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REVIEW ARTICLE

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The evolving landscape of prostate cancer somatic mutations

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Abstract

Background: The landscape of somatic mutations in prostate cancer (PCa) has quickly evolved over the past years.

Results: This evolution was in part due to the improved quality and lower cost of genomic sequencing platforms available to an ever-larger group of clinicians and researchers. The result of these efforts is a better understanding of early and late mutations that are enriched or nearly exclusive to treated PCa. There are, however, some important limitations to the current knowledge. The expanding variety of next-generation sequencing (NGS) assays either capture a wide spectrum of mutations but at low coverage or are focused panels that cover a select number of genes, most often cancer-related, at a deep coverage. Both of these approaches have their advantages, but ultimately miss low-frequency mutations or fail to cover the spectrum of potential mutations. Additionally, some alterations, such as the common *ETS* gene fusions, require a mixture of DNA and RNA analysis to capture the true frequency. Finally, almost all studies rely on bulk PCa tumor samples, which fail to consider tumor heterogeneity. Given all these caveats, the true picture of the somatic landscape of PCa continues to develop.

Summary: In this review, the focus will be on how the landscape of mutations evolves during disease progression considering therapy. It will focus on a select group of early and late mutations and utilize SPOP mutations to illustrate recurrent alterations that may have clinical implications.

KEYWORDS
gene fusion, mutations, prostate cancer, SPOP

1 | FIRST SNAPSHOT OF PROSTATE CANCER (PCa) SOMATIC MUTATIONS

There are two major classes of significant mutations: inactivating mutations (in tumor suppressors) and activating mutations (in oncogenes). Inactivation comes from point mutations, or most often 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 structural rearrangements leading to gene fusions.

PCa can be defined by several types of somatic mutations which have been known since the 1980 and 1990s, including chromosome 8p loss, 8q (*MYC* locus) gain, 10p (*PTEN* locus) loss, 17q (*TP53* locus) loss, and androgen receptor (*AR*) alterations. With the advent of

high-throughput transcriptomics and next-generation sequencing (NGS), a clearer picture of the landscape of PCa somatic alterations has emerged. These include the *ETS* gene fusions—most commonly the *TMPRSS2-ERG* gene fusion—and *SPOP* mutations, the most frequent the recurrent point mutation in PCa (Figure 1).

2 | EARLY SOMATIC PCa MUTATIONS

2.1 | NKX3.1

During the course of PCa progression, there are some early events that are considered gate-keeper events. Based on many mouse models, usually only one of the alterations is sufficient to lead to cancer. One of the most typical areas of genomic loss is at chromosome 8p. He et al. identified a prostate-specific gene, *NKX3.1*, which is homologous to the Drosophila NK homeobox gene family.[2] *NKX3.1* is expressed at high levels in normal prostate and is activated in response to androgen in LNCaP cells. The authors mapped *NKX3.1* to chromosome band 8p21, a region that was previously noted to undergo loss, and proposed a potential tumor suppressor role. Loss of *NKX3.1* expression was demonstrated by Bowen, et al. in 6%–22% of primary PCa specimens and 78% of metastases.[3]

2.2 | PTEN

Another very common somatic loss occurs at 10q23. The distal region of 10q is lost in a number of cancers such as glioblastoma and breast cancer. Early studies using restriction fragment length polymorphism (RFLP) assays located the loss at 10q24, but a series of papers in the 1990s targeted 10q23.1 as a potential site for a tumor suppressor gene. In 1990, Carter et al. reported 10q loss in around 30% of localized PCa.[4] In 1995, Gray et al. suggested the critical area for a potential tumor suppressor was in 10q23-24, which was lost in 62% of the 37 PCa cases they examined.[5] In 1996, Ittmann was the first to propose that 10q23.1 demonstrates increased loss in advanced PCa. His critical work also recognized that prior studies using array comparative genomic hybridization (aCGH) approaches—state-of-the-art at the time—may have missed the 10q23.1 region, as deletions in some cases were small.

Mapping 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 *PTEN* (phosphatase and tensin homolog deleted on chromosome 10).[6] Mutations were detected in brain and breast cancer cell lines and xenografts. The PCa cell lines tested demonstrated either mutations (i.e., LNCaP, DU145) or homozygous deletions (i.e., NCIH660, PC-3). Since the initial work, few inactivating *PTEN* mutations have been detected in PCa.[7] However, *PTEN* (10q23) loss is common in localized PCa and increases in frequency during disease progression. PTEN plays a critical role in regulating the PI3K-AKT pathway such that loss leads to downstream activation. More recent studies support the nonclonal loss of *PTEN* in hormone-naïve tumors[8] with enrichment corresponding with PCa progression.

