

Prostate Cancer: Basic Biology in the New Millennium

Understanding Why It's So Complicated and
What the Opportunities for Chemotherapy
Might Be

Perry Karfunkel
Lahey Clinic Burlington, MA



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Prostate Cancer in the U.S.

- 180,000 new cases in 2008; 218,000 in 2010
- 28,000 men died of it in 2008; 32,000 in 2010
- Father pos. → 2.5 odds ratio for his son
- Brother pos. → 3.4 odds ratio
- Two particular genotype changes increase the risk of “highly aggressive” tumor:
 - ILB+3954: TT vs. CC single base pair change
 - IL8-47: CT vs. CC single base pair

Prostate Cancer is Multifocal

When a prostate gland is removed from a man who's had a positive biopsy, it is often found to have multiple, isolated sites of cancer within that gland: 25% of such men have at least four isolated sites of cancer and those sites are often found to have different Gleason staging.

Prostate Cancer is Multifocal

Thus, unlike breast cancer or pancreatic cancer or cancer of the colon, the malignant change that occurs in prostate cancer seems to represent a change within a man's prostate gland in a general way: that (or those) change(s) allowing multiple, individual, isolated cells to start malignant growth.

Euonymus









Mendel's Pea Plants: 1

	Tall TT	Tall TT
Short tt	Tall Tt	Tall Tt
Short tt	Tall Tt	Tall Tt

Mendel's Pea Plants: 2

	Tall Tt	Tall Tt
Tall Tt	TT	Tt
Tall Tt	Tt	tt

Gleason staging

- Stage 1= normal tissue; glands well-formed and closely packed
- Stage 2= well-formed glands but reduction of the stroma between these
- Stage 3= glands darker-staining than usual with some glandular cells invading the stroma
- Stage 4= few recognizable glands; many cells invading the surrounding stroma
- Stage 5= sheets of prostate cells without recognizable glands seen

Epigenetics

Changes in gene expression that occur without changes in DNA sequence: these may involve

DNA methylation

Histone modification

Interference RNA

Epigenetics: DNA methylation

- DNA methyl transferase is an enzyme that adds a methyl group to a cytosine nucleotide of DNA which is itself adjacent to a guanine nucleoside
- This occurs particularly frequently near promoter genes: the methylation silences the genes
- Methylation of the promoter regions of tumor-suppressor genes therefore allows tumors to develop and grow without restriction
- Normally-methylated (and therefore silenced) onco-genes such as HRAS become activated when they lose their methylation

Epigenetics: DNA methylation

- The prostate cancer tissue-culture cell line LnCaP is sensitive to suppression by GnRH agents (Lupron, Eligard).
- The variant line LnCaP-r10 is hormone resistant. DNA methyl-transferases (DNMT's) are up-regulated in that line.
- Men with sequence polymorphism in DNMT3b have an increased frequency of tumor-suppressor gene hypermethylation and have a 2.8-fold increased risk of developing prostate cancer.

Epigenetics: DNA methylation

- **GSTP's** (glutathione S-transferase P's) are a family of four classes of enzymes that protect cells against reactive-oxygen damage to DNA and subsequent carcinogenesis: GSTP1 is the gene that encodes for this family.
- The silencing of GSTP1 by hyper-methylation suppresses the production of these enzymes (GSTP's) that are protective : this is the most common epigenetic change associated with prostate cancer.
- GSTP's can be detected in a man's semen and there was a time that it was thought that they could be a screening assay for prostate cancer

Epigenetics: DNA methylation

- The GSTP1 gene is hyper-methylated in cancerous prostate cells but not in BPH cells or non-cancerous cells within a surgically resected prostate gland that contains cancer. This hyper-methylation blocks the normal cancer-protection of the gene products.
- GSTP1 is somewhat hyper-methylated in PIN cells within a biopsy core

Epigenetics: DNA methylation

- **RARb-2** is a tumor-suppressor gene that blocks prostate cancer; its function is blocked by hypermethylation
- **RARb-2** shows the same pattern of progressive hypermethylation as one examines BPH cells, PIN cells, cancerous cells within a prostate that has cancer within it but not in non-cancerous cells within that gland

Epigenetics: DNA methylation

- DNA methyl-transferase is itself a potential target for chemotherapy.
- The base analogues decitabine and zebularine block tumor activity.
- Green tea is an anti-oxidant that prevents hypomethylation of histones; it silences genes for growth and it suppresses both BPH and prostate cancer, but it increases the risk of colon cancer.

