by enzymes to alter chromatin, however, further studies linking obesity to prostate-specific histone modifications need to be performed to determine how obesity impacts the nature of these alterations.

Interestingly, in our gene set enrichment analysis of normal prostate tissue we did not identify a relationship between BMI and chromatin remodeling gene expression, suggesting that characteristics specific to tumor tissue may render susceptibility to changes influenced by obesity. Along these lines, our group previously demonstrated that obesity is linked to worse cancer prognosis primarily in men with tumors harboring the *TMPRSS2:ERG* gene fusion (29), 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 potential epigenomic rewiring observed in obese patients.

To our knowledge this is the first human study to look at gene expression differences in prostate tissue by obesity status and to relate such differences to prostate cancer outcomes. One previous study evaluated gene expression profiles of prostate tumor and matching normal tissue according to BMI at the time of radical prostatectomy (RP) and found an association of high BMI with altered levels of lipid metabolism and cholesterol homeostasis genes; however, this study was limited to 12 patients (*29*). A second study focused on gene expression changes in human periprostatic adipose tissue by BMI status among a small number (N=18) of prostate cancer patients undergoing prostatectomy surgery (*30*). These authors found altered expression of genes involved in adipogenic/antilipolytic, proliferative/anti-apoptotic, and mild immunoinflammatory processes in the periprostatic adipose tissue of obese subjects.

Our study is the largest to date to evaluate gene expression signatures of obesity among patients with prostate cancer within two large, established cohort studies with long-term follow-up. Strengths of our study include its prospective design and validated data on anthropometric measures. In addition, the patients were well-characterized with respect to clinical and pathologic measures, including re-review of Gleason score. Long-term follow-up allows for the ascertainment of lethal prostate cancer as the outcome, which is the most clinically relevant endpoint for prostate cancer.

Due to the lack of public data sources with both gene expression and BMI data, we were unable to validate our results in an independent cohort. Thus, future gene expression studies are required to confirm these findings. In addition, the cohort used is almost exclusively white, and we do not know whether our conclusions would apply to men of other ethnic groups. A potential limitation of the study is the use of BMI as a measure of obesity. BMI is an imperfect measure of body fatness because it does not distinguish between fat mass and lean mass. 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 (*31*).

Finally, we cannot completely rule out that obesity affects prostate cancer outcomes at least in part through its effect on detection and treatment of the disease, rather than through true biological differences in the tumors themselves. Obesity may make screening and detection of prostate cancer more difficult due to lower serum prostate-specific antigen (PSA) concentrations, lower accuracy of the digital rectal examination, and larger prostate size among obese individuals, which may delay diagnosis and treatment (*32*). 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 screening and PSA screening eras and noted that the association remained largely unchanged (*5*). While obese patients may receive different treatments than non-obese patients (*32*), our study includes men who primarily underwent prostatectomy as curative treatment, which limits the possible impacts of treatment differences that are observed in the overall patient population.

In conclusion, this analysis provides the first comprehensive look at obesity-associated gene expression alterations in prostate tumor tissue. These results improve our understanding of the biology of aggressive prostate cancer and provide additional support for a causal relationship between obesity 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.

Materials and Methods

Study population

This study was nested among prostate cancer patients enrolled in two prospective studies: the PHS and HPFS. The PHS I and II began in 1982 and 1997 respectively as randomized trials of aspirin and dietary supplements enrolling 29,067 U.S. male physicians for the primary prevention of cardiovascular disease and cancer (*33-36*). The HPFS is an ongoing prospective cohort study of the causes of cancer and heart disease among 51,529 U.S. male health professionals initiated in 1986 (*4*). In both studies, participants were followed with regular questionnaires to collect self-reported data on diet, lifestyle behaviors, medical history, and disease outcomes, including prostate cancer. Incident prostate cancer cases were confirmed by review of medical records and pathology reports. The study was 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 each subject.

Following confirmation of diagnosis, archival formalin-fixed paraffin-embedded (FFPE) prostate tissue specimens collected during RP or transurethral resection of the prostate (TURP) were retrieved from treating hospitals. Study pathologists provided a standardized histopathologic review of each case including Gleason grading (*37*). Gene expression profiling was performed on a subset of the tumor cohort based on an extreme case sampling design. In total, 402 prostate cancer patients diagnosed between 1982 and 2005 were included in the study, comprising 113 lethal cases (developed metastatic disease or died from prostate cancer) and 289 indolent cases (survived at least 8 years after prostate cancer diagnosis without any evidence of metastases). For a subset of these men (N=200), we also profiled adjacent normal tissue.

Whole-transcriptome gene expression profiling

Gene expression profiling of archival FFPE tissue was performed as previously described (*38*). Briefly, two to three 0.6-mm cores were sampled from regions of high-density tumor and

adjacent normal prostate tissue. RNA was extracted using the Biomek FX^P automated platform with the Agencourt FormaPure kit (Beckman Coulter). Whole-transcriptome amplification was performed using WT-Ovation FFPE System V2 (NuGEN) and the amplified cDNA was hybridized to a GeneChip Human Gene 1.0 ST microarray (Affymetrix). For the expression profiles generated, we regressed out technical variables and then shifted the residuals to have the original mean expression values, and normalized using the robust multi-array average method (*39, 40*). 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; this resulted in 20,254 unique gene names. Gene expression data are available through Gene Expression Omnibus accession number GSEXXXXX.

Anthropometric data

Self-reported questionnaire information on height and weight was used to calculate BMI. Participants provided height and weight at enrollment and weight information annually (PHS) or biannually (HPFS) thereafter. In HPFS, self-reported measurements of weight were tested against standardized technician measures and showed high validity, with a Pearson correlation of 0.97 (*41*). In this study, we used BMI information from the closest questionnaire prior to prostate cancer diagnosis. The mean prediagnosis BMI was 25.4 kg/m^2 (range = 19.0 to 36.8 kg/m^2) and the mean time between BMI measurement and prostate cancer diagnosis was 1.3 years (range = 0 to 11.3 years). Since the proportion of men in our study above the World Health Organization cut-off for obesity (BMI ≥ 30 kg/m^2) was low, we divided BMI into the following categories for subsequent analyses: 18.5 to < 25 kg/m^2 (healthy weight), 25 to < 27.5 kg/m^2 (overweight), and ≥ 27.5 kg/m^2 (very overweight or obese).

Clinical and follow-up data

Information about prostate cancer diagnosis including age and date of diagnosis, PSA level at diagnosis, and pathologic and clinical stage was abstracted from medical records and pathology reports. Men diagnosed with prostate cancer were followed through questionnaires for details of their clinical course, including subsequent treatments, changes in PSA, and development of metastases. Deaths were ascertained through repeated mailings, telephone calls to non-respondents, and searches of the National Death Index. Date and specific cause of death was assigned after review of death certificates, information from family, and medical records. Lethal prostate cancer was defined as distant metastases or prostate cancer-specific death. Follow-up is complete through March 2011 for PHS and December 2011 for HPFS.

Statistical analysis

ANOVA and χ^2 tests were used to assess differences in clinical characteristics according to BMI categories. Linear regression and χ^2 test for trend were used to examine linear trends in clinical characteristics across BMI categories.

