Molecular Pathology of Prostate Cancer

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KEYWORDS

• Prostate cancer • AR • ERG • PTEN • Intratumoral heterogeneity • Metastasis • Morphology

Key points

- Numerous profiling studies have delineated the molecular blue print of prostate cancer in the past years.
- Pertinent genomic alterations include recurrent rearrangements (in particular gene fusions involving erythroblast transformation-specific transcription factors) and copy number alterations (eg, copy number loss of *PTEN*, copy number gain of *AR*).
- DNA repair gene alterations are common and can be present as germline and somatic variants. These mutations have important prognostic and predictive implications.
- Primary prostate cancers are often multifocal and composed of independently arising tumor cell clones, which has major implications for diagnosis and treatment.
- Metastatic prostate cancer is a highly heterogeneous disease. Lineage plasticity characterized by the loss of prostatic lineage markers is a common feature of therapy-resistant prostate cancer.

ABSTRACT

Molecular profiling studies have shed new light on the complex biology of prostate cancer. Genomic studies have highlighted that structural rearrangements are among the most common recurrent alterations. In addition, both germline and somatic mutations in DNA repair genes are enriched in patients with advanced disease. Primary prostate cancer has long been known to be multifocal, but recent studies demonstrate that a large fraction of prostate cancer shows evidence of multiclonality, suggesting that genetically distinct, independently arising tumor clones coexist. Metastatic prostate cancer shows a high level of morphologic and molecular diversity, which is associated with resistance to systemic therapies. The resulting high level of intratumoral heterogeneity has important implications for diagnosis and poses major challenges for the implementation of molecular studies. Here we provide a concise review of the molecular pathology of prostate cancer, highlight clinically relevant alterations, and discuss opportunities for molecular testing.

OVERVIEW

Prostate cancer (PC) is the most common noncutaneous malignancy in men in the United States and makes up almost 20% of all newly diagnosed cancer cases.[1] The initial presentation and clinical course of PC can vary greatly between patients.

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The clinical spectrum ranges from indolent disease with an exceedingly low risk of progression to highly aggressive disease variants with early recurrence and high rates of cancer-related death.[2–4] Given this disease heterogeneity, understanding factors that predict the future clinical behavior of PC in an individual patient has been of the highest interest in the field. For decades, the assessment of histopathologic features such as Gleason grade and grade group tumor grade, tumor volume, and tumor stage have been the most pertinent prognostic parameters on which clinical decision-making is based. This factor strongly emphasizes the important relevance of the pathologist in the care of PC patients. Over the past years, molecular diagnostic applications have been penetrating more and more into the daily practice of genitourinary pathology. Many of these novel molecular tools have the potential to improve diagnostic accuracy and predictive values and ultimately lead to better clinical outcomes. In the multidisciplinary care for patients with PC, pathologists will play an essential role in bridging molecular studies and clinical decision-making.

In this review, we aim to provide a concise overview of relevant molecular alterations in PC and highlight opportunities for precision pathology in clinical practice, as well as delineate the challenges posed by the complex biology of PC.

PROSTATE CANCER ETIOLOGY AND GERMLINE ALTERATIONS

Although the etiologic factors that contribute to PC initiation remain a matter of intensive research, recent studies have highlighted the role of chronic unresolved inflammation, infection, and persistent epithelial cell injury as well as the exposure to dietary carcinogens, in particular heterocyclic amines in the pathogenesis of PC.[5–8] These exposure risk factors (which are to some extent modifiable) need to be evaluated in the context of germline genetic risk predisposition. Inherited genetic risk factors are an important determinant for PC development. Indeed, genome-wide association studies have revealed numerous genetic risk variants linked to PC.[9,10] Interestingly, some variants involve genes that regulate inflammation and pathogen response (eg, *RNASEL*, *MSR1*) highlighting the interplay between environment and host factors.[2] Although the individual contribution of these low penetrant risk alleles might be limited, models combining multiple risk loci can identify individuals with more than 5-fold increased risk for developing PC.[11] Importantly, a recent study demonstrated that certain germline risk alleles determine somatic epigenome alterations in PC suggesting that germline alterations can influence a plethora of somatic changes.[12] In addition, several highly penetrant germline variants, in particular in *HOXB13* (G48E) and *BRCA2* were identified.[13,14] Although these alterations are relatively uncommon, they are associated with a substantial (5- to 7-fold) increased risk for developing PC.[14,15] For current clinical practice, it is relevant to highlight the association of germline mutations as they relate to familial tumor syndromes. In particular, germline alterations in *BRCA1* and *BRCA2* in addition to genes associated with Lynch Syndrome can be found in PC patients which warrants germline genetic testing and genetic counseling of family members in a subset of PC patients (discussed elsewhere in this article).[13]