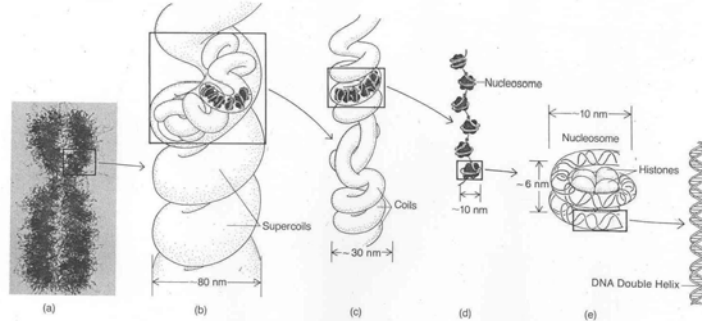
Histones

Histones are positively charged protein molecules around which DNA strands are wound. These create beaded clusters called nucleosomes.

Histones: Wessells & Hopson: Biology

Figure 9-4 A CHROMOSOME UNWOUND: FROM "COLORED BODY" TO DNA.

A chromosome (a) contains highly coiled and supercoiled DNA strands (b). (c) A close-up of a section of the chromosome shows supercoils, each made up of coils of DNA complexed with proteins. (d) The coils of DNA have a "beads-on-a-string" arrangement of nucleosomes. (e) A detailed view of the nucleosomes shows the DNA strands wrapped around clusters of 8 proteins called histones. The DNA double-helix structure is shown in (e).



Epigenetics: Histone acetylation

- Acetylation of an N-terminal lysine amino acid creates an open or non-compact core unit of DNA-histone; such acetylation is brought about by a histone acetyl-transferase
- Removal of an acetyl group from lysine is brought about by a histone-deacetylase
- The highest levels of histone-deacetylase are found in hormone-refractory cancer cells

Epigenetics: Histone acetylation

- **HDAC-1**: a histone-deacetylase enzyme that is linked to gene silencing
- It represses genes for cell differentiation
- It represses genes for growth-control of cells
- It is found at low levels in BPH cells and in normal-appearing cells in a prostate that has tumor within it
- It is found at high levels in malignant cells within a prostate that has tumor within it
- It is found at moderate levels in PIN cells within a prostate core at the time of a punch-biopsy

Epigenetics: Histone methylation

- Histone methylation is brought about by a histone methyl-transferase enzyme, and this also impacts on cancer cell behavior.
- In prostate cancer patients, those patients who have the highest level of the enzyme EZH2 (which methylates lysine 2 on the histone H3) also have the highest level of hormone-resistance in their metastatic cells. Its presence in the primary cancer predicts PSA rises post-operatively, with a 30% risk of surgical failure to cure in these men.

Epigenetics: histone modification

- Assessment of global histone acetylation in 3 residues on H3 and H4 and of histone methylation at other domains has been used by researchers at UCLA to stratify post-prostatectomy patients as to their risk of cancer recurrence.
- This is true for patients with low Gleason scores, which raises the potential for identifying those patients who would benefit from second-line therapy post-operatively even in spite of their low Gleason scores pre-operatively.

Initial Changes in Prostate Cancer: Changes in Cell Adhesion and Migration

- Matrix metalloproteinase enzymes: MMP's
- Metal-ion binding proteases that break down matrix stromal connective tissues: the enzyme that breaks down tadpole tail connective tissue, the family that frees tumor cells bound to their stroma, allowing cells to spread from a tumor focus
- MMP-9 allows cancer cells to migrate in culture; Rx of bosutinib inhibits this

Initial Changes in Prostate Cancer: Changes in Cell Adhesion and Migration

- Prostate-derived Ets Factor: E26 factor
- Downregulates MMP-9
- Increased Ets factor correlates with increased aggressiveness of phenotype
- Re-introduction of Ets factor in cells in culture makes those cells less invasive

