Molecular Pathology of Prostate Cancer

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Introduction

Biology of Prostatic Epithelium

- The prostate is not fully developed until after puberty. The normal adult prostate epithelium consists of a single layer of columnar luminal cells situated above a single layer of cuboidal basal cells (Fig. 14.1). Rare neuroendocrine cells are interspersed throughout the epithelium.
  - Basal cells separate luminal cells from the basement membrane and often extend cytoplasmic projections that intercalate between the basolateral aspects of the luminal cells.
  - Basal cells express nuclear p63, keratins 5 and 14, and many have low levels (albeit non-negative) of AR, NKX3.1, and HOXB13. They do not express PSA/KLK3.
  - Basal cells are traditionally thought to be the stem/progenitor cells of prostate epithelium; in mice, especially when the tissue is damaged or inflamed, they can proliferate and differentiate into luminal cells.
    - Prostatic epithelial cell turnover is slow in normal conditions; proliferation is very infrequent in normal luminal cells, occurring more frequently in normal-basal cells.
    - Most proliferation in normal epithelium is found in the basal cell compartment (e.g., 70% of cells expressing Ki67).
    - Putative multipotent basal progenitor cells are enriched near the proximal ducts/urethra.
  - Loss of basal cells is a hallmark feature of prostate adenocarcinoma.
  - Mature luminal cells carry out the differentiated functions of the prostate, including secretion of PSA and other components into the acinar lumens to contribute to the ejaculate fluid.
  - Luminal cells express high levels of "differentiation markers" including the androgen receptor (AR), prostate-specific antigen (PSA, encoded by KLK3) prostate-specific acid phosphatase, NKX3.1, HOXB13, keratins 8 and 18, and FOXA1.
  - Maintenance of this differentiated status requires androgens; castration results in decreased expression of androgen-regulated genes (e.g., NKX3.1 and KLK3/PSA), apoptosis of many luminal cells, an atrophic cuboidal appearance of the remaining luminal cells, and a prominence of the basal layer.

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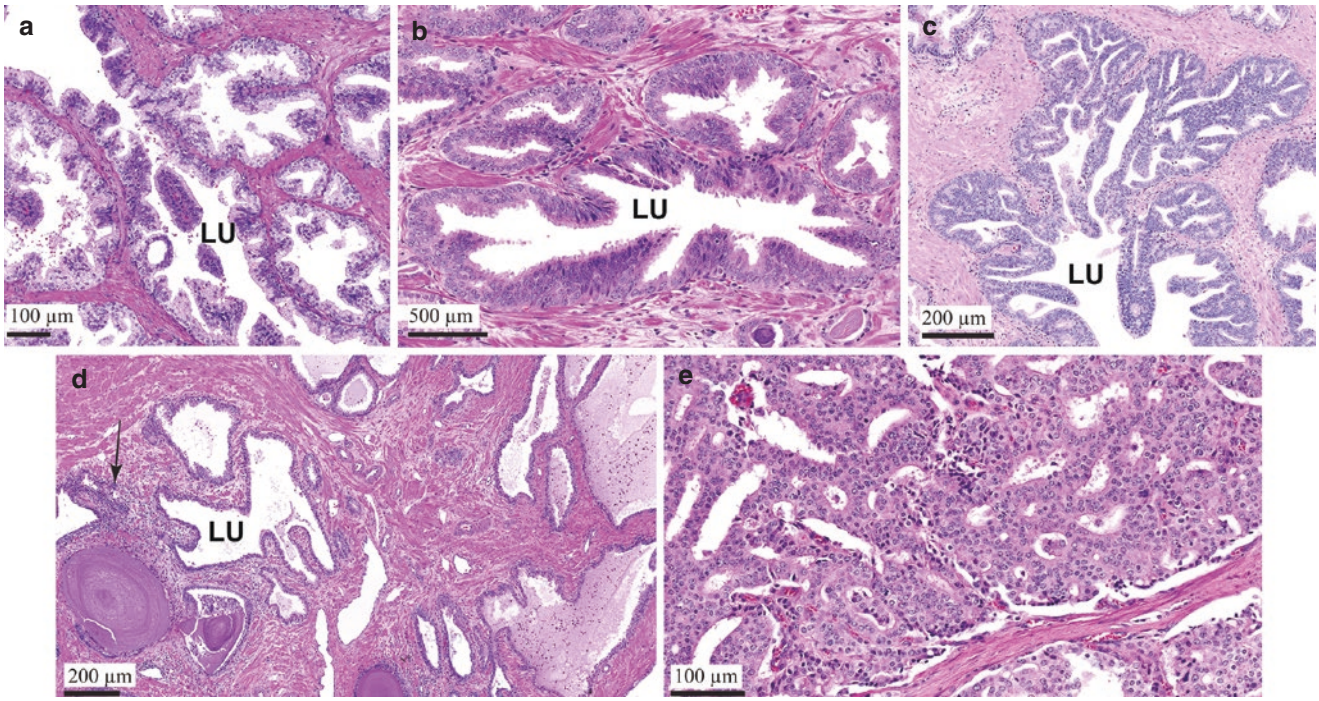


Fig. 14.1 Histopathology of normal, preneoplastic, intraductal, and invasive adenocarcinoma. (**a**) Normal appearing epithelium and stroma. (**b**) High-grade PIN. (**c**) Intraductal carcinoma. (**d**) Simple atrophy/PIA. (**e**) Invasive adenocarcinoma of the prostate, Gleason pattern 4, large cribriform

    - Atrophy occurring in luminal cells after androgen withdrawal or blockade is considered "hormonal" or diffuse atrophy.
  - The nature of the "true" long-lived stem cells in the prostate is somewhat controversial; most evidence suggests this activity is predominantly in the basal compartment with enrichment toward the urethra, but some studies indicate that both basal and luminal cells can each self-renew; and at times, give rise to both cell types.
  - In regions of focal atrophy (that are not associated with androgen withdrawal/blockade and often accompanied by chronic inflammation; referred to as proliferative inflammatory atrophy or PIA), there are variable numbers of "intermediate" luminal cells that have reduced yet variable levels of AR and differentiation markers, and many express keratins typical of both basal and luminal cells (e.g., keratin 5); they show a relatively high proliferative fraction and these cells appear to be efficient progenitor cells in stem-like cell assays.
  - Recent studies using single-cell RNA sequencing have shown cellular heterogeneity within the luminal populations:
    - One population is mainly secretory, and the other is secretory but contains more stem cell properties.
  - Neuroendocrine cells are very rare, encompassing <1% of all epithelial cells.
  - They express chromogranin A, synaptophysin, neuron-specific enolase, neural cell adhesion molecule, forkhead-box A2, and CXC chemokine receptor 2; they are negative for AR and PSA.
  - Primary prostatic adenocarcinomas (by far the most common histological type) nearly always (except for very rare primary tumors characterized by p63 nuclear positivity) have phenotypic features of luminal cells, suggesting the cell of origin for prostate cancer is a luminal cell.
- The prostatic stroma consists of abundant smooth muscle cells along with nerves (controlling smooth muscle function during ejaculation), blood vessels, indistinct fibroblasts, and scattered immune cells.

Epidemiology and Etiology of Prostate Cancer

- Incidence and Mortality.
  - Prostate cancer (prostatic adenocarcinoma) is the most common noncutaneous malignancy in men.
  - While low-grade prostate cancers (Gleason score 6 or grade group 1) may remain clinically indolent for many years, higher grade lesions may progress to lethal metastatic disease and death.
  - Prostate cancer is second only to lung cancer in cancer-related deaths in males, with 34,130 men estimated to die of this disease in the United States in 2021.
  - In the United States, the lifetime risk of a prostate cancer diagnosis is roughly 1 in 9, yet the risk of dying is roughly 1 in 41;

  - This indicates the need to determine which tumors are potentially aggressive and life threatening and which are not.
  - Globally, there are more than 1.2 million new cases and deaths exceed 350,000 annually.
  - The incidence of prostate cancer has been rising in a number of Asian countries.
- The major risk factors for the development and progression of prostate cancer include advancing age, family history/germline genetics, and race.
- The sharp increased risk with age results in particularly high levels of cases in regions with high life expectancy.
- The disease disproportionately impacts African American/Black men, with an approximately twofold higher incidence and mortality compared with non-Hispanic White men.
  - Recent results indicate that, despite the increased incidence and mortality, when controlling for grade and stage of disease, and access to high-quality care, the rate of progression to metastatic disease and death rate from prostate cancer in Black men is not different than in White men.
  - Recent studies also suggest improved outcomes after radiation therapy for Black men as compared with White men after treatment for localized disease.
- Environmental Factors
  - Environmental exposures are implicated in prostate cancer since men who emigrate from South East Asia to North American or Australia develop a higher risk of prostate cancer within 1 generation;
  - Dietary factors are believed to underlie these risks.
    - Diets rich in red meats and well done meats have been implicated.
  - The most well-recognized precursor to invasive prostate cancer is high-grade prostatic intraepithelial neoplasia (PIN).
  - Chronic inflammation may drive disease development through increased oxidative damage and sublethal and lethal injury to epithelial cells leading to regeneration and development of PIA (Fig. 14.2).
  - PIA lesions contain intermediate/progenitor liminal cells that may lead to PIN, and/or at times directly to early invasive carcinomas.
  - Recent evidence suggests that bacterial infection in association with chronic inflammation may at times drive the development of TMPRSS2:ERG gene fusions in PIA lesions.
  - Obesity and weight gain are associated with increased disease recurrence after primary treatment.

