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Genome-wide methylation analysis identifies involvement of TNF-α mediated cancer pathways in prostate cancer

Sun Jung Kim [a,d], William Kevin Kelly [b,1], Alan Fu [a], Kenneth Haines [c], Aaron Hoffman [a,2], Tongzhang Zheng [a], Yong Zhu [a,*]

[a] Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT, USA
[b] Department of Surgical Medicine, Yale University School of Medicine, New Haven, CT, USA
[c] Department of Pathology, Yale University School of Medicine, New Haven, CT, USA
[d] Department of Life Science, Dongguk University-Seoul, Seoul, Republic of Korea

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ABSTRACT

Altered signaling pathways resulting from aberrant changes in epigenetic parameters may play a pivotal role in carcinogenesis. To identify biological pathways likely to be affected by methylation-mediated alterations in gene expression in prostate cancer, we performed a genome-wide methylation analysis of 27,578 CpG sites, corresponding to 14,495 genes on a pooled sample of 12 pairs of prostate tumor and adjacent normal tissues. In all, 972 CpG sites were significantly hypermethylated while 209 sites were hypomethylated in prostate tumor tissue (FDR adjusted p-value < 0.05; fold change $\geqslant 2$) corresponding to 1043 unique genes, which is consistent with genome-wide gene-specific hypermethylation patterns previously observed in multiple cancer models. Global hypomethylation in prostate tumor was also detected by measuring methylation changes in ALU repeat sequences. Pathway analysis of the genes with altered methylation patterns identifies the involvement of a cancer related network of genes whose activity may be heavily regulated by TNF-α in prostate tumorigenesis. Our results suggest that epigenetic dysregulation of cellular processes relevant to TNF-α-dependent apoptosis and electrophile detoxification may be intimately involved in prostate carcinogenesis. These findings may lend credence to the possibility of using tumor-specific alterations in methylation patterns as biomarkers in estimating prognosis and assessing treatment options for prostate cancer.

1. Introduction

Epigenetic changes via the alteration of methylation patterns of CpG sites have been previously associated with prostate cancer development [1–3], and many genes have been shown to be epigenetically regulated during prostate tumor initiation and progression [4,5]. Epigenetic alteration of at least two different categories of genes – proto-oncogenes and tumor suppressors – may contribute to prostate cancer development. During the progression of cancer, proto-oncogenes, such as *WNT5A*, *CRIP1*, *S100P*, and *KRAS*, usually undergo hypomethylation, which results in their up-regulation [6,7]. Conversely, tumor suppressors,

Abbreviations: EST, expressed sequence tag; FFPE, formalin-fixed paraffin-embedded; PrCa, prostate cancer; RT-PCR, reverse transcription–polymerase chain reaction.

* Corresponding author. Address: Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT 06520, USA. Tel.: +1 203 785 4844; fax: +1 203 737 6023.
E-mail address: yong.zhu@yale.edu (Y. Zhu).
[1] Present address: Department of Medical Oncology and Urology, Thomas Jefferson University, Philadelphia, PA, USA.
[2] Present address: Department of Epidemiology, Tulane School of Public Health and Tropical Medicine and Tulane Cancer Center, New Orleans, LA, USA.

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such as *RASSF1A*, *SLC5A8*, and *TFPI2*, undergo hypermethylation, leading to down-regulation of gene expression [8–10].

Various approaches have been carried out to search for genes that are epigenetically regulated in prostate cancer. Individual tumor suppressors and proto-oncogenes that contain CpG islands within their promoter regions have been examined for changes in methylation in normal prostate and cancer cells or tissues [11,12]. In addition, genes displaying altered expression in malignant cells cultured with the demethylating agent, 5-aza-2′-deoxycytidine, have been identified as potential candidates in the epigenetic model of prostate cancer development. A systemic, genome-wide analysis of differentially methylated CpG sites in prostate cancer has also been performed using methylation-sensitive restriction enzymes followed by PCR amplification. The methylated amplicons were then subjected to microarray hybridization [13]. This study discovered that 27 of the top 100 hypermethylated genes were homeobox or T-box genes, which is consistent with current literature on methylation patterns in other cancers, including those of the lung, breast, and colon. Recently, a sequencing-based genome-wide methylation analysis method was developed (Illumina Infinium Methylation Assay) that has been successfully used in a study of leukemia [14] and an analysis of sex differences in genome-wide methylation patterns [15]. However, no previous study of prostate cancer has been reported using this method.

In the current study, an Illumina Infinium Methylation Assay covering 27,578 CpG sites associated with 14,495 genes was performed to identify genes that are differentially methylated in prostate cancer using formalin-fixed paraffin-embedded prostate tumor and normal tissues. Expression levels of selected genes were then measured by real-time quantitative RT-PCR analysis to confirm the methylation data. Identified genes with differential methylation patterns in tumor vs. normal tissue were further analyzed using bioinformatic tools to detect cancer-related pathways that may play a role in prostate tumorigenesis.