2.3 | ETS fusions

Before NGS, the discovery of recurrent ETS gene fusions represented one of the first recurrent gene fusions observed in solid cancer. The most frequent of these in PCa is the *TMPRSS2-ERG* fusion,[9] and since its initial discovery in 2005, a great deal has been learned about this molecular event. The *TMPRSS2-ERG* fusion is an early event, referred to as a truncal lesion. During the course of disease progression, some tumors become AR insensitive the frequency of *ETS* remains approximately the same, but the ability to detect these fusions may become more difficult as the 5′ prime driver (*TMPRSS2*) is androgen-regulated and ERG protein expression may be more difficult to detect.

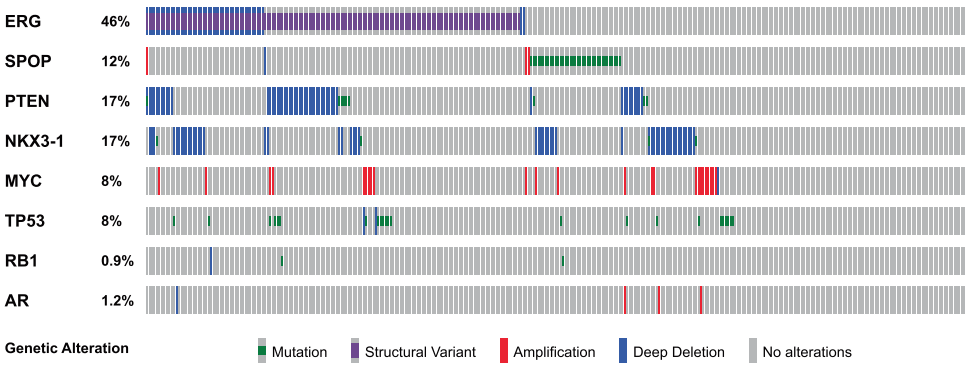

FIGURE 1 A landscape of common somatic alterations in localized prostate cancer from the Cancer Genome Atlas (TCGA) prostate cancer study.[1] Early somatic mutations include the mutually exclusive TMPRSS2-ERG (ERG) gene fusions and SPOP recurrent mutations. PTEN loss (10p23) is another early somatic loss as is NKX3.1. MYC (8q gain) is seen in early localized prostate cancer and associated with more aggressive disease. TP53, RB1, and AR alterations can be observed but are more highly enriched in advanced disease after ADT (oncoprint generated from cBioportal)

The ETS gene fusions do not create a chimeric protein but instead overexpress an ETS transcription factor in a normal, albeit truncated form. Most often these fusions involve the 5′-untranslated region of *TMPRSS2* (21q22.3) with ETS transcription factor family members, either *ERG* (21q22.2), *ETV1* (7p21.2),[9] or *ETV4*,[10] suggesting a novel mechanism for overexpression of the ETS genes in PCa. Since the initial discovery of ETS fusions in PCa, a number of studies have identified fusion events involving additional ETS family members (i.e., *ELK4*[11,12]), novel 5′ (upstream) partners, and a class of non-ETS based fusions.[13]

Though the largest category of ETS fusions involves *TMPRSS2* there remain other, less common, fusion events. Interestingly, the ETS family member fusions involve a diverse set of 5′ upstream partners, as exemplified by *ETV1* having at least nine different fusion partners. In addition to *TMPRSS2*, three other androgen-responsive 5′ partners *SLC45A3*,[14,15]*HERPUD1*,[16] and *NDRG1* [17] have been found to fuse with *ERG*. However, like *TMPRSS2*, many of the 5′ partners appear to fuse to multiple ETS family members, such as *SLC45A3* (*-ERG*, *-ELK4*, *-ETV1*, and *-ETV5*), which is also androgen-responsive. The majority of these AR-regulated promoters confer an organ and tissue specificity to these gene fusions. Interestingly, as these events occur as early as the precursor lesion, high-grade PIN, they suggest one of the first hormonally regulated mutations in PCa development. This may have implications in how individual men respond to endogenous hormone and/or hormone manipulation as part of systemic treatment for more aggressive PCa.

NGS has further discovered RAF kinase gene fusions, *SLC45A3-BRAF*, *ESRP1-RAF1*, and *RAF1-ESRP1* in advanced PCA.[18] Although rare, detected in ~1%–2% of PCa, RAF kinase fusions represent the first "driver" fusion in PCa that do not involve an ETS family member.

The *TMPRSS2-ERG* fusion is an early event observed in approximately 20% of high-grade PIN lesions intermingled with PCa that carried the same fusion pattern. Immunohistochemistry can be used to detect elevated ERG protein expression, and this can be seen in the area of high-grade PIN, but not in directly adjacent benign prostate tissue.

The prevalence of *TMPRSS2-ERG* in PCa has been reported to range from 40% to 70%, depending on the clinical cohorts investigated. The first large clinical study on a German prostatectomy cohort reported approximately 50% of cases exhibited a *TMPRSS2-ERG* fusion.[19] Multiple, retrospective studies from PSA-screened prostatectomy cohorts have reported frequencies of the *TMPRSS2-ERG