Common Structural and Epigenetic Changes in the Genome of Castration-Resistant Prostate Cancer


Abstract

Progression of primary prostate cancer to castration-resistant prostate cancer (CRPC) is associated with numerous genetic and epigenetic alterations that are thought to promote survival at metastatic sites. In this study, we investigated gene copy number and CpG methylation status in CRPC to gain insight into specific pathophysiological pathways that are active in this advanced form of prostate cancer. Our analysis defined and validated 495 genes exhibiting significant differences in CRPC in gene copy number, including gains in androgen receptor (AR) and losses of PTEN and retinoblastoma 1 (RB1). Significant copy number differences existed between tumors with or without AR gene amplification, including a common loss of AR repressors in AR-unamplified tumors. Simultaneous gene methylation and allelic deletion occurred frequently in RB1 and HSD17B2, the latter of which is involved in testosterone metabolism. Lastly, genomic DNA from most CRPC was hypermethylated compared with benign prostate tissue. Our findings establish a comprehensive methylation signature that couples epigenomic and structural analyses, thereby offering insights into the genomic alterations in CRPC that are associated with a circumvention of hormonal therapy. Genes identified in this integrated genomic study point to new drug targets in CRPC, an incurable disease state which remains the chief therapeutic challenge. Cancer Res; 72(3); 616–25. ©2011 AACR.

Introduction

Despite the effectiveness of castrating therapies in slowing the growth of metastatic prostate cancer, therapeutic resistance eventually develops. Unchecked, metastatic castration-resistant prostate cancer (CRPC) is lethal, causing approximately 30,000 annual deaths in the United States (1). Tissue from metastatic CRPC is difficult to obtain for genetic characterization because biopsies can be technically challenging and are generally unnecessary for routine clinical decision making. Considerably less is therefore understood about the later acquired genetic alterations that emerge in the context of the selection pressure of an androgen-deprived milieu.

To address this, rapid autopsy programs were developed to provide high-quality metastatic tissue for research (2–4). Tissue obtained from these studies has been used for genomic analysis and to create cell lines and xenograft models (2). In addition, improvements in DNA and RNA array–based techniques have allowed for the acquisition of high-resolution quantitative tumor genome information from small amounts of tissue. This includes the quantification of global gene copy number (CN), expression level, and gene CpG methylation status.

Recent studies of metastatic CRPC have examined the variation in androgen receptor (AR) splicing (5), AR signaling (6), tumor heterogeneity (7), and has identified new targets for therapy (8). Similarly, a number of recent studies have implicated local androgen production by tumor cells (9–11). Genomic studies across different platforms, as described here, allow a more thorough investigation and yield a more complete picture of the genetic changes that occur in CRPC. In this study, 15 metastatic CRPC tumors obtained at autopsy were profiled using comprehensive high-resolution array-comparative genomic hybridization (aCGH) and methylation arrays to describe the most common CN aberrations in metastatic CRPC and their concordant methylation levels.

Materials and Methods

Clinical profile of cases

Fifteen tumors were obtained from rapid autopsies of 14 patients, including 2 metastases from 1 individual carried out...
from 1996–1999. Clinical characteristics have been reported (2); all had metastatic CRPC, had received hormonal therapy, and all but one had received chemotherapy.

To corroborate findings from the metastatic cohort, a separate cohort of 36 intermediate grade primary prostate tumors obtained at prostatectomy were also obtained. Patient clinical characteristics and methods used to isolate DNA have been reported (12). With a median of 60.2 months follow up, 14 patients had biochemical evidence of recurrent disease, whereas 22 did not.

**Tissue processing**

The tissue procurement procedure has been described (2). Metastatic liver (\(N = 7\)) or soft-tissue (\(N = 8\)) lesions were used for genomic analysis, including 2 separate soft-tissue deposits from the same patient, both of which were used. A single pathologist (MR) sectioned 15 tissue slices at 15 \(\mu\)m from areas with extensive tumor involvement. DNA was extracted with a Wizard Genomic DNA Isolation kit (Promega) and purified by phenol/chloroform extraction. All DNAs were of sufficient quality and quantity for genomic analysis by gel chromatography and UV-Vis spectrophotometry.

