The genomic complexity of prostate cancer

Rami Aqeilan, PhD
The Lautenberg Center for Immunology and Cancer Research
Hebrew University-Hadassah Medical School
Jerusalem
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Prostate Cancer

Definition

• Relevance
  – Most common noncutaneous malignancy in men
• Incidence
  – Nearly 200,000 new cases per year in U.S.
• Mortality
  – 32,000 deaths in the United States each year
  – Second most common cause of cancer death in men
• Morbidity
  – Single histologic disease
  – Ranges
    • From indolent, clinically irrelevant
    • To virulent, rapidly lethal phenotype: castrate-resistant prostate cancer (CRPC).

Small, E., Cecil Textbook of Medicine, Prostate Cancer, 2004, WB Saunders, an Elsevier imprint
Theodorescu, D., Prostate Cancer: Management of Localized Disease, www.emedicine.com, 2004²
Prostate-specific antigen (PSA) as biomarkers for PC

1926 - Alexander and Ethel Guthman described elevated acid phosphatase activity in prostate cancer
1936 - PSA discovered [PMID: 4986767]
1941 - Charles B. Huggins publishes studies showing relationship between testosterone and prostate cancer
1980 - PSA found to be elevated in men with prostate cancer
1985 - PSA test approved by FDA for monitoring cancer recurrence
1994 - PSA test approved by FDA for screening in conjunction with a digital rectal exam. [FDA Announcement]
2005 - Discovery of antibodies that act as a new biomarker for prostate cancer
2005 - Discovery of TMPRSS2:ERG gene fusion in PCs
Prostate Cancer
Epidemiology

- Prostate-specific antigen (PSA) assay has affected incidence of prostate cancer
- Incidence
  - Prior to PSA
    - 19,000 new cases / year in US
  - 1993
    - 84,000
  - 1996
    - 300,000
  - Since 1996
    - 200,000 per year
    - A number that more closely estimate the true annual incidence of clinically detectable disease

Small, E., Cecil Textbook of Medicine, Prostate Cancer, 2004, WB Saunders, an Elsevier imprint
Prostate Cancer

Pathophysiology

• Adenocarcinoma
  – 95% of prostate cancers
    • Developing in the acini of prostatic ducts
• Rare histopathologic types of prostate carcinoma
  – Occur in approximately 5% of patients
  – Include
    • Small cell carcinoma
    • Mucinous carcinoma
    • Endometrioid cancer (prostatic ductal carcinoma)
    • Transitional cell cancer
    • Squamous cell carcinoma
    • Basal cell carcinoma
    • Adenoid cystic carcinoma (basaloid)
    • Signet-ring cell carcinoma
    • Neuroendocrine cancer

Proposed cellular model of prostate carcinogenesis and metastases

Prostate carcinogenesis

- Self-renewal ability
  - Multipotent prostatic stem cell
    - CD133⁺, CD44⁺, αβ₁, integrin⁺, KIT, CK5⁺, AR⁻ and (Sca-1 in mouse)
  - PTEN⁺/⁻, Nkx3.1⁺/⁻, p27⁻/⁺
  - Tumorigenic PC stem/progenitor cell
    - CD133⁺, CD44⁺, αβ₁, integrin⁺, CK5⁺, AR⁺ and (Sca-1 in mouse)

- Accumulating of genetic alterations

- Cellular senescence and apoptosis
  - PTEN⁺/⁻, p53⁻/⁺, pRb⁻
  - Activation of diverse oncogenic signaling elements (hedgehog, EGFR, RTKs, PI3K/Akt and NF-kB)

- Total PC cell mass
  - Tumor formation

- EMT program

- Total PC cell mass
  - Invasive tumor

- Primary PC progression

Metastases

- Dissemination through the peripheral circulation

- Metastatic initiating cell

- Metastases
  - Metastases at distant sites
    - Adrenal gland
    - Lung
    - Bone
    - Brain
    - Liver
Androgen-dependent and independent PC

In advance disease, androgen-deprivation therapy results in rapid response; however, nearly all patients eventually progress to metastatic castration-resistant prostate cancer (CRPC)
Common chromosomal fusions in prostate cancer

Among men in the United States, prostate cancer accounts for more than 200,000 new cancer cases and 32,000 deaths annually\(^1\). Although androgen deprivation therapy yields transient efficacy, most patients with metastatic prostate cancer eventually die of their disease. These aspects underscore the critical need to articulate both genetic underpinnings and novel therapeutic targets in prostate cancer.

