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The Genomics of Prostate Cancer: emerging understanding with technologic advances

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With the advent of next-generation sequencing technologies and large whole-exome and genome studies in prostate and other cancers, our understanding of the landscape of genomic alterations has dramatically been refined. In additional to well-known alterations in genomic regions involving 8p, 8q, 10q23, common ETS translocations and androgen receptor amplifications, newer technology have uncovered recurrent mutations in SPOP, FOXA1, MED12, IDH and complex large scale genomic alterations (eg, chromoplexy). This review surveys the enhanced landscape of genomic alterations in clinically localized and advanced prostate cancer.
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The genomics of prostate cancer (PCA) has been difficult to study compared with some other cancer types for a multitude of reasons, despite significant efforts since the early 1980s (Figure 1). First, the anatomic location of the prostate gland has initially made it challenging to obtain samples for research. This was largely overcome with the widespread acceptance of the nerve sparing radical prostatectomy.[1] Second, unlike melanoma, lung and breast cancers, PCA does not lend itself to simple growth in culture. From the earliest attempts to grow PCA *in vitro* to develop short-term cultures or to develop karyotypes, it was clear that cells would not readily grow and most often undergo senescence or are overtaken by benign fibromuscular stromal cells. The effects have severely hampered PCA research limiting model systems for understanding the basic biology and genomics of the disease. Recent work in the development of PCA organoids has reignited interest in developing novel model systems for biologic and genomic studies that more closely reflect the PCA observed in patients.[2] Third, it has been traditionally difficult to obtain samples from advanced disease. However, now improved care for men with advanced castration-resistant prostate cancer (CRPC) has led to longer survival. As a consequence, there is more acceptance for metastatic bone and soft tissue biopsies making CRPC samples available for genomic studies.[3]

The advent of high-throughput technologies including comparative genomic hybridization (CGH), fluorescence *in situ* hybridization (FISH), array CGH (aCGH), gene expression profiling and more recently the introduction of massively parallel genomic sequencing referred to as next-generation sequencing (NGS) has led to a deeper understanding of cancer genomics in general. The increased developments in the field of computational biology that have paralleled these advances have also led to widely available genomic data sets that have encouraged researchers from many fields to contribute to our understanding of genomics.

This review will provide an overview of the PCA genomic landscape with an emphasis on the cardinal mutations and alterations observed to be consistently seen in PCA (Figure 1), both hormone naive localized PCA and CRPC.

Cardinal PCA mutations

Cancer is a genetic disease with two major classes of significant mutations: inactivating mutations (in tumor suppressors) and activating mutations (in oncogenes). Inactivation often comes from structural rearrangements involving loss of genomic DNA resulting in deletions (large or focal) or rearrangements. In both cases, a gene or groups of genes are disrupted. These events can be either mono- (heterozygous) or bi-allelic (homozygous). Activation can occur through amplification, point mutation or

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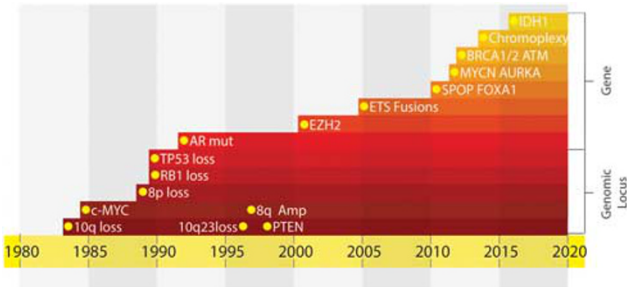

Figure 1 Timeline of cardinal genomic lesions in PCA.

structural rearrangements leading to gene fusions. The cardinal recurrent alterations in PCA include 8p, 8q (C-MYC locus), 10p (PTEN locus), 17q (TP53 locus) and androgen receptor (AR).