2. Materials and methods

2.1. Study subjects

Study subjects were identified via the Yale Cancer Center Rapid Case Ascertainment system. All patients consented to the donation of removed tissue to the Yale-New Haven Hospital (YNHH) tissue bank and samples were obtained according to protocols approved by the Research Ethics Board from YNHH, New Haven County, Connecticut and the Connecticut Department of Public Health HIC. Seventeen pairs of formalin-fixed paraffin-embedded (FFPE) prostate cancers (PrCa) and their corresponding adjacent normal tissue specimens were obtained from patients who had undergone surgery between 2005 and 2009 at YNHH. Five-micrometer-thick sections were cut from the FFPE tissues and mounted on microscope slides. PrCa specimens were subjected to histological examination by an expert pathologist for independent confirmation of the Gleason grades. Gleason grades varied between specimens, with a composite score ranging from 6 to 9. The average Gleason grade of the specimens with reported scores was 7.4, with an average primary score of 3.5 and an average secondary score of 3.9. One specimen did not have an affiliated Gleason grade as it was obtained post-hormone treatment. As chemo- and radiotherapy have previously been implicated in the alteration of methylation patterns, no subjects who have received either type of treatment were included in the study.

2.2. Isolation of genomic DNA and total RNA

Regions of tumor tissue with distinct Gleason grades and adjacent normal tissue were identified following sectioning of FFPE blocks and hematoxylin and eosin staining. Regions with neoplastic compositions of 80% or greater were marked as tumor tissue. 1–3 cm^2 sections of tissue from 2 to 7 slides were manually microdissected using a needle and collected into 1.5 ml microtubes. Genomic DNA was isolated using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA) and total RNA was isolated using the miRNeasy FFPE Kit (Qiagen) according to the supplier's protocols.

2.3. Genome-wide methylation assay

A total of 50 ng of chromosomal DNA was prepared for each tissue type following DNA isolation. Tumor and matching adjacent normal tissue from twelve specimens were combined by tissue type for the purposes of CpG methylation microarray analysis, with each specimen contributing an equal amount of DNA. The Illumina Infinium Methylation Assay was used to detect genome-wide methylation (27,578 CpG sites, spanning 14,495 genes). The CpG sites were located within the proximal promoter regions, ranging from 0 to 1499 bp from the transcription start site (average distance: 389 ± 34 bp). A methylation index (β) was outputted for each site, which is a continuous variable ranging between 0 and 1, representing the ratio of the intensity of the methylated-probe signal to the total locus signal intensity. A β value of 0 corresponds to no methylation while a value of 1 corresponds to 100% methylation at the specific CpG locus measured. These values were then used to calculate a ratio of relative methylation between normal and tumor tissue, with higher values corresponding to greater levels of methylation in tumor tissue relative to normal tissue. All array data have been uploaded to the Gene Expression Omnibus (GEO) database, and can be accessed via their website (http://www.ncbi.nlm.nih.gov/geo/; accession number GSE26319).

2.4. Pathway analysis

To identify pathways displaying tumor-specific altered methylation patterns with potential roles in prostate cancinogenesis, functional categorization and pathway construction were performed using the Ingenuity Pathway Analysis (IPA) software tool produced by Ingenuity Systems. IPA utilizes an extensive database of functional interactions which are drawn from peer-reviewed publications and are manually maintained [16]. *p*-Values

for individual networks were obtained by comparing the likelihood of obtaining the same number of transcripts or greater in a random gene set as are actually present in the input file (i.e. the set of genes differentially methylated in normal and tumor tissue) using a Fishcer's exact test, based on the hypergeometric distribution. Because certain genes were tested at more than one CpG site, the standard IPA-generated network output was altered to accommodate genes displaying differential methylation levels at multiple CpG sites. The highest confidence functional network was designated as the top network.

2.5. Real-time RT-PCR

Expression levels of 10 selected genes were measured by real-time quantitative RT-PCR analysis to check for consistency between the microarray data and gene expression. cDNA was first synthesized from 100 ng of total RNA using the Affinity Script Multiple Temperature cDNA Kit (Stratagene, Santa Clara, CA) with random primers. One microliter of the cDNA was used for the quantitative real-time RT-PCR, and duplicate reactions were performed for each sample using the Kapa SYBR Fast qPCR Kit (Kapa Biosystems, Woburn, MA) with gene-specific primers on an ABI 7500 instrument (Applied Biosystems, Foster City, CA). The primers used for these selected genes were listed in Supplemental Table 1. RNA quantity was normalized using GAPDH content, and gene expression was quantified according to the $2^{-\Delta Ct}$ method.

2.6. Methylation measurement for ALU repetitive sequences

Sodium bisulfite modification of genomic DNA was carried out using the EZ DNA Methylation Gold Kit (Zymo Research Corp, Orange, CA) according to the manufacturer's protocol using 0.1 mg of purified DNA. The methylation status of the ALU repetitive sequence, and the gene in prostate tissue was assayed by real-time methylation-specific PCR using the Kapa SYBR Fast qPCR Kit (Kapa Biosystems) as previously described [17].

