

# Integrative analysis of genetic and epigenetic alterations in lethal metastatic prostate cancer

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## ABSTRACT

**Background and Objectives:** Cancer cells appear to establish their malignant phenotype through the acquisition of numerous somatic genetic and epigenetic genome alterations. Recent evidence suggests that such genetic and epigenetic alterations may cooperate to drive cancer initiation and progression.

**Methodologies:** We have developed a novel technology platform for highly integrative, parallel analysis of allele-specific genome-wide copy number and DNA methylation alterations. This technology features the use of methyl-binding domain polypeptides from the MBD2 protein to selectively enrich for methylated DNA fragments followed by analysis with high-density oligonucleotide SNP arrays (Affymetrix SNP 6.0 platform). Comparison with the total input fraction using novel computational methods allows parallel analysis of both allele-specific copy number and DNA methylation.

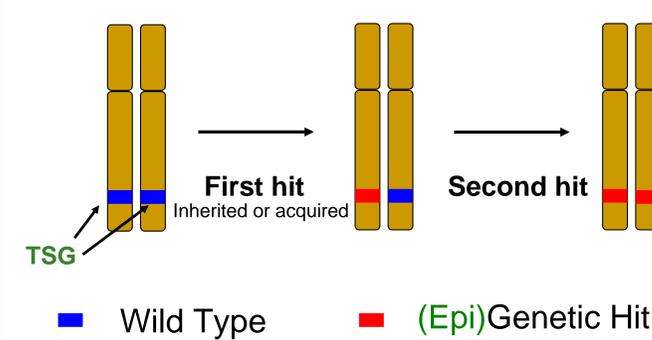
**Results:** Applying this technology to multiple distinct prostate cancer metastatic deposits and matched normal tissues from each of 14 subjects from a rapid autopsy series, we observe that both copy number and DNA methylation alterations are clonally maintained during metastatic dissemination, with evidence of clonal evolution driving some heterogeneity. Furthermore, this analysis revealed several genomic loci that exhibit bi-allelic inactivation of putative tumor suppressor regions through a cooperation of copy number loss and DNA methylation in a recurrent fashion. Finally these analyses have helped us to develop a genome-wide map of total and allele-specific DNA methylation (e.g., CpG island hypermethylation, imprinting/loss of imprinting) as well as total and allele-specific copy number alterations in lethal metastatic prostate cancer.

**Conclusions:** Epigenetic alterations in DNA methylation can cooperate with genetic alterations such as copy number gains and losses during progression to lethal metastatic prostate cancer.

**Impact:** These analyses will help us to better understand the cooperation between genetic and epigenetic processes during the formation of lethal metastatic prostate cancer and may also help to identify novel targets for prostate cancer treatment and diagnosis and risk stratification.

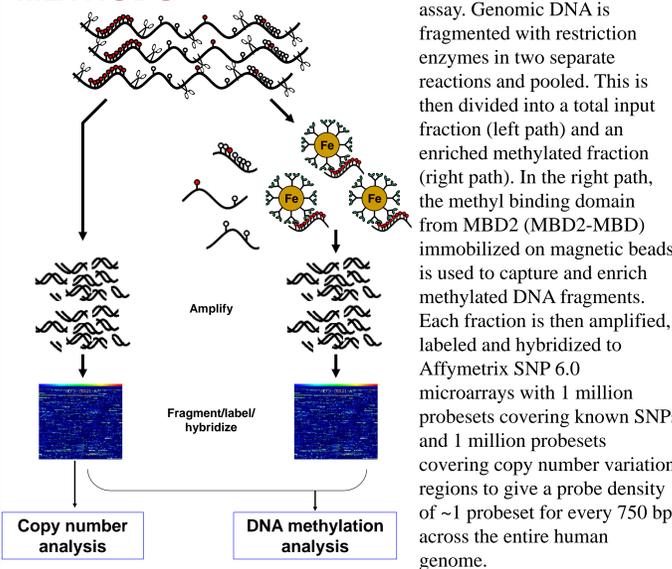
**Keywords:** DNA methylation, Copy Number alteration, biomarker, prostate cancer

## BACKGROUND



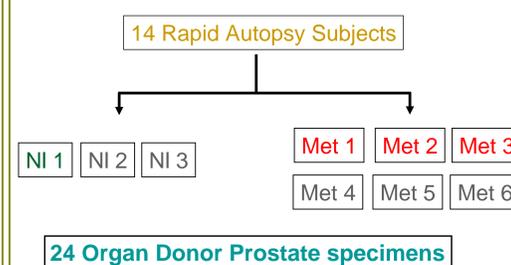
**Figure 1.** The modern version of the Knudson two-hit hypothesis can be interpreted to mean that carcinogenesis is promoted by the acquisition of two hits, one to each allele, to completely inactivate tumor suppressor genes (TSG). Each hit can come from a variety of mechanisms including genetic (mutations, copy number alteration, etc.) and epigenetic (DNA methylation, chromatin alteration, miRNA, etc.).