Clinical Features

- The widespread use of prostate-specific antigen (PSA) serum testing starting in the 1990s in the US revolutionized the early detection of prostate cancer, resulting in a

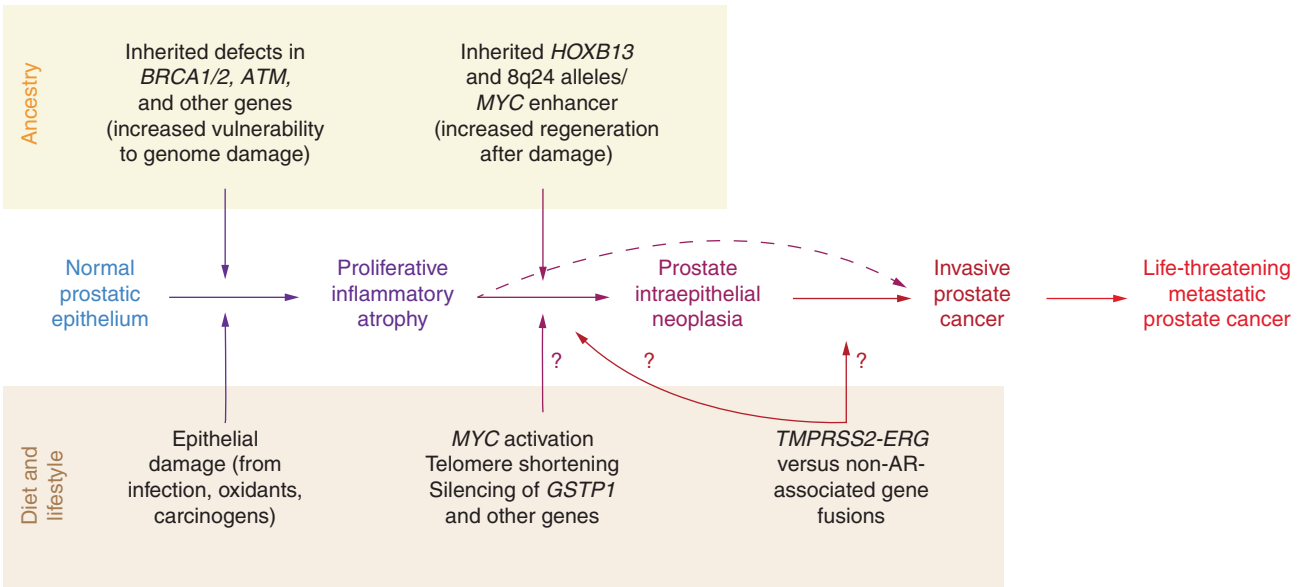


Fig. 14.2 Diet, lifestyle, and ancestry converge to produce proliferative inflammatory atrophy to drive the molecular pathogenesis of prostate cancer. Inherited vulnerability to cell and genome damage repair and response sensitizes prostate cells to infections, inflammation, and carcinogens, leading first to proliferative inflammatory atrophy and then to neoplastic transformation and malignant progression. Gene rearrangements could occur via AR-dependent mechanisms, like TMPRSS2-ERG, or non-AR-dependent mechanisms. (From Nelson 2022 J Clin Invest. with permission)

marked increase in detection, and a stage migration at diagnosis from predominantly metastatic to mostly clinically localized.
- The death rate for prostate cancer has been decreasing over the last few decades, and some of this decrease is likely attributable to PSA screening and early detection followed by radical treatment of primary cancers with surgery, and/or radiation therapy, the latter with or without combined androgen deprivation.
- Despite the success of PSA-driven early detection and treatment, PSA testing has also led to overtreatment of nonlife threatening disease (Grade Group 1 or GS6) in which men are subjected to potential serious side effects from radical treatment, yet do not stand to benefit because their disease would not progress to a symptomatic or life-threatening aggressive form in their lifetime.
- To decrease overtreatment, recommendations regarding screening changed (US Preventative Task Force 2012 grade D recommendation) such that there is now less PSA screening, especially in men over age 75, as well as an increasing use of active surveillance in men that are diagnosed with low grade (Grade Group 1) disease.
  - However, recent data indicate that while the incidence of localized prostate cancer decreased after recommendations to reduce PSA screening were implemented, there has been an increase in men presenting with metastatic disease, indicating a potentially clinically detrimental cost of such reduced screening.
- Advances in prostate imaging, especially multiparametric MRI, are improving diagnostic accuracy and increasing the safe use of active surveillance.
- Newer types of imaging are promising to improve this even further, including PET imaging for PSMA and using combined information from both mpMRI and PET-PSMA imaging.
- Clinically, the major known determinants of indolent versus aggressive disease and treatment decisions are largely based on the pathological grade from needle biopsies, as well as the serum PSA and clinical and pathological disease stage.
- For patients with intermediate risk disease, the clinical course is quite variable and not well predicted by Gleason grading and clinical staging.
  - A number of single biomarkers such as PTEN loss by IHC or FISH, and Ki67 Index, as well as a number of commercial RNA-based multiplex assays (e.g., Genomic Health Oncotype Dx, Myriad Prolaris, and GenomeDx Decipher) can provide additional prognostic information beyond typical clinico-pathological variables; however, none are used routinely or widely in standard clinical practice.
- For clinically localized prostate cancer, radical prostatectomy or radiation therapy (with or without combined androgen deprivation treatment) remains the mainstay of treatment.
- After primary treatment, combined histopathological and clinical features are often used in algorithms such as the Cancer of the Prostate Risk Assessment Post-Surgical score (CAPRA-S) as prognostic tools; studies are continuing to determine whether the addition of biomarkers, such as those indicated above, can add prognostic value beyond these features.
- Approximately 30% of men with intermediate or high-risk adenocarcinoma that are treated with curative intent experience disease recurrence, which generally starts out as biochemical recurrence (increased serum PSA).
- Many men with biochemical recurrence are treated with combined androgen deprivation therapy (> 99% of primary adenocarcinomas express high levels of the androgen receptor), and some of these recurrences become castration resistant even prior to metastatic disease development. Others, whether treated or not for biochemical recurrence, develop distant metastatic disease.
- The most frequent site of metastasis is bone, with lymph nodes and liver also being involved commonly. Other metastatic sites may include the lungs and adrenals.
- Patients with metastatic disease (or at times with local recurrence or biochemical recurrence) are treated with combined androgen deprivation therapy, which results in initial responses in nearly all men; however, nearly all men progress to castration resistant metastatic disease (many are subsequently treated with second and third line hormonal therapies that can provide benefit but are not curative).
  - Taxane-based chemotherapies are often used in this setting, but responses are generally not durable.
  - Immune checkpoint blockade treatments have generally been ineffective so far in prostate cancer, except in rare cases usually associated with tumors with mismatch repair defects and a high mutational burden.
  - Some men with homologous recombination repair defects, such as those caused by BRCA2 mutations, appear to benefit from PARP inhibitors.
  - An additional promising approach in the CRPC setting is the administration of intermittent high dose testosterone, which can paradoxically result in treatment responses in approximately 20–30% of men, even after several lines of prior hormonal or other therapies. As with other treatments in late-stage disease, resistance does develop over time.
- A small subset of men develop neuroendocrine carcinoma (with a spectrum including overt small cell neuroendocrine carcinoma (SCNC), very rare large cell neuroendocrine carcinoma, to very high grade poorly adenocarcinomas with prominent neuroendocrine features), or an otherwise androgen receptor reduced or neg-

ative disease; this can occur very rarely in a primary hormone naive state, or more commonly after a number of rounds of androgen deprivation therapy. Current estimates range from between 5 and 20% of late-stage cases. In almost all cases, these tumors appear to arise from lineage plasticity occurring in a preexisting clonally related concomitant adenocarcinoma. Mechanistic studies, along with molecular studies of clinical samples, suggests that combined complete inactivation of RB1 and TP53 mutations are key drivers of this transition. Since these tumors are highly resistant to all standard chemotherapies, the biological/molecular nature of these lesions is under intense study (see below).

Histopathology of Prostate Cancer

Precursor Lesions

- PIN (prostatic intraepithelial neoplasia) is defined as the presence of cells with morphological features of adenocarcinoma, often with cellular crowding and pseudostratification, present within preexisting ducts and acini (Fig. 14.1b). The diagnosis of high-grade PIN in almost all cases requires marked nucleolar enlargement in at least 10% of the cells.
- Low-grade PIN has similar features but lacks the pervasive nucleolar enlargement.
- Most early prostatic adenocarcinomas are likely derived from high-grade PIN (HGPIN), although some have been associated more directly with PIA without HGPIN.
- At times it may be difficult to distinguish high-grade PIN from intraductal spread of adenocarcinoma.

Intraductal Carcinoma (IDC-P)

- Intra-acinar/intraductal spread of prostatic adenocarcinoma (Fig. 14.1c) occurs frequently in cases from grade groups 3–5. In prostatectomies, it can be recognized by the expansion of preexisting ducts and acini by carcinoma cells, which often show a cribriform or solid pattern.
  - Intraductal carcinoma has strict diagnostic criteria when diagnosed on needle biopsy. However, this criteria likely results in an underestimate of actual intracinar/intraductal spread of preexisting adenocarcinoma into benign glands/acini, and efforts are underway to better distinguish HGPIN from intraductal carcinoma molecularly.
    - The presence of IDC-P is a prognostic marker that is often associated with higher grade cancer, higher cancer-specific mortality, as well as distant metastasis at initial presentation.
    - Loss of PTEN is also a promising, albeit not highly sensitive, biomarker to distinguish intraductal carcinoma vs. high-grade PIN, since it is common to lose PTEN in intraductal carcinoma, but not in HGPIN.

Acinar Adenocarcinoma

- Most prostate cancers are acinar adenocarcinomas that arise from the peripheral zone of the prostate, less commonly from the transition zone (the site of most benign prostatic hyperplasia) and very infrequently from the central zone (Fig. 14.3).
- Most cases are multifocal that often have proven to be clonally distinct; often, there is a dominant nodule that is also the highest grade lesion.
  - The increased sophistication of molecular diagnostic techniques has allowed for the molecular distinction of separately arising lesions within the prostate.
  - These findings have confirmed the tumor heterogeneity in prostate cancer even within the same patient.
  - ERG IHC is a useful marker for a more rapid assessment of multifocality in prostate cancer.
  - A recent study using a combination of multiple proteins and DNA markers identified interfocal molecular heterogeneity in ~60% of primary prostate tumor samples as well as ~10% collision tumors as evidenced as discordant ERG/SPINK1 status.
- Diagnosis
  - Criteria for invasive carcinoma include several features that together aid in the final diagnosis.
  - A characteristic hallmark in almost all carcinomas is that many of the tumor cells contain enlarged prominent nucleoli.
    - Tumor cells also frequently show hyperchromasia, and in almost all cases, nuclear enlargement.
    - In well differentiated carcinomas, atypical glands are often smaller than benign/normal glands, have straight liminal borders and infiltrate into the stroma between benign glands.
  - Diagnostically, specific features for carcinoma include perineural invasion, glomeruloid formations, mucinous fibroplasia, or seminal vesicle invasion.
  - In difficult cases, one can employ basal cell-specific staining to demonstrate the absence of basal cells (keratins 5/14 or p63; or a combination of AMACR, p63, and basal cell keratins referred to as a PIN4 stain).