2.7. Statistical analysis

The Infinium Methylation Chip data were analyzed using Illumina's GenomeStudio software. Due to the multiple comparisons inherent in the microarray analysis, adjustments were made to control for false discoveries using the Benjamini-Hochberg method previously described in order to obtain a false discovery rate-adjusted differential score and *p*-value for each observation [18]. Observations with adjusted *p*-values equal to or greater than 0.05 were removed and thus precluded from further analysis. Following adjustment, remaining genes were defined as differentially methylated if they displayed at least a twofold difference in DNA methylation levels between tumor and adjacent normal tissue in order to further reduce the number of false positive observations, and to enrich for biologically relevant methylation changes. Student's *t*-test was used to detect differences in mean levels of methylation for ALU, and the expression level between the normal and cancer tissues using SPSS for Windows, release 17.0 (SPSS Inc., Chicago, IL). *p*-Values < 0.05 were considered to be statistically significant.

3. Results

3.1. Analysis of genome-wide methylation

The genome-wide methylation analysis found hypermethylation in 972 CpG sites and hypomethylation in 209 CpG sites, corresponding to 1043 unique genes. These results suggest that the number of genes epigenetically inactivated in prostate tumorigenesis greatly outnumbers the number of epigenetically activated genes as illustrated in Fig. 1. Moreover, hypermethylation seemed to be a general pattern appearing across entire chromosomes, with no discernible differences appearing in pericentromeric or subtelomeric regions (Supplemental Fig. 1).

As expected, genes that were previously shown to be hypermethylated in prostate cancer [13,18] were also hypermethylated in our sample, including *GSTP1* (8.8-fold), *APC* (4.1-fold), *CDKN2A* (4.9-fold), and *CD44* (8.9-fold). In addition, several genes with known hypermethylation in other cancer types were also hypermethylated in our sample. These included *RUNX3* (2.7-fold) [19], *RASSF1* (5.2-fold) [20], and *WT1* (7.7-fold) [21]. The highest level of hypermethylation was for the *D4S234E* gene (28.4-fold), which was recently shown to be a p53-responsive gene involved in inducing apoptosis in response to DNA damage.

3.2. Pathway analysis of the differentially methylated genes

All 1181 sites fitting our significance criteria for differential methylation were examined for functional interrelatedness using the Ingenuity Pathway Analysis software tool. The highest functional network ($P = 1.0E^{-40}$) resulting from differential gene methylation was designated as "cancer, cellular movement, hematological system development and function" (Fig. 2). Interestingly, TNF-α, the key extrinsic mediator of the TNF-induced model of cellular apoptosis, features most prominently in the network and appears to function as a master regulator of a network of several gene transcripts potentially relevant to prostate tumor development and progression. The transcripts displaying the most significant levels of altered methylation within this network were *GSTM1* (20.47-fold increase) and *SOCS1*, which showed both hypermethylation (2.6-fold increase) and hypomethylation (8.17-fold decrease) at the two CpG sites tested. *SOCS1* expression has previously been established to be upregulated in prostate tumor tissue and may contribute to prostate carcinogenesis and tumor progression by inhibiting TNF-α-dependent apoptosis. *GSTM1*, which plays a key role in the detoxification of harmful electrophiles has been consistently found to be hypermethylated in prostate tumor and likely offers a protective effect against prostatic exposure to electrophilic carcinogens. Notable cancer-related genes within the TNF-α-dependent apoptosis pathways that were hypermethylated in prostate tumor included *BCL2* (7.14- and 4.67-fold increase) and *BAK1* (14.75-fold increase), both of which have been found to be underexpressed in prostate tumor. Significantly hypomethylated cancer-relevant genes included *TNFR2* (3.0-fold decrease), which encodes TNF-α receptor 2, a mediator of both pro- and anti-tumoral processes, and *RELB* (2.14-fold decrease), previously found to enhance tumor growth in prostate cancer. Representative hypermethylated and hypomethylated genes with fold changes from the top network were included in Table 1.

3.3. Expression confirmation of the differentially methylated genes

Expression of selected hypermethylated genes was determined by real-time RT-PCR using 17 pairs of normal and tumor tissues, including the 12 pairs previously used in the genome-wide methylation assay. To examine whether our tumor tissues displayed consistency between mRNA levels and DNA methylation, 10 of the top hypermethylated genes were chosen for RT-PCR expression analysis. Six of these genes (*APC*, *GSTP1*, *BCL2*, *CD38*, *DRD2*, and *CACNA1G*) were previously established methylation markers in cancer or other diseases, and four genes (*GPR83*, *ADCY4*, *LOC63928*, and *D4S234E*) were newly identified genes from our analysis and their methylation patterns have not been previously examined. As shown in Supplemental Fig. 2, all of the 10 genes except for one (*APC1*) were down-regulated in our tumor tissues, although differences in mRNA expression were only statistically significant for *GSTP1*, *BCL2*, *ADCY4*, *CACNA1G*, *LOC63928*, and *D4S234E*.