10q Loss is Common in PCA

Atkin and Baker[4] were the first to describe in human PCA samples chromosome 10 deletion at 10q24. A prior study on the PCA cell line LNCaP had reported 10q24 loss.[5] Later reports confirmed this 10q24 locus as a site for current deletion in PCA.[6–8] Mitelman's group demonstrated 10q loss (10q24) occurred in 5/33 (15%) PCA cases studied.

TP53 (Cellular Tumor Antigen p53)

Studies based on the restriction fragment length polymorphism (RFLP) approach helped discovery and define the role of TP53 as a tumor-suppressor altered in multiple cancer types including lung, colon, breast, bladder and brain cancers.[9–11] Using this approach, Carter *et al*[12] defined recurrent LOH on a number of loci in PCA including the 17p locus harboring TP53. Isaacs *et al*[13] demonstrated for the first time the presence of p53 mutations in cell lines (eg, PC-3 and DU145) and two primary human PCA samples. They also demonstrated the functional role of wild-type (WT) p53 has as a tumor suppressor. This study helped define p53 as a tumor suppressor in PCA. Subsequent studies demonstrated that in larger numbers of patient samples, p53 mutations accumulated with disease progression.[14,15] Specifically, Navone *et al*[15] in 1993 detected p53 mutations in 23 of 53 AR (43%) 'independent' PCA (referred to today as CRPC) in contrast to no mutations seen in 44 AR-dependent PCA samples. Enrichment for p53 mutations with PCA disease progression has been confirmed in numerous studies as a consistent event. In 2016, Robinson *et al*[3] reported 53% mutations in the Stand Up 2 Cancer/ Prostate Cancer Foundation CRPC500 study cohort (referred hereon as CRPC500 cohort).

RB1 (Retinoblastoma-Associated Protein)

RB1 was originally localized to 13q14.1 by cytogenetic analysis from patients with retinoblastoma[16] and cloned by Friend *et al* in 1986.[17] In 1990, Bookstein *et al*[18] reported RB1 loss in the DU145 PCA cell line and functionally associated loss with increased tumorigenicity, which could be reversed by reintroduction of RB1. Early studies suggested RB1 loss ranging from around 30 to 60% using RFLP analysis for the 13q RB1 locus.[19–21] In the CRPC500 cohort, RB1 is reported lost in 21% of cases.[3]

8p Loss

The first report of 8p loss in PCA was by Konig *et al*[22] in 1989 on the LNCaP cell line. Numerous groups confirmed this observation in primary and advanced PCA.[20,23–34] Although candidate tumor-suppressor genes had been proposed in this region,[35] it was not until 1997 that He *et al*[36] identified in a prostate-specific gene, NKX3.1, which was homologous to the *Drosophila* NK homeobox gene family. NKX3.1 is expressed at high levels in normal prostate and is androgen sensitive as they determined by androgen stimulation of LNCaP cell lines. They mapped NKX3.1 to chromosome band 8p21, a region that was previously noted to undergo loss. He *et al* proposed a potential tumor-suppressor role for NKX3.1.

Androgen Receptor

Mutations have been long known to exist in the AR. AR mutations occur and result in a germline disorder called androgen insensitivity syndrome, an X chromosome-linked inherited disorder (reviewed in Hughes *et al*[37] and Shukla *et al*[38]). Mutations in the ligand-binding domain of the AR receptor were first observed in an androgen-responsive PCA cell line, LNCaP.[39] Newmark *et al*[40] reported the first AR mutations associated with primary PCA. Frequent AR mutations were observed in CRPC (50%) demonstrating for the first time that AR resistance via mutation occurs with AR-targeted therapy.[41] Another mechanism for AR resistance can be explained by AR gene amplification. aCGH and FISH technology helped define 4-fold to over 20-fold AR amplifications in hormone-treated PCA patients but not in untreated hormone naive PCA.[42] With the development of tissue microarray (TMA) technology, larger numbers of clinical samples could be detected on a single slide. Using TMAs, Bubendorf *et al*[43] queried the AR status on 371 PCA samples by FISH. AR was determined to be amplified in 23% of the 47 CRPC cases in contrast to 2 of 205 (1%) of the primary hormone naive PCA cases. In more recent studies using NGS, the AR aberration frequencies show the same patterns. In the TCGA study of 333 hormone naive PCA, no AR mutations were detected.[44] In studies where tumors were evaluated after androgen deprivation therapy (ADT), AR mutations and amplification frequencies were in the range of the initial reports.[3,45–48] Other mechanism of AR

resistance have been proposed including v7 AR splice variants[49] and lineage plasticity to AR indifferent CRPC.[45,50]