Gene Set Enrichment Analysis (GSEA) (*9*) was performed on gene expression profiles of tumor and adjacent normal prostate tissue to identify predefined sets of functionally related genes correlated with prediagnosis BMI. The analysis included 589 predefined Gene Ontology (GO) Biological Process gene sets from the Broad Institute Molecular Signature Database v4.0. Genes were ranked based on a signal-to-noise metric comparing individuals in the highest BMI category (very overweight/obese) to those in the lowest BMI category (healthy weight). 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. Significance was estimated by comparing the observed score to the distribution of scores from 10,000 phenotype-based permutations. To account for multiple hypothesis testing, the normalized enrichment score (NES) and false discovery rate (FDR) were used to identify the

top GO biological processes differentially expressed by obesity status. Gene sets with an FDR less than 0.25 were considered for subsequent analyses. Enrichment Map (*42*) was used to uncover gene set redundancy and aid in interpretation of the GSEA results. To build the gene set networks an overlap coefficient cut-off of 0.5 was used.

The genes identified in the enrichment analysis were used to create a "metagene" score. A score was computed for each sample by averaging the normalized (mean centered and variance scaled) expression values of all member genes. Score values ranged from -1.2 to 1.5 (median = 0.0; Q1, Q3 = -0.2, 0.2). A t-test was used to compare the mean score between tumor and adjacent normal tissue. To test the relationship between the score and BMI we used Pearson correlation.

ANOVA and χ^2 tests were used to assess differences in clinical characteristics according to quartiles of the "metagene" score. Linear regression and χ^2 test for trend were used to examine linear trends in clinical characteristics across score quartiles. Logistic regression was used to calculate odds ratios and 95% confidence intervals for the association between the "metagene" score and lethal prostate cancer. The score was modeled as continuous (per 0.1 units) or categorical (quartiles). We tested for linear trend across score categories by modeling the quartiles as a continuous variable (quartile 1 = 0, quartile 2 = 1, quartile 3 = 2, quartile 4 = 3). All models were adjusted for age and year at diagnosis (continuous). We further adjusted for Gleason grade (continuous: <7 = 0, 3+4 = 1, 4+3 = 2, >7 = 3) to test whether the score is an independent predictor of lethal prostate cancer.

Finally, we evaluated whether the "metagene" score attenuated the association between BMI (continuous, per 5 kg/m^2) and lethal prostate cancer in logistic regression models. We considered age and date at diagnosis and Gleason grade as covariates in this analysis. We also

applied a formal mediation analysis to calculate the percentage of the association between BMI and lethal prostate cancer explained by the score (*11*). We modeled the score and BMI as continuous variables and considered age and date at diagnosis and Gleason grade as covariates. Mediation analyses were performed using SAS version 9.3. R version 3.1.0 was used for all other analyses.

Supplementary Materials

Table S1. Top 20 Gene Ontology biological process gene sets overexpressed in tumor tissue of very overweight/obese compared to healthy weight patients.

Table S2. Top 20 Gene Ontology biological process gene sets underexpressed in tumor tissue of very overweight/obese compared to healthy weight patients.

Table S3. Top 20 Gene Ontology biological process gene sets overexpressed in adjacent normal tissue of very overweight/obese compared to healthy weight patients.

Table S4. Top 20 Gene Ontology biological process gene sets underexpressed in adjacent normal tissue of very overweight/obese compared to healthy weight patients.

Table S5. Top 20 Gene Ontology biological process gene sets overexpressed in tumor tissue of very overweight/obese compared to healthy weight patients among a subset of the study population that have both tumor and adjacent normal data.

Table S6. Top 20 Gene Ontology biological process gene sets underexpressed in tumor tissue of very overweight/obese compared to healthy weight patients among a subset of the study population that have both tumor and adjacent normal data.

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Figures and Tables:

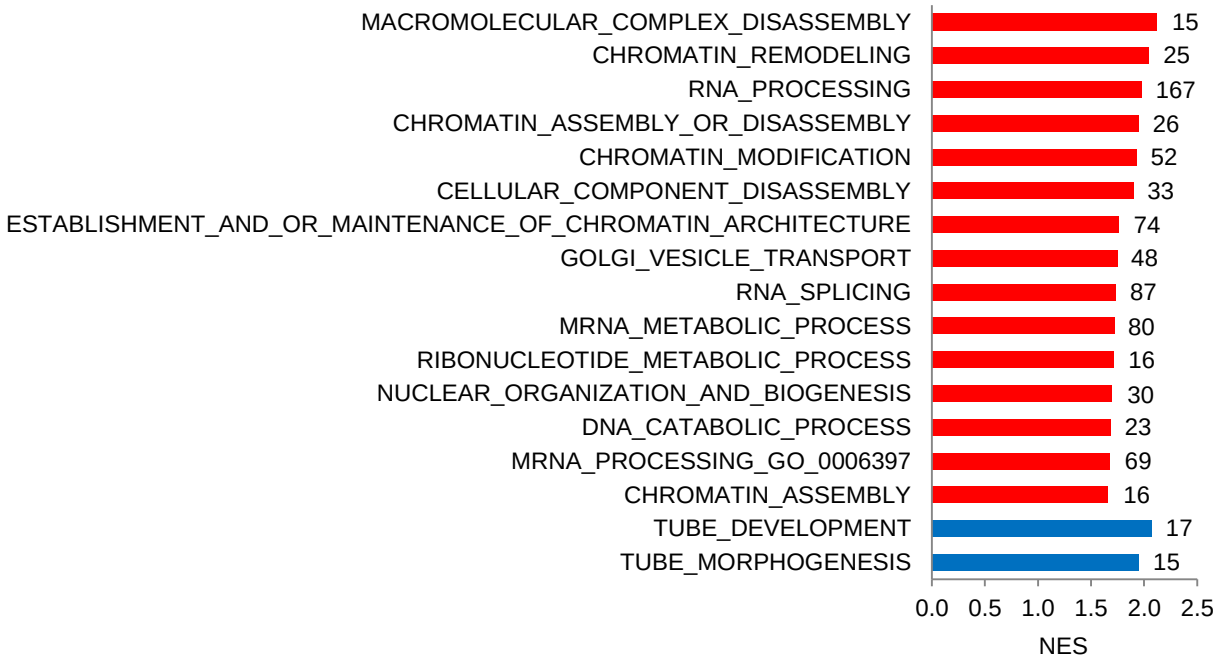

Figure 1. Top Gene Ontology biological process gene sets enriched in tumor tissue of overweight/obese patients compared to healthy weight patients. Gene sets with a false discovery rate less than 0.25 are shown. Gene Ontology terms are ordered according to the normalized enrichment signal. Numbers next 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.