**Array comparative genomic hybridization**

aCGH was carried out using Human Genome microarrays (Agilent), consisting of 244,000 60-mer oligonucleotide DNA probes with approximately 9 kb spatial resolution, with pooled male lymphocyte DNA (single lot, Promega) as a reference sample. Labeling and microarray processing were done according to the manufacturer's protocol using 500 ng of genomic DNA. After 60-hour hybridization, microarrays were scanned at 10 \(\mu\)m resolution using an Agilent-G25005B scanner. All samples passed Agilent quality control assessment. Feature-level data was abstracted with Agilent software. CN was expressed as the log2 ratio of tumor:control DNA fluorescence intensity.

**DNA methylation profiling**

DNA was bisulfite converted using the EZ DNA Methylation Kit (Zymo). DNA was then whole-genome amplified, enzymatically fragmented, precipitated, resuspended, and hybridized overnight at 48°C to an array containing 27,578 locus-specific oligonucleotide primers (Illumina). After hybridization, the C or T nucleotides were detected by fluorescent single-base primer extension using an Illumina BeadStation GX scanner.

Data were analyzed with Genome Studio software (v2009.1; Illumina) which assigns a quantitative measure of the methylation level (\(\beta\) value) for each CpG site, corresponding to the ratio between the fluorescence signal from the methylated allele (C) and the sum of the fluorescence signals of the methylated (C) and unmethylated (T) alleles for that CpG site. The methylation status for each site ranges from 0 (completely unmethylated) to 1 (completely methylated). Conservative \(\beta\) value cutoffs of \(>0.8\), \(<0.8\) to \(>0.2\), and \(<0.2\) were used, respectively, to call a CpG locus homozygously methylated, heterozygously methylated, or unmethylated. Hierarchical clustering of \(\beta\) values used the Pearson correlation as the similarity measure and complete linkage as the agglomeration method and was analyzed with R software. Only probes in which 50% or more of samples had nonmissing data and which had SD greater than the 25th percentile were used for whole-genome clustering (20,675 of 27,578), and in which not all samples had the same methylation status (513 of 27,578) for exploration of probes differentially methylated across samples. Using methylated and unmethylated status encoded as 1 and -1, respectively, hierarchical clustering was done separately with Cluster and Treeview softwares (13) using Euclidean distance as the similarity measure and Ward's agglomeration method (14).

**Validation of candidate gene copy changes**

A subset of samples showing AR copy gain or PTEN deletion by aCGH were selected for validation using the TaqMan Gene Copy Number Assay (Applied Biosystems) with the UCSF Genome Core. Reference samples included pooled male lymphocyte DNA (Promega), DNA from 2 castration-sensitive primary prostate tumors known to harbor normal AR and PTEN CN by aCGH (unpublished data), and AR and PTEN copy-normal DNA (Coriell; ref. 15). The internal reference gene was RnaseP. Ten nanograms of DNA was used per sample and run in quadruplicate per manufacturer's instructions. CN analysis was done using CopyCaller software (Applied Biosystems).

**Gene expression**

A subset of the metastases (5 liver, 8 soft tissue) were analyzed at the University of Michigan by Dr. Tomlins (data available on Oncomine; ref. 16) on Agilent Whole-Human Gene Expression 44K microarrays against pooled benign prostate tissue (Clontech). RNA preparation has been previously described (17). Hierarchical clustering was done using log2 ratio values, Euclidean distance, and Ward's agglomeration method (14). Only those probes which had at least 50% of samples with nonmissing data were considered (466 of 507 probes).

**Pathway analysis**

The Panther libraries (18–21) were used to identify pathways containing genes of interest. A binomial statistical tool is used to compare classifications of multiple clusters of lists to a RefSeq-based reference list to statistically determine whether specific classification categories are over- or underrepresented, using the Bonferroni method to adjust for multiple testing.

**Statistical analysis**

CN data was mapped to the human genome sequence hg18 freeze and log2 intensity ratios were segmented using circular binary segmentation to translate noisy intensity measurements into regions of equal CN (22). The median absolute deviation (MAD), scaled by the factor 1.4826, of the difference between the observed and segmented values was used to estimate the sample-specific experimental variation. Outlier probes were identified as those which were more than 4 sample-specific MAD away from their segment value. For each sample, a segment was declared gained or lost if the segment value was more than 2 times the sample MAD from the median segment value of the autosomes. Y chromosome probes were...
excluded. Probe-wise frequency plots of CN alterations were based on gain/loss/normal status.