PRECURSORS LESIONS

Although the definition of the cell of origin of PC is a matter of ongoing debate in the literature, almost all primary PCs show features of prostatic luminal epithelial cell differentiation, which is characterized by the expression of the androgen receptor (AR) and AR target genes such as prostate-specific antigen (PSA). Collectively, these findings suggest that a luminal cell phenotype is the dominant cellular differentiation in primary PC.[16–18] Precursor lesions of PC have been studied extensively over that past decades.[19–21] Currently, the most widely accepted PC precursor lesion is high-grade prostatic intraepithelial neoplasia (HG-PIN), which is characterized by cytologically atypical cells confined to preexisting ducts and acini by intact basal cells.[19,20] HG-PIN often shares molecular alterations with adjacent invasive carcinoma, which was originally used to define HG-PIN as a precursor lesion.[21–23] However, more recent in-depth genomic studies of this putative precursor lesion have suggested that at least a subset of lesions with morphologic features of HG-PIN are in fact invasive carcinoma cells, retrogradely colonizing preexisting ductal and acinar spaces.[21,24–26] These lesions appear morphologically as HG-PIN, but are on the molecular level consistent with invasive carcinoma.[24] This observation has important implications for screening and provides some explanation for the association between the extent of HG-PIN and the risk for subsequent cancer detection on repeat biopsies.[21,27]

THE MULTIFOCAL AND MULTICLONAL NATURE OF PROSTATE CANCER

It is well-established that primary PCs often show several distinct tumor nodules.[28–30] Indeed, multifocal tumor lesions can be found in up to 80% of radical prostatectomy specimens.[31–34] Individual

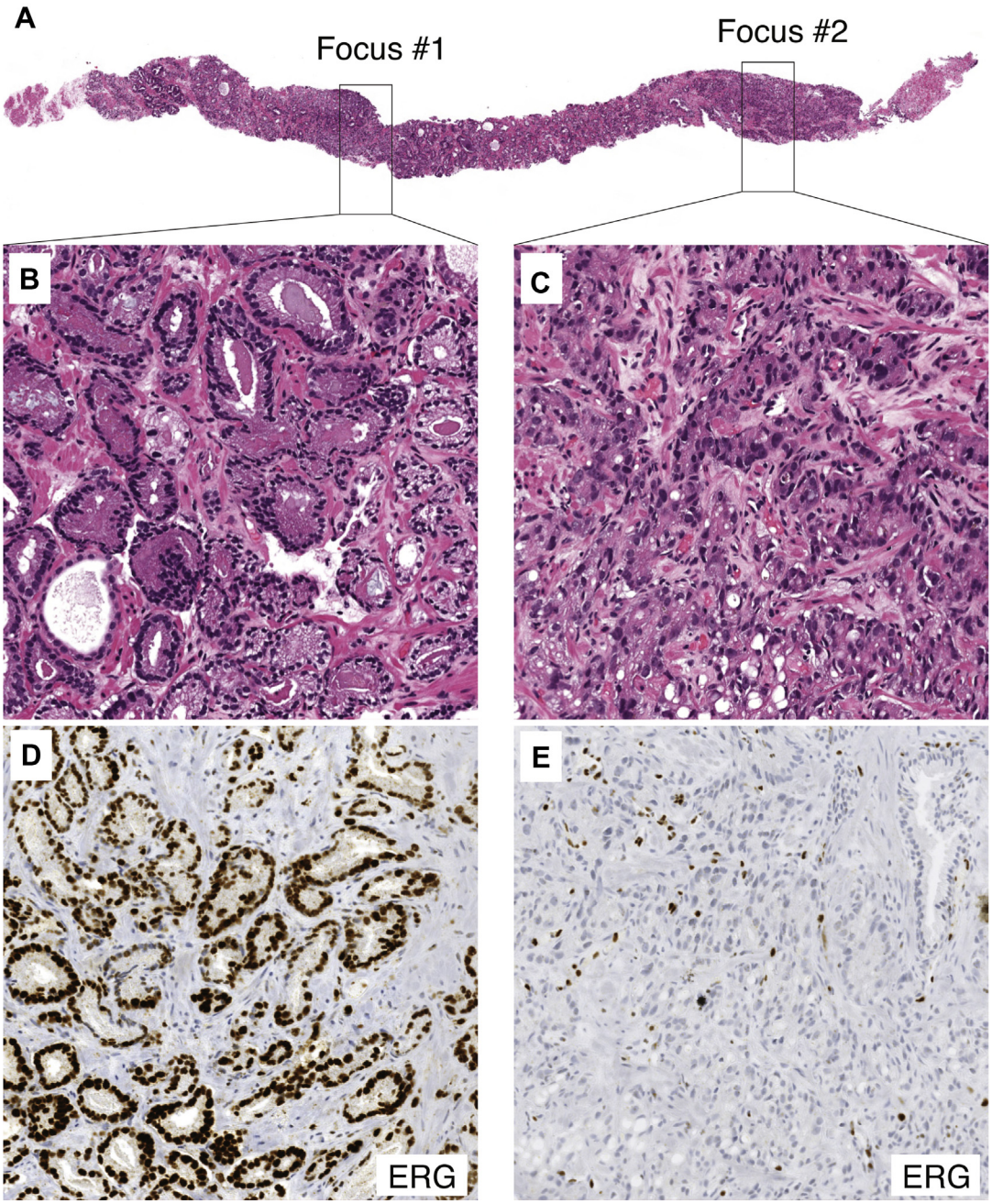

Fig. 1. Intratumoral heterogeneity in primary PC. Primary PCs are often multifocal and multiclonal. This is illustrated by a needle core biopsy [original magnification 20x] (*A*), which shows 2 noncontiguous tumor foci. Notably, the 2 tumor foci differ in tumor grade (focus 1, Gleason score 3 + 3 = 6 [original magnification 20x] [*B*]; focus 2, Gleason score 4 + 4 = 8 (original magnification 20x) [*C*]) and ERG rearrangement status assessed by ERG IHC (focus 1, ERG positive [original magnification 20x] [*D*], focus 2, negative (original magnification 20x) [*E*]), strongly suggesting that these 2 lesions are of independent clonal origin.