10q23 (PTEN)

The distal region of 10q is lost in a number of common cancers such as glioblastoma and breast cancer. Early studies using RFLP assays located the loss at 10q24, but a series of articles in the 1990s targeted 10q23.1 as a potential site for a tumor-suppressor gene. In 1990, Carter *et al*[12] reported 10q loss in around 30% of localized PCA. In 1995, Gray *et al*[51] suggested the critical area for a potential tumor suppressor was in 10q23–24 lost in 62% of the 37 PCA cases they examined. In 1996, Ittmann was the first to propose 10q23.1 demonstrating increased loss in advanced PCA. He also suggested that prior studies using aCGH approaches may have missed the 10q23.1 region as deletions in some cases were small.

Mapping of multiple cancers including brain, breast and prostate, Ramon Parson's group pinpointed a minimal area of genomic deletion at 10q23.1 leading to the cloning of the candidate tumor-suppressor gene phosphatase and tensin homolog deleted on chromosome ten (PTEN).[52] Mutations were detected in brain and breast cancer. All PCA cell lines tested demonstrated either mutations (ie, LNCaP and DU145) or homozygous deletions (ie, NCI-H660 and PC-3). Since the initial work, few inactivating PTEN mutations have been detected in PCA.[53] PTEN has a critical role in regulating the PI3K-AKT pathway such that loss leads to downstream activation.

MYC Amplification (8q24)

C-Myc is a transcription factor with a wide range of functions including modulation of protein synthesis, cell cycle and metabolism. C-myc, the protein encoded by the MYC oncogene on 8q24, was observed in 1986 as overexpressed in human primary PCA. Flemming *et al*[54] reported c-myc overexpression at the transcript level using Northern blot technology. In 1997, Jenkins *et al*[55] conducted the first extensive study using FISH at 8q24 to demonstrate gene amplification of MYC. Amplification of MYC was observed in 25% of the clinically localized PCA tumors but in 46% of the advanced PCA samples examined, suggesting that MYC amplification corresponds to disease progression. Interestingly, they also observed that in the localized samples, MYC amplification was often only amplified in a subset of the tumor cells in the lesion consistent with genomic heterogeneity. MYC amplification is one of the genomic events that appears to be significantly different in CRPC *vs* primary PCA.

The importance of co-occurring molecular alterations is well illustrated by the amplification of MYC and activation of the PI3K-pathway. Clegg *et al*[56] observed that there is a statistically significant association between PI3K pathway alterations (ie, PTEN, PI3CA, AKT1, AKT2 and AKT3) and MYC amplification with 27% and 70% co-occurrence in localized and metastatic PCA, respectively. To determine the potential impact of these co-occurring genomic alterations, they developed a series of genetically engineered mouse models (GEMs) to explore the relationships of the individual and co-occurring alterations. Using mice with either PTEN loss or AKT overexpression and crossing them, respectively, with high MYC overexpressing mice [57] in a prostate conditional context, they demonstrated that the addition of MYC leads to an acceleration of PIN and adenocarcinoma. Interestingly, whereas RAD001, a rapamycin analog, can inhibit the formation of PIN in prostate conditional AKT-activated GEMs, RAD001 did not abrogate the development of PIN in mice expressing both AKT and MYC. This suggests that MYC acts in a manner that is independent from mammalian target of rapamycin C1 (mTORC1) activation. These important studies begin to reveal the complexity of co-occurring genomic alterations in cancer, the additional challenges to therapeutic strategies and the need to better understand them through model systems.