Probes in which CN aberrations were significantly associated with one group versus another were identified based on segment values with original log2 ratios for outlier probes and using moderated t statistics as implemented in the limma package (23) in Bioconductor (24). Q values were computed from the nominal P values to adjust for multiple testing by controlling the false discovery rate (25). A Q value cutoff of 0.05 or less was used to declare a probe to be significant.

For the AR gene aberration analysis all 20 probes mapping to the AR gene had the same CN status for all samples (copy normal or amplified). These AR probes were excluded when carrying out probe-wise tests of association of CN with AR and also when testing association of chromosomal arms with AR. The sample profiles were clustered using gain, loss, and copy-normal status encoded as 1, −1, and 0, respectively. Hierarchical clustering was done as discussed above.

To analyze the correlation between gene expression and CN, each expression probe was mapped to the closest CN probe. Only expression probes which were less than 50 kb from the closest CN probe and in which there were 5 or more samples with CN aberration, identified as having absolute CN segment value greater than 0.3, were considered. A Spearman correlation coefficient was computed for each probe. P values, calculated using asymptotic t approximation, identified significant correlations for a precompiled candidate list of genes involved in androgen and phosphatidyl-inositol-3-kinase (PI3K) signaling (Supplementary Table S1). Q values, computed to correct for multiple testing, identified significant correlations for noncandidate genes. P and Q values cutoffs 0.05 or less were, respectively, considered significant.

All analyses were done using R software in the UCSF Biostatistics Core. The aCGH and methylation microarray data have been deposited in National Center for Biotechnology Information Gene Expression Omnibus (ref. 26; accession no. GSE34174).

Results

**Oligonucleotide aCGH**

Overall, 217,960 (92.8%) probes had a CN aberration (gain or loss) in at least one of the 15 metastatic samples. Specifically, 119,405 (50.84%) probes had amplification and 180,372 (76.8%) probes had deletion in 1 or more samples. The absolute log2 ratio for copy change relative to the control ranged from −6.41 (homozygous loss) for AK054947 on chromosome 6, to +5.88 (gain of 59 copies) for KIF4A, located 2.56 Mb downstream of AR on chromosome X.

Figure 1 shows the probe-wise frequency of CN changes across the entire genome for all metastatic samples. A frequency value of 66% or more (i.e., ≥10 of the samples) was set to identify commonly amplified or deleted probes. After filtering out probes in intergenic regions, 495 unique genes (79 amplified and 416 deleted) met this frequency cutoff (Gene list, Supplementary Table S2).

**Validation of copy number gains and losses**

The CN of 2 candidate genes, AR and PTEN, was assayed using a TaqMan PCR assay. These genes were selected because both have been shown to be amplified (AR) or deleted (PTEN) in prostate cancer, both were frequently aberrant in the metastatic aCGH analysis (AR, 73%; PTEN 87%), and both are present at normal CN in available controls (15). One metastatic sample harboring AR copy gain and one harboring PTEN deletion as determined by aCGH were selected (Fig. 2).

For the metastatic sample, the mean aCGH log2 ratio across all probes in the AR gene was 3.41 (range: 2.65–4.08), corresponding to a mean gain of 11 copies per cell. By PCR, this sample had 8 copies (range: 7.65–9.17) per cell (Fig. 2A and B). Similarly, the mean aCGH log2 ratio across all probes in the PTEN gene for the selected sample was −2.11 (range: −0.54 to −2.73), indicating deletion of one or both copies per cell. By PCR, this sample had 0.55 copies (range: 0.54–0.6), corresponding to heterozygous PTEN deletion with some evidence of both copies being lost (Fig. 2C and D).

**Correlation of copy number results with expression**

Of the 495 commonly aberrant genes identified by aCGH, 318 (64%) were both represented on the expression array and had available expression data. After inclusion of candidate genes involved in androgen and PI3K signaling and correction for multiple testing for noncandidate genes, 77 genes were identified that had a significant correlation of gene CN to expression (Spearman correlation range: 0.5–0.89, Q < 0.05, Gene list, Supplementary Table S1).