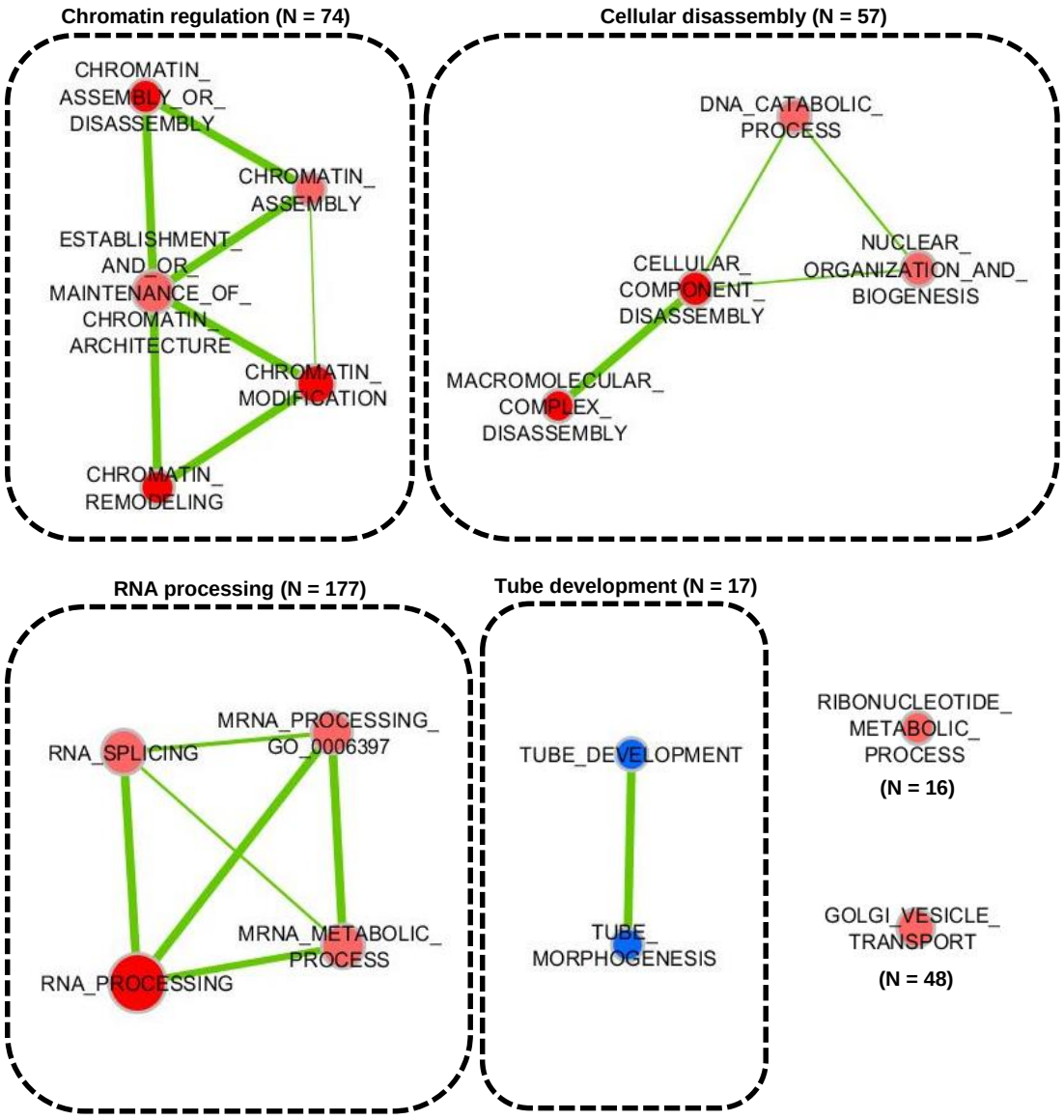

Figure 2. Enrichment Map of Gene Ontology biological process gene sets enriched in tumor tissue of overweight/obese patients compared to healthy weight patients. Gene sets with a false discovery rate less than 0.25 are shown. Each gene set is a node and links represent gene overlap between sets. The 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.

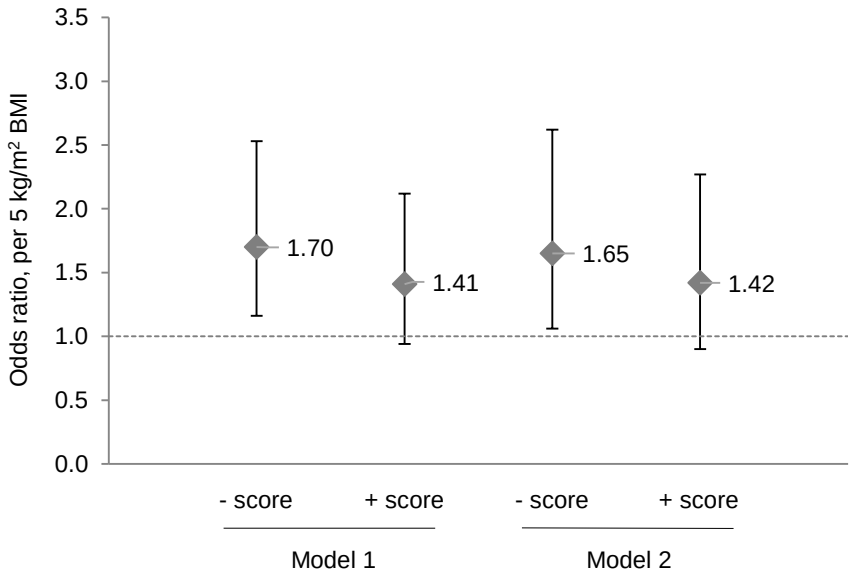

Figure 3. Odds ratios and 95% confidence intervals for lethal prostate cancer according to prediagnosis body mass index. Model 1 is adjusted for age and year at diagnosis (continuous). Model 2 is additionally adjusted for Gleason grade (continuous: <7 = 0, 3+4 = 1, 4+3 = 2, >7 = 3). For each model the odds ratio for lethal prostate cancer is illustrated with and without adjustment for the chromatin gene score (continuous).

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.

Characteristic	All men (N=402)	Prediagnosis BMI		
		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)
Age at diagnosis, years, mean (SD)	65.7 (6.5)	65.8 (6.5)	66.3 (6.5)	64.5 (6.2)
Year of diagnosis, N (%)				
Before 1990 (pre-PSA era)	45 (11.2)	27 (14.1)	10 (7.9)	8 (9.5)
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)
Pathologic TNM stage, N (%)[b]				
T2 N0 M0	218 (59.4)	111 (62.7)	67 (58.8)	40 (52.6)
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)
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)
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)
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)
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.

Abbreviations: 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 the 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)**
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)
Year of diagnosis, N (%)					
Before 1990 (pre-PSA era)	45 (11.2)	11 (10.9)	8 (8.0)	13 (13.0)	13 (12.9)
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)
Pathologic TNM stage, N (%)[b]					
T2 N0 M0	218 (59.4)	57 (61.3)	60 (63.8)	58 (63.0)	43 (48.9)
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)
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)
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)
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)
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.

Abbreviations: SD = standard deviation, Q1 = lower quartile, Q3 = upper quartile.

Table 4. Odds ratios and 95% confidence intervals for lethal prostate cancer according to the 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)	4.13E-08	1.18 (1.09, 1.28)	4.31E-05
Categorical					
Quartile 1 (low)	15	ref	1.03E-07	ref	8.11E-05
Quartile 2	23	2.13 (1.02, 4.57)		2.03 (0.88, 4.81)	
Quartile 3	25	2.25 (1.08, 4.82)		2.04 (0.89, 4.79)	
Quartile 4 (high)	50	6.78 (3.42, 14.16)		5.01 (2.31, 11.38)	

[a]Quartiles modeled as 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 adusted for Gleason grade (continuous: <7 = 0, 3+4 = 1, 4+3 = 2, >7 = 3).