tumor foci can be separated spatially and show distinct morphologic features.[32] More recent genomic studies have shown that up to 70% of cases of multifocal PC consists of genomically distinct tumors with nonoverlapping mutation profiles.[29,35–38] This finding suggests, that a prostate gland can harbor multiple separate tumors that likely arise independently and show

distinct molecular alterations and biological behavior.[29,35,38,39] The observation that primary PCs can be composed of distinct tumor clones sets PC apart from most other solid tumors. The resulting high level of spatiogenomic heterogeneity represents a major challenge for primary PC diagnosis. As shown in **Fig. 1**, a given prostate core biopsy can sample 2 tumor foci, with distinct morphologies (see **Fig. 1**). In situ assays, such as immunohistochemical staining for ERG, which can be used to infer the clonal relationship demonstrate that in this biopsy core 2 genomically distinct tumors were represented. This scenario is not uncommon. In fact, a recent study showed that around 25% of biopsies with noncontiguous core involvement tumors sample 2 separate tumor clones.[40] This finding illustrates the complexity of multifocality and multiclonality in PC core biopsies. It is therefore important to consider this high level of intratumoral heterogeneity when selecting biopsy samples for molecular analysis. Using a targeted sequencing approach, the heterogeneity of genomic alterations in biopsy samples and matched radical prostatectomy samples was studied recently.[41] Of a total of 22 genomically distinct tumor lesions, only 10 were represented on diagnostic biopsy samples.[41] More broadly, this finding also implies that systematic needle biopsies are probably insufficient to detect all relevant tumor clones and subclones.[39,42] This finding is particularly relevant for clinical practice, where the primary tumor sample information is often used to make decisions about actionable alterations in distant metastases.[43]

In addition, these observations challenge the assumptions of both standard template biopsy, as well as image-guided targeted biopsies and call into question the concept of a "dominant lesion," which is defined solely by size or histologic criteria, being largely responsible for a patient's clinical course. This finding is supported by studies showing that certain genomic and molecular features, rather than size or histology alone, can identify tumor foci that are more likely to contribute to disease progression.[35,38,39,44] For instance, we reported a case several years ago, in which we were able to demonstrate that the lethal metastatic cell clone in a patient who died of PC originated from a small well differentiated (Gleason pattern 3) lesion in the primary tumor. Importantly, this small low-grade lesion that showed evidence for molecular alterations that are tightly linked to aggressive disease, such as genomic alterations in *PTEN* and *TP53*, was associated with a bulky clonally distinct and higher grade tumor that did not contribute to the lethal tumor burden.[38] The study of intratumor heterogeneity in PC can be technically challenging and although the literature on intratumor heterogeneity in PC has expanded dramatically over the past years, future studies are needed to more directly address the clinical challenges that arise from the multiclonal nature of PC.

CLINICALLY RELEVANT MOLECULAR ALTERATIONS

Recent large-scale profiling studies have laid out a blueprint of the genomic and transcriptomic landscape of PC.[45–50] Although the overall point mutation rate in PC is relatively low, copy number alterations and structural rearrangements are very common and often involve driver gene changes. We have summarized several key pathways that are frequently altered in PC and contribute to tumor progression and therapy resistance.

GENOMIC ALTERATIONS

Androgen Receptor

The vast majority of PCs crucially depend on AR signaling.[51] The AR is a nuclear hormone receptor that is required for maintaining prostatic differentiation, but is subverted in PC to fuel cancer growth.[52] Although initial and often profound responses to therapies that lower testosterone levels or interfere with AR signaling are common, most PCs become refractory to these interventions and progress to castration-resistant PC (CRPC). Importantly, despite AR pathway inhibition, tumor cells maintain aberrant AR activity through a number of genomic and nongenomic mechanisms. These include high-level copy number gains of the *AR* gene itself or its distant enhancer and gain-of-function mutations in AR.[48,52,53] Collectively, more than 60% of metastatic CRPC (mCRPC) cases show evidence for genomic *AR* alterations.[48,52,53] In addition, the *AR* gene locus can give rise to constitutively active *AR* splice variants.[54] Importantly, *AR* genomic alterations and splice variants can be detected in blood-based assays from cell free DNA and circulating tumor cells.[54–58] Their presence has been associated with resistance to first- and second-line hormonal therapies and is currently explored as a predictive biomarker for advanced PC.[54,55] In addition to alterations in the *AR* gene itself, several other genes involved in AR signaling including *FOXA1*, *MED12*, *ZBTB16*, *NCOR1*, and *NCOR2* harbor genomic alterations in PC.[48] Because these AR alterations are almost exclusively present in treatment-refractory metastatic disease, there is currently limited experience with tissue-based assessment.[59] However, with

increasing frequency of biopsies from mCRPC, investigation of the AR signaling axis will likely become important for clinical management in the future (discussed elsewhere in this article).