We next investigated whether CN aberrations in these correlated genes were present in unmatched primary tumors, and if so, whether the aberrations were associated with recurrence after prostatectomy. A higher prevalence of CN aberrations was observed for the correlated genes in recurrent cases (26.8%, N = 14 recurrent cases) than for nonrecurrent tumors (17%, N = 22 nonrecurrent cases, Fisher’s exact test, P < 0.001). This comparison held for the entire 495 copy aberrant gene set,
with 26.1% of genes aberrant in the recurrent group compared with 15.5% in the nonrecurrent group \((P < 0.001)\).

The most common copy gains and losses, defined by a frequency \(66\%\) or more occurred on 6 specific chromosomes \((6, 8, 10, 13, 16, \text{and } X)\). Mapping of correlated genes (CN and expression) was done to evaluate whether focused regions of alteration could be identified for these commonly aberrant chromosomes and, in so doing, identify potential "driver" genes, whose expression correlates with their tumor genomic copy status, from potential "passenger" genes, whose expression levels do not. This proved to be most useful on chromosome 8 (Fig. 3).

**Genomics of AR amplification**

Because AR overexpression is key to CRPC growth and because AR amplification and expression were significantly correlated \((P = 0.03)\), the degree of genomic AR amplification was investigated. AR amplification was present in 11 of 15 (73%) of the metastatic samples. Across the 20 AR microarray probes, the average \(\log_2\) ratio for copy gain was 1.7 in the AR-amplified subset compared with 0.16 in the remaining 4 samples (referred to as AR copy normal). Overall, the maximum AR \(\log_2\) ratio detected for a single probe was 4.08, corresponding to 17 AR gene copies per cell.

To further explore the differences between AR copy-normal and AR-amplified samples, genome topography plots were constructed for each subset (Fig. 4). Despite the small sample size, it is evident that AR-amplified samples have a higher overall frequency of amplification across their entire tumor genome, with \(66\%\) or more of samples having copy gain in chromosomes 7q, 8q, and X. Between the 2 groups there was no significant difference in arm-wise chromosomal deletions, and no single probe was found to be significantly different after correction for multiple testing.

It is plausible that the AR copy-normal tumors have activated the AR pathway by means other than AR amplification, thus a supervised analysis was undertaken to investigate the CN of 197 genes that regulate AR signaling (27). Retinoblastoma 1 (RB1) and PTEN, considered to have a role in AR regulation, were deleted and underexpressed in both cohorts.

---

**Figure 2.** Copy number validation with PCR. **A,** AR-amplification by TaqMan PCR confirms gain in a metastatic CRPC tumor. Due to location on the X chromosome, normal males harbor only one copy of AR. CSPC (castration-sensitive prostate cancer) and germline DNA, controls. **B,** aCGH of same CRPC tumor profiled in A, showing focal AR amplification. **C,** PTEN deletion by TaqMan PCR confirms loss in a metastatic CRPC tumor. **D,** aCGH of same CRPC tumor profiled in C, showing focal PTEN deletion.

---

www.aacrjournals.org Cancer Res; 72(3) February 1, 2012 619

Downloaded from cancerres.aacrjournals.org on October 22, 2017. © 2011 American Association for Cancer Research.
Four genes were found to have discordant copy status between the 2 AR groups. Amplification of the AR coactivator NCOA2 was significantly associated (P = 0.002) with the presence of AR amplification, whereas deletion of large tumor suppressor, homolog 2 (LATS2) and pleiomorphic adenoma gene-like 1 (PLAG1) were detected in 75% of AR copy-normal samples, but in none of the AR-amplified tumors. DnaJ (Hsp40) homolog, subfamily C, member 3 (DNAJC3) was found to be deleted in all AR copy-normal samples, but in only 36% of AR-amplified samples.

To parse out the evolution of NCOA2 and AR amplification, a comparison of this dataset with genome-wide CN data from 36 primary prostate tumors was done. NCOA2 is amplified in 21% of primary tumors which subsequently recurred biochemically, compared with 9% of nonrecurrent tumors (Fisher’s exact test, P = 0.36); AR was not amplified in any primary prostate tumors.

Methylation signature of metastatic CRPC

Average methylation values were higher for the metastatic samples than those reported for benign prostate tissue analyzed on the same platform (28). The average β value was 0.38 (0.34–0.41) across all of the metastatic samples, compared with 0.3 for benign prostate tissue. When restricted to CpG islands (N = 20,006 probes), the average β value per

Figure 3. Mapping of frequently copy aberrant genes. Mapping reveals 2 amplicons and 2 loci of deletion on chromosome 8. Positively correlated genes are found above the dashed horizontal line. Correlated deleted genes: BIN3, RHOBTB2, CHMP7, LOXL2, FUT10, and RBM13. Correlated amplified genes: TERF1, RPL7, STAU2, COX6C, and GRHL2. X-axis, chromosome position. Y-axis, Q value for CN: expression correlation.