## METHODS

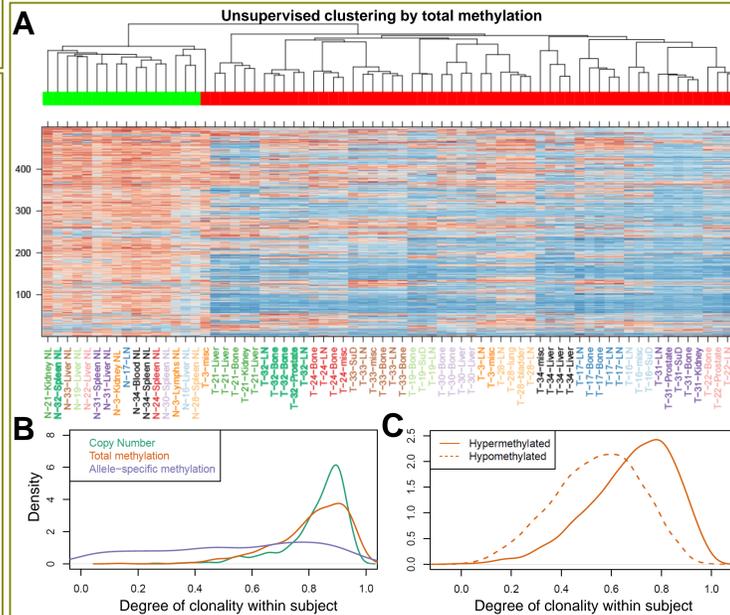


**Figure 2.** MBD-SNPchip assay. Genomic DNA is fragmented with restriction enzymes in two separate reactions and pooled. This is then divided into a total input fraction (left path) and an enriched methylated fraction (right path). In the right path, the methyl binding domain from MBD2 (MBD2-MBD) immobilized on magnetic beads is used to capture and enrich methylated DNA fragments. Each fraction is then amplified, labeled and hybridized to Affymetrix SNP 6.0 microarrays with 1 million probesets covering known SNPs and 1 million probesets covering copy number variation regions to give a probe density of ~1 probe for every 750 bp across the entire human genome.

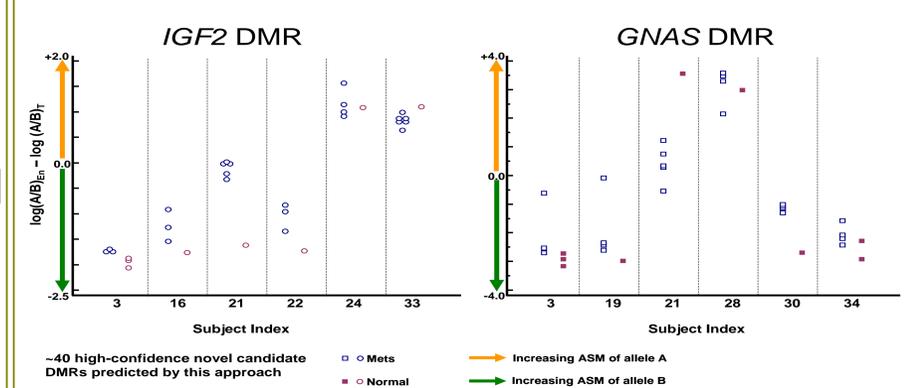
## STUDY DESIGN



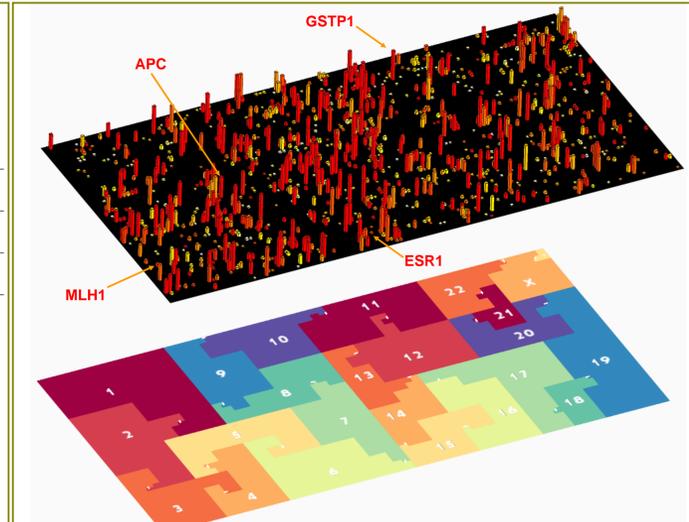
**Figure 4.** Overall study design. At least 1, but up to 3 normal germline specimens, and at least 3, but up to 6 anatomically distinct metastatic deposits from each of 14 rapid autopsy subjects that died of hormone-refractory metastatic prostate cancer were included; Normal prostate tissues from 24 organ donors were also included.