Erythroblast Transformation-Specific Transcription Factors

The most common recurrent genomic alterations in PC are rearrangements involving erythroblast transformation-specific transcription factors, which include ERG, ETV1, ETV4, ETV5, and FLI1. In up to 80% of cases erythroblast transformation-specific genes become juxtaposed to androgen-regulated genes through genomic rearrangements resulting in their overexpression in PC.[60,61] By far the most common rearrangement comprises the 5′ end of the androgen regulated of *TMPRSS2* fused to *ERG*. *TMPRSS2-ERG* rearrangements seem to be an early event in PC development and functionally contribute to cell invasion and transcriptional reprogramming.[62,63] This scenario suggests that structural alterations likely represent key driver events in PC. Importantly, a large fraction of these structural alterations shows a complex chain architecture involving numerous genome fragments often encompassing multiple driver genes. Such complex chained rearrangements (also termed "chromoplexy") are unlikely to arise from sequential independent events but rather suggest a coordinated underlying mechanism. This finding supports a model of punctate evolution in which a large number of driver alterations are generated in a small number of events rather than gradual accumulation over time.[64] There is a growing body of literature suggesting that transcriptional processes rather than DNA replication may represent important events resulting in genomic instability in PC.[65,66] For instance, it was noted that androgen signaling can induce DNA double-strand breaks, in a process that involves class 2 topoisomerase activity.[67] Such androgen-induced breaks can seed rearrangements (eg, recurrent rearrangement between *TMPRSS2* and *ERG*) and are likely also involved in general genomic instability in PC.[65,66] Indeed, several studies have highlighted that sites of genomic rearrangements in PC are enriched for AR-binding site and numerous complex rearrangements involve at least one androgen-regulated gene.[64,68,69]

The prognostic relevance of ERG rearrangements has been analyzed in numerous studies, but ERG as single marker did not show any robust association with disease outcomes or aggressive PC phenotypes.[70–72] ERG expression, however, can serve in certain settings as a helpful marker to determine the presence of PC and as shown elsewhere in this article (see **Fig. 1**) is extremely valuable for assessing clonality.

DNA Repair

As noted elsewhere in this article, despite the relatively low mutation rate, copy number and structural variants are very common in PC, suggesting potential alterations in pathways involved in DNA repair.[46,48,64,73] Genes encoding for proteins involved in both single- and double-strand break sensing and repair have been found to harbor both somatic and germline alterations in men with PC. Sequencing studies over the past years have demonstrated that key DNA repair genes including *BRCA2*, *ATM*, *CHEK2*, *BRCA1*, and *ATR* show somatic inactivating mutations, which are enriched in advanced metastatic PC.[13,15,74,75] Collectively, around 20% of all metastatic PC cases show alterations in DNA damage-response (DDR) genes. Of particular interest is that a large fraction of these alterations is present already in the germline DNA with cumulatively around 10% of men with advanced PC harboring germline mutation in *BRCA2*, *ATM*, and *BRCA1*.[13] Importantly, men with germline alterations in these genes are more likely to show disease progression and adverse outcomes.[13,15,75–77] Given the importance for patient management as well as the implications for cancer risk in other family members, genetic testing for germline DDR gene alterations followed by appropriate genetic counseling should be considered in men with localized disease with a Gleason score of 8 or greater (grade group $\geq$4) and/or a PSA of 20 or greater and any patient with metastatic disease.[59] Testing for DNA repair gene alterations also provides important predictive information. Tumors with certain DDR gene defects show a high sensitivity to poly (ADP-ribose) polymerase inhibitors and platinum compounds (such as cisplatin).[78] Several clinical trials have shown high response rates in men with DDR defects to the poly (ADP-ribose) polymerase inhibitors olaparib and rucaparib, which prompted the recent US Food and Drug Administration approval of these drugs for mCRPC.[79,80] Although responses seem to be robust in cases with *BRCA2* mutations, it is unclear if this finding can be extrapolated to other DDR gene alterations.[81–83] More broadly, targeting DNA repair defects will likely become an important therapy option for advanced PC. With an increasing number of highly specific inhibitors to key DNA repair proteins, appropriate patient selection and the development of robust companion diagnostic tests will be extremely important.[84] In

addition to homologous DNA repair defects, alterations in mismatch repair (MMR) genes (including MSH2, MSH6, MLH1 and PMS2), which results in microsatellite instability have been observed in primary PC (<3%) and mCRPC (approximately 10%). In localized PC, MMR alterations are associated with higher Gleason grade and are enriched for cases with ductal morphology.[85,86] Germline alterations of MMR genes are less common than other homologous repair gene alterations but should be considered owing to their association with the Lynch syndrome. The rationale that MMR generates hundreds to thousands of somatic mutations that encode potential neoantigens led the US Food and Drug Administration to approve pembrolizumab for all tumor types with MMR alterations.[87] Although MMR-deficient PC cases might be rare, their identification is therapeutically meaningful because they can show durable responses to anti-programmed cell death and programmed cell death ligand 1 therapies.[88]