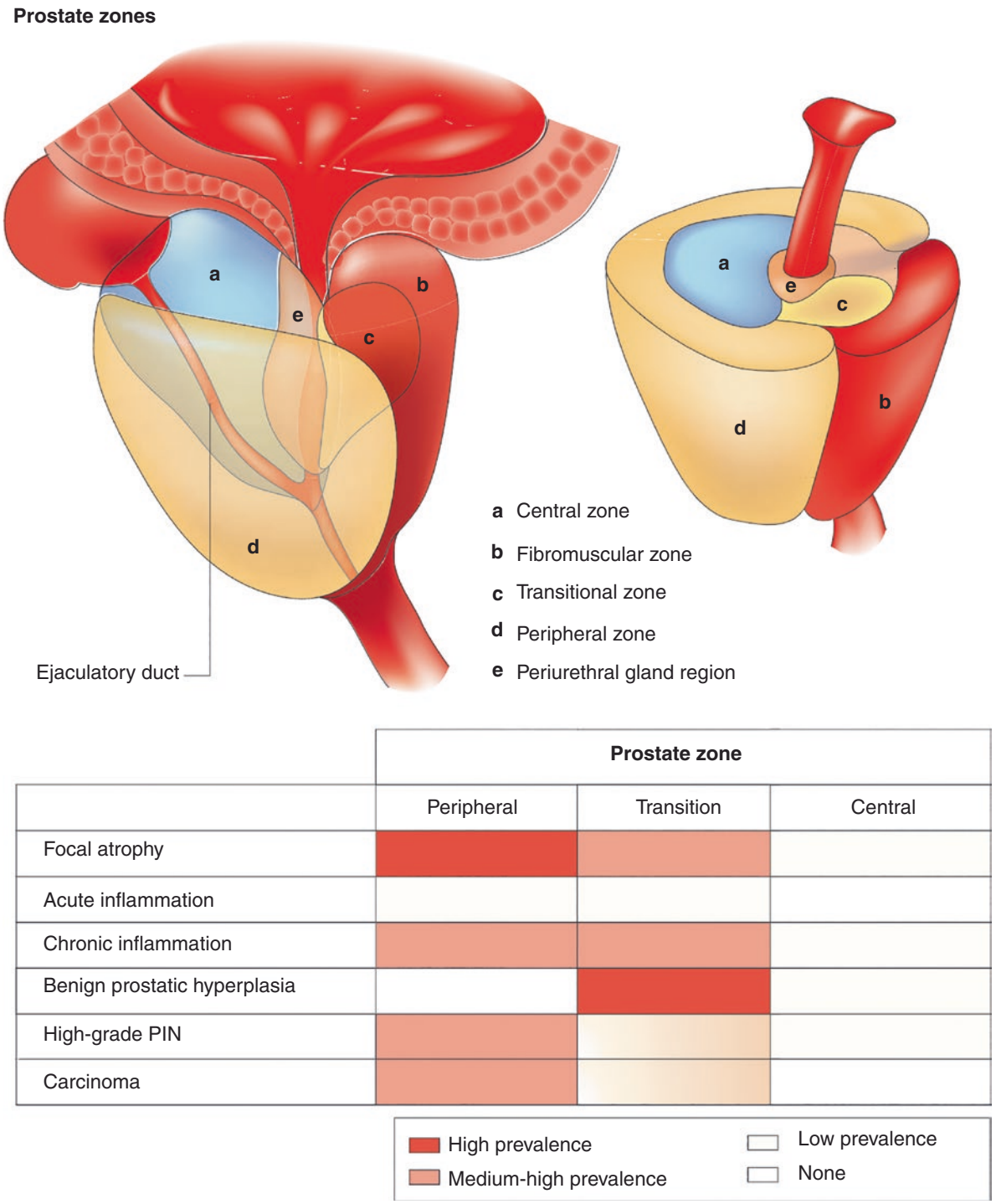


Fig. 14.3 Zonal predisposition to prostate disease. Most cancer lesions occur in the peripheral zone of the gland, fewer occur in the transition zone and almost none arise in the central zone. Most benign prostate hyperplasia (BPH) lesions develop in the transition zone, which might enlarge considerably beyond what is shown. The inflammation found in the transition zone is associated with BPH nodules and atrophy, and the latter is often present in and around the BPH nodules. Acute inflammation can be prominent in both the peripheral and transition zones, but is quite variable. The inflammation in the peripheral zone occurs in association with atrophy in most cases. Although carcinoma might involve the central zone, small carcinoma lesions are virtually never found here in isolation, strongly suggesting that prostatic intraepithelial neoplasia (PIN) lesions do not readily progress to carcinoma in this zone. Both small and large carcinomas in the peripheral zone are often found in association with high grade PIN, whereas carcinoma in the transition zone tends to be of lower grade and is more often associated with atypical adenomatous hyperplasia or adenosis, and less often associated with high grade PIN. The various patterns of prostate atrophy, some of which frequently merge directly with PIN and at times with small carcinoma lesions, are also much more prevalent in the peripheral zone, with fewer occurring in the transition zone and very few occurring in the central zone. Upper drawings are adapted from an image on Understanding Prostate Cancer website. PIN, prostatic intraepithelial neoplasia. (From De Marzo 2007 Nat Rev. Cancer, with permission)

  - There are a number of histological features, that if present as the sole finding on needle biopsy, can make it difficult to render a clear diagnosis. These include atrophic carcinoma, foamy gland carcinoma, pseudohyperplastic carcinoma, and mucinous carcinoma.
  - Very rare tumors express nuclear p63 diffusely. These distinct lesions have bland nuclei and so far have not been shown to be aggressive.
- Other histological patterns of acinar adenocarcinoma include atrophic glands, pseudohyperplastic adenocarcinoma, microcystic, and foamy gland carcinoma. The primary significance of these patterns are that each can be misconstrued as benign on needle biopsies, as they may mimic benign glands.
- Adenocarcinomas frequently show mucinous differentiation and at times may show prominent extracellular mucin.
- Carcinomas may also contain signet ring-like cells, which often, albeit not always, appear as Gleason pattern 5. These cells accumulate lipid and not mucin.

Ductal Adenocarcinomas

- Tumor cells are columnar with hyperchromasia, basally located nuclei, and a pseudostratified appearance.
- The glands may be cribriform, or show prominent papillary infoldings with fibrovascular cores.
- Most are found mixed with acinar adenocarcinomas (usually grade group 3 or higher), but in rare cases these may be present as a lone component at the prostatic urethra as distinct papillary lesions seen on cystoscopy and found by transurethral resection.
- There is generally no known histological or molecular distinction between prostatic ducts and acini, unless one observes a long duct radiating from the urethra outward. Thus, other than convention and the fact that at times they appear to arise near the urethra, there is not a strong biological basis for referring to these as ductal versus adenocarcinomas.
- These tumors tend to present with relatively low PSA levels for their volume and behave somewhat aggressively, often with visceral metastases.

Rare Subtypes

Neuroendocrine Carcinoma (NEPC)

- These come in two major types, those that are very well differentiated and traditionally considered carcinoid tumors and those that are poorly differentiated, which include a spectrum from SCNC to large cell neuroendocrine carcinoma (LCNC, much more rare).
- Carcinoid tumors of the prostate are very rare and will not be considered further.
- Most primary SCNC are mixed with acinar or other subtypes. While they may express androgen receptor (AR), it is usually at low levels and most show low or absent PSA expression, although other prostate restricted markers such as NKX3.1 may still be expressed; again, often at low levels.
- An evolving panel of neuroendocrine markers is being employed to better classify these lesions; newer markers include loss of YAP1, loss of cyclin D1, loss of RB1, and strong expression of FOXA2 and INSM1. Traditional markers such as chromogranin, synaptophysin, and CD56 may be positive, but not in all cases.
- Molecular studies have shown that NEPC can be driven to arise from adenocarcinoma cells by transdifferentiation/lineage plasticity after concomitant inactivation of both alleles of RB1 and TP53 with upregulation of SOX2 and EZH2.
- It is still possible that a small subset of NEPC may arise directly from prostatic basal cells and/or from pre-existing neuroendocrine cells in the tumor.
- Another recent study has found extensive reprogramming of the FOXA1 transcriptome in a series of NEPC xenografts that was required for maintenance of the NEPC phenotype.
- SCNC and LCNC are extremely aggressive lesions and most patients succumb to metastatic disease within a few years of diagnosis.

Other Histological Variants/Patterns of Differentiation

- Many primary acinar adenocarcinomas contain neuroendocrine cells (staining positive for chromogen and/or synaptophysin that are present in numbers from a scattered few to relatively frequent), but these tumors do not behave like SCNC and LCNC.
- Some poorly differentiated tumors with prominent Gleason pattern 5 sheet-like differentiation appear to be hybrids with parts of the tumor showing evidence of neuroendocrine differentiation with low/negative AR staining and signaling (e.g., PSA and/or NKX3.1 expression) and others showing retained strong AR staining and signaling.
- Some poorly differentiated carcinomas, along with SCNC, have been referred to as “anaplastic” or more recently, “aggressive variant” carcinomas, although these terms also relate to clinical behavior as very aggressive; and, they tend to be at least somewhat responsive to platinum based therapies.

- Other rare histological subtypes of prostatic carcinoma include sarcomatoid carcinoma, PIN-like ductal carcinoma, pleomorphic giant cell adenocarcinoma, and squamous carcinoma.

Grading of Adenocarcinoma

- Grading of adenocarcinoma of the prostate has been based on the Gleason system for several decades. Since 2005, several modifications have been made by the International Society of Urological Pathology (ISUP 2014) and more recently the Genitourinary Pathology Society (GUPS).
- The system is based on the fact that there are consistent glandular architectural patterns of invasive prostatic adenocarcinoma and that more than one pattern is often present in a given tumor lesion.
- Each pattern is given a numeric value, from 1 to 5, based on increasing levels of architectural distortion starting from glands that appear nearly benign, to those consisting of sheets of cells lacking acinar formation.
- Traditionally, to arrive at a Gleason score, one takes the most common pattern and adds it to the second most common pattern (e.g., 3 + 4 = 7).
- In needle biopsies, however, one now takes the most common and the highest grade.
- The adoption of grade groups (GGs) has occurred that start at GG1 (Gleason score of 6 in GUPS system) and end at GG5.
- In prostatectomies, the grade can include a tertiary pattern and if this is deemed greater than 5%, then it becomes incorporated as the secondary grade.
- More recent developments have added an estimation of the percentage of pattern 4 in Gleason 7 cancers and the presence of cribriform patterns (Fig. 14.1e), although precisely how to define this, and the ability to distinguish it from intraductal carcinoma, is still somewhat in flux.
- Currently, there are a few differences between the ISUP 2019 and GUPS systems. For example, the 2019 ISUP allows for some grade 3 + 4 = 7 lesions to be included as GG1, whereas the GUPS system does not.
  - Therefore, for precise communication with clinicians, pathologists should designate which system they are using when reporting grade groups.
- Clinical progression is uncommon in low-grade (e.g., Gleason 6 = GG1 and low volume GG2) cases, and many men now elect not to undergo immediate definitive treatment but instead opt for "active surveillance".
- Despite this, within the middle of the grade groupings, there is a wide variation in disease progression and additional tools and treatment approaches are needed.
  - Such tools are being developed and evaluated, including a number of molecular biomarkers.