Figure 4. Genome topography of CRPC stratified by AR copy number (CN) status. Y-axis, fraction of the samples altered. X-axis, probes across the genome. Green, gains; red, losses. Vertical dashed lines represent the centromeres.
sample was 0.27 (0.23–0.31) compared with 0.16 for benign tissue, and for non-CpG islands (N = 7,572 probes), the average methylation was 0.64 (0.59–0.68) compared with 0.58 for benign tissue.

For the metastases, 9,217 probes (33.4%) were methylated in at least one sample. The average number of methylated probes per patient was 4,744 (17.2%, range: 3,522–6,388). Of all 27,578 CpG sites assayed, there were 1,361 sites (4.9%) that were methylated across all samples, corresponding to 1,133 different genes (7.3% of all genes on the array, Supplementary Table S3).

Hierarchical clustering was done to establish a comprehensive methylation signature of metastatic CRPC. Figure 5A shows that the majority of probes (blue) are unmethylated. After subtracting probes with identical methylation status across all samples, there were 513 probes which showed differential methylation among the samples (Fig. 5B). Two different patterns of CpG methylation emerged, with the majority of samples having a mixed methylation phenotype (hyper and hypomethylated) and a subset of samples (Met29_23M, 25_46M, and 12_6M) evincing a hypomethylated phenotype relative to the rest. This hypomethylated set did not segregate by clinical factors (Gleason score, PSA at diagnosis, time from diagnosis to death, and type of therapy received) nor genomic factors (metastatic site, whole-genome CN, and AR amplification status). Three classes of genes were unmethylated in this subset whereas methylated in more than 90% of the other samples, including the homeobox genes (HOXC11, HOXD3, HOXB2, and HOXD4), 2 PI3K isoforms (PIK3CG and PIK3CD), as well as 4 kallikrein isoforms, (KL, KLOC1, KLK4, and KLK5).

**Genomics of the androgen biosynthesis pathway**
Both the CN and the methylation gene sets were significantly enriched for genes responsible for androgen synthesis and metabolism. Three of 20 genes in this pathway, HSD17B2, HSD17B3, and LIPA, were both simultaneously copy lost and methylated in the promoter region. Of the gene set immediately involved in testosterone synthesis, methylation of CpG sites in the gene body of both CYP17A1 and HSD17B3 was observed (Fig. 6). AR was unmethylated at all 11 CpG sites investigated.

**Genomics of p53/IGF-1/protein kinase B signaling cascade**
The p53 pathway and the IGF-1/protein kinase B signaling cascade pathways share a number of similar aberrant genes, including deletion of PTEN and methylation within the gene body and promoter of multiple subcomponents of the PI3 kinase as well as promoter methylation of AKT3. Two tumor suppressors in the p53 pathway, RB1 and TNFRSF10C, were found to be both deleted as well as exhibit promoter methylation.

**Genomics and site of metastasis**
To investigate whether structural, epigenetic, and/or transcriptional changes play a role in determining the site of metastatic spread, hierarchical clustering of samples was carried out individually for CN, methylation, and expression. Neither CN, methylation nor expression separated the samples by metastatic site (Fig. 5 and Supplementary Fig. S1).

**TMPRSS2-ERG and TMPRSS2-ETS2 mapping**
Genomic alterations involving TMPRSS2, ERG, and ETS2 were previously reported in 8 of these 15 samples (29). Because...
These genes are found in close proximity on chromosome 21, high-resolution mapping of this locus was done to explore whether the deletion of intergenic DNA between TMPRSS2 and ERG or ETS2 and specific breakpoints in these regions were detectable using aCGH.

In total, genomic deletions in this region were detectable in 12 of the 15 samples assessed, including the 8 detected previously by FISH (Fig. 7), one which harbored only a small deletion in TMPRSS2, and 3 additional samples which harbored deletion of the entire genomic segment that included TMPRSS2, ERG, and ETS2. Most of the intergenic deletions encompass ETS and introns 1–3 of ERG. Of note, ETS2 was unmethylated in all samples, TMPRSS2 promoter methylation was present in 1 sample, and ERG promoter methylation was present in 6 samples. No clear correlation of ERG methylation and other genomic aberrations was found. More detailed information about the aCGH and methylation results is in Supplementary Table S4.