Cell Cycle

P53 is a stress-induced transcription factor that regulates cell cycle arrest, senescence, and apoptosis among many other cellular pathway.[89] *TP53* is mutated in approximately 10% of primary PCs, but shows a strong enrichment in mCRPC, with up to 50% of metastatic PCs harboring *TP53* alterations. *TP53* mutations seem to be early truncal events in PC and are associated with high-grade disease and adverse clinical outcomes.[38,44,90] However, it is important to note that not all *TP53* mutations lead to a loss of tumor suppressive function. Rather, some *TP53* mutations can result in a specific functional gain that contributes to tumor progression.[91] In PC, there is a near even split between classical loss-of-function mutations (homozygous deletions, truncating mutations) and missense mutations, which can potentially result in context-dependent gain- or loss-of-function and dominant negative phenotypes.[48,91,92] Although the clinical relevance of different *TP53* alterations is unclear, ongoing preclinical studies and early clinical trials are currently testing novel approaches to restore p53 function or specifically target *TP53* mutant cancers.[93]

RB1 is an important tumor suppressor gene involved in cell cycle regulation, but also controls independent protumorigenic transcriptional programs.[94,95] Although RB1 alterations are relatively rare in primary PC (1%), *RB1* loss (most commonly through copy number alterations) is enriched in advanced CRPC (9%).[96,97] In fact, a recent large-scale genomics study showed that RB1 alterations have the strongest association with poor outcomes in metastatic PC.[88] Notably, concomitant *RB1* and *TP53* loss is a common feature of small cell neuroendocrine PC (NEPC). Whereas in murine PC models deletion of *Rb1* and *Tp53* results in high-grade carcinoma with neuroendocrine differentiation,[98] these two genomic alterations by themselves seem to be insufficient to induce a small cell phenotype in human PC.[99,100]

PI3K/PTEN

Alterations in the tumor suppressor gene *PTEN* occur in up to 20% of localized prostate and 40% of mCRPC. Most cases with *PTEN* loss harbor copy number changes, resulting in a complete absence of PTEN protein expression.[101–104] Therefore, *PTEN* status can be interrogated robustly using genetically validated immunohistochemical approaches.[102–104] Several studies have highlighted the prognostic potential of PTEN immunohistochemistry in large retrospective studies, demonstrating that PTEN loss is associated with increased rates of biochemical recurrence and shorter survival in contemporary biopsy and radical prostatectomy cohorts.[103,105,106] Furthermore, there is evidence that PTEN loss in lower grade tumors is associated with the presence of adjacent higher grade lesions.[107] In addition to its role as a prognostic marker, PTEN loss can potentially also serve as a predictive biomarker for therapies targeting the PI3K-AKT axis.[108]

Wnt

Wnt comprises a group of signaling pathways involved in cell proliferation, cellular homeostasis, stem cell renewal and cell migration.[109,110] Wnt signaling pathways have been shown to harbor genomic alterations in 10% to 20% of cases with advanced PC, with an enrichment in activating mutations in *CTNNB1* and *RSPO2* and inactivating mutations in *APC*, *RNF43*, and *ZNRF3*.[48] These alterations are associated with adverse histopathologic findings and earlier clinical progression. Importantly, the wnt pathway has been mechanistically implicated in regulating AR signaling and tumors with wnt pathway activating mutations show decreased responsiveness to second-generation hormonal therapies abiraterone and enzalutamide.[111,112] In addition, nongenomic alterations of wnt signaling, in particular increased expression of wnt signaling intermediates such as WNT5A and DKK1 are present in a large fraction of mCRPC.[113,114] These findings open up new opportunities for cotargeting of wnt together with other signaling pathways in PC.

EPIGENETIC ALTERATIONS

In addition to genomic changes, epigenetic alterations are increasingly recognized as important driver alterations in PC.[115,116] The spectrum of epigenome modifications is broad and encompasses all potentially heritable changes that alter gene expression.[115,116] Likely owing to the availability of robust analytical tools, DNA methylation changes have been extensively explored in PC over the past decade.[115–118] The methylation of cytosine in the context of CpG dinucleotides is an important regulator of gene transcription and defines cellular identity. Alterations in DNA methylation patterns are universal in PC and occur early during PC initiation.[115] Although DNA methylation changes are potentially reversible, several studies have documented that cancer specific DNA methylation marks are highly recurrent (eg, hypermethylation of *GSTP1* can be found in up to 90% of PCs) and remarkably stable and maintained throughout disease progression.[116,119] These findings suggest that DNA methylation changes could serve as valuable biomarkers in PC. Indeed, numerous studies have evaluated methylation-based biomarkers for PC detection in the blood and urine,[119–122] and a commercial test (ConfirmMDx, MDxHealth, Irvine, CA) is available for assessing the risk of cancer detection on repeat biopsies.[123] Because PC is characterized by a paucity of recurrent genomic alterations, DNA methylation changes could be used as attractive cancer-specific analytes for the early detection of PC, as well as disease monitoring in the metastatic setting.[55,124,125] Although most of these assays are currently in early stages of clinical development and validation, there will likely be a wave of innovative DNA methylation-based biomarkers with diverse clinical applications in the near future.