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REVIEW

Role of diet in prostate cancer: the epigenetic link

DP Labbé[1,2], G Zadra[1,3], EM Ebot[4], LA Mucci[4,5], PW Kantoff[1], M Loda[1,3] and M Brown[1,2]

Diet is hypothesized to be a critical environmentally related risk factor for prostate cancer (PCa) development, and specific diets and dietary components can also affect PCa progression; however, the mechanisms underlying these associations remain elusive. As for a maturing organism, PCa's epigenome is plastic and evolves from the pre-neoplastic to the metastatic stage. In particular, epigenetic remodeling relies on substrates or cofactors obtained from the diet. Here we review the evidence that bridges dietary modulation to alterations in the prostate epigenome. We propose that such diet-related effects offer a mechanistic link between the impact of different diets and the course of PCa development and progression.

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INTRODUCTION

In the United States, an estimated 233 000 new prostate cancer (PCa) cases will be diagnosed and 29 480 patients will die from PCa in 2014, making this disease the most commonly diagnosed cancer and the second leading cause of cancer-related death in American men.[1] In Europe, PCa is estimated to be the third leading cause of cancer-related death in men for 2014, behind lung and colorectal cancers.[2] There are a few confirmed risk factors for PCa incidence overall, of which age is the most important: PCa is uncommon before 50 years of age and is rarely lethal before 60 years. In fact, 70% of PCa-related deaths occur after age 75.[3] African ancestry and a positive family history are also among the risk factors associated with PCa, and now numerous genetic risk loci have been validated in multiple studies.

The incidence of PCa worldwide can vary by as much as 50-fold between low- and high-risk populations. The large disparity in PCa incidence between the Eastern and the Western hemispheres, a trend observed even before the adoption of prostate-specific antigen testing in developed countries,[4] points to a key role of environmental factors, such as diet, as an etiologic factor in this disease.[5,6] This association is further supported by observations from Japanese immigrants in Los Angeles County, in whom PCa rates are almost quadrupled compared with Japanese living in their homeland and almost match the incidence rate seen in California native residents.[7]

PCa is characterized by complex genomic alterations that are highly heterogeneous and vary greatly from patient to patient, as well as within the same tumor focus. Such disparities can be partly explained by an underlying genomic instability.[8] In addition, PCa has been described as an 'epigenome catastrophe', because various changes in DNA methylation patterns can be detected well before the cancer becomes invasive,[9] suggesting that epigenetic changes are pivotal events in tumor initiation.[10,11] Interestingly, diet can induce various epigenetic modifications that result in global alterations in chromatin packaging; such stable and heritable changes regulate the access of the transcriptional machinery to target genes, and thereby modulate gene expression profiles.[9,12]

Here we introduce some of the evidence that supports the thesis that diet impacts PCa initiation and progression, and examine the hypothesis that these diet-related effects are, in part, mediated by epigenomic alterations.

DIET AND PCa: THE EPIDEMIOLOGICAL EVIDENCE

The impact of diet on cancer growth was first described in landmark studies at the beginning of the 20th century by researchers such as Peyton Rous, who reported that some tumors have a delayed growth and retarded development when transplanted to previously underfed hosts, whereas other tumors are unaffected by the host's diet.[13] We now know that not all cancer types are equally sensitive to dietary modulation,[14] a phenotype that may be attributed in part to defined genetic alterations.[15]

An increasing number of epidemiological and molecular studies point to a link between diet and PCa, particularly for cancers that are more aggressive. Despite this, the role of specific dietary components in PCa development and progression is still unclear. In 2007, the World Cancer Research Fund/American Institute for Cancer Research reported that a diet rich in foods containing lycopene/cooked tomatoes or selenium (nota bene, selenium content in food is mirrored by the soil's selenium abundance) has a protective effect against PCa, whereas diets high in calcium have been associated with increased risk for PCa.[16]

Following this line of reasoning, the role of lycopene and tomato products in PCa prevention has been extensively studied and, although evidence is mixed, available data suggest an inverse association between increased consumption and PCa.[17] In the prospective Health Professionals Follow-up Study, consumption of tomato products was shown to be inversely associated with the incidence of total PCa as well as of advanced stage disease.[18] Also of interest, low levels of selenium have been associated with increased risk of PCa, particularly in relation to advanced or

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aggressive disease.[19] However, selenium supplementation did not significantly reduce the risk of developing PCa in the SELECT randomized trial, indicating that whether selenium intake is obtained directly from the diet or as supplements may impact differently PCa risk.[20] With limited evidence, other potential protective dietary elements include vitamin E, cruciferous vegetables, soy/isoflavones, polyphenols, fish/marine omega-3, coffee and vitamin D.[21–23] Conversely, a number of epidemiological studies have reported an increased risk of PCa for extreme categories of calcium intake,[24] with stronger associations for the risk of advanced or lethal disease.[18] The effect of folate intake (including folic acid supplementation) on PCa risk is conflicting. Although dietary and total folate intake is not associated with PCa risk, high circulating folate levels are associated with an increased risk of PCa,[25] a risk further heightened in patients of African ancestry.[26] With limited evidence, a high dietary intake of red meat and heterocyclic amines, saturated and monounsaturated fats, as well as the essential alpha-linolenic fatty acid (FA) promotes PCa development.[21,23]

FEEDING PCa

Evidence from preclinical models

The impact of diet on PCa progression has been evaluated in various mouse models (see the excellent review by Irshad and Abate-Shen[27] for a detailed overview of the strengths and limitations of each mouse model). It has been shown that a high-carbohydrate/high-fat diet enhances the growth of human PCa cell xenografts in mice.[28,29] In the Hi-Myc transgenic mouse model of PCa, a low-fat diet delays tumor progression,[30] whereas Hi-Myc mice maintained on a calorie-restricted diet display a reduced incidence of *in situ* adenocarcinoma compared with overweight controls (10% kcal from fat) or with mice on a diet-induced obesity regimen (60% kcal from fat).[31] Importantly, calorie-restricted mice do not develop invasive adenocarcinoma, and the frequency of invasive adenocarcinoma is significantly lower in mice fed a low-fat diet compared with mice on the diet-induced obesity regimen. Increased feeding of mice is correlated with greater activation of growth factor signaling,[31] and the greater frequency of prostate adenocarcinoma occurrence in the transgenic adenocarcinoma of the mouse prostate (TRAMP) model has also been attributed to excessive calorie retention.[32] Moreover, a high-fat diet in LADY (12 T-10) transgenic mice is correlated with increased neuroendocrine differentiation, a marker of aggressive PCa.[33]

Similarly, $PTEN^{PE-/-}$ (PE, prostate epithelium) mice that are fed an omega-3 FA-rich diet display reduced PCa growth, slower histopathological progression and increased survival, whereas mice fed on an omega-6 FA-rich diet exhibit the opposite result. Insertion of an omega-3 desaturase (which converts omega-6 into omega-3 FA) into the $PTEN^{PE-/-}$ background rescues the phenotype of mice that are fed the high omega-6 diet.[34] Along the same lines, Yue *et al.*[35] recently observed that esterified cholesterol specifically accumulates in high-grade PCa and metastases, and that this accumulation results from the hyper-activation of the PI3K/AKT pathway following the loss of *PTEN*. Inhibiting acyl-coenzyme A (CoA):cholesterol acyltransferase (*ACAT-1*) results in a net depletion of stored cholesteryl ester, which impedes cell proliferation, migration and even tumor growth in murine xenograft models. Although the underlying mechanism responsible for this unforeseen phenotype, where cholesteryl ester fuels PCa growth, still remains to be fully defined,[35] these observations are further strengthened by the recent findings that ACAT-1 expression can serve as a prognostic marker that readily distinguishes indolent from aggressive PCa.[36]