Recent years have heralded a marked expansion in our understanding of the somatic genetic basis of prostate cancer. Of considerable importance has been the discovery of recurrent gene fusions that render ETS transcription factors under the control of androgen-responsive or other promoters\(^2\)\(^–\)\(^5\). These findings suggest that genomic rearrangements may comprise a major mechanism driving prostate carcinogenesis. Other types of somatic alterations also engage important mechanisms\(^6\)\(^–\)\(^8\); however, the full spectrum of prostate cancer genomic alterations remains incompletely characterized. Moreover, although the androgen signalling axis represents an important therapeutic focal point\(^9\)\(^,\)\(^10\), relatively few additional drug targets have yet been elaborated by genetic studies of prostate cancer\(^11\). To discover additional genomic alterations that may underpin lethal prostate cancer, we performed paired-end, massively parallel sequencing on tumour and matched normal genomic DNA obtained from seven patients with ‘high-risk’ primary prostate cancer.

**Landscape of genomic alterations**

All patients harboured tumours of stage T2c or greater, and Gleason grade 7 or higher. Serum prostate-specific antigen levels ranged 2.1 to 10.2 ng ml\(^{-1}\) (Supplementary Table 1). Three tumours contained chromosomal rearrangements involving the *TMPRSS2* (transmembrane protease, serine 2)–*ERG* (v-ets erythroblastosis virus oncogene homologue (avian)) loci as determined by fluorescence *in situ* hybridization (FISH) and PCR with reverse transcription (RT)–PCR\(^2\) (Table 1 and Supplementary Table 1). We obtained approximately 30-fold mean sequence coverage for each sample, and resequenced somatic mutations in more than 80% of the genome (described in Supplementary Information). Circos plots\(^12\) indicate genomic rearrangements and copy number alterations for each state cancer genome are shown in Fig. 1.

We identified a median of 3,866 putative somatic base mutations (range 3,192–5,865) per tumour (Supplementary Table 2); the estimated mutation frequency was 0.9 per megabase (see Supplementary Methods). This mutation rate is similar to that observed in myeloid leukaemia and breast cancer\(^13\)\(^–\)\(^16\) but 7–15-fold lower than reported for small cell lung cancer and melanoma\(^17\)\(^–\)\(^19\). The mutation rate at CpG (that is, cytosine–phosphate–guanine) dinucleotide was more than tenfold higher than at all other genomic positions.
Point Mutation rate

0.9 mutation per megabase

Graphical representation of the first seven sequenced prostate cancer genomes

Complex structural rearrangements in prostate cancer

Disruption of CADM2 and the PTEN pathway by rearrangements

**CADM2, cell adhesion molecule 2**

- Rearranged in 3 out of the seven PC cases
- Using another cohort, CADM2 showed rearrangement in 6/90 independent cases

Not much is known:
- Putative tumor suppressor in PC cells (reduced expression by IHC and its ectopic expression suppresses cell growth)
- Its expression is regulated in part by promoter methylation
Rearrangements disrupting *PTEN* and *MAGI2*

- Rearranged in 4 out of the seven PC cases, including all three tumors with *TMPRSS2-ERG* rearrangements.
- *MAGI2* (membrane associated guanylate kinase, WW and PDZ domain containing 2) is PTEN-interacting protein.
- Direct or indirect loss of PTEN function thus dysregulating PI3 kinase pathway in PC
Association between rearrangement breakpoints and genomewide transcriptional/histone marks in PC