TRANSCRIPTOMIC ALTERATIONS

Extensive expression profiling efforts in PC have highlighted transcriptional alterations that can provide relevant prognostic information. Several expression signatures have been turned into commercial "genomic classifiers" (ie, Polaris, OncotypeDx, Decipher) that provide prognostic value in addition to existing clinicopathologic data (reviewed in[126,127]). Although these assays have been validated extensively in retrospective cohorts, prospective studies are currently missing and the clinical settings in which these assays are most impactful has yet to be defined.[126,128] In addition, other expression signatures, such as the PAM50 classifier, which was originally developed to subtype breast cancers into luminal and basal types, has been suggested to provide prognostic as well as predictive information for response to hormonal therapy in PC.[129] This intriguing finding suggests that certain transcriptional changes seem to be associated with more aggressive disease phenotypes, irrespective of the tissue of origin.[130] Although most expression analyses focus on annotated protein coding genes, more recent studies have investigated long noncoding RNAs, which do not contain protein coding sequences, but can have important structural functions.[131] In PC, several long noncoding RNAs with potentially useful biomarker properties have been identified (NEAT1, PCA3, PCAT-1, and SChLAP1).[132–137] The detection of PCA3 in the urine can, for instance, improve the performance of cancer detection in PSA screening cohorts.[136] SCHLAP1 expression, in contrast, hand has been described as a robust prognostic marker and high SChLAP1 levels are associated with increased rates of recurrence and mortality.[132,133] Interestingly, SCHLAP1 expression has been associated with more aggressive morphologic variants of PC including intraductal and cribriform morphologies.[138]

ASSESSING METASTATIC PROSTATE CANCER

HISTOMORPHOLOGIC FEATURES OF METASTATIC PROSTATE CANCER

Over the past decades, the vast majority of PC pathology was limited to the assessment of prostate biopsies and prostatectomy specimens. However, with improved methodologies for obtaining metastatic PC biopsies from both soft tissue and bone and the increasing importance of the assessment of molecular alterations in metastases for patient management, it is likely that the number of metastatic PC specimens seen by surgical pathologists will greatly increase in the coming years. With the evolution of novel therapies for PC, the spectrum of morphologies of metastatic PC has greatly changed. Our group has recently studied the morphologic features of metastatic PC obtained in a large rapid autopsy cohort and found a broad spectrum of variant histologies (**Fig. 2**). This encompasses common PC morphologies such as acinar and cribriform architectures, but also poorly differentiated and anaplastic carcinomas. In addition, squamous features and neuroendocrine differentiation (carcinoid-like pattern and small cell carcinoma) are present in a large number of mCRPC (see **Fig. 2**). These diverse patterns found in mCRPC, which often show minimal to no resemblance to primary PC, highlight the difficulties in assessing PC in the metastatic setting.