Artificial Intelligence in Prostate Cancer Histopathology

- This field is moving rapidly and recent work indicates that AI-based systems can perform as well or better than expert genitourinary pathologists at diagnosing and grading prostate cancer on needle biopsies.
- Many additional studies are underway to determine precisely how AI-based technologies, using digitally scanned slides, will augment the ability of pathologists to diagnose, grade, and predict outcomes and response to treatments worldwide.

Molecular Features of Prostate Cancer

Germline Alterations

- While there is not a specific gene, such as *APC* for hereditary colorectal cancer, that when inherited in mutant form severely increases the risk of prostate cancer, family and twin studies implicate a strong hereditary contribution.
- Large-scale genome-wide association (GWAS) studies have implicated many loci and some genes and variants have consistent associations from multiple studies including:
  - *HOXB13* (17q21)
    - Encodes a homeobox transcription factor that is expressed in adult tissues in a prostate and distal GI tract-specific manner.
    - Germline mutations/variants associated with increased risk of prostate cancer are enriched in the conserved homeodomain that interacts with homeobox cofactor MEIS1.
    - The *HOXB13* G84E variant is higher in men of European ancestry among affected men and those diagnosed at a younger age or with a family history of prostate cancer.
      - May be associated with pseudo-hyperplastic features, less frequent ERG rearrangements, and more SPINK1 overexpression.
      - A recent study shows that wild-type HOXB13 binds to HDAC3, repressing lipogenic regulators, whereas HOXB13 G84E does not; this was reported to result in increased expression of key prostate cancer growth regulators including FASN (encoding fatty acid synthase).
    - *HOXB13* G132E is associated with increased risk in Japanese and Chinese men.

- An African variant (X285K, a stop-loss mutation resulting in a longer protein) is associated with early onset and increased disease aggressiveness.

- *MYC*
  - *MYC* is located on chromosome 8q24, a region that undergoes somatic copy number increases in aggressive prostate cancer.
  - Several inherited variants located on chromosome 8q24 near *MYC* have been associated with an increased risk for prostate cancer (approximately 15 independent risk variants).
    - The majority of these are more frequent in African American men than men with European ancestry.
      - One such rare variant (rs72725854 [A>G/T] (~6% frequency of the African ancestry specific "T" risk allele) is localized within a prostate cancer-specific enhancer region that can modulate expression of *MYC*, and several nearby long noncoding RNAs including *PCAT1* and *PVT1,* sensitizing then to androgen regulation.
- DNA repair genes: studies implicate germline mutations in DNA repair related genes that impart increased risk of overall and aggressive (higher grade) cancers.
  - Homologous recombination (HR) pathway for double-strand DNA repair: *BRCA2*, *BRCA1*, *ATM*, *CHEK2*, *PALB2* (and other repair genes).
    - A recent study estimating the prevalence of germline *BRCA2* mutations in the United Kingdom resulted in an estimated 8.6-fold increased risk of prostate cancer by age 65, which corresponds to an absolute risk of 15% by age 65.
    - Germline mutations in DNA repair genes including *BRCA1*, *BRCA2*, *ATM,* and *CHEK2* have been associated with more aggressive prostate cancer and worse outcomes.
      - In patients with biallelic inactivation of *BRCA2* in their cancers, approximately 50% inherited a mutated inactive allele.
        - *CHEK2*
          - *CHEK2* encodes a cell cycle checkpoint kinase that is activated by DNA damage and leads to either cell cycle arrest until the DNA is repaired, or apoptosis.
          - Germline mutations in *CHEK2* are associated with a higher risk of prostate cancer (found in 1–2% of cases).
            - One of the most common *CHEK2* mutations, c.1100delC, is enriched in lethal prostate cancer in European American patients compared to indolent prostate cancer or patients from other origins.
            - A recent study involving a small number of prostate cancer patients suggested frequent co-occurrence of germline *CHEK2* mutations and somatic *CDK12* mutations, indicative of a potential synergistic effect.
        - *PALB2*
          - Encodes a protein that links BRCA1 and BRCA2 during the HR process of DNA double-strand break repair.
          - Germline mutation prevalence is approximately 0.29% in a Polish population and was associated with more aggressive disease, lower 5-year survival, and a higher all-cause mortality rate.
    - Castration-resistant prostate cancer patients carrying germline homologous recombination defects showed better response to platinum treatment.
    - Prostate cancer patients carrying germline or somatic mutations in DNA repair genes, especially *BRCA2*, showed higher sensitivity to PARP (poly-ADP ribosylase) inhibitors (e.g., olaparib and rucaparib).
  - DNA mismatch repair genes (MMR).
    - *MLH1*, *MSH2*, *MSH6,* and *PMS2* – canonical genes.
    - Germline mutation frequency is approximately 1% in advanced prostate cancer and much less so in localized disease.
    - Several studies have reported potentially favorable responses to checkpoint blockade immunotherapy in prostate cancer patients with MMR-deficiency/MSI-H, potentially through a higher presence of tumor-infiltrating T cells recognizing neoepitopes in those tumors with a high mutational burden.
  - *AR*
    - Studies have shown that in populations with a higher incidence of prostate cancer (African Americans), *AR* may have shorter polymorphic polyglutamine repeats, which are associated with increased receptor activity.
    - This is in contrast to populations with a low incidence of prostate cancer (Asians) who have been reported to have longer polymorphic polyglutamine repeats.
    - This has led to speculation that the length of these repeats affects prostate cancer susceptibility—however, there is conflicting evidence for this.

  - *TP53*
    - A recent study found germline *TP53* mutations occur in ~0.6% of prostate cancer patients; and for Li-Fraumeni syndrome (LFS) patients with a germline *TP53* mutation, the incidence of prostate cancer is 25-fold higher compared to the general population.
    - Tumors harboring *TP53* germline mutations often present with higher grade and stage, with 2/3 of them also possessing a somatic second allele inactivation.
    - Mutational hotspots in these prostate tumors are different from the classical LFS *TP53* mutations.
- Clinical relevance on hereditary cancer genetic testing
  - Due to the high prevalence of hereditary genetic mutations in high grade and mCRPC, germline genetic testing is rapidly developing and helps to determine optimal disease management options for screening, active surveillance, and precision therapies.
  - Testing has progressed from single-gene to multigene panels.
    - Common testing options include genes in the DNA damage repair pathways, *TP53* and *HOXB13*.
  - Multiple organizations, including the National Comprehensive Cancer Network, have provided guidelines for germline testing criteria for prostate cancer.
    - Guidelines are consistent in recommending genetic testing to men with prostate cancer with any of the following characteristics: metastatic disease, high or very high risk (based on stage and Gleason pattern), Ashkenazi Jewish ancestry, or intraductal or cribriform histology, or family history of mutations in known related cancer-risk genes.
  - Sample types mainly include saliva, blood, cheek, and/or buccal swabs and sometimes skin punch biopsies.
    - Some institutions also use other methods such as immunohistochemistry to detect germline mutations including MMR for high-grade prostate cancer (GS9-10).

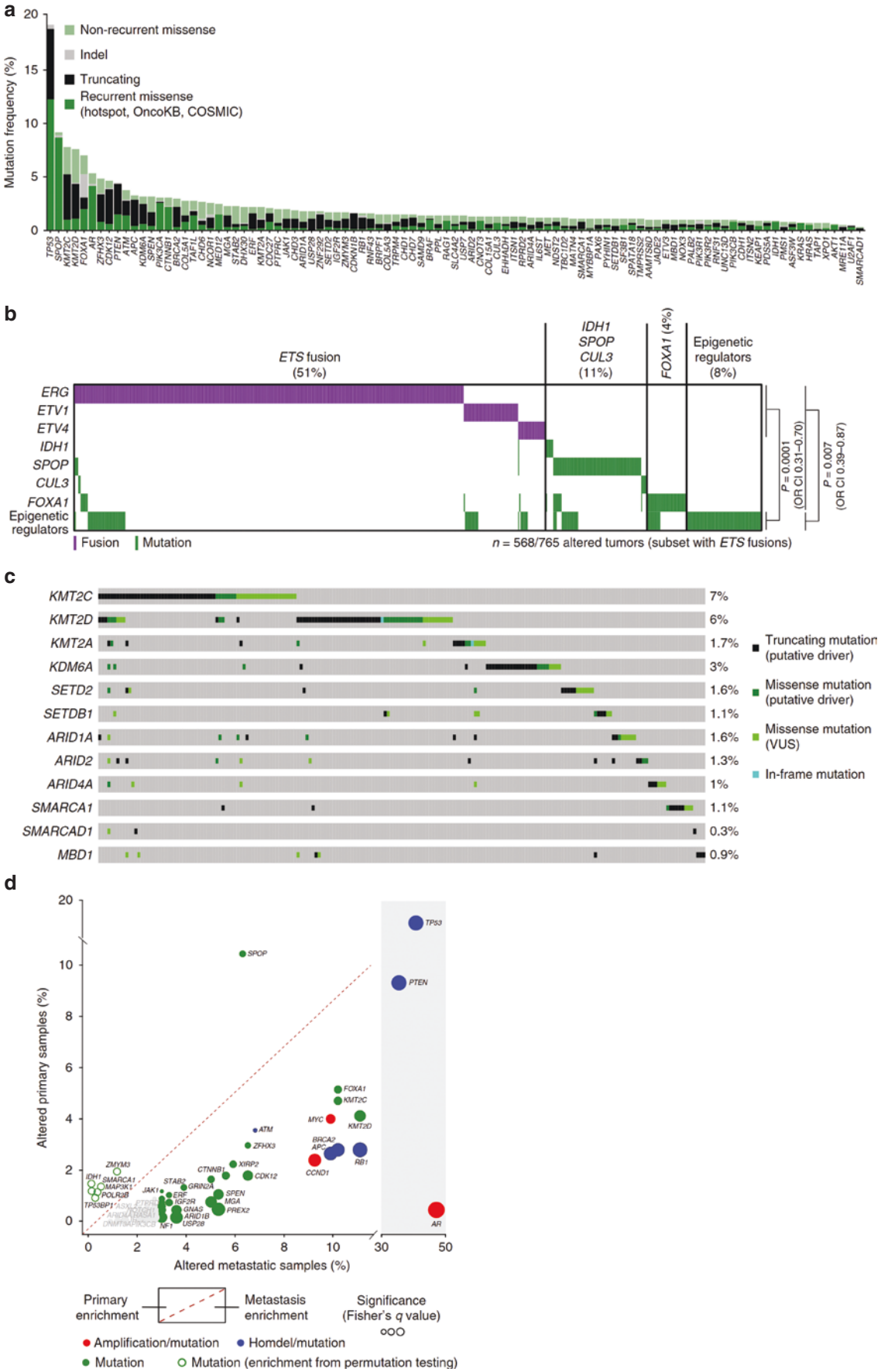
Somatic Genomic Alterations in Prostate Cancer

- As in other cancers, there is a stepwise acquisition of molecular alterations during the development and progression of prostate cancer.
- Whole genome and whole exome sequencing efforts revealed gene fusions to ETS family members, with *TMPRSS2–ERG* rearrangements as the most common somatic genomic alterations. A number of additional driver genes undergo recurrent point mutations (e.g., *SPOP*, *FOXA1*, *TP53*, *PTEN; KMT2C, KDM6A, CHD1, ATM,* etc.) (Fig. 14.4).
- There are frequent copy number alterations and complex genome rearrangements.
- The importance of epigenetic mechanisms in tumorigenesis is well established.