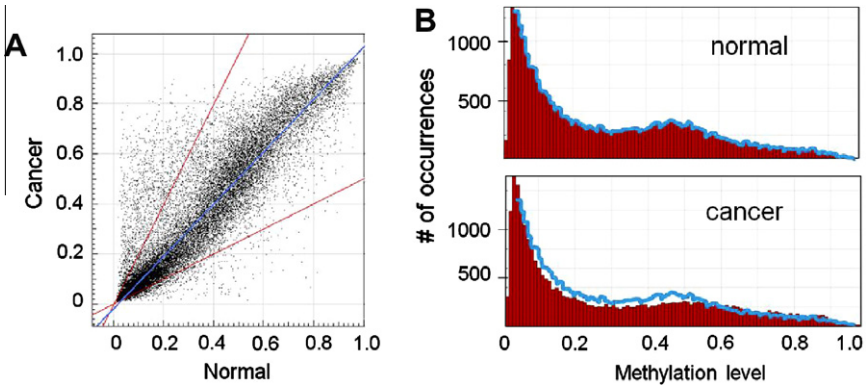

Fig. 1. Genome-wide hypermethylation in prostate cancer. (A) Methylation histogram of normal (*X*-axis) vs. cancer (*Y*-axis). The methylation level of 27,578 CpG sites was measured by Infinium methylation assay and is presented as a value from 0 (completely unmethylated) to 1.0 (completely methylated). Best fit and twofold difference lines were added. (B) Number of occurrences (*Y*-axis) vs. methylation level (*X*-axis). The curved line following the number of occurrences of each methylation value in the normal tissue has been superimposed onto the cancer tissue histogram to highlight the difference in normal vs. tumor methylation (i.e. there are more occurrences of the lower values in normal tissue than in tumor tissue, and vice versa for the higher values, indicating that the tumor tissue DNA is generally more methylated).

3.4. Global hypomethylation of prostate cancer genome

Seventeen pairs of prostate tumor and adjacent normal tissue were used in real-time methylation-specific PCR assay to evaluate the methylation status of ALU sites. The results showed that cancer tissues were significantly less methylated at these sites than the corresponding normal tissue (p-value = 0.013), indicating that DNA is globally hypomethylated in prostate tumor tissues (Fig. 3).

4. Discussion

In our analysis of prostate tumor methylation patterns, we found that ALU repetitive sequences, which may lead to genomic instability when actively expressed, were hypomethylated in prostate cancer. Across the entire genome, however, hypermethylation was more prevalent in prostate tumor tissue than hypomethylation, which is consistent with genome-wide methylation patterns in other cancer types, including tumors of the breast [22] and lung [23], particularly at gene-associated CpG sites.

Our investigation on the biological significance of epigenetic dysregulation for prostate carcinogenesis yielded some interesting findings from the pathway based analysis. Notable is the observation of altered tumor-specific methylation patterns within a network of genes whose activity may be heavily influenced by TNF-α. TNF-α, a pleiotropic cytokine whose expression has been detected in prostate tissue, is the key mediator of the TNF-α-dependent apoptosis pathways, and has been found to be both contributive to and protective against prostate tumorigenesis [24]. Our analysis indicates that all three TNF-α-mediated apoptosis-relevant pathways – the MAPK pathway, the classical NF-kB pathway, and the TNF-α-induced caspase-dependent death signaling pathway – are epigenetically dysregulated in prostate tumor tissue. *BCL2*, which encodes an integral outer mitochrondrial membrane protein that inhibits caspase activity and suppresses apoptosis [25], was found to be hypermethylated at both CpG sites (7.14- and 4.67-fold increase). Although our results are seemingly paradoxical in the light of *BCL2*'s role as a suppressor of apoptotic activity, they are largely corroborated by previous studies that have reported the frequent silencing of *BCL2* in prostate tumor due to aberrant promoter methylation [2,26]. It is possible that the expected reduction in expression resulting from *BCL2* promoter hypermethylation is offset by methylation-dependent silencing of the BCL-2 antagonists, *BIK* (2.62-fold increase) and *BAK1* (14.75-fold increase), the latter of which has been found to be underexpressed in prostate tumor tissue [27].

TNFRSF1B, which exhibited a 3.0-fold decrease in methylation in prostate tumor, encodes TNF-receptor 2 (TNFR2), a membrane-bound receptor of TNF-α that acts as an upstream mediator between TNF-α and downstream apoptotic processes [28]. TNFR2's precise functional role is as yet unclear, but it is generally recognized that TNFR2 activation is pro-apoptotic. Although its function may appear to contradict our own finding, TNFR2 has been found to contribute to both pro- and anti-tumoral effects in multiple cancer models and is overexpressed in prostate tumor but down-regulated in many other cancer types. Interestingly, TNFR2-associated apoptosis is enhanced by lipid raft associated proteasomal degradation of the anti-apoptotic mediator, TRAF2. This is consistent with our methylation results, which show *RFTN1*, a gene that encodes a protein which may be critical for the formation and maintenance of lipid rafts [29], is hypermethylated in prostate tumor (5.50-fold increase). Overall, our findings support TNFR2's role as a complex mediator of cancer-relevant cellular processes whose functional role may be heavily reliant upon the specific disease and biomolecular context.