SCNAs define PCA risk progression

CGH identified somatic copy number alterations (SCNAs) in a high percentage (~75%) of localized PCA. Losses were found to be five times more common than gains and most often involved 8p (32%), 13q (32%), 6q (22%), 16q (19%), 18q (19%) and 9p (16%). These early genome-wide studies also suggested that the pattern of copy number alterations changes with disease progression. For example, gains of 7, 8q and X were more often observed in the CRPC state.[23] La Pointe *et al*[58] proposed a subclassification of PCA risk based on aCGH data with three potential risk groups. More recently, refined maps of SCNAs have demonstrated significant association with Gleason grade prognostic groups [59] and overall SCNAs as a measure of PCA relapse.[60] In recent work in populations of CRPC, distinct SCNAs in AR and MYC have been confirmed from prior studies.[3] Therefore, the overall burden and specific genomic alterations may potentially become useful PCA biomarkers.

ETS rearrangements in PCA

There is an emerging view of PCA suggesting a high degree of molecular complexity involving common recurrent gene fusions,[61] large complex genomic rearrangements[62] and common recurrent mutations.[44,62–64] This heterogeneity might be best viewed as a collection of more homogenous subgroups defined by SCNAs, mutations and

rearrangements. The most common gene fusion in PCA is the *TMPRSS2:ERG* fusion.[61] The discovery of the ETS family transcription factor gene fusions in 2005 dramatically changed the view that gene fusions predominately occurred in sarcomas and hematopoietic malignancies.[61] Cancer outlier profile analysis on existing PCA expression microarray experiments nominated two consistently high-scoring, and mutually exclusive candidates across 50% of PCA samples, which were members of the ETS family of transcription factors, *ERG* and *ETV1*. Further experiments revealed fusions of the 5'-untranslated region of *TMPRSS2* (21q22.3) with the *ETS* transcription factor family members, either *ERG* (21q22.2), *ETV1* (7p21.2)[61] or *ETV4*,[65] suggesting a novel mechanism for overexpression of the ETS genes in PCA. In addition to *TMPRSS2*, three other androgen-responsive 5' partners *SLC45A3*,[66,67] *HERPUD1*[68] and *NDRG1* [69] have been found to fuse with *ERG*.

Recent advances in next-generation transcriptome sequencing facilitated the discovery of RAF kinase gene fusions, *SLC45A3-BRAF*, *ESRP1-RAF1* and *RAF1-ESRP1* in advanced PCA.[70] Although rare, detected in ~1–2% of PCAs, RAF kinase fusions represent the first 'driver' fusion in PCA that do not involve an ETS family member.

Emerging Understanding of PCA Genomic Complexity

Genomic rearrangements appear to be nonrandom, locus specific and depend, in part, on the proximity of chromosomal regions in the nucleus.[71] Moreover, there is a mounting evidence suggesting that transcription factors are associated with DNA double-strand breaks, thus predisposing transcribed regions to genomic rearrangements. For example, both androgen and estrogen signaling recruits the enzyme topoisomerase 2B (TOP2B) to target gene promoters, which creates DNA double-strand breaks and facilitates transcription.[72,73] The AR and TOP2B are co-expressed in human PCA precursor lesions in which *TMPRSS2:ERG* rearrangements are known to occur, suggesting a critical role of TOP2B in the recurrent ETS rearrangements. Three recent studies have also shown that androgen signaling promotes *TMPRSS2:ERG* fusion formation,[74–76] in part, by recruiting DNA break-inducing enzymes (eg, activation of induced cytidine deaminase) to translocation breakpoint sites.[75] More recently, Berger *et al*[62] demonstrated that rearrangement breakpoints were enriched near open chromatin, AR and ERG DNA-binding sites in the setting of the ETS gene fusion *TMPRSS2:ERG*, but inversely correlated with these regions in tumors lacking ETS fusions. Hence, transcription factors can contribute to the formation of genomic rearrangements by facilitating the juxtaposition of chromosomal loci and recruiting enzymatic machinery involved in DNA breaks to these target loci.