The human data

In an elegant *ex vivo* study, Aronson *et al.*[37] randomized men with PCa (but not currently under treatment) to either a low-fat (15% kcal) high-fiber and soy-supplemented diet or a typical high-fat (40% kcal) Western diet for 4 weeks; they found that proliferation of LNCaP cells grown in a medium containing 10% human serum from these patients is significantly inhibited only in the presence of serum from men maintained on a low-fat diet for 4 weeks. Consistent with this, obesity is correlated with a lower risk of early stage PCa, as well as an elevated risk of aggressive PCa.[38] In a meta-analysis, Cao and Ma[6] reported that an elevated body mass index of 5 kg/m^2 is associated with a 20% higher PCa-specific mortality. Obesity dysregulates a number of key hormonal pathways and it has been proposed that lower sex hormone-binding globulin, adiponectin and higher insulin, growth hormone, insulin-like growth factor 1 (IGF-1) may also contribute to the development of high-grade tumors in obese patients. In particular, the growth hormone/IGF-1 pathway, known to have a role in the metabolic syndrome (that is, increased blood pressure, high blood sugar level, abnormal cholesterol levels, excess in waist body fat), is implicated in PCa progression.[39–44] Interestingly, high circulating IGF-1 levels are more strongly associated with low-grade than high-grade PCa. This result may reflect a greater dependency of differentiated neoplastic cell on circulating IGF-1 compared with undifferentiated cells that may be less responsive due to a constitutively active PI3K/AKT pathway.[45] In addition, among men diagnosed with PCa in the Physicians' Health Study, excess body weight and a high plasma concentration of C-peptide (a surrogate for insulin levels) both predispose men to an increased likelihood of dying of the disease, further suggesting a role for insulin in PCa progression in obese men.[46] Finally, men with hypercholesterolemia are also more at risk of developing aggressive PCa, a trend reverted by statins' intake.[47]

Collectively, these results obtained from preclinical models and human data demonstrate that both diet and obesity can alter PCa risk and progression. Obviously, the influence of these factors on PCa development is complex and involves a large number of 'classical' signaling pathways (reviewed by Venkateswaran and Klotz[48]). In this review, we propose that diet also alters the prostate epigenome and affects the course of the disease.

THE ALTERED EPIGENOME OF PCa

Epigenetic marks, including DNA methylation and histone modifications, are critical for maintaining a carefully regulated state for the cell. These marks affect local as well as global chromatin packaging, which in turn dictates the sets of active and inactive genes at any given time. It is now clear that cancer development is at least supported,[49] if not initiated,[11] by alterations of the epigenome, which then leads to transcriptional rewiring. Epigenetic modifications observed in PCa evolve throughout disease progression.

DNA methylation in eukaryotes is defined as methylation of the fifth carbon on cytosine residues in CpG dinucleotides (5-methylcytosine). These covalently added methyl groups project into the major groove of DNA and alter transcription.[50] In PCa, genome-wide DNA methylation of cytosine residues in CpG dinucleotides is greatly impaired as the disease progresses to a metastatic stage and leads to global hypomethylation,[51] which can enable the transcription of normally unexpressed proviral and retrotransposon repeats,[52,53] followed by disruption of nearby genes and a predisposition to genomic instability.[53,54] Specific promoter hypomethylation can also reactivate proto-oncogenes such as the urokinase-type plasminogen activator (*PLAU*),[55,56] the matrix metalloproteinase-2 (*MMP2*)[56] or the heparanase (*HPSE*),[57] known to be implicated in tumor invasion and metastasis. On the other hand, promoter hypermethylation and silencing of specific