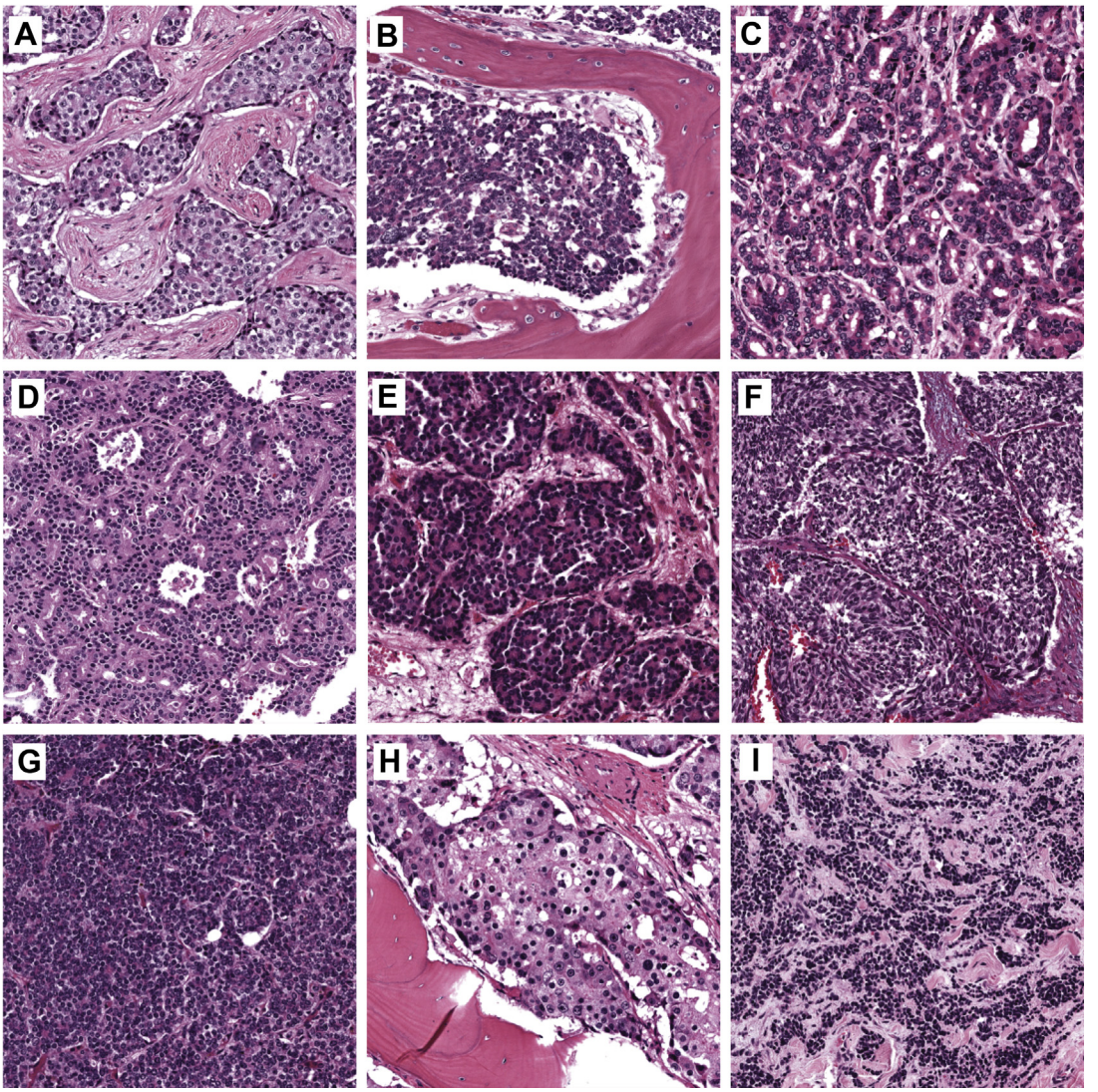

Fig. 2. The broad morphologic spectrum of metastatic PC. Histomorphologically, mCRPC can range from high grade carcinoma with squamous features [original magnification 20x] (*A*), high-grade carcinoma with pleomorphic giant cells [original magnification 20x] (*B*), adenocarcinoma [original magnification 20x] (*C*), adenocarcinoma with cribriform architecture [original magnification 20x] (*D*), carcinoid-like tumors [original magnification 20x] (*E*), high-grade neuroendocrine tumors with spindle cell morphology [original magnification 20x] (*F*), poorly differentiated carcinoma not otherwise specified [original magnification 20x] (*G*, *H*), and small cell neuroendocrine carcinoma [original magnification 20x] (*I*).

Although in the majority of cases AR expression will still be maintained and AR-regulated lineage markers such as PSA, PSAP, and NKX3.1 are positive, recent studies have shown that a substantial number of PCs loose AR expression and are subsequently negative for PSA and NKX3.1 (discussed elsewhere in this article).[139] HOXB13 has been suggested as a robust marker for prostatic differentiation in the metastatic setting[140]; however, similar to other prostate lineage markers, HOXB13 expression can be lost in tumors with neuroendocrine differentiation.

LINEAGE PLASTICITY

The widespread clinical use of highly potent AR-targeting therapies, including enzalutamide and abiraterone, has greatly shaped the phenotypic landscape of metastatic PC.[125,139,141,142] Increasingly recognized in clinical practice is a subset of