Although not a representative molecule within the canonical TNF-α pathways, SOCS1 nonetheless plays a role in regulating TNF-α-dependent apoptosis via the inhibition of pro-apoptotic mediators [30]. Our finding of altered *SOCS1* methylation levels is thus consistent with the overarching model of the epigenetic dysregulation of TNF-α-dependent apoptosis in prostate tumor. Similarly, *RELB*, a proto-oncogene previously implicated in tumorigenesis in human prostate tissue, is also hypomethylated in our prostate tumor sample. Of the genes within our network, *GSTM1*, which encodes a key Phase II enzyme responsible

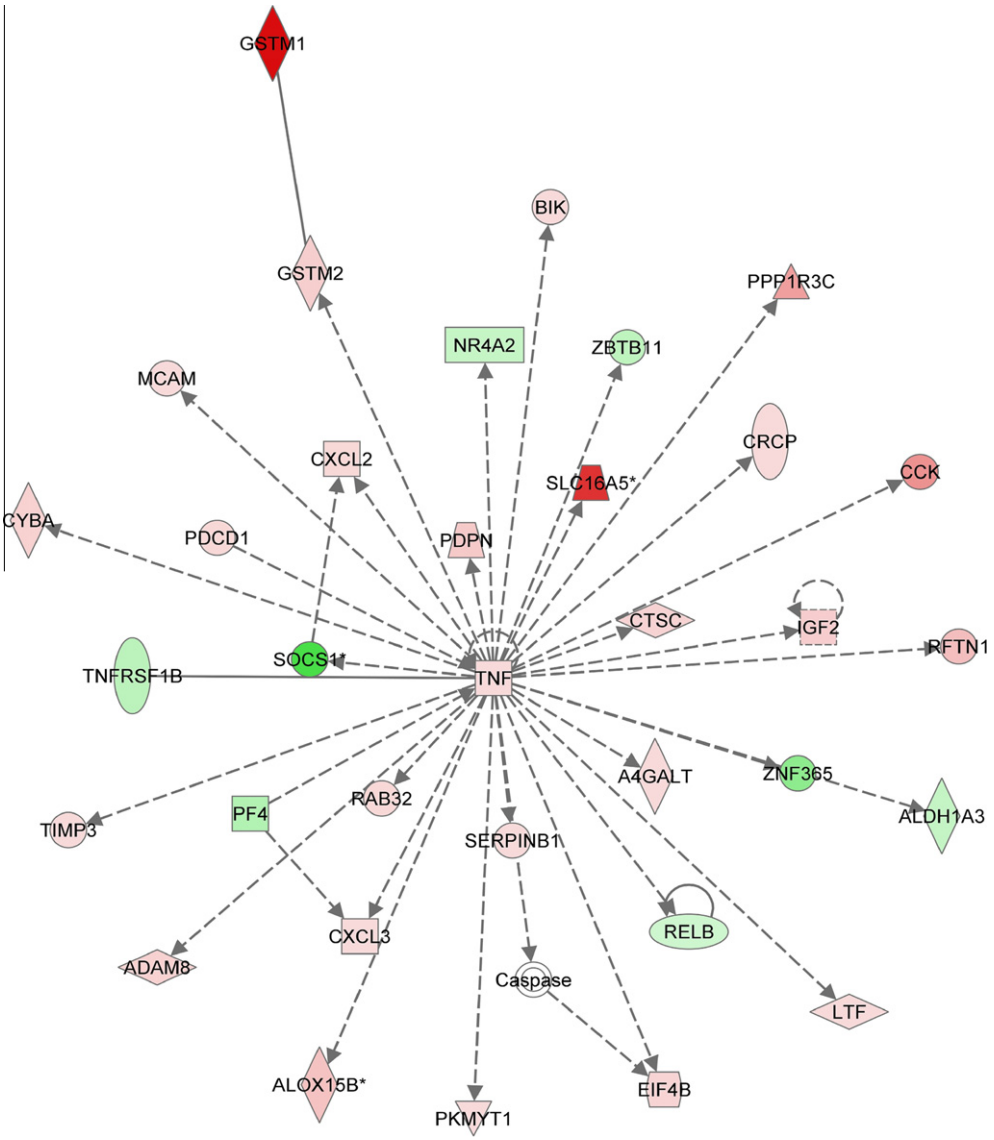

Fig. 2. Highest confidence network of genes displaying altered methylation levels in prostate tumor. According to IPA, the network is relevant to ''cancer, cellular movement, hematological system development and function''. Genes that were hypermethylated in prostate tumor are shaded in red, while those that were hypomethylated are shaded in green, with intensity signifying the magnitude of methylation change. Each interaction is supported by at least one literature reference, with solid lines representing direct interactions, and dashed lines representing indirect interactions.

for detoxification of eletrophilic compounds, displayed the greatest degree of increased methylation in prostate tumor tissue (20.47-fold). Notably, *GSTP1*, *GSTM2*, and *GSTM5* all displayed significantly increased levels of methylation in our prostate tissue sample. These findings are consistent with prior literature reporting prostate tumor-specific underexpression and hypermethylation of members of the GST superfamily [31], suggesting that methylation-induced gene silencing of Phase II metabolic enzymes may play an important role in prostate carcinogenesis.