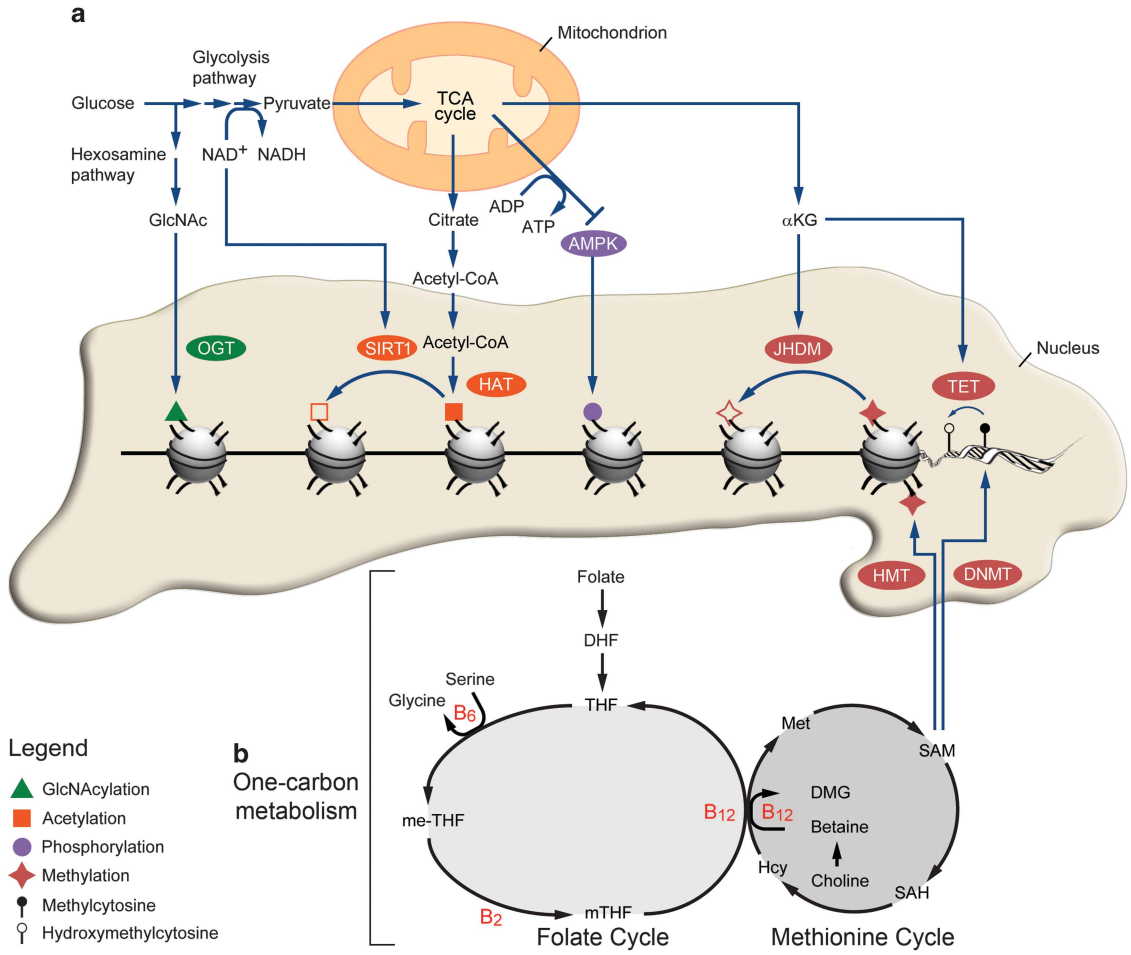

Figure 1. From metabolism to epigenetic remodeling. (**a**) SIRT1 activity depends on the NAD^+/NADH ratio modulated by glycolysis, while *O*-linked *N*-acetylglucosamine transferase uses GlcNAc produced by the hexosamine pathway. Pyruvate entering the tricarboxylic acid (TCA) cycle produces alpha-ketoglutarate, a critical cofactor for Jumonji domain-containing histone demethylase and TET. Acetyl-CoA is converted from the citrate generated by the TCA cycle and used as a donor by histone acetyltransferases. Finally, the increase in ATP/ADP ratio from the TCA cycle also inactivates AMPK. (**b**) SAM acts as a methyl donor for histone methyltransferases and TET and is obtained through the coordinate action of the folate and methionine cycles, termed one-carbon metabolism. αKG: Alpha-ketoglutarate; AMPK: 5′ AMP–activated protein kinase; ADP: adenosine diphosphate; ATP: adenosine triphosphate; B_2: vitamin B_2; B_6: vitamin B_6; B_{12}: vitamin B_{12}; DHF: dihydrofolate; DMG: dimethylglycine; DNMT: DNA methyltransferases; GlcNAc: N-acetylglucosamine; HAT: histone acetyltransferases; Hcy: homocystein; HMT: histone methyltransferases; JHDM: Jumonji domain-containing histone demethylase; OGT: O-linked N-acetylglucosamine transferase; me-THF: 5,10-methylenetetrahydrofolate; Met: methionine; mTHF: 5-methyltetrahydrofolate; NAD+: nicotinamide adenine dinucleotide (oxidized); NADH: nicotinamide adenine dinucleotide (reduced); SAH: S-adenosylhomocysteine; SAM: S-adenosylmethionine; SIRT1: sirtuin histone deacetylase 1; TCA: tricarboxylic acid; TET: ten eleven translocation; THF: tetrahydrofolate.

genes such as that for the detoxification enzyme *GSTP1* is observed in more than 75% of high-grade prostatic intraepithelial neoplasms and in almost all prostate carcinomas (95%),[58] and possibly sensitizes cells to DNA damage. In fact, hypermethylation of the *GSTP1* promoter is a highly specific PCa marker and is rarely detected in benign prostatic hyperplasia[58,59] and normal prostatic tissues.[59,60]

Global patterns of histone acetylation and methylation are also affected throughout PCa progression and can predict the risk of PCa recurrence.[61–63] Bert *et al.*[64] compared the long-range epigenetic remodeling that occurs in different PCa cell lines with that in normal primary cell lines. They used coordinate assessment of histone modifications, DNA methylation profiles and RNA expression; they identified 35 long-range epigenetic activation domains, each about 1 Mb long, and found that a total of 251 genes were activated within these domains—these include oncogenes and genes for microRNAs and PCa biomarkers (for example, *KLK3*, *PCA3*). In particular, alterations of histone marks in PCa cells were characterized either by an enrichment of active histone marks (H3K9ac and H3K4me3) or by the replacement of repressive marks (H3K27me3) by active marks (H3K9ac).[64]

This comprehensive analysis also revealed that, on a genome-wide scale, a subset of long-range epigenetic activation domains were not characterized by promoter hypomethylation, but rather by an extensive DNA hypermethylation in the CpG islands of promoter regions. On the basis of these findings, the authors propose that DNA hypermethylation of promoter regions can prevent the binding of transcriptional repressors, thereby facilitating transcriptional activity.[64] Their findings support a complex interaction between DNA methylation and the histone code in regulating gene transcription.

Together with the report that chromatin modifiers such as *CHD1*, *CHD5* and *HDAC9* are mutated in an important subset of primary PCa,[65] the above results demonstrate that the epigenome undergoes a complex and dynamic remodeling throughout disease progression.

EPIGENETIC MODIFICATIONS AND DIET

A fundamental feature of epigenetic remodeling is its reliance on substrates or cofactors obtained from the diet (Figure 1). When under situations of metabolic stress, the energy-sensing serine-threonine kinase 5′ AMP-activated protein kinase (AMPK) phosphorylates histone H2B at serine 36 and triggers a cell survival program.[66] Histone H2B is also targeted by an *O*-linked *N*-acetylglucosamine (*O*-GlcNAc) residue on serine 112, a glucose-dependent modification that is often located near transcribed genes.[67] The activity of sirtuin histone deacetylase (SIRT) is dictated by the ratio of oxidized and reduced nicotinamide adenine dinucleotide (NAD^+/NADH), which can be modulated by fasting,[68] calorie restriction[69] or dietary supplementation of NAD^+ precursors.[70] Interestingly, in PCa, levels of both NAD^+ and GlcNAc metabolites are altered following seminal vesicle invasion or lymph node metastasis.[71] Alpha-ketoglutarate, an intermediate of the tricarboxylic acid cycle, is also a critical cofactor for histone demethylation by Jumonji domain-containing histone demethylase,[72] as well as for DNA demethylation by ten eleven translocation (Tet) proteins[73] (see the excellent review by Lu and Thompson[74] for details about these metabolite-dependent epigenetic modifications). In addition, the two most well-studied epigenetic processes, namely, methylation and acetylation, are also deeply connected to the diet.

Methylation: an epigenetic modification governed by one-carbon metabolism

DNA and histone methylation by DNA methyltransferases and histone methyltransferases, respectively, requires the transfer of a methyl group (catalyzed by a methyltransferase) from the methyl donor *S*-adenosylmethionine (SAM). Although DNA methylation is usually associated with transcriptional inhibition, the effect of histone methylation depends on the location of the methyl-lysine residue on the histone tail and also on the degree of methylation.[75] SAM is derived from methionine, an essential amino acid that can either be obtained from the diet *per se* or can be generated from homocysteine in a process that utilizes carbon derived from dietary folate, choline or betaine (also a product of choline oxydation) in a vitamin B12-dependent reaction.[76] This cyclic cellular process is termed one-carbon metabolism and is a bicyclic metabolic pathway that refers to the folate and methionine cycles (Figure 1). One-carbon metabolism integrates the donation of carbon units from nutrient inputs into essential cellular processes such as the regulation of redox balance, maintenance of the nucleotide pool, biosynthesis of proteins and the regulation of epigenetic modifications (reviewed by Locasale[77]). Erythrocyte levels of SAM can be altered by dietary intake of fat as well as of calories.[78] Evidence of a link between high serum levels of homocysteine (or deficiency in either folate or vitamin B12) and neural tube defects in the fetus during early stages of pregnancy led to mandatory worldwide folic acid fortification.[79] Finally, because one-carbon metabolism is central to cellular growth and proliferation, folate antagonists—first described in 1948 by Farber and Diamond[80] as a promising treatment for pediatric acute lymphoblastic leukemia—are also used as chemotherapeutic agents.

The yellow agouti (A^{vy}) mouse carries an intracisternal A particle (*IAP*) retrotransposon into the 5′ end of the *agouti* (*A*) gene and is a viable model for determining the impact of diet on epigenetic marks. When unmethylated and active, a cryptic promoter located within the 5′ end of *IAP*'s long terminal repeat hijacks the transcriptional control of the *agouti* gene and leads to ubiquitous expression of the agouti signaling protein; under normal conditions, this protein is restricted to hair cycle-specific patterns.[81] This yields mice that have a yellow coat color and develop multiple health issues such as type II diabetes, obesity and a higher frequency of tumor formation,[82] and serves as a phenotypic readout for a ready assessment of the methylation status of a promoter under different environmental conditions.