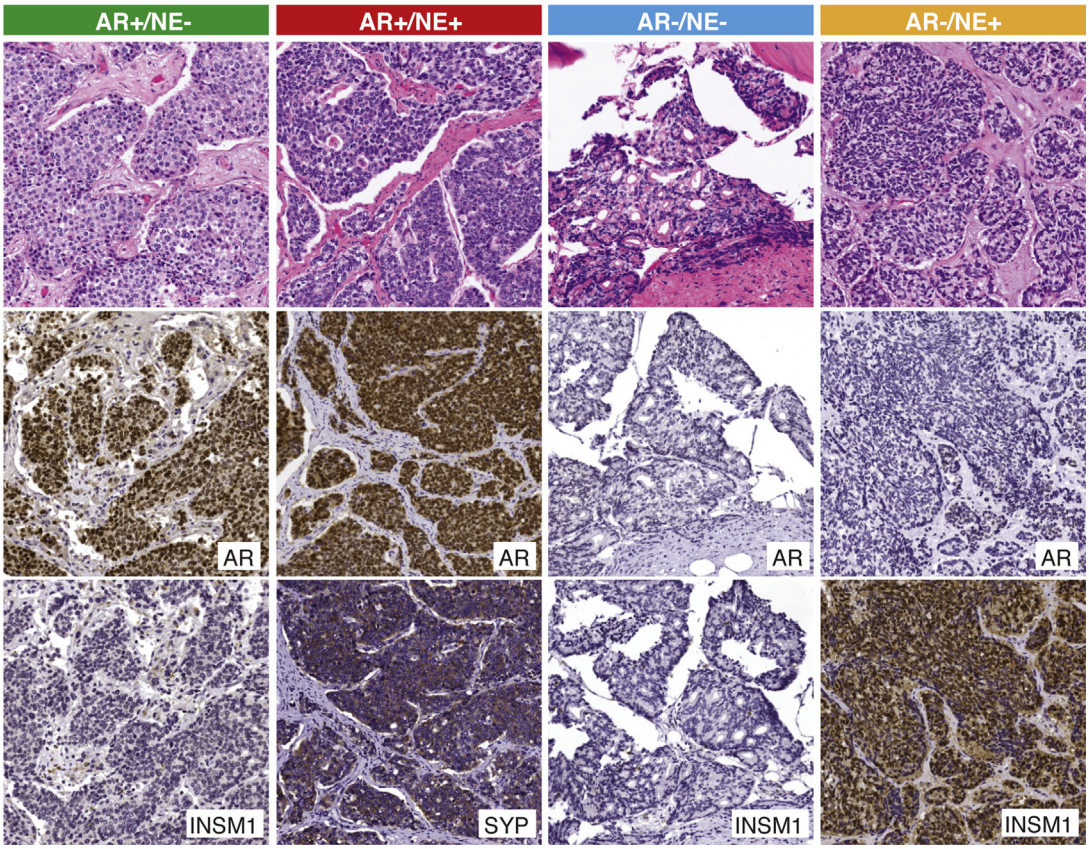

Fig. 3. Molecular subtypes of metastatic PC. Based on the expression of AR and AR target genes as well as neuroendocrine markers (NE; eg, INSM1; SYP, synaptophysin) metastatic treatment refractory PCs can be subdivided into 4 molecular subtypes characterized by AR expression in the absence of NE marker expression, expression of AR and NE markers (in particular SYP), absence of AR and NE markers (double-negative PC [DNPC]) and neuroendocrine PC (NEPC) characterized by absence of AR but strong NE marker expression.

patients with advanced CRPC who show evolution to a rapidly progressing disease that is refractory to hormone therapy and exhibits a visceral dissemination pattern.[143,144] Such tumors often show loss of AR and gain of neuroendocrine marker expression. Morphologically, this clinically aggressive variant of PC can show features of poorly differentiated carcinoma or even small cell morphology[143] (**Fig. 3**). Although de novo small cell carcinoma of the prostate is exceedingly rare,[145] therapy-related NEPCs are present in up to 20% of patients undergoing contemporary AR targeted therapies.[144,146,147] Recent preclinical and clinical evidence suggest that these tumors likely arise through trans-differentiation of a preexisting adenocarcinoma as an adaptive resistance mechanism to AR targeted therapies.[143,145,147–149] The accurate distinction between high-grade adenocarcinoma and NEPC is of clinical importance since the management for these entities differs; whereas NEPC is mostly resistant to conventional androgen deprivation therapies, these tumors show sensitivity to platinum-based chemotherapies or other targeted therapies.[143,150] While there is currently no well-established panel of immunohistochemical markers, the use of prostate lineage markers AR, PSA and NKX3.1 together with neuroendocrine markers, in particular INSM1, synaptophysin and FOXA2 have shown value in assessing neuroendocrine differentiation in both de novo and treatment associated NEPC.[151,152] In addition to tumors that loose AR and gain neuroendocrine marker expression, a third molecular subtype of metastatic PC was recently described. These double negative PC (DNPC) are characterized by the absence of both AR and neuroendocrine marker and make up around 20% of all mCRPC (see **Fig. 3**).[139,149] Although the biology of these tumors is currently under investigation, they show a dependence on MAPK and FGF signaling and likely represent a distinct subgroup with unique therapeutic

vulnerabilities. Lastly, a group of mCRPC termed amphicrine PC shows co-expression of AR and a subset of neuroendocrine markers (in particular synaptophysin, see **Fig. 3**).[149] It is presently unclear if these tumors represent a distinct subgroup or an intermediate in the transdifferentiating from a conventional adenocarcinoma to a NEPC. Assessment of AR and neuroendocrine markers on metastatic biopsies will be likely become very important in the diagnostic workup for patients with mCRPC. Although currently used mostly in the setting of clinical trials, the important information gained from such tissue-based studies will be extremely relevant for the optimal diagnostic management in the future. However, before widespread clinical use, markers and assays used in this setting will need to be carefully validated in multi-institutional studies.

SUMMARY

There is no doubt that the use of molecular testing in PC will improve patient outcomes; however, their clinical implementation will likely be challenging. Challenges will arise from the complex biology of PC, in particular the high level of intertumoral and intratumoral heterogeneity, but also from the necessity of a coordinated multidisciplinary approach to the care of PC patients. The plethora of prognostic tests available for localized PC will require an active interface between urologists and pathologists in determining the clinical significance of different assay platforms and most importantly the accessibility of tissue. The complex presentation of metastatic PC and the increased appreciation of resistance mechanisms that involve loss of classical lineage features will necessitate an even more focused interaction between pathologists and oncologists. In addition, an increasing interaction between pathology and radiology will likely be at the center of PC clinical diagnostics in the future. The major improvements in multiparametric MRI imaging have allowed to visualize tumors more robustly and will enable a more focused sampling. It will be important in future studies to correlate these histomorphologic and molecular findings with imaging studies. Similarly, advanced functional imaging will open a real-time window to studying tumor heterogeneity in the setting of metastatic disease. However, these powerful new technologies will be most clinically useful when combined with advanced tissue based and liquid biopsy approaches. This process will require novel computational frameworks to analyze the complex multidimensional data generated as part of such diagnostic workup studies.[153] Therefore, in the paradigm of multidisciplinary care, the role of the pathologist as a steward who bridges molecular diagnostic and clinical care will be ever more important.

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