 TMPRSS2-ERG

Fabio Vandin, Javed Siddiqui

CRPC. Integrating exome copy number analysis identified disruption and confirmed the monoclonal origin of lethal prostate cancer; however, nearly all patients eventually progress to CRPC. Treatment-naive, high-grade localized prostate cancers. We identified low overall mutation rates even in heavily treated CRPCs (2.00 per megabase) and confirmed the monoclonal origin of lethal prostate cancer. Similarly, we demonstrate that recurrent mutations in the AR, such as the interaction of the MLL complex with the AR, which is required for AR-mediated signalling. We also identified novel recurrent mutations in the AR collaborative factor FOXA1, which is mutated in 5 of 147 (3.4%) prostate cancers (both untreated localized prostate cancer and CRPC), and showed that mutated FOXA1 represses androgen signalling and increases tumour growth. Proteins that physically interact with the AR, such as the ERG gene fusion product, FOXA1, MLL2, UTX (also known as KDM6A) and ASXL1 were found to be mutated in CRPC. In summary, we describe the mutational landscape of a heavily treated metastatic cancer, identify novel mechanisms of AR signalling deregulated in prostate cancer, and prioritize candidates for future study.

Although localized prostate cancer is highly curable, more than 32,000 US men die annually of metastatic disease. Androgen-deprivation therapy results in rapid responses in men with metastatic prostate cancer; however, nearly all patients eventually progress to CRPC. Although CRPC was thought to be androgen-signalling independent, recent evidence demonstrates that androgen signalling is often maintained through varied mechanisms (reviewed in refs 1, 5). Gene expression and copy number profiling studies have identified recurrent gene fusions, chromosomal gains and losses, and deregulated pathways in prostate cancer. Resequencing studies have characterized the mutational spectrum of prostate cancer1,2, and the genomes of seven exomes of xenografts from 16 CRPC cases were reported3.

We sequenced the exomes of 50 lethal heavily pre-treated CI patients (patient identifiers WA2–WA60) obtained at rapid autopsy, including three distinct sites in the same patient, and 11 treatment-naive, high-grade localized prostate cancers (patient identifiers T1–T97) (Supplementary Table 1). Sequencing results, including covariate statistics, mutation rates, validation rates, mutational spectrum, firmation of the monoclonal origin of CRPC, and overlap with mutations observed in previous studies are provided in Supplementary Results, Supplementary Figs 1–6 and Supplementary Tables 2–6.

We used exome sequencing data to identify somatic copy number alterations4 (see Methods, Supplementary Fig. 7 and Supplementary Tables 7–9), and as shown in Supplementary Fig. 8 we identified recurrent aberrations previously associated with prostate cancer development and progression (Supplementary Results). We additionally performed array comparative genome hybridization (aCGH) copy number and gene expression profiling on a matched cohort of breast, local prostate tissues, localized prostate cancers (3/59 sequenced) and 35 CRPCs (31/35 sequenced) (Supplementary Table 10). Profiles uploaded into Oncomine (http://www.oncomine.com) for automated data processing, analysis and visualization, and are available for exploration. aCGH profiles were similar to copy number analysis by sequencing and to other prostate cancer profiling studies available on Oncomine (Supplementary Fig. 9). Global gene expression profiles similar to previous studies (analyses available in Oncomine), with mutations described in Supplementary Results and Supplementary Fig. 10. Finally, we performed transcriptome sequencing of 11 prostate cell lines to identify likely somatic variants (see Supplementary Re Supplementary Methods and Supplementary Tables 11–15).