A major hallmark of the epigenome is its considerable plasticity during embryogenesis, which enables the differentiation of a single totipotent cell into more than 200 different cell types.[83] Wolff *et al.*[84] published a landmark study in which pregnant non-agouti (*a*/*a*) mothers mated with A^{vy}/*a* males were fed a methyl-supplemented diet (enriched in choline, betaine, folic acid, and vitamin B12), and found that fewer A^{vy}/*a* dams fed *in utero* with the methyl-supplemented diet had a yellow coat color and that this decrease was mirrored by an increased methylation of the A^{vy} proximal long terminal repeat.[85,86] In fact, the darkness of the coat color of the A^{vy}/*a* dams was directly correlated with the degree of methylation of the A^{vy} allele.[87]

In contrast, maternal exposure to bisphenol A (BPA) 2 weeks before mating and throughout gestation and lactation led to an increase in the proportion of A^{vy}/*a* dams that had a yellow coat color and carried a hypomethylated A^{vy} allele. This effect was negated when the BPA diet was supplemented with methyl donors.[88] Alternatively, peri-conceptional feeding of a methyl-deficient diet to female sheep resulted in adult offspring with CpG islands that were hypomethylated or unmethylated relative to animals fed on the control diet. Methyl-deficient diets also led to several health issues, ranging from higher body weight, increased fat, insulin resistance or elevated blood pressure in adult offspring.[89] Similarly, early peri-conceptional exposure to famine during the Dutch Hunger Winter in World War II led to hypomethylation of the imprinted*IGF2* gene in individuals compared with their same-sex siblings, a feature that was maintained for more than 60 years after the event itself.[90] Loss of *IGF2* imprinting is also a feature observed in PCa tissues,[91] as well as in proximal and distal tumor-associated tissues.[92]

Together, these results suggest that dietary modulation of rate-limiting factors of one-carbon metabolism generates long-lasting alterations in the methylation profile, and thus leads to phenotypic changes, in a given organism.

Histone acetylation is a nutrient-sensitive epigenetic mark

Acetylation of lysine residues on histones by histone acetyltransferases neutralizes the basic charge of the lysine, decreases electrostatic affinity between histone proteins and DNA and favors gene transcription via facilitated recruitment of the transcriptional machinery.[93] Lysine acetylation on proteins not only triggers gene transcription, but is also a critical posttranslational modification that regulates the activity of core metabolic enzymes.[94] Analysis of mass spectrometry data reveals that almost every enzyme involved in FA metabolism, glycogen metabolism, glycolysis, gluconeogenesis, the tricarboxylic acid cycle and the urea cycle is acetylated,[95] and functional analysis further documents a complex layer of regulation for protein lysine acetylation of metabolic enzymes. The acetylation status of these metabolic enzymes is responsive to environmental cues—such as the levels of amino acids, FAs or glucose—and modulates the activity and stability of the enzymes.[95]

Fluctuation in protein acetylation in response to dietary factors can be attributed, in part, to the availability of the acetyl group itself, which is obtained from the metabolite acetyl-CoA. Under nutrient-rich conditions, acetyl-CoA is generated by the ATP-citrate lyase (ACL), which catalyzes the conversion of citrate derived from the tricarboxylic acid cycle.[96] Alternatively, acetyl-CoA can be generated through the action of acetyl-CoA synthetases (ACECSs) from the pool of acetate, CoA and ATP. The activity of ACECSs is tightly regulated through reversible acetylation. Under low-nutrient conditions, the NAD^+/NADH ratio increases, activates SIRT1, which in turn de-acetylates and triggers ACECSs activity.[97] Therefore, the pool of acetyl-CoA, which is

governed by nutrient availability, controls the acetylation of metabolic enzymes as well as of histones at any given time.

Along these lines, studies in yeast reveal that levels of acetyl-CoA—which vary depending on the metabolic state—dictate cell growth, in part through the acetylation of histones at growth genes.[98] In yeast, this growth regulation mechanism may be balanced by the competition between histone acetylation and *de novo* FA biosynthesis for the same nucleocytosolic supply of acetyl-CoA, which normally matches growth signals with the required output in macromolecules.[99] In mammalian cells, histone acetylation is similarly dependent on the availability of acetyl-CoA, and inhibiting generation of acetyl-CoA through ACL knockdown thus results in global histone hypoacetylation.[96]

This critical mechanism for regulating cell growth is hijacked by the master transcription factor and proto-oncogene c-Myc, which is implicated in up to 70% of human cancers; Myc overexpression or deregulation results in cancer cells that become addicted to nutrients.[100] Specifically, Myc deregulation leads to the uptake of glucose and glutamine, which are carbon sources used to generate citrate (and consequently acetyl-CoA) through ACL activity.[101] Myc thus increases *de novo* FA biosynthesis and histone acetylation from glucose-derived acetyl groups.[102] Deregulation of cell metabolism by Myc leads to alteration of chromatin structure[103] combined with the generation of the biomass required for supporting uncontrolled cell growth.[104]

PCa: THE IMPACT OF DIET ON THE EPIGENOME

Several studies report a role for dietary components in the remodeling of the cancer epigenome (reviewed by Supic *et al.*[105]). In the context of PCa, the phytoestrogen genistein has the capability to partially demethylate CpG islands in the promoter region of specific genes such as *GSTP1*, leading to increased protein expression.[106] In PCa cell lines, genistein treatment also increases/restores expression of various tumor suppressors including *PTEN*, *p53*, *CYLD, p21WAF1/CIP1* and *p16INK4a*.[107,108] This feature is attributed to the coordinated demethylation and acetylation of H3K9 residues[107] or to increased expression of histone acetyltransferases that result in the enrichment of acetylated histones H3 and H4.[108] Similarly, the flavone apigenin also increases the acetylation of histones H3 and H4 *in vitro* and, when fed orally, significantly impedes PCa tumor growth *in vivo*. In this case, the phenotype is attributed to a marked reduction in histone deacetylase (HDAC) activity as well as in HDAC1 and HDAC3 protein expression.[109] Together, these results suggest that specific dietary molecules can alter PCa progression, in part by remodeling the epigenome. In addition, manipulating the content of dietary methyl donors or dietary fat alters the prostate epigenome and the course of the disease.