ETS Gene Fusions

- The ETS (E26 transformation-specific) gene family encodes a group of transcription factors that all share a conserved ETS domain responsible for DNA-binding activity.

Fig. 14.4 Mutational significance in 1013 prostate cancers and enrichment of genomic alterations in metastatic tumors. (**a**) Recurrently mutated genes ($n = 97$). Genes are ordered by frequency, and mutations are stratified by mutation type and, for missense mutations, by recurrence. Recurrence is defined via http://cancerhotspots.org/, http://oncokb.org/, and COSMIC; truncating mutations are defined as frameshift, nonsense, splice, and nonstop. (**b**) Mutations in epigenetic regulators and chromatin remodelers are significantly enriched in ETS-fusion-negative tumors. P values are calculated using a two-tailed Fisher's exact test and shown for ETS fusions as compared to all epigenetic mutations (including those co-occurring with SPOP and CUL3) and for ETS fusions as compared to non-overlapping mutations in epigenetic modifiers only. (**c**) Cohort-wide view of mutations in epigenetic regulators and chromatin remodelers, which affect 20% of samples. Samples are shown from left to right (only the 202 tumors with alterations are shown, out of 1013), and gene alterations are color-coded by mutation type and, for missense mutations, by assumed driver status; mutations are assumed to be drivers if they have been previously reported and entered into COSMIC or annotated in OncoKB or variants of unknown significance (VUS). (**d**) Most genomic alterations are enriched in metastatic disease. Alteration percentages in metastatic samples ($n = 333$) are shown on the x axis, and those in primary samples ($n = 680$) are shown on the y axis. The significance of enrichment (two-sided Fisher's test q value or weighted permutation test) is shown by the size of the dots. Genes in bold have a significant enrichment of mutations using Fisher's test and weighted permutation test correcting for mutation burden. (From Armenia 2018 Nat. Genet. with permission)


a
Non-recurrent missense
Indel
Truncating
Recurrent missense
(hotspot, OncoKB, COSMIC)
Mutation frequency (%)
b
ETS fusion
(51%)
IDH1
SPOP
CUL3
(11%)
FOXA1 (4%)
Epigenetic
regulators
(8%)
ERG
ETV1
ETV4
IDH1
SPOP
CUL3
FOXA1
Epigenetic
regulators
P = 0.0001
(OR CI 0.31–0.70)
P = 0.007
(OR CI 0.39–0.87)
Fusion
Mutation
n = 568/765 altered tumors (subset with ETS fusions)
c
KMT2C 7%
KMT2D 6%
KMT2A 1.7%
KDM6A 3%
SETD2 1.6%
SETDB1 1.1%
ARID1A 1.6%
ARID2 1.3%
ARID4A 1%
SMARCA1 1.1%
SMARCAD1 0.3%
MBD1 0.9%
Truncating mutation
(putative driver)
Missense mutation
(putative driver)
Missense mutation
(VUS)
In-frame mutation
d
Altered primary samples (%)
Altered metastatic samples (%)
TP53
PTEN
SPOP
FOXA1
KMT2C
KMT2D
MYC
ATM
ZFHX3
BRCA2
APC
CCND1
RB1
AR
CTNNB1
XIRP2
CDK12
SPEN
MGA
PREX2
GNAS
ARID1B
USP28
NF1
STAB2
GRIN2A
IGF2R
ERF
JAK1
IDH1
SMARCA1
MAP3K1
POLR2B
TP53BP1
ZMYM3
Primary
enrichment
Metastasis
enrichment
Significance
(Fisher's q value)
Amplification/mutation
Homdel/mutation
Mutation
Mutation (enrichment from permutation testing)

  - These proteins play an important role in normal development with distinct spatial-temporal-specific expression patterns.
- Five ETS genes, *ERG*, *ETV1*, *ETV4*, *ETV5,* and *FLI1*, have been identified to be rearranged in prostate cancer, leading to overexpression of transcripts with truncations at the 5′ ends.
  - *ERG* (21q22) is the most commonly rearranged member, ranging from 20 to 50% of both localized and metastatic prostate cancer.
  - Significant variation of the prevalence among men in different racial/ancestral and ethnic backgrounds is seen.
    - The prevalence is approximately 50% in White men of European ancestry, as low as 17% in Asians, and approximately 25% in Black men (African American and African Caribbean).
  - *ETV1* (7p21) is the second most common rearranged gene, found in up to 8–10% of all prostatic adenocarcinomas.
  - *ETV4* (17q21), *ETV5* (3q27), and *FLI1* (11q24) are rearranged in 1–5% of cases.
- The most common 5′ fusion partner (~85% of all cases harboring ETS rearrangements) is *TMPRSS2* (21q22), which encodes an androgen-inducible, prostate-restricted transmembrane serine protease.
  - Other less frequent 5′ fusion partners are diverse.
- In addition, some primary prostatic carcinomas overexpress full-length *ETV1*, *ETV4,* and *FLI1* without detectable gene rearrangement.
- For *TMPRSS2-ERG*, gene fusions occur through two predominant mechanisms: interstitial deletion on chromosome 21 or translocations without intervening genetic loss.
- The fusion of the gene leads to the androgen-mediated overexpression of the particular ETS transcription factor via the *TMPRSS2* regulatory region, which leads to incomplete cellular differentiation and modified AR transcriptional output, enhanced NOTCH signaling, as well as increased cellular migration and invasion.
- Very recent work suggests ERG expression may facilitate prostate carcinogenesis by blocking oncogene-induced senescence in prostatic luminal cells.
- The ETS gene fusions occur as very early events in the development of prostatic adenocarcinomas, either at the stage of PIN or right at the onset of invasion, although rare examples have been reported in low-grade PIN and PIA lesions.
- Diagnostic implications
  - *TMPRSS2–ERG* fusions (and hence ERG protein overexpression) have a >95% specificity for prostate cancer, or high-grade PIN.
    - Positive staining of ERG protein by immunohistochemistry can be useful as an aid to diagnosis in lesions suspicious for, but not diagnostic, of cancer by H&E alone (negative staining does not help in these cases).
- Prognostic implications.
  - While there is some evidence to indicate that the mechanism of gene fusion (i.e., deletion versus translocation) may relate to outcome, in general there is not an increased risk of aggressive disease in tumors that are ETS gene fusion positive.
  - Animal studies have shown synergy in disease progression in combination with PTEN loss, although in humans, tumors with PTEN loss that are ERG negative are associated with a higher rate of death due to prostate cancer than PTEN-negative and ERG-positive lesions.

Other Apparently Mutually Exclusive (with ETS Alterations) and Truncal Somatic Mutations

- *SPOP*
  - Located at chromosome 17q21
  - *SPOP* encodes the substrate-recognition component of a Cullin3-based E3-ubiquitin ligase.
  - Structurally, SPOP protein contains 3 domains, MATH, BTB, and BACK; the MATH domain is essential for substrate recognition, whereas the later two can interact with their counterparts in another SPOP protein and facilitate homodimerization, which is critical for the ubiquitin ligase function.
  - Upon ubiquitylation, many SPOP substrates are targeted to the 26S proteasome and degraded.
  - In terms of single point mutations, *SPOP* is the most commonly mutated gene in primary prostate cancer, occurring in ~10% of the cases, and less frequently mutated in metastatic disease (~5%).
    - This lower frequency in metastatic lesions may relate to the fact that SPOP-mutated tumors tend to be more responsive to hormonal therapies (see below).
  - SPOP mutations and ETS rearrangements are generally mutually exclusive in prostate cancer (Fig. 14.4), while gene deletions of *CHD1*, a chromatin remodeler, and SPOP paralogue SPOPL have been seen concurrently in SPOP mutant cancers.
    - Mutations in other genes in the ubiquitin-proteasome (USP) and ligase family also occur in both primary and metastatic prostate cancer, with a frequency of approximately 1–2%.
  - Point mutations in *SPOP* are always restricted to the substrate-binding cleft within the MATH domain. As a