In summary, ETS fusions are the most common recurrent genetic mutation identified in PCA. Although a number of ETS and non-ETS family members have been observed to be fused with TMPRSS2 or other 5' partners, the vast majority of fusions involve TMPRSS2:ERG. This fusion can be studied in large numbers, as it was identified in approximately 45% of all prostate-specific antigen (PSA) screened PCA.

Molecular landscape of PCA in the NGS era

Comprehensive NGS studies characterizing the PCA genomic landscape have identified a few highly recurrent somatically mutated genes (including *SPOP*, *TP53*, *PTEN* and *FOXA1*, all $<15\%$), with recurrent broad copy number alterations (CNAs; ie, 8p loss and 8q gain), but relatively few focal and/or high-level CNAs (most commonly focal *PTEN*, *TP53* and *RB1* losses). The recent TCGA publication of 333 PCA genomes, transcriptomes and methylomes solidifies the idea of PCA molecular subclasses.[77] One major advantage of NGS generated tumor data sets is the possibility to computationally assess tumor cellularity (fraction of tumor cells in cells constellation whose DNA was analyzed, also referred to as tumor purity) by exploiting single base resolution data and to infer tumor aneuploidy, as accomplished by various tools including ABSOLUTE[78] and CLONET.[79] In addition, NGS data make it possible to assess the clonality of each single somatic lesion (fraction of tumor cells that harbor a specific somatic lesion) allowing for the definition of the first primary PCA evolution chart[63] and of phylogenic trees to trace tumor history in one patient when for instance multiple metastatic biopsies are sequenced.[48,80] Altogether these computational assessments from large collection of prostatectomies samples characterized through whole-exome sequencing (WES) or whole-genome sequencing (WGS) deepened our recognition of earlier *vs* later events in prostate carcinogenesis.

Insights into Genomic Complexity

With the advent of WGS, insights into global genomic rearrangement are possible. Berger *et al*[62] published the first seven whole genomes revealing structurally complex rearrangements.[62] The associations were significantly enriched for disruption of tumor-suppressor genes. There was also an observation that the rearrangements in the ETS rearrangement cancers tended to occur in the proximity of transcriptionally open chromatin marks suggesting that subclasses of PCA may act differently based on early genomic alterations. In a significantly larger WGS study, Baca *et al*[63] extended these observations based on 57 PCAs. Using statistical arguments and

simulations, they found that the many complex rearrangements likely originated within a single-cell cycle, providing a proliferative advantage to a cancerous cell causing multiple oncogenic events. This was termed 'chromoplexy' to refer to these distinctive genomic restructuring events. They demonstrated that this process is distinct from the phenomenon of chromothripsis.[81] Chromoplexy is active across the majority of PCAs and in several non-prostate tumor lineages. Similarly, in a recent publication Fraser and colleagues[82] describe results from a large WGS effort on localized, intermediate-risk PCA that includes specific single-nucleotide variants and rearrangement signatures with DNA footprints associated with ageing and clustered mutation phenomenon called kataegis. This large WGS study demonstrates a high burden of complex rearrangements, including samples with evidence of chromothripsis (chromosome shattering). Although not reported, their study also demonstrated chromoplexy (chains of balanced rearrangements) altogether suggesting highly altered 3D structure of PCA cell genome (personal communication).

Another observation from WGS analysis revealed that while the ETS fusion cases demonstrated a high frequency of intergenic gene rearrangements, a second group of around 10% of PCA demonstrated high frequencies of intragenic rearrangements. This later class is best defined as harboring SPOP mutations and CHD1 loss.[83] This recent work had demonstrated that SPOP mutations are associated with a homology repair defect phenotype.