Dietary modulation of one-carbon metabolism to influence PCa development

As described above, one-carbon metabolism is central to DNA and histone methylation, as it generates SAM, the ultimate methyl donor. As in earlier studies with use of the A^{vy}/a model,[84] Shabbeer *et al.* used the Hi-Myc mouse model to investigate the impact of excess dietary methyl groups on PCa progression.[110] Overexpression of nuclear Myc protein is frequently detected in prostatic intraepithelial neoplasms, and in a majority of primary carcinomas and metastatic samples,[111] making the Hi-Myc mouse a particularly appropriate mouse model for the study of PCa. Mice were fed a control diet or a 'methyl' diet enriched in choline, betaine, folic acid, vitamin B12 and also in L-methionine and zinc sulfate while *in utero*[112] and during the first month of postnatal life, at which time all mice were fed the control diet. Although given only *in utero* and during early postnatal life, the methyl diet had a long-lasting effect on PCa development. At 5–7 months of age, no invasive adenocarcinoma was detected in prostates from Hi-Myc mice that were fed the methyl diet compared with a high incidence of invasive cancer in the control group. However, this difference in incidence was not observed in younger mice (at 3–5 months of age), suggesting that the methyl diet has an impact on the transition from mPIN to invasive adenocarcinoma, possibly via epigenomic changes.[112] These counterintuitive results indicate that timing might be critical in the context of modulating one-carbon metabolism, and can lead one to hypothesize that the methyl donor diet, if administered during the development of adenocarcinoma, would instead fuel uncontrolled tumor growth by maintaining a hyperactive one-carbon metabolism.

Along the same lines, Bistulfi *et al.* investigated the effects of manipulating dietary folate during disease progression in the TRAMP model, which relies on inactivation of pRb, p53 and PP2A following prostate-specific expression of SV40 large T and small t antigens.[113] TRAMP mice were fed one of three different diets at weaning: a folate-deficient diet, a folate-supplemented diet or a diet containing the recommended amount of folic acid for rodents.[114] Although folate supplementation had little to no effect on tumor growth, folate deficiency clearly improved PCa histopathological parameters compared with the control group, suggesting that folate might be a rate-limiting agent but only when it is under a certain threshold. Depletion of folate from the diet slowed the progression of cancer[114] and the robust arrest of disease progression was attributed by the authors to the secretory function of the prostate, which produces massive amounts of polyamines and exports them into reproductive fluids.[115] Indeed, no reduction in levels of polyamine was found in mice that were fed the folate-deficient diet, although polyamine synthesis draws on pools of SAM through the activity of SAM decarboxylase. This observation suggests that preferential use of SAM for polyamine synthesis under conditions of low folate in the prostate impedes other SAM-related pathways, such as the DNA methylation of CpG islands.[114] Consistent with this, a choline- and methionine-deficient diet led to increased expression of *Igf2* in the prostate of wild-type mice, a result that was mirrored by epigenetic changes at the gene promoter.[116]

In humans, the role of folate in PCa is unclear, although some evidence points to a positive association between high levels of circulating folate and PCa progression.[117] However, before considering the influence on the epigenome of dietary modulation of one-carbon metabolism, it is important to keep in mind that long-term deficiency of dietary methyl donors has important adverse effects. Folate depletion blocks *de novo* biosynthesis of thymidylate, leading to misincorporation of uracil into the DNA and culminating in single-strand DNA breaks[118]—as a consequence, prolonged dietary deficiency of methyl donors in mice leads to the development of intestinal tumors,[119] liver tumors and even to spontaneous mortality.[116] Thus, further experiments aimed at determining the timing, length and extent of a dietary intervention, to effectively impact the course of the disease while keeping side effects to a minimum, are warranted.

The cross talk between lipids and the prostate epigenome

As discussed above, manipulating dietary fat alters the progression of PCa in animal models. In 2010, Llaverias *et al.*[120] showed that increasing both dietary fat and dietary cholesterol significantly accelerates tumor progression in the TRAMP model, but the issue of whether cholesterol *per se* has a role in this aggravated phenotype was left unresolved. Pommier *et al.*[121] attempted to deconvolute these results using a mouse with a double knockout of the genes for the Liver X receptors alpha and beta ($Lxr\alpha\beta^{-/-}$), which encode nuclear receptors central to cholesterol homeostasis. The dorsal prostate lobes of $Lxr\alpha\beta^{-/-}$ mice fed on a standard diet were histologically similar to those of wild-type mice. But when $Lxr\alpha\beta^{-/-}$ mice were fed a high-cholesterol diet,

they accumulated intra-prostatic cholesteryl ester associated with mPIN development; gene expression analysis revealed that two prostatic tumor suppressor genes, *Nkx3.1* and *Msmb*, were downregulated in these mice. This event was attributed to an increase in the H3K27me3 mark at *Nkx3.1* and *Msmb* promoters, possibly a consequence of upregulation of the well-known prostate oncogene histone methyltransferase *Ezh2*.[121,122] Both *LXRβ* downregulation and *EZH2* upregulation have also been reported in human PCa.[123,124] Together with the recent report of abnormal cholesteryl ester accumulation in primary and metastatic human PCa (probably as a consequence PI3K/AKT hyperactivation following *PTEN*-loss),[35] these findings support a role for dietary cholesterol in influencing the prostate epigenome as well as disease progression of PCa.

Aside from dietary cholesterol, *de novo* lipid synthesis may also contribute to the regulation of epigenetic marks, especially histone acetylation. Indeed, *de novo* lipid synthesis is an important hallmark of PCa and correlates with tumor progression and poorer prognosis.[125] Use of an AMPK activator to block *de novo* lipogenesis impedes PCa growth and has been described as a promising treatment avenue, with or without the combined use of AR antagonists.[126] Along these lines, Kee *et al.* demonstrated that overexpression of the enzyme spermidine/spermine N^1-acetyltransferase (SSAT) leads to the diversion of pools of nucleocytosolic acetyl-CoA to polyamine catabolism. In the TRAMP model, overexpression of SSAT leads to a 70% decrease in the availability of acetyl-CoA and resulted in a genitourinary tract that is four times smaller than in control TRAMP mice.[127] It is thus tempting to speculate that *de novo* lipid synthesis observed in PCa also supports cell growth, in part, through global acetylation reprograming.[128]

CONCLUSIONS AND FUTURE DIRECTIONS

Mounting evidence implicates specific diets and dietary components in affecting the course of PCa and the risk of developing the disease. As PCa is considered to be an 'epigenetic catastrophe'[9] and because epigenetic marks rely on substrates or cofactors that are obtained from the diet, we suggest that the impact of diet on PCa development is, at least in part, linked to epigenomic remodeling.

Despite the promising results described here, a number of critical elements remain to be experimentally validated before the causality between diet and the prostate epigenome is established; these include the generation of a comprehensive epigenomic map of both healthy and neoplastic prostatic tissues from different models that are fed on controlled diets, and the metabolomics profile of matching tissues. Such an undertaking would facilitate the determination of the strength of the relationship between diet and the prostate's epigenome. Importantly, results obtained from PCa models should be carefully interpreted relative to their respective oncogenic drivers. Indeed, integrative metabolomic analysis recently revealed that PCa models driven by AKT1 are associated with the accumulation of aerobic glycolysis metabolites, while on the other hand MYC-driven PCa models are associated with dysregulated lipid metabolism.[129] Also, with the emergence of epigenetic-based PCa biomarkers (reviewed by Valdés-Mora and Clark[130]), the identification of common dietary- and cancer-dependent epigenetic alterations could be useful for patient risk stratification as well as for the development of specific dietary guidelines for defined patients.

Recently, epigenetic inhibitors that target DNA methyltransferases (azacitidine, decitabine) or HDAC (vorinostat, romidepsin) have been tested in clinical trials and approved by the US Food and Drug Administration for use in treating defined cancers.[131] Thus, deconvoluting the specific role of diet in rewiring the prostate's transcriptional network may yield critical information and may uncover dietary-related epigenetic pathways that can be therapeutically targeted to prevent or treat PCa.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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