result, heterodimers formed by wild-type SPOP and mutant SPOP can lead to unstable substrate recognition and thus less ubiquitination (removing the brake for degradation of oncogenic proteins via a dominant-negative effect). Another mutant (Q165P) impairs dimerization and substrate degradation.
    - Several oncogenic proteins in prostate tumorigenesis were found to be SPOP substrates, including AR and its co-activators TRIM24, SRC-3, and BET proteins, which leads to upregulation of AR signaling.
        - Since AR signaling is key in SPOP mutant prostatic carcinomas, patients carrying SPOP mutations tend to respond better to androgen deprivation therapies in various clinical settings, including neo-adjuvant treatments for primary tumors, CSPC and CRPC.
    - Wild-type SPOP is known to facilitate homologous recombination during double-stranded DNA break repair by promoting degradation of 53BP1 which induces NHEJ and inhibits HR. SPOP mutants, therefore, can induce HR defects and chromosomal instability as 53BP1 is no longer degraded. Since this is similar to loss of BRCA1, this may lead to increased sensitivity to PARP inhibition and radiation therapy.
    - PD-L1 has also been identified as a SPOP substrate, thus tumors with mutated SPOP showed elevated PD-L1, potentially making them more susceptible to PD-1/PD-L1 inhibitors.
- *FOXA1*
  - Located at chromosome 14q21.
  - Generally mutually exclusive to ETS gene rearrangements.
  - Encodes a transcriptional pioneer factor that induces an open chromatin conformation and subsequent recruitment of transcription factors such as AR.
  - Under physiological conditions, FOXA1 induces a prostatic luminal cell phenotype.
  - FOXA1 is overexpressed at the mRNA level in a stepwise manner going from normal epithelium to primary tumors and then to metastasis.
  - FOXA1 mutations occur in the protein coding regions in 10–13% of prostate cancers across all stages.
    - Many FOXA1 mutations, which are mainly missense and in-frame indels, occur in the forkhead (FKHD) DNA-binding domain and frequently reside within its wing 2 region that directly contacts DNA.
        - Wing 2-associated mutations in FOXA1 can lead to faster nuclear de-compacting activity and thus promote an oncogenic luminal AR transcription program.
    - The second most frequent mutations consist of frameshift truncations toward the C terminal regulatory domain; the resulting truncated protein is able to replace the wild-type protein and drive a WNT metastasis program and is enriched in mCPRC cases.
  - Other common FOXA1 alterations are structural variants that are mainly in the forms of tandem duplications and translocations without changing the protein coding sequence of FOXA1.
    - Both types of structural variants can lead to FOXA1 overexpression.
    - These are present in approximately 8% of primary cancers and enriched in up to 22% mCRPC cases.
    - Therefore, the overall cumulative frequency of genomic alterations in mCRPC is ~35% for FOXA1.
  - One recent study reported overexpression of FOXA1 at mRNA level in NEPC, although to a less extent compared to prostatic adenocarcinomas, and its importance in maintaining neuroendocrine features through its binding to relevant regulatory elements in the genome.
- *CDK12*
  - A tumor suppressor gene located on chromosome 17q12.
  - Encodes cyclin-dependent kinase 12, which heterodimerizes with cyclin K, functioning in DNA repair, splicing, and differentiation.
  - Recurrent deleterious CDK12 mutations occur in 2%–4% primary prostate cancers and in 4.7%–11% of mCRPCs; they can be monoallelic or biallelic.
  - Carcinomas with biallelic inactivation of CDK12 show a distinct form of genetic instability; while they are baseline diploid, there are numerous focal copy number gains representing tandem duplications, without high-level amplifications or widespread deletions (Fig. 14.5).
  - Cases may contain high neoantigen burdens from gene fusions from focal tandem duplications, imparting increased immunogenicity.
    - It is not clear yet whether these tumors consistently contain increased tumor-infiltrating lymphocytes, since results so far have been mixed.
  - CDK12 alterations are associated with a high Gleason score at diagnosis and worse survival.
  - A clinical trial conducted on mCRPC patients in a Chinese population reported a higher prevalence (15.4%) of CDK12 loss-of-function alterations than Western populations and an unfavorable response to abiraterone.

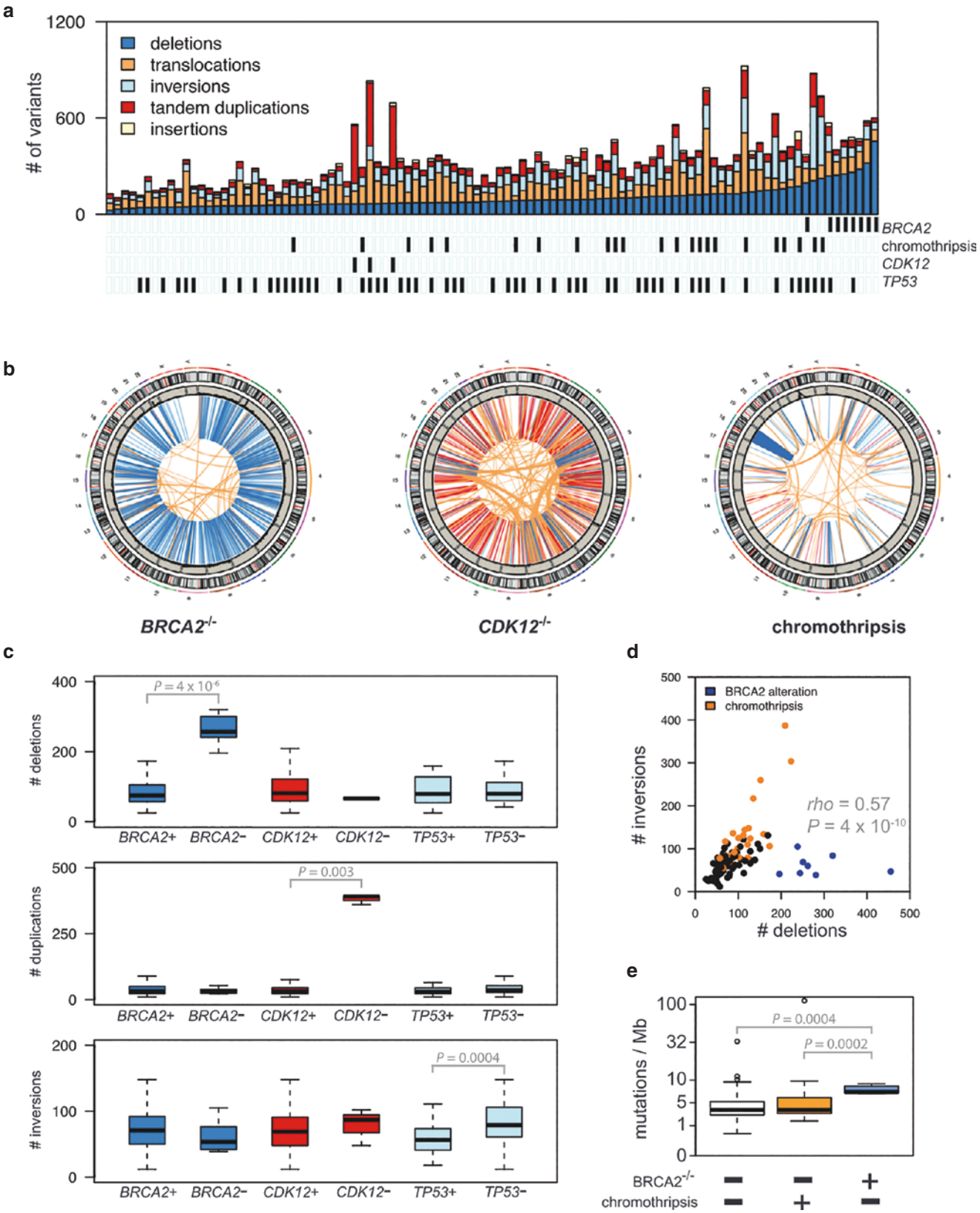


Fig. 14.5 DNA Repair Alterations Are Associated with Structural Variation Frequency (**a**) Top: structural variant frequency by sample, sorted by deletion frequency. Bottom: presence of chromothripsis or biallelic inactivating alterations in BRCA2, CDK12, or TP53. (**b**) Circos plots illustrating BRCA2 inactivation (left), CDK12 inactivation (center), and chromothripsis (right). Colors as in (**a**). (**c**) Box and whiskers plots showing association between biallelic inactivating alterations in BRCA2, CDK12, or TP53 and the frequencies of deletions, tandem duplications, and inverted rearrangements respectively. (**d**) Counts of inverted rearrangements and deletions per sample. Samples with biallelic BRCA2 loss drawn in blue, samples bearing chromothripsis drawn in orange. (**e**) Box and whisker plots showing mutation frequency in the presence of biallelic loss of BRCA2 and chromothripsis. (From Quigley 2018 Cell with permission)

- *IDH1*
  - Located on chromosome 2q34.
  - Encodes cytoplasmic isocitrate dehydrogenase 1 (IDH1) that catalyzes the decarboxylation of isocitrate to generate α-ketoglutarate (α-KG) while replenishing the NADPH pool using NADP(+).
  - Missense mutations of *IDH1* have been found in 1–2% of prostate cancers with almost all occurring in codon 132 (R132).
    - Such mutations confer a novel function of the IDH1 protein (neomorphic) that instead of generating α-ketoglutarate and NADPH, it converts isocitrate to d-2-hydroxyglutarate (2-HG) and consumes NADPH.
  - Prostate tumors harboring *IDH1* R132 mutations show high levels of genome-wide hypermethylation with numerous epigenetically silenced genes.
    - This occurs through 2-HG-mediated inhibition of α-KG-dependent DNA demethylases including the TET family proteins.
  - *IDH1* mutant prostate tumors usually do not possess other commonly observed oncogenic drivers such as ETS gene fusions and have been proposed as a distinct molecular subtype of prostate cancer; however, it is difficult to be sure of this since IDH1 mutations are so rare.

Other Genetic Alterations in Prostate Cancer

- Telomere shortening
  - Somatic telomere shortening occurs in most cases of high-grade PIN and adenocarcinoma.
  - Such shortening can lead to chromosome instability.
  - A prognostic biomarker has been proposed which consists of a combination of telomere shortening in stromal cells in the immediate tumor microenvironment and variability in telomere length in tumor cells.
- Tumor Suppressor Genes/Chromosomal deletions
  - Chromosome 8p
    - Deletions and loss of heterozygosity on the short arm of chromosome 8 (8p) are very common in prostate cancer.
    - The most well-studied gene in this area is *NKX3.1*.
      - *NKX3.1* codes for a prostate-restricted homeobox protein involved in developmental regulation and protection against oxidative damage from free radical effects.
      - Loss of *NKX3.1* generally involves one allele only (can be germline or somatic but is usually somatic).
      - Since its expression is maintained (albeit at somewhat reduced levels compared to normal luminal cells) in the vast majority of prostatic adenocarcinomas and is not seen in most other tumor types, NKX3.1 has proven useful as part of a panel of IHC stains, as a marker for prostate cancer, in cases in which very high-grade cancers are present at the bladder neck that are difficult distinguish between prostate and bladder cancer, as well as in metastatic lesions of unknown primary origin.
  - *PTEN*
    - A tumor suppressor gene located on 10q23.
    - PTEN is a lipid and protein phosphatase whose best-known function is to dephosphorylate PIP3, which counterbalances PI3 kinase—a protein involved in the PI3K–AKT-mTOR pathway important for cell growth, proliferation, and survival.
    - Inactivated biallelically in 20–50% of all prostate cancers, with higher rates in high grade and metastatic disease.
      - In the majority of cases, there are large deletions encompassing the *PTEN* locus, which are either homozygous or accompanied by a mutation in the other allele.
        - The significance of single copy loss is still unclear.
    - Loss of PTEN by FISH (chromosome 10q) or IHC is associated with higher Gleason score and advanced stage.
    - Loss of PTEN is associated with a poor prognosis, including an increased rate of biochemical recurrence, shorter time to metastasis, and decreased survival, the latter mostly occurring in ERG-negative cases.
    - If PTEN loss is detected in a lower grade tumor, the chances of there being a higher grade tumor present nearby is higher.
      - Some labs are employing IHC for PTEN (an excellent surrogate for genomic loss) in all GG1 cancers.
    - Loss of PTEN is often seen concurrently with ETS rearrangements.
    - PTEN loss has also been associated with an immunosuppressive tumor microenvironment in prostate cancer.
    - PTEN status as a predictive biomarker has also been examined:
      - Loss has been associated with less effective AR-targeted therapy, although its loss does not preclude such therapy.
      - In early-phase clinical trials, PTEN loss has been associated with response to AKT inhibitors.