SPOP Mutations Define a Distinct Molecular Subclass of PCA

Recurrent missense mutations in *SPOP* are the most common point mutations in primary PCA, occurring in about 10% of both clinically localized and metastatic CRPC.[3,44,62,84,85] The most common *hot spot SPOP* mutations occur at F133, Y87, F102 and W131 and often co-occur with specific genomic features including deletions at 5q21, 6q15 and 2q21.[83,84] Molecularly, human PCA can be classified into those harboring rearrangements in *ETS* transcription factors (eg, *TMPRSS2-ERG*) and those lacking *ETS* rearrangements. *SPOP* mutant PCA also defines characteristic genomic rearrangements, gene expression profiles and methylation patterns.[44,83–85] *SPOP* mutations occur early in the natural history of PCA solely as heterozygous missense mutations with dominant negative, selective loss of function toward the remaining WT allele.[63,79,83,86]

SPOP encodes the substrate recognition component of a CUL3-based E3 ubiquitin ligase, and PCA-derived *SPOP* mutants appear to act as dominant negatives with selective loss-of-function.[83] Known substrates of *SPOP* are numerous, and the specific substrates that are deregulated by *SPOP* mutations are starting to be defined. These include the chromatin-associated oncogene DEK,[86] the oncogenic co-activator TRIM24[86,87] and the AR itself.[88,89]

Initial models have established the role of *SPOP* mutation as a driver of prostate neoplasia *in vivo*, and studies exploring the downstream effects of *SPOP* mutations have largely relied on overexpression of mutant *SPOP* protein in cell lines with alterations outside the genetic context of *SPOP* mutant PCA.[86,89–92]

Blattner *et al*[93] reported the development of the first conditional mouse model showing that *SPOP* mutation drives prostate tumorigenesis *in vivo.* Using *in vitro* models derived from these mice, they demonstrated that mutant *SPOP* activated both PI3K/mTOR signaling and AR signaling, effectively uncoupling the normal negative feedback between these two pathways. Together, these findings show that *SPOP* mutation drives prostate neoplasia *in vivo* through deregulation of the PI3K/mTOR and AR pathways, and underscore the critical role of these two signaling pathways across molecular subtypes of human PCA (Figure 2). More recent work has also suggested a genetic predisposition to this distinct subclass of PCA and this may be modulated by AR signaling and DNA repair alterations.[94]

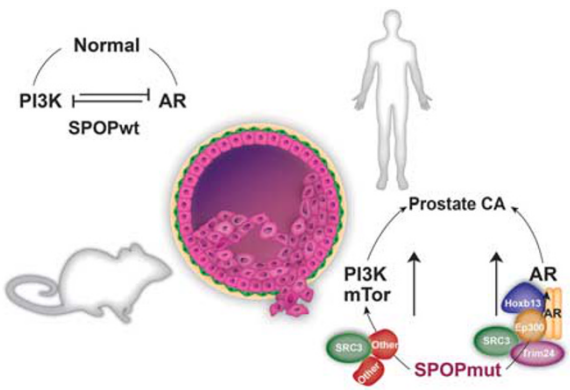

Figure 2 Schematic of human SPOP mutation potential to drive prostate tumorigenesis. Mouse model work shows that human SPOP mutation can drive prostate tumorigenesis through coordinate deregulation of PI3K/mTOR and AR pathways.

Genomic alterations and resistance

It has been long observed that AR mutations are only seen in the setting of treated PCA. Recent genomics analysis has provided a deeper understanding as to the extent and repertoire of alterations associated with resistance.

Multiple studies have now assessed the molecular landscape of lethal CRPC (predominantly from rapid autopsy samples).[47,95–99] WES and targeted NGS studies have demonstrated that *AR* is the most recurrent alteration (approximately 50%) in CPRC but not in untreated PCA (from unrelated patients or previous studies), consistent with the known role of

AR in mediating CRPC progression.[100–106] Nevertheless, numerous genes are altered more frequently in CRPC *vs* untreated PCA (ie,*TP53* and *PTEN*), and additional potential low-frequency driving alterations are observed more frequently in lethal CRPC than untreated PCA (ie, deleterious *APC* alterations in 20% of CRPC and 3–5% of untreated PCA from TCGA and our previous untreated PCA studies).[47,63,64,106] Similarly, single gene, functional and profiling studies have identified pathways that may mediate progression to CRPC along with AR pathway deregulation, such as the WNT/APC/CTNNB1 or PI3K pathways,[47,106–111] however, it is unclear if these alterations were present before ADT, mediated progression to CRPC or acquired after CRPC progression.