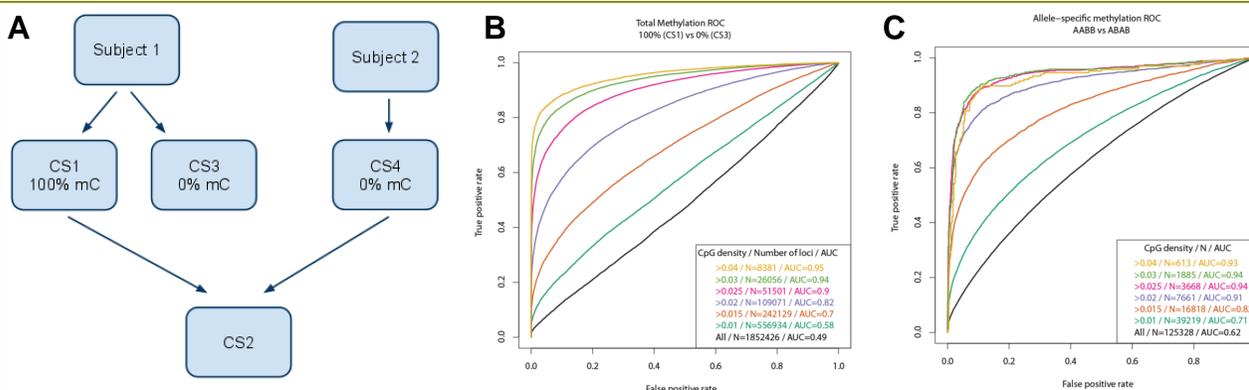
**Figure 6.** Alterations in DNA methylation patterns are clonally maintained during metastatic dissemination to a degree similar to genetic alterations in copy number, with hypermethylation changes showing higher maintenance than hypomethylation changes.



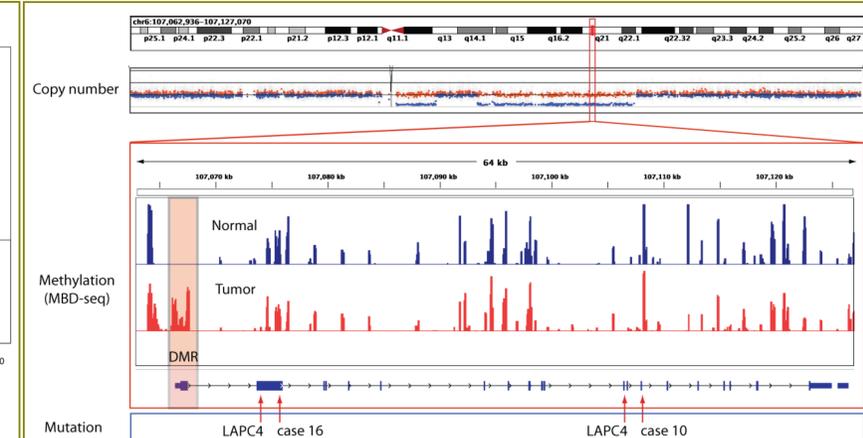
**Figure 5.** Identification of known and novel putative imprinted regions. Note presence of loss of imprinting in a subset of prostate cancer specimens.



**Figure 7.** The genome-wide DNA hypermethylation "Cityscape" in lethal metastatic prostate cancer. The genome was organized into an urban "cityscape" with regions from each chromosome arranged into "neighborhoods" according to the Hilbert plotting algorithm (bottom). For each region, the frequency of hypermethylation compared to the organ-donor normals is represented by the height of each bar (top). The consistency of hypermethylation across all metastases from the subject with the most pronounced methylation is color-scaled, with red representing high consistency, and lighter colors representing less consistency/maintenance. Several genes such as *GSTP1* and *APC* (marked in the top panel) are highly consistently and frequently hypermethylated in the metastases, while other genes can be infrequently but consistently hypermethylated (e.g. *MLH1*, *ESR1*). Methylation consistency may distinguish drivers vs. passengers.



**Figure 3.** Validation of the MBD-SNP-chip approach with engineered control specimens. (A) To test the effectiveness of MBD-SNP-chip, we assembled 4 control specimens: CS1 consisted of completely in vitro methylated (M.SssI treated), whole genome amplified, normal prostate DNA from organ donor Subject 1. CS3 and CS4 consisted of whole genome amplified, completely unmethylated DNA from normal prostate of organ donor Subjects 1 & 2. CS2 was constituted by a 1:1 mix of CS1 and CS4. CS1 and CS3 could be used as controls to create ROC curves for total methylation and CS2 provided both positive and negative controls for allele specific methylation (ASM) depending upon the genotype in subjects 1 and 2 at each locus. (B,C) For regions with CpG density greater than 2.5%, we achieved area under the ROC (AUC) > 0.9 for total methylation (B), and > 0.94 for allele specific methylation (C).



**Figure 8.** Integrative analyses are beginning to reveal novel putative tumor suppressors that can be inactivated by multiple mechanisms, including copy number loss, DNA methylation, and mutation, as is shown here for a region of chr 6 that is commonly deleted in prostate cancers.

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