- *CDKN1B*
  - *CDKN1B* encodes p27, a cyclin-dependent kinase inhibitor, which can show single copy genomic loss or at times mutations and/or biallelic inactivation; even without genetic alterations, there is commonly decreased p27 protein in PIN and adenocarcinoma lesions.
  - One mechanism by which p27 is also downregulated is by the PI3K–AKT signaling pathway.
  - Loss of p27 has been associated with a poor prognosis in prostate cancer in a number of studies.

- Other tumor suppressor genes
  - Deletions and/or mutations of tumor suppressor genes common to other cancers are also seen in prostate cancer.
    - *TP53* (mutations are present in approximately 5% of primary tumors but in upward of 50% of mCRPC).
      - Associated with elevated genomic inversion events in mCRPC.
      - Missense mutations in *TP53* often associated with p53 protein overexpression have demonstrated prognostic value including an association with biochemical recurrence and prostate-specific death in localized primary tumors.
    - Single copy loss of RB1 is common in primary tumors, but biallelic inactivation of *RB1* is more common in advanced disease; although loss of both RB1 alleles is infrequent except in SCNC, where it is present in >80% of cases.
  - DNA damage response pathways
    - Multiple studies have uncovered that mutations in DNA damage response (DDR) pathways are commonly observed in prostate cancer, both germline (discussed above) and somatically.
    - Overall mutations of DDR genes are present in 10–19% of primary localized prostate cancers and are further enriched (23–27%) in mCRPC.
    - When DNA damage occurs, the cell has a cascade of pathways to sense the damage, transduce the signal, and resolve the damage depending on the type of lesion:
      - When the DNA lesion is limited to one strand, including single-strand breaks (SSBs), intrastrand cross-links and base mismatches, the cell responds with base excision repair (BER), nucleotide excision repair (NER), and mismatch repair (MMR) pathways, respectively.
      - Double-strand breaks (DSBs) can be repaired mainly by two pathways, homologous recombination repair (HR) and nonhomologous end-joining (NHEJ).
        - Error-free HR uses sister chromatids as a template to repair the break, yet NHEJ is error-prone and repairs the DSBs by ligating the DNA ends.
      - *BRCA2*, *CDK12*, *ATM*, *FANCA*, *PALB2*, *RAD50*, and *BRCA1* are the most frequently mutated HR genes and *MSH2*, *MLH1*, *MSH6*, *PMS2* are the most frequently mutated MMR genes in prostate cancer.
    - *BRCA1/2*
      - *BRCA1* is located on chromosome 17q and *BRCA2* on 13q.
      - The encoded proteins BRCA1 and BRCA2 play an important role in DNA repair, specifically in HR for double-strand breaks.
      - Alterations in *BRCA1/2* are usually homozygous deletions or loss-of-function mutations:
        - *BRCA1* is altered in ~1% of prostate cancer in both localized and metastatic stages.
        - *BRCA2* gene alterations are found in 3% of primary prostate cancer and 5.3–13% of mCRPC, one of the most frequently altered DDR genes.
          - mCRPC tumors harboring biallelic *BRCA2* mutations show significantly higher genomic deletion events as well as tumor mutational burden.
      - Several clinical trials, including ongoing ones, have shown promising responses to PARP inhibitors (PARPi) in metastatic prostate cancer patients with *BRCA1/2* mutations (germline or somatic).
    - *ATM*
      - Located on chromosome 11q.
      - Encodes a kinase that senses DSBs and initiates DDR by phosphorylating various proteins in relevant pathways.
      - Inactivating mutations of ATM represent the second most frequently mutated DDR gene in both localized prostate cancer (4%) and mCRPC.
    - *PALB2*
      - Also involved in the Fanconi anemia pathway, if germline homozygously inactivated, leads to FA phenotype.
      - Somatically mutated or biallelically inactivated in ~2% of patients across all stages of prostate cancer.
    - Mismatch repair (MMR) pathway genes
      - The MMR system is responsible for repairing base–base mispairs and small insertions/deletions of DNA mainly occurring during DNA replication.
      - There are 8 genes encoding protein components of the MMR system, among which *MLH1*, *MSH2*, *MSH6*, and *PMS2* are most frequently mutated in prostate cancer.

- Overall mutation prevalence of MMR genes is less than 5% and is often associated with higher Gleason score and advanced disease at diagnosis.
  - Homozygous deletion and hypermutation are two common types of alterations of MMR genes in prostate cancer.
- A large portion of prostate tumors harboring MMR gene mutations demonstrate MMR protein(s) loss and/or microsatellite instability high (MSI-H) and a high tumor mutation burden.
- Several studies have reported potentially favorable responses to checkpoint blockade immunotherapy, such as pembrolizumab, in prostate cancer patients with MMR-deficiency/MSI-H, potentially through a higher presence of tumor-infiltrating T cells.

- Genes in the Fanconi anemia (FA) pathway
  - The Fanconi anemia DNA repair pathway is responsible for recognizing and resolving interstrand cross-links (ICL) of DNA during replication.
  - The FA pathway thus includes many genes in the HR pathway, such as *FANCD1/BRCA2*, *FANCN/PALB2,* and *FANCS/BRCA1* whose significance in prostate cancer is discussed elsewhere in this chapter.
  - *FANCA* is an FA/HR gene that is recurrently mutated in prostate cancer (in 3–8% of all cases), mainly in the form of missense mutations and homozygous deletions.
    - FANCA protein mainly interacts with BRCA1 during the HR process.
    - Recent studies reported that prostate cancer patients possessing biallelic *FANCA* loss showed response to PARPi.
- CDK12 is discussed elsewhere in this chapter.

- Hormonal Pathway Genes
  - *AR*
    - The androgen receptor (encoded by *AR*) is a ligand regulated (physiologically by testosterone and dihydrotestosterone) prostate master transcription factor.
    - Upon ligand binding, AR is translocated to the nucleus and binds to thousands of sites throughout the genome (these sites together constitute the AR "cistrome").
    - AR is highly expressed in normal prostatic luminal cells and is associated with prostatic epithelial and stromal morphogenesis and epithelial cellular differentiation. Binding to its main ligand, DHT, is required for luminal cell survival (for many luminal cells) and for proper differentiated function.
    - The protein product is expressed in most prostatic adenocarcinomas and its inhibition by castration, medical castration, or by AR antagonists is a key well-known treatment for locally advanced and metastatic prostate cancer.
    - AR "constitutive" activation is found in the majority of mCRPC—those cancers that no longer respond to castrate levels of testosterone and DHT in the circulation.
      - *AR* gene amplification, activating point mutations, and AR enhancer amplification are only seen to any degree in mCRPC. These alterations are accompanied by high levels of *AR* mRNA and protein expression (much higher than in primary tumors).
      - These alterations are thought to increase the sensitivity to very low androgen levels, which are derived from the adrenals and at times have been shown to be produced endogenously by the tumor.
      - These findings regarding AR support the concept of oncogene addiction in prostate cancer.
        - In this case the need for androgen signally for proliferation and prevention of cell death is inherent to prostatic cancer cells.
      - AR amplification, which is commonly seen in mCRPC, increases the sensitivity to lower levels of AR ligands in circulation.
        - Detection of amplified *AR* in circulating tumor cells (CTCs) or circulating tumor DNA (ctDNA) in clinical trials has been associated with treatment resistance to enzalutamide and abiraterone.
      - AR variants (AR-Vs)
        - AR-Vs are truncated AR proteins lacking the AR ligand-binding domain, potentially resulting from rearrangements in the *AR* gene and/or alternative splicing of the *AR* mRNA.
        - Without the ligand-binding domain, AR-Vs can be constitutively activated and drive the AR-dependent transcriptional programs even in the absence of ligands.
        - AR-V7 is one of the most well-studied AR-Vs that is rare in primary prostate cancer but is commonly seen in patients treated with hormonal deprivation therapies, especially in those with mCRPC.
        - Detection of AR variants, especially AR-V7, in mCRPC tumor samples or CTC from mCRPC patients has been associated with

treatment resistance to enzalutamide and abiraterone as well as favorable response to taxane-based therapies.

- *HSD3B1*
  - encodes 3β-hydroxysteroid dehydrogenase-1, an enzyme that catalyzes the initial rate-limiting step in converting dehydroepiandrosterone (DHEA) to testosterone (T) and dihydrotestosterone (DHT),
  - A germline variant or somatic mutation of this gene at nucleotide position 1245 from A to C can lead to resistance to protein degradation by the ubiquitin proteasome system.
    - HSD3B1 (1245C) is thus called an "adrenal permissive" allele as it increases potent AR ligand (T/DHT) production using adrenal androgen precursors.
      - The allele frequency of HSD3B1 (1245C) ranges from 8 to 34% depending on the ancestry, in that it is higher in men from Europe, and lower in men from Asia.
      - The 1245C allele is also selected for in patients undergoing androgen deprivation therapy, either through acquiring somatic mutations or loss of heterozygosity.
    - A few clinical studies have shown potential prognostic value for the 1245C allele after ADT as patients with low-volume prostate cancer carrying 1245C showed worse outcome and shorter survival.
    - The presence of the 1245C allele in mCRPC patients is also associated with poor outcome after they were treated with enzalutamide and abiraterone, suggesting potential predictive value.