A key limitation of our study design is seen in the inability to assess methylation levels with respect to tumor grade, thus precluding an early examination of potential methylation patterns specific to cancer initiation vs. those specific to tumor progression. The use of pooled DNA samples also makes it unfeasible to detect the frequency of methylation in tumor samples. In addition, because a single gene may contain multiple promoter-specific CpG sites, this study and any other study of this type is limited by sparse knowledge on the relative importance of specific CpG sites with respect to gene expression and function. Nonetheless, the degree of consistency between our findings and prior literature in the sampling of cancer-relevant genes we examined gives bearing to the potential of using tumor-specific alterations in methylation pattern as a prognostic tool in prostate cancer development and progression. Because of the inherent limits of the IPA software, for genes tested at two or more CpG site, the CpG site corresponding to the highest methylation change was used for the purposes of network generation. However, the

Table 1
Genes on top network displaying differential methylation in prostate tumor.

Symbol	Accession	Brief relevant functions	Fold change[a]
A4GALT	NM_017436.4	Enzyme that catalyzes formation of P^k antigen	+2.0
ADAM8	NM_001109.2	Cell adhesion in neurogeneration	+3.9
ALDH1A3	NT_035325.6	Aldehyde detoxification, androgen-responsive	−2.6
ALOX15B	NM_001141.1	Fatty acid hydroperoxide production	+5.1, +2.7
BIK	NT_011520.11	BCL-2 antagonist, apoptosis-inducing	+2.6
CCK	NM_000729.3	Pancreatic enzyme secretion and gastrointestinal motility	+9.3
CRCP	NM_014478.3	Mediates intracellular cAMP levels	+2.1
CTSC	NM_001814.2	Thiol protease, protein turnover	+3.7
CXCL2	NM_002089.1	Hematoregulatory chemokine, angiogenic	+3.2
CXCL3	NM_002090.2	Hematoregulatory chemokine, angiogenic	+3.0
CYBA	NM_000101.2	α subunit of cytochrome b, microbicidal activity	+3.8
EIF4B	NM_001417.2	Required for mRNA binding to ribosomes	+3.5
GSTM1	NM_000561.2	Phase II metabolic enzyme – hypermethylated in PrCa	+20.5
GSTM2	NM_000848.2	Phase II metabolic enzyme	+4.1
IGF2	NM_000612.2	Growth factor, imprinted gene – may be involved in PrCa progression	+4.2
LTF	NM_002343.2	Iron binding and transport	+2.1
MCAM	NM_006500.2	Cell adhesion – hypermethylation associated with advanced PrCa tumor stage	+2.7
NR4A2	NM_006186.2	Nuclear receptor and transcription factor – suppresses apoptosis	−2.5
PDCD1	NM_005018.1	Part of immunoglobulin superfamily – possibly apoptosis-inducing	+3.2
PDPN	NM_006474.4	A type-I integral membrane glycoprotein-involved in tumor invasion	+4.5
PF4	NM_002619.1	A small cytokine belonging to the CXC chemokine family- has a role in inflammation and wound repair	−3.4
PKMYT1	NM_182687.1	A member of the serine/threonine protein kinase family-has a role in cell cycle regulation	+2.9
PPP1R3C	NM_005398.3	A protein phosphatase-regulates a wide variety of cellular functions by reversible protein phosphorylation	+8.2
RAB32	NT_025741.14	A member RAS oncogene family	+3.3
RELB	NM_006509.2	v-rel reticuloendotheliosis viral oncogene homolog B	−2.1
RFTN1	NM_015150.1	Raft protein-plays a role in the formation and/or maintenance of lipid rafts	+5.5
SERPINB1	NM_030666.2	Serpin peptidase inhibitor	+2.4
SLC16A5	NM_004695.2	A member of solute carrier family	+17.2, +4.2
SOCS1	NT_010393.15	Suppressor of cytokine signaling 1	−8.2, +2.6
TIMP3	NM_000362.4	An inhibitors of the matrix metalloproteinases	+2.2
TNF	NM_000594.2	A group of cytokines family that can cause apoptosis	+2.0
TNFRSF1B	NM_001066.2	Tumor necrosis factor receptor superfamily member 1B	−3.0
ZBTB11	NM_014415.1	Zinc finger and BTB domain containing 11	−2.9
ZNF365	NM_199451.1	zinc finger protein 365	−5.0

[a] Certain genes contain more than one CpG site with differential methylation.

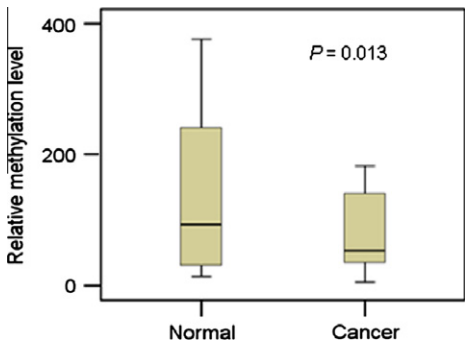

Fig. 3. Global hypomethylation of the prostate cancer in the ALU sequence. Seventeen pairs of normal and cancer tissue were examined by methylation-specific PCR each in duplicate and the average relative methylation level is presented.

network generated in this manner is, in theory, structurally identical to that which would be generated if all CpG sites were taken into account.