Discussion

In this study, a high-resolution integrated analysis of structural and epigenetic changes was done on metastatic CRPC tumors. Although small, the sample set is a rare resource for which whole-genome analysis can provide insight into the lethal form of prostate cancer. Multiple lines of evidence support the copy changes observed. First, many of the common copy changes observed, including AR gain and loss of PTEN and RB1 are consistent with those reported in previous studies (8, 30, 31). Second, a novel PCR-based tool was used to qualitatively confirm the CN aberrations observed, showing for the first time the feasibility and application of this specific approach for validating genomic changes observed with large-scale arrays. Further work is needed to determine the quantitative accuracy of this approach. Third, the gene set identified as commonly aberrant in these metastatic samples was more frequently aberrant in a separate set of primary prostate tumors which recurred after surgery compared with those that did not recur. Lastly, mapping of the locus of deletion between TMPRSS2 and ETS2 was consistent with prior FISH results in this sample set (29) and also shows the usefulness of high-resolution aCGH in mapping specific intrachromosomal translocation breakpoints.

AR amplification was common, and AR expression correlated strongly with copy change (Spearman correlation 0.53, Q = 0.05). When compared with AR-unamplified tumors, AR-amplified tumors were found to commonly carry complications of broad areas along chromosomes 7, 8, and X. Among these, amplification of the AR coactivator and putative oncogene NCOA2 was significantly associated (P = 0.002) with the presence of AR amplification. NCOA2 amplification has been reported to be amplified in 17% of metastatic tumors; in this study it was amplified in 60% of samples (8). This difference could be due to different statistical methods used to analyze aCGH or the relatively small sample sizes in each study. It has been suggested that AR and NCOA2 genes cooperate in early prostate cancer (32); however, it seems likely that tumors first acquire NCOA2 amplification along with broad amplifications on chromosome 8q. Work by us and others has shown gain of 8q to be a common event but AR gain rare in primary tumors (33). NCOA2-amplified tumors may then be essentially “primed” for increased androgen responsiveness, which occurs once AR becomes amplified at a later time point. An examination of either metastatic castration-sensitive or newly castration-resistant tumors would help further define this evolution and could potentially aid in identifying patients more or less likely respond to AR-targeted therapies.

Although 4 patients in this study lacked AR amplification, they nonetheless died of metastatic CRPC. Although no single gene was uniquely amplified in this subset, statistically significant losses were observed in genes critical to the repression of AR activity. Specifically, 3 putative tumor suppressors were deleted including LAT2, a negative regulator of AR (34), PLAG1, a transcription factor thought to function as a repres- sor of AR transcriptional activity (35), and DNAJC3, which codes for the c-subunit of the HSP40 complex which prevents nuclear AR translocation (36). Loss of any of these genes is postulated to derepress AR activity and lead to increased AR-mediated signaling, thereby potentially conferring a selective tumor growth advantage that could occur in the absence of AR gene amplification. These cases also raise the possibility that the progression of tumors without AR amplification may nevertheless be driven by aberrations of AR signaling as opposed to completely AR-independent pathways. Further study is needed to see whether this observation holds in larger sample sets.

Although gene amplification and deletion have been described as mechanisms of genomic change in CRPC, relatively little is known about CpG methylation and its effect on the development and progression of this disease. Here, for the
first time, a comprehensive methylation signature of metastatic CRPC is established (Fig. 5) using a high-resolution array. Overall, the results show that whereas the majority of probes are unmethylated, CRPC is slightly hypermethylated compared with benign tissue. This hypermethylation occurs at individual CpG loci, both within and outside the context of CpG islands. Whether CRPC is globally hypo- or hypermethylated relative to earlier prostate tumors is not established in this study.

Although methylation signatures appeared similar for the majority of samples, at least 3 samples (Mets 29_23, 25_46, and 12_6) were hypomethylated. These tumors did not differ by clinical or pathologic characteristics nor by the CN or methylation status of the DNA methyltransferase gene (DNMT). Three classes of genes were differentially methylated between these 2 methylation subsets, including homeobox genes implicated in tumorigenesis (37), PI3K isoforms known to be involved in protein translation (38), as well as multiple kallikrein isoforms, proteases implicated in tumor invasion and metastasis, of which PSA is a member (39). Whether these methylation changes would influence response to therapies, including inhibitors of PI3K/AKT/mTOR and/or serve as biomarkers for response to other therapies is worthy of future investigation.