AR Indifferent PCA: Another Mechanism for Resistance

Treatment-related neuroendocrine prostate cancers (NEPC) are clonally derived from a castration-resistant adenocarcinoma precursor (see recent review Rickman *et al*[112]). Although they retain PCA genomic alterations, NEPC tend to lose expression of common prostate markers and are often treated with regimens similar to small cell lung cancer. There is a spectrum of disease within CRPC, with small cell carcinoma being the extreme and mixed and intermediate atypical phenotypes also observed which can retain AR expression and harbor overlapping molecular features. Clinical features associated with platinum sensitivity (termed 'anaplastic' or 'aggressive variant' PCA) have been defined for patient selection in Phase 2 chemotherapy studies and share pathologic and molecular features of NEPC. The cell plasticity associated with NEPC is associated with decreased or absent expression of the AR and downstream androgen-regulated genes such as *PSA*. Moreover, data from pre-clinical models also favor a trans-differentiation model of treatment-related NEPC. For example, adenocarcinoma cells (eg, LNCaP) display a neuroendocrine phenotype similar to the few established NEPC cell lines that have been described (eg, NCI-H660)[50,113,114] following exposure to a variety of stimuli (eg, androgen deprivation[115] or treatment with cAMP,[116] IL-6[117] or fractionated ionizing radiation).[118] Similar trans-differentiation results have been observed *in vivo*. For example, a well-characterized patient-derived prostate adenocarcinoma xenografts (LTL331) implanted into the subrenal capsule of mice develops small cell NEPC following castration (LTL331R)[119] and which phenotypically resembled other lines that were generated from bone fide NEPC tumor tissue (eg, LTL352, LTL370,[119] LuCAP-49,[120] UCRU-PR-2[121] and WISH-PC2).[122] Genomic profiling at various time-points during trans-differentiation suggests clonal evolution from the adenocarcinoma LTL331 to the NEPC LTL331R tumor.[119]

Activation of Myc family oncogenes commonly occur across poorly differentiated neuroendocrine carcinomas and in small cell lung cancer, up to 20% harbor genomic amplification of N-myc, C-Myc and L-Myc, or a recurrent fusion involving *MYCL1* (9%).[123–126] N-Myc amplification occurs in high-risk neuroblastoma and a subset of NEPC and SCLC. N-Myc has also been shown to be amplified and overexpressed in NEPC and can drive the NEPC phenotype.[50,127,128] Recently, Dardenne *et al*[129] showed using a variety of pre-clinical models including isogenic cell lines, xenografts, GEMM and mouse tumor organoids cultures, that N-Myc overexpression is associated with highly proliferative, invasive PCA with pathologic features of NEPC. Dardenne *et al* further showed that the N-Myc interacts and cooperates with enhancer of zeste homolog 2 (EZH2) to drive NEPC transcriptional programs including an abrogation of AR signaling, enhanced AKT and EMT signaling, repression of polycomb repressive complex 2 (PRC2) target genes and expression of neuroendocrine markers. Lee *et al*[127] used a forward engineering approach of benign human prostate cell that overexpress N-Myc in the context of myristoylated AKT and provided compelling data showing that N-Myc overexpression resulted in castrate-resistant tumors with NEPC or adenocarcinoma histology foci. These complementary results solidify N-Myc as a driver of the NEPC phenotype. In both studies, N-Myc was shown to form a complex with Aurora kinase A (Aurora-A) that stabilized N-Myc and that was sensitive to allosteric inhibitors to Aurora-A. These results showed the feasibility of exploiting the mutual dependence of N-Myc and Aurora-A to revert their oncogenic functions.