Initial Changes in Prostate Cancer: Changes in Cell Adhesion and Migration

- Cadherins: calcium-dependent adhesion proteins
- Cadherin-11: found in osteoblastic metastases of prostate cancer in bone
- Down-regulation of cadherin-11 decreases cell motility and invasiveness in culture
- Up-regulation increases cell motility and invasiveness

Initial Changes in Prostate Cancer: Changes in Cell Adhesion and Migration

- Catenins: a series of proteins found just below the cell membrane which are linked to the intracellular component of cadherins on one side and to the cytoskeletal actin filaments that extend further into the cytoplasm of the cell on the other side.
- Aberrant levels of E-cadherin and/or catenin expression (compared to normal tissue) is found in breast cancer, colorectal cancer and esophageal cancer

Initial Changes in Prostate Cancer: Changes in Cell Adhesion and Migration

- Cqtenin immuno-histological staining is strong in well-differentiated cancers of breast, bladder, lung, and pancreas that maintain their cell adhesiveness and are less invasive but is reduced in poorly-differentiated tumors which have lost their cell-cell adhesion and show strong invasive behavior.
- The restoration of E-cadherin-catenin expression and function can be brought about by various drugs, including those used for chemotherapy: tamoxifen, taxol, retinoic acid and progestagens, insulin-like growth factor, as well as aspirin

Breakout from Androgen Sensitivity: “Castration Resistance”

- AZPG1: Androgen-related zinc alpha-2 glycoprotein
- TMPRSS2: trans-membrane protease serine-2
- GATA: G-A-T-A transcription factors
- KLK-2: Kallikrein-2 and Kallikrein-3 (PSA)
- TRPV2: transient receptor-potential vanilloid-2

Neuroendocrine Malignant Cells: the newest focus in many kinds of Cancer

- Specifically stainable by chromogranins
- 50% of all adenocarcinomas show the presence of neuroendocrine cells: lung, colon, as well as prostate (most lung cancers are not adenocarcinomas)
- Can found either intercalated in “composite” tumors or in side-by-side “collision” tumors
- Neuroendocrine cells responsive to their own specific chemotherapy: should you treat both cell types in mixed tumors or just the predominant type ?

Prostate Cancer: Metastases

- 83%: Bone
- 62%: Lymph nodes
- 65%: Liver
- 50%: Lung
- 18%: Brain
- 30%: Dura
- 8%: Pancreas

Prostate Cancer: Metastases

In any one patient with metastatic prostate cancer, some of those metastases show Gleason Stage 4 tissue, others show Gleason Stage 5 tissue.

Prostate Cancer: Metastases

In a single patient studied for androgen-receptor staining, both the staining intensity and the percent of cells in any one metastasis which do stain for androgen receptor can vary widely: his prostate tumor itself (after he had received androgen-ablation therapy) showed 12% staining intensity, but that seen in his various metastases varied:

Bone metastases:	22% intensity
Lung metastases:	24% intensity
Liver metastases:	46% intensity
Lymph node metastases:	71% intensity

Metastatic Spread

- Runx2: originally found in “runt” mice
- Runx2 is a transcription factor abnormally expressed in cancer cell lines metastatic to bone.
- Src kinases: originally found in sarcoma cells
- Src kinases: control gene expression to favor “ruffling” of cell membranes to facilitate cell migration- bosutinib is a drug that blocks Src kinases and reduces the extent to which an implanted tumor will generate metastases

Metastatic Spread

- RANKL (receptor activator nuclear factor K-B ligand) is responsible for osteoclast formation, differentiation and survival. It creates holes within bones in which metastases can grow.
- Denosumab is a human monoclonal antibody against RANKL; its administration delays bone metastatic spread in men with castration-resistance by blocking this “cavity building.”
- Soledronic acid is a bisphosphonate that also blocks bone-resorption by osteoclasts.

Terminal Cancer: “Cancer-induced Cachexia”

- A wasting process generated by cytokines
- “I don’t have the energy to go on...”
- “I don’t want to go on if it means living this way, feeling this weak...”
- Mitochondrial dysfunction leads to muscle wasting
- Society on Cachexia and Wasting Disorders
- SARM’s: selective adrenergic receptor modulators