From our exome data, we identified nine genes that were significantly mutated (false discovery rate ≤ 0.10) (Fig. 1 and Supplementary Table 16), four of which have been reported as recurrently mutated prostate cancer: TP53, AR, ZFHX3, RB1, PTEN and APC. Three significantly mutated genes do not have described roles in prostate cancer: AMEL, OR5L1 and CDK12. MLL2 encodes an H3K4-specific histone methyltransferase that is recurrently mutated in multiple cancers and was recently identified as significantly mutated in ovarian serous carcinoma5,6. Additionally, using several approaches, we identified multiple significantly mutated pathways, including WNT signalling and a PTEN interaction network (Supplementary Fig. 11 and Supplementary Tables 17 and 18); observations on significantly mutated genes and pathways are provided in the Supplementary Results.

*These authors contributed equally to this work.

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1Michigan Center for Translational Pathology, University of Michigan Medical School, Ann Arbor, Michigan 48109, USA. 2Department of Pathology, University of Michigan Medical School, Ann Arbor, Michigan 48109, USA. 3Howard Hughes Medical Institute, University of Michigan Medical School, Ann Arbor, Michigan 48109, USA. 4Comprehensive Cancer Center, University of Michigan Medical School, Ann Arbor, Michigan 48109, USA. 5Howard Hughes Medical Institute, University of Michigan Medical School, Ann Arbor, Michigan 48109, USA. 6Department of Pathology, University of Michigan Medical School, Ann Arbor, Michigan 48109, USA. 7Howard Hughes Medical Institute, University of Michigan Medical School, Ann Arbor, Michigan 48109, USA. 8Compendia Bioscience, Ann Arbor, Michigan 48104, USA. 9Division of Biostatistics, Yale School of Public Health, New Haven, Connecticut 06520, USA. 10Department of Computer Science & Center for Computational Molecular Biology, Brown University, Providence, Rhode Island 02912, USA. 11Department of Internal Medicine, University of Michigan Medical School, Ann Arbor, Michigan 48109, USA. 12Department of Urology, University of Michigan Medical School, Ann Arbor, Michigan 48109, USA.

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Major findings in metastatic CRPCs

• Low overall mutation rate
• Disruption of *CHD1* that defines a subset of ETS gene family fusion-negative PCs.
• Recurrent mutations in multiple chromatin and histone-modifying genes.
• Identify novel mechanisms of AR signaling deregulated in PCs; *FOXA1* and *MLL2*. 
Integrated mutational landscape of lethal metastatic CRPC

CRPC harbours mutational aberrations in chromatin/histone modifiers that physically interact with the AR

Supplementary Figure 8. Comparison of copy number aberrations identified by exome sequencing in castrate resistant prostate cancer (CRPC) and localized prostate cancer. Exomes of 50 CRPC (WA3-WA60; three foci from WA43) and 11 high-grade untreated localized prostate cancers (T8-T97) were sequenced for determination of somatic mutations and copy number alterations. Genome wide copy number analysis of each sample was performed using exome sequencing. For all genes, the sum of somatic copy number calls (+/-1: one copy gain or loss, respectively; +/-2: high level copy gain/loss, respectively) across a) all profiled samples, b) only CRPC samples or c) only localized prostate cancers was plotted and ordered by genome location (WA43-24 and -71 are excluded from a and b). Genes in peaks of copy changes are indicated.
Integrated mutational landscape of lethal metastatic CRPC

Architecture of 5q21 deletions in PC

**CHD1** deregulation deletion in ETS fusion negative PCs

Supplementary Figure 13. Deregulation of genes at 5q21, including **CHD1**, confirmed by matched aCGH and gene expression profiling. Genome wide copy number analysis of high-grade localized prostate cancer and castrate resistant prostate cancer by exome sequencing identifies peak of copy number loss on chr 5q21 centered on **CHD1**. A subset of samples used for exome sequencing, and additional benign prostate tissue samples are indicated in black or gray type, respectively. Samples with focal deletion of **CHD1** (5q21) or other genes within 5q21 (5q21) by aCGH are indicated with green or red background, respectively, according to the legend. The adjoining plot shows the genome wide copy number plot for T56, which harbors a focal, high level deletion on 5q21 including **CHD1**.