Although N-Myc is overexpressed in the majority of NEPC cases, there is a spectrum of N-Myc expression in CRPC samples with 20% of CRPC tumors demonstrating transcript levels in the range of NEPC.[45,129] N-Myc overexpression in prostate adenocarcinoma was sufficient to drive the NE phenotype.[129] Specifically, RNA-seq analyses from multiple stable LNCaP or 22Rv1 cell populations overexpressing N-Myc showed that N-Myc overexpression resulted repression of AR signaling and activation of PRC2 signaling and gene expression signatures associated with RB1 loss,[129] all of which are molecular features associated with NEPC.[45,50,127] These results suggest that N-Myc is at least one driver of NE plasticity in PCA. Recent pre-clinical data also suggest that lineage plasticity can be observed in the context of *RB1/TP53* loss.[130,131] EZH2 inhibitors can also sensitizes NEPC tumor cells to enzalutamide. This suggests that reversing or delaying lineage plasticity with this type of epigenetic approach may provide a clinical benefit to a larger number of patients than previously appreciated and would extend clinical responses to antiandrogen therapy in the case of PCA.

Heterogeneity

The Impact of Multifocality and Heterogeneity on Tracking Lethal CRPC

At radical prostatectomy (RP), ~80% of patients harbor multiclonal (also referred to as multifocal) PCA, where spatially distinct tumor foci, which may show similar morphology and/or grade (Gleason score), are present in the same prostate.[132,133] Multifocal PCA represents clones of independent origin, as supported by numerous approaches, including *ERG* rearrangement status (by FISH or IHC);[27,134–145] in contrast, lethal, metastatic CRPC appears uniformly *ERG* rearrangement positive or negative in all sites in a given patient, consistent with clonal origin, although extensive subclonal structure is present.[47,95,96,98,99,146,147]

ERG rearrangement status (ERG^+ or ERG^-) is a useful clonal marker to demonstrate spatially distant multifocal tumors.[135,137,141,142,144,145,148] Several anecdotal NGS studies (n-values ≤ 5–10) have added complexity to tracking the eventual CRPC clone through identifying intrafocal heterogeneity at RP.[95,98,99,149–152] These series of locally advanced PCA vary from reporting little divergence to complete lack of shared alterations between the index focus and lymph node metastases and/or CRPC. Haffner *et al*[73] tracked the lethal clone in a single patient. Remarkably, they found that at RP, a small, organ confined low-grade (Gleason score 6) area of a large, high-grade primary tumor was the only area that harbored all alterations present in distant CRPC and lethal metastases. Critically, these alterations were absent from the vast majority of the primary tumor and lymph node metastasis at RP. Hence, in this patient, the lethal CRPC clone arose from a small, low-grade area of a histologically defined single index focus, rather than the higher grade area or concurrent lymph node metastasis. Whether this 'n of 1' case represents the exception, rather than the rule, can only be assessed in a large cohort of paired RP and CRPC specimens, rather than locally advanced PCA.

Gundem *et al*[80] recently explored PCA clonal evolution in 10 men with heavily treated CRPC at rapid autopsy. Like other published rapid autopsy series, this cohort did not represent a clinical trial and did not include patients treated with current second line agents targeting AR signaling (enzalutamide and abiraterone). With these caveats, their study presents a key snapshot of heavily treated lethal CRPC. In their study, primary prostate tumors (retained during treatment of advanced disease) demonstrated the presence of a large 'trunk' of mutations seen subclonally. Among the mutations found in the trunk, a subset of potential driver mutations was observed in a more pure, clonal form in the metastatic lesions. They demonstrate the feasibility of tracking clonal mutations in metastases back to initiating lesions. Currently on-going studies from the SU2C-CRPC500 cohort are actively addressing this in a more formal manner.

In summary, the advent of the radical prostatectomy coupled with advances in genomics led for steady increase in our understanding of PCA genomics. More recently, the examination of patients treated for advanced CRPC either at time of autopsy or through metastatic biopsies is leading to important insights into tumor plasticity and resistance. Future advances in cancer mouse models and organoids derived from patients will enable the development of new treatment strategies.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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