- Oncogenes
  - *MYC*
    - *MYC* is located at 8q24 and in primary untreated tumors, low-level amplification is associated with high Gleason score, advanced stage, and disease progression.
    - Overexpression of MYC mRNA and protein, decoupled from 8q24 gain, arises as an early event in prostate cancer, including almost all PIN lesions.
    - Upwards of 80–90% of all prostate cancers overexpress MYC mRNA and protein.
      - MYC protein is also highly expressed in late-stage castration-resistant disease.
    - MYC overexpression results in a profound transcriptional reprogramming of prostate luminal epithelial cells characterized by the induction of genes related to nucleolar function, ribosome biogenesis, and cell proliferation.
      - MYC expression also reprograms the AR cistrome, blunting AR-induced gene expression at loci associated with classic AR signaling, which appears to occur by preventing pause release at AR target genes, but not by reducing AR binding to such regions.
        - Further, MYC overexpression increases AR binding at loci associated with FOXA1 occupancy.
    - The structural organization of the 8q24 locus has come into sharp focus recently. Epigenetic regulation of MYC mRNA overexpression has recently been tied to long- range interactions between distant enhancer regions and the MYC promoter. The region contains a number of noncoding RNAs that may be coexpressed with MYC including *PCAT1* and *PVT1*.
    - Upon androgen deprivation, MYC upregulation is often seen, which may contribute to development of castration resistance; conversely, supraphysiological levels of testosterone can lead to MYC downregulation and tumor regression in some patients.
      - One mechanism of such suppression appears to be that androgen disrupts the interaction between a super enhancer and the MYC promoter by redistributing and/or sequestering transcriptional coactivators between these two regions.
    - FISH for chromosome 8q24 amplification, encompassing the MYC locus, has shown prognostic value.
      - When present in combination with PTEN loss, MYC copy number gain is associated with higher Gleason score as well as prostate cancer-specific death.
  - *EZH2*
    - EZH2 is a histone lysine methyltransferase involved in chromatin remodeling as part of the PRC2 polycomb repressive complex.
    - It is overexpressed in all phases of prostate cancer including the precursor lesion, high grade PIN.
    - EZH2 promotes proliferation, invasion, and tumorigenicity of prostate cancer cells.
    - Upregulation of EZH2 in prostate cancer can result from:
      - Gene amplification.
      - By deletion of its negative regulator mir-101.
      - Transcriptional regulation by *ETS* gene family members.
      - Transcriptional regulation directly by MYC.
      - Downregulation of other negative regulators mir-26a and mir-26b, which are themselves negatively regulated by MYC.
      - Gain of function mutations.

    - Noncanonical functions of EZH2 have been identified recently including transcriptionally activating AR and posttranslationally methylating FOXA1 protein to improve protein stability and promote oncogenic phenotypes.
  - *SPINK1*
    - SPINK1 is a protein with a high homology to the epidermal growth factor receptor (EGFR) and has been found to be overexpressed in some prostate cancers, particularly in *ETS*-fusion negative cases.
    - Prostate cancers harboring SPINK1 overexpression have been associated with faster progression to biochemical recurrence and castration resistance.
    - Androgen deprivation therapy can induce SPINK1 upregulation.
- Genome/chromosome alterations
  - In general, the tumor mutational burden in primary prostatic adenocarcinomas tends to be quite low except in rare cases of mismatch repair deficiency.
    - mCRPC, however, does possess a higher tumor mutational burden compared to mCSPC and primary tumors (Fig. 14.4).
  - Copy number alterations, including gains and losses, are common in prostate cancer, although there appears to be a high fraction of grade group 1 cancers that are relatively "quiet" in this regard with few copy number changes.
    - The fraction of the genome altered can be prognostic.
  - Complex chromosome alterations
    - Chromoplexy
      - defined as complex genomic structure rearranged chromosome segments formed in a chain in an interdependent manner.
      - More frequently observed in ETS-rearragement positive tumors.
      - May account for loss of tumor suppressor genes and upregulation of known oncogenes.
      - Mechanistically may result from AR-induced double-stranded break and TOP2B-mediated chromatin reorganization.
    - Chromothripsis
      - Complex genomic structures formed by up to thousands of shattered chromosomal segments in a single catastrophic event; usually involve only one chromosome or one arm of a chromosome.
      - A recent report suggested ~50% prevalence of chromothripsis events in prostate cancer, which can contribute to oncogene amplification and loss of tumor-suppressor genes, similar to chromoplexy.
      - TP53 inactivation and polyploidy are two potential predisposing factors for chromothripsis.

Epigenetic Alterations in Prostate Cancer

- Three major epigenetic marks are found to be commonly altered in prostate cancer including histone acetylation, histone methylation, and DNA methylation.
- Each mark has its corresponding regulation machinery consisting of epigenetic writers, erasers, readers, preservers, and remodelers.
- Collectively as a group, epigenetic machinery genes are the most frequently mutated in prostate cancer, found in ~15–20% of all cases and mostly are potentially inactivating.
- Mutations in these epigenetic machinery genes are significantly associated with higher Gleason score at diagnosis, and are significantly enriched in tumors without ETS gene fusions or other known drivers.
- *SChLAP1*
  - Long-noncoding RNA *SChLAP1* has been found to be overexpressed in ~25% of prostate cancer and has shown an antagonistic effect on the function of chromatin-remodeling complex SWI/SNF by interfering with its genomic binding ability.
  - Overexpression of SChLAP1 in prostate cancer has been associated with higher Gleason score and pT stage, intraductal/cribriform histology, increased biochemical recurrence, metastasis, and prostate cancer-specific lethality.
- DNA methylation
  - DNA hypermethylation is one of the most consistent epigenetic alterations in prostate cancer.
  - Many DNA hypermethylation alterations are associated with higher grade and/or stage, disease recurrence, as well as lethal prostate cancer and NEPC.
  - CpG hypermethylation
    - Involves the methylation of deoxycytidine residues within CpG dinucleotides, usually in the upstream regulatory regions of specific genes and often leads to gene repression.
    - The most well understood gene affected in prostate cancer is *GSTP1*.
      - *GSTP1* encodes a protein that is part of a family of enzymes that counteract damage from reactive chemical species via a glutathione-mediated conjugation mechanism.
      - CpG hypermethylation results in silencing of the gene and increased sensitivity to genetic damage from oxidative stress.
      - This somatic genome alteration has been found in approximately 90–95% of all prostate cancers

and can be detected in blood, urine, and prostatic fluid.
    - CpG hypermethylation of *GSTP1* is present in ~70% of PIN and between 4 and 6% of prostate atrophy lesions but is not present in normal appearing prostatic epithelium, even in the microscopic vicinity of carcinoma.
    - Prostate cancers that retain GSTP1 expression are substantially enriched in African American patients, especially those with positive ERG expression.
  - Other genes known to be affected recurrently by CpG hypermethylation in prostate cancer include:
    - *APC*
    - *RASSF1a*
    - *ENDRB*
    - *PTGS2*
    - *MDR1*
  - There is a global reduction of 5-hydroxymethylcytosine in prostate cancer
    - 5-hydroxymethylcytosine (5hmC) is one of the major oxidized products of 5-methylcytosine, the most common DNA methylation mark.
    - This oxidative reaction is catalyzed by a family of TET proteins (10–11 translocation), TET1-3, that are mutated at times in prostate cancer.
    - The resulting 5hmC could be detected, excised, and repaired with nonmethylated cytosine through the base excision pathway, leading to DNA demethylation.
    - This reduced DNA demethylation may then contribute to the DNA hypermethylation commonly seen in prostate cancer, as well as in many normal stem cell compartments.
- Global DNA hypomethylation in repetitive elements is also observed in prostate cancer, usually at later stages of disease; the clinical significance of this type of epigenetic change is under active research.

- Histone Modifications
  - Histones, the "DNA packaging protein," can be subjected to a variety of post-translational modifications including methylation and acetylation.
  - Histone acetylation generally is associated with transcriptional activation while de-acetylation is correlated with transcriptional repression.
  - Histone methylation can be associated with either activation or repression.
  - H3K27me3 global reduction:
    - is highly correlated with the 5hmC amount in prostate cancer and is associated with the stem cell/progenitor cell phenotype.
    - occurs as early as in PIN and is continuously present in more advanced stages of prostate cancer; mechanistically linked to MYC overexpression.

Tumor Microenvironment in Prostate Cancer

- Growing evidence has suggested a pivotal role of the tumor microenvironment including the immune cell populations for prostate cancer initiation and progression.
- Prostate cancer is generally considered "immune cold" with low levels of inflammatory infiltrates in the tumors and often show only very limited responses to immune checkpoint blockade.
- Recent studies have offered some insights into the potential mechanisms leading to such an "immune desert model" for prostate cancer.
  - Prostate tumors usually possess a low tumor mutational burden, especially in primary lesions, unless they harbor mutations in MMR genes (discussed above), resulting in low mutation-related tumor-specific neoantigens that can be recognized as foreign by the immune system.
  - Prostate cancer cells generally express little-to-no PD-L1, one of the critical immune co-inhibitory checkpoint molecules, on their cell surface, suggesting that the lack of immune recognition is not from PD-L1 upregulation of tumor cells.
  - Alternatively, some studies indicated a repressed adaptive immune microenvironment for prostate cancer.
    - Cytotoxic CD8+ T cells in prostate tissues from prostate cancer patients often concurrently express PD-1, suggestive of an "exhaustion phenotype" of T cells.
    - The number of FOXP3+ regulatory T cells (Treg) is found to be increased somewhat in prostate cancer samples.
    - Innate immune cells, including mast cells in benign tissues and protumorigenic M2 macrophages, may contribute to prostate cancer progression.
    - MHC molecules (major histocompatibility complex) that facilitate immune system recognition by presenting foreign molecules including neoantigens on the cell surface are found to be downregulated in prostate cancer cells.
- The detailed immune landscape in the tumor microenvironment of prostate cancer across disease stages is still an active area of research with advances in multiplex phenotyping techniques, single cell transcriptomics, and spatial transcriptomics promising to markedly augment our knowledge in this area in the near future.

Prognostic Utility of Somatic Tissue-based Genetic Testing

- DNA Based Testing
  - Many patients with high grade and metastatic cancers are having tumor tissues tested for somatic DNA alter-

ations including mutations, gene fusions, and copy number alterations using panel-based testing.
  - Commercial examples of such tests include those from Foundation Medicine, Tempus, and Caris.
  - While none of these are employed as standard of care, increased use is occurring to determine whether patients may be candidates for PARP inhibitors (e.g., with mutations in genes involved in HR repair defects) or checkpoint in inhibitor therapies (e.g., MMR defects).
- RNA Based Testing
  - An emerging understanding of prostate cancer biology through microarray studies and RNA sequencing efforts has led to develop multiple tissue-based testing for prognosis and risk stratification.
  - These include Prolaris (Myriad Genetics), OncotypeDx Genomic Prostate Score, and Decipher (GenomeDx).
  - These tests use RT-PCR or microarrays to measure expression of a panel of genes in various pathways, mainly cell proliferation/cell cycle (Prolaris) but also androgen signaling, stromal response and cellular organization (GenomeDx).
    - OncotypeDx GPS was designed for needle biopsies while Prolaris Decipher has been used for both biopsies and radical prostatectomy samples.
  - These tests provide a score that shows prognostic values in terms of biochemical recurrence, metastasis, as well as prostate cancer-specific lethality. While none are used routinely in clinical practice, they show potential for molecular profiling to augment our ability to tailor patients for adjuvant therapies and for selection for specific treatments in clinical trials.

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