Hierarchical clustering by CN, expression (Supplementary Fig. S1), and methylation (Fig. 5) was unable to establish a genomic signature of a liver or a soft-tissue metastasis. The finding that tumors do not cluster by tissue type is in concor-
dance with work showing that metastatic prostate tumors have monoclonal origins (7). Although multiple genomic changes must occur to allow a tumor cell to metastasize to a distant site, the clustering in this study implies that for a metastatic tumor, few if any specific CN or methylation changes are needed to allow it to establish itself in liver tissue compared with soft tissue. Whether this is true of other metastatic sites including bone is not known. The small sample size and large number of comparisons with whole-genome data may miss small, consistent changes across tumors. Further study is needed in larger metastatic sets to determine the gene(s)/mechanism(s) that program a cancer cell to hone to a particular organ.

Integrating the CN and methylation analyses, it was found that CpG methylation occurs more frequently for genes commonly deleted than for those commonly amplified. The observation that CN and methylation work in concert more frequently for genes deleted than for those amplified suggests that gene silencing may occur through heterozygous loss of one allele and CpG methylation of the remaining allele. This cooperation may be essential for inhibiting expression of critical tumor suppressors, such as RB1, which was both frequently deleted and methylated in CRPC. This points to CpG methylation as a possible therapeutic target in CRPC, for which there are already available hypomethylating agents (e.g., 5-azacytidine). More work fully dissecting the functional significance of the commonly observed epigenetic and structural
changes is needed, and control tumors of earlier stage and consistent grade will help identify which epigenetic and structural changes occur early or late in the development of CRPC.

Intracrine androgen signaling is implicated in prostate cancer progression (11), and the integrated analysis here suggests that methylation and CN changes may occur concurrently to both promote the local production and inhibit local breakdown of potent androgens (Fig. 6). Recent work suggests that promoter methylation reduces gene transcription, whereas gene body methylation increases transcription (40–42). Thus, as a hypothesis generating exercise in the absence of expression data for these genes, methylation of CpG sites in gene promoters was interpreted as reducing gene transcription, whereas methylation of gene body CpGs was interpreted as increasing transcription. With this interpretation, we posit that despite almost half of the metastatic tumors exhibiting heterozygous deletion of CYP17A1, a gene critical to androgen synthesis and the target of multiple inhibitors currently in clinical trials (43, 44), tumors are nonetheless able to upregulate CYP17A1 through methylation of CpG sites in the gene body. Resistance to CYP17A1 inhibitors may be epigenetically mediated, and this clinically suggests that resistance to CYP17A1 inhibitors could be attenuated through the use of hypomethylating agents.

This genomic study, which to the best of our knowledge includes the most comprehensive methylation analysis of metastatic CRPC, presents evidence of specific pathway biomarkers that may be useful for assessment of prognosis and stratification for therapy if validated in larger clinical study sets. Furthermore, these observations suggest that the application of agents targeting genes both structurally and epigenetically altered may delay the progression of castration-resistant disease.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

The authors thank Ravi Nagarajan for assistance with methylation experiments, and Catherine Burke for gene mapping.

Grant Support

The work received support from UCSF REAC Career Award (P.L. Paris), UCSF Faculty Fund (T.W. Friedlander/C.J. Ryan), and UCSF Bernard Schwartz Fund (T.W. Friedlander).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

Received June 23, 2011; revised November 16, 2011; accepted November 17, 2011; published OnlineFirst December 7, 2011.

References


Common Structural and Epigenetic Changes in the Genome of Castration-Resistant Prostate Cancer

Terence W. Friedlander, Ritu Roy, Scott A. Tomlins, et al.

Cancer Res  Published OnlineFirst December 7, 2011.

Updated version  Access the most recent version of this article at: doi:10.1158/0008-5472.CAN-11-2079

Supplementary Material  Access the most recent supplemental material at: http://cancerres.aacrjournals.org/content/suppl/2011/12/07/0008-5472.CAN-11-2079.DC1

E-mail alerts  Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions  To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions  To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.