In summary, we present in this study a genome-wide methylation profile of prostate cancer using the most comprehensive methylation measurement technique currently available. Our findings lend credence to the hypotheses that epigenetic dysregulation of important cancer-relevant molecules, including those pertinent to detoxification and TNF-α-dependent apoptosis, may be involved in prostate carcinogenesis, and that tumor-specific alterations in methylation patterns have the potential to serve as prognostic biomarkers in prostate cancer development and progression. Further investigations into the mechanisms leading to differential methylation observed in our study, as well as associations of methylation status with prostate tumor stage and progression may provide additional insights which could prove useful in estimating prognosis and determining treatment options.

Conflict of interest

All authors declare that there are not any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.canlet.2010.12.010.

References

[1] W. Nelson, A. De Marzo, S. Yegnasubramanian, Epigenetic alterations in human prostate cancers, Endocrinology 150 (2009) 3991–4002.
[2] N. Cho, B. Kim, M. Choi, E. Yoo, K. Moon, Y. Cho, D. Kim, G. Kang, Hypermethylation of CpG island loci and hypomethylation of LINE-1 and Alu repeats in prostate adenocarcinoma and their relationship to clinicopathological features, J. Pathol. 211 (2007) 269–277.
[3] C. Cooper, C. Foster, Concepts of epigenetics in prostate cancer development, Br. J. Cancer 100 (2009) 240–245.
[4] S. Yegnasubramanian, M. Haffner, Y. Zhang, B. Gurel, T. Cornish, Z. Wu, R. Irizarry, J. Morgan, J. Hicks, T. DeWeese, W. Isaacs, G. Bova, A. De Marzo, W. Nelson, DNA hypomethylation arises later in prostate cancer progression than CpG island hypermethylation and contributes to metastatic tumor heterogeneity, Cancer Res. 68 (2008) 8954–8967.
[5] D. Mishra, Z. Chen, Y. Wu, M. Sarkissyan, H. Koeffler, J. Vadgama, Global methylation pattern of genes in androgen-sensitive and androgen-independent prostate cancer cells, Mol. Cancer Ther. 9 (2010) 33–45.
[6] Q. Wang, M. Williamson, S. Bott, N. Brookman-Amissah, A. Freeman, J. Nariculam, M. Hubank, A. Ahmed, J. Masters, Hypomethylation of WNT5A, CRIP1 and S100P in prostate cancer, Oncogene 26 (2007) 6560–6565.
[7] L. Benbrahim-Tallaa, R. Waterland, M. Styblo, W. Achanzar, M. Webber, M. Waalkes, Molecular events associated with arsenic-induced malignant transformation of human prostatic epithelial cells: aberrant genomic DNA methylation and K-ras oncogene activation, Toxicol. Appl. Pharmacol. 206 (2005) 288–298.
[8] L. Liu, J. Yoon, R. Dammann, G. Pfeifer, Frequent hypermethylation of the RASSF1A gene in prostate cancer, Oncogene 21 (2002) 6835–6840.
[9] J. Park, W. Zheng, D. Kim, J. Cheng, N. Kumar, N. Ahmad, J. Pow-Sang, Candidate tumor suppressor gene SLC5A8 is frequently down-regulated by promoter hypermethylation in prostate tumor, Cancer Detect. Prev. 31 (2007) 359–365.
[10] T. Ribarska, M. Ingenwerth, W. Goering, R. Engers, W. Schulz, Epigenetic inactivation of the placentally imprinted tumor suppressor gene TFPI2 in prostate carcinoma, Cancer Genom. Proteom. 7 (2010) 51–60.
[11] S. Takahashi, S. Inaguma, M. Sakakibara, Y. Cho, S. Suzuki, Y. Ikeda, L. Cui, T. Shirai, DNA methylation in the androgen receptor gene promoter region in rat prostate cancers, Prostate 52 (2002) 82–88.
[12] A. McKie, D. Douglas, S. Olijslagers, J. Graham, M. Omar, R. Heer, V. Gnanapragasam, C. Robson, H. Leung, Epigenetic inactivation of the human sprouty2 (hSPRY2) homologue in prostate cancer, Oncogene 24 (2005) 2166–2174.
[13] K. Kron, V. Pethe, L. Briollais, B. Sadikovic, H. Ozcelik, A. Sunderji, V. Venkateswaran, J. Pinthus, N. Fleshner, T. van der Kwast, B. Bapat, Discovery of novel hypermethylated genes in prostate cancer using genomic CpG island microarrays, PLoS One 4 (2009) e4830.
[14] S. Deneberg, M. Grövdal, M. Karimi, M. Jansson, H. Nahi, A. Corbacioglu, V. Gaidzik, K. Döhner, C. Paul, T. Ekström, E. Hellström-Lindberg, S. Lehmann, Gene-specific and global methylation patterns predict outcome in patients with acute myeloid leukemia, Leukemia 24 (2010) 932–941.