- **a.** Genome wide analysis by aCGH identified a similar peak of copy number loss on 5q21 (upper panel, sum log2 copy number across all samples plotted) centered on **CHD1**. The expanded view is as in Figure 2a, except the area (absolute Log2 ratio) and color intensity (Log 2 ratio; copy number loss in blue) of each box are proportional to binned copy number for that gene according to the legend. ETS+ and ETS+ samples are indicated in black or gray type, respectively. Samples with focal deletions of **CHD1** (5q21) or other genes within 5q21 (5q21) by aCGH are indicated with green or red background, respectively, according to the legend.
- **b.** Co-expression of **CHD1** and ETS family members. Heatmap of **CHD1**, ETS genes (ERG, ETV1, ETV5) and SPINK1 gene expression for that gene according to the legend. ETS+ and ETS+ samples are indicated in black or gray type, respectively. Samples with focal deletions of **CHD1** (5q21) or other genes within 5q21 (5q21) by aCGH are indicated with green or red background, respectively, according to the legend. The adjoining plot shows the genome wide copy number plot for T56, which harbors a focal, high level deletion on 5q21 including **CHD1**.
- **c.** Expression of **PJA2** stratified by benign prostate tissue and prostate cancer (including localized and CRPC). ETS+ and **CHD1** status was determined, with black and green indicating ETS+ and **CHD1**, respectively.
- **d.** Expression of **PJA2** stratified by benign prostate tissues (orange), localized prostate cancers (cyan) and CRPCs (black). T65 is indicated in red. 

[www.nature.com/nature]
CHROMODOMAIN HELICASE DNA-BINDING PROTEIN 1 (CHD1)

• Encodes an ATP chromatin-remodeling enzyme

• *CHD1* is involved in assembly, shifting and removal of nucleosomes from the DNA double helix to keep it in an open and transcriptionally active state

• *CHD1* is essential to maintain open chromatin of pluripotent embryonic stem cells.

• It associates with the promoters of active genes through binding to the H3K4-trimethylated histones.
Effect of CHD1 depletion on the transcription of AR-dependent genes

Effect of CHD1 on expression of known tumor suppressor genes of prostate cancer cells

Significantly mutated genes in PC

SPOP, the most frequently mutated gene in ETS- tumors
Recurrent mutations in the AR collaborating factor *FOXA1* promote tumour growth and affect AR signalling

Integrated Analysis of Genomic Deletions and Rearrangements Reveals Signatures of Concurrent Alterations

Chromoplexy

• Interdependent genomic restructuring driving prostate cancer progression

• A complex chains of DNA rearrangements accompanied by significant DNA deletions that spanned breakpoints from distinct fusions

• Is prevalent in highly expressed genomic regions of ETS fusion-positive prostate tumors

• Induce coordinated dysregulation of multiple cancer genes, including deletion of tumor suppressors and generation of oncogenic ETS fusions
Chromoplexy May Coordinately Dysregulate Multiple Cancer
Chromotripsy: A massive genomic rearrangement acquired in a single catastrophic event during cancer development
Manifestations of Chromoplexy Vary by ETS Fusion Status

ETS-fusion positive, CHD1 wild-type (ETS⁺, CHD1⁺)
- PR-07-4610
- P05-3852
- P07-4941
- P01-28
- P08-1042
- P08-492
- PR-07-4814
- PR-08-2153

ETS-fusion negative, CHD1-deleted (ETS⁻, CHD1⁻)
- PR-08-556
- P07-837
- P08-1541
- P05-620

B

C

D

A Continuum Model for the Genomic Evolution of Prostate Cancer

Conclusions

• Low overall point mutation rate

• High prevalence of complex structural rearrangements; closed chain and copy neutral (Chromoplexy)

• Many rearrangements may occur preferentially in genes that are localized with transcriptional or chromatin compartments

• Novel mechanisms of AR signaling deregulation in PC & new subsets of ETS-/CHD1- tumors
Your best bid!