[15] J. Liu, M. Morgan, K. Hutchison, V. Calhoun, A study of the influence of sex on genome wide methylation, PLoS One 5 (2010) e10028.
[16] S.E. Calvano, W. Xiao, D.R. Richards, R.M. Felciano, H.V. Baker, R.J. Cho, R.O. Chen, B.H. Brownstein, J.P. Cobb, S.K. Tschoeke, C. Miller-Graziano, L.L. Moldawer, M.N. Mindrinos, R.W. Davis, R.G. Tompkins, S.F. Lowry, A network-based analysis of systemic inflammation in humans, Nature 437 (2005) 1032–1037.
[17] J. Roman-Gomez, A. Jimenez-Velasco, X. Agirre, J.A. Castillejo, G. Navarro, E. San Jose-Eneriz, L. Garate, L. Cordeu, F. Cervantes, F. Prosper, A. Heiniger, A. Torres, Repetitive DNA hypomethylation in the advanced phase of chronic myeloid leukemia, Leuk. Res. 32 (2008) 487–490.
[18] N. Verkaik, G. van Steenbrugge, W. van Weerden, M. Bussemakers, T. van der Kwast, Silencing of CD44 expression in prostate cancer by hypermethylation of the CD44 promoter region, Lab. Invest. 80 (2000) 1291–1298.
[19] E. Wolff, G. Liang, C. Cortez, Y. Tsai, J. Castelao, V. Cortessis, D. Tsao-Wei, S. Groshen, P. Jones, RUNX3 methylation reveals that bladder tumors are older in patients with a history of smoking, Cancer Res. 68 (2008) 6208–6214.
[20] F. Reu, D. Leaman, R. Maitra, S. Bae, L. Cherkassky, M. Fox, D. Rempinski, N. Beaulieu, A. MacLeod, E. Borden, Expression of RASSF1A, an epigenetically silenced tumor suppressor, overcomes resistance to apoptosis induction by interferons, Cancer Res. 66 (2006) 2785–2793.
[21] M. Kaneuchi, M. Sasaki, Y. Tanaka, H. Shiina, H. Yamada, R. Yamamoto, N. Sakuragi, H. Enokida, M. Verma, R. Dahiya, WT1 and WT1-AS genes are inactivated by promoter methylation in ovarian clear cell adenocarcinoma, Cancer 104 (2005) 1924–1930.
[22] Y. Ruike, Y. Imanaka, F. Sato, K. Shimizu, G. Tsujimoto, Genome-wide analysis of aberrant methylation in human breast cancer cells using methyl-DNA immunoprecipitation combined with high-throughput sequencing, BMC Genomics 11 (2010) 137.
[23] D. Shames, L. Girard, B. Gao, M. Sato, C. Lewis, N. Shivapurkar, A. Jiang, C. Perou, Y. Kim, J. Pollack, K. Fong, C. Lam, M. Wong, Y. Shyr, R. Nanda, O. Olopade, W. Gerald, D. Euhus, J. Shay, A. Gazdar, J. Minna, A genome-wide screen for promoter methylation in lung cancer identifies novel methylation markers for multiple malignancies, PLoS Med. 3 (2006) e486.
[24] R. van Horssen, T. Ten Hagen, A. Eggermont, TNF-alpha in cancer treatment: molecular insights, antitumor effects, and clinical utility, Oncologist 11 (2006) 397–408.
[25] R. Youle, A. Strasser, The BCL-2 protein family: opposing activities that mediate cell death, Nat. Rev. Mol. Cell Biol. 9 (2008) 47–59.
[26] J.R. Carvalho, L. Filipe, V.L. Costa, F.R. Ribeiro, A.T. Martins, M.R. Teixeira, C. Jerónimo, R. Henrique, Detailed analysis of expression and promoter methylation status of apoptosis-related genes in prostate cancer, Apoptosis 15 (2010) 956–965.
[27] X. Shi, L. Xue, J. Yang, A. Ma, J. Zhao, M. Xu, C. Tepper, C. Evans, H. Kung, R. deVere White, An androgen-regulated miRNA suppresses Bak1 expression and induces androgen-independent growth of prostate cancer cells, Proc. Natl. Acad. Sci. USA 104 (2007) 19983–19988.
[28] D. Hui, N. Satkunam, M. Al Kaptan, T. Reiman, R. Lai, Pathway-specific apoptotic gene expression profiling in chronic lymphocytic leukemia and follicular lymphoma, Mod. Pathol. 19 (2006) 1192–1202.
[29] K. Saeki, Y. Miura, D. Aki, T. Kurosaki, A. Yoshimura, The B cell-specific major raft protein, Raftlin, is necessary for the integrity of lipid raft and BCR signal transduction, EMBO J. 22 (2003) 3015–3026.
[30] A. Kimura, T. Naka, S. Nagata, I. Kawase, T. Kishimoto, SOCS-1 suppresses TNF-alpha-induced apoptosis through the regulation of Jak activation, Int. Immunol. 16 (2004) 991–999.
[31] D. Bostwick, I. Meiers, J. Shanks, Glutathione S-transferase: differential expression of alpha, mu, and pi isoenzymes in benign prostate, prostatic intraepithelial neoplasia, and prostatic adenocarcinoma, Hum. Pathol. 38 (2007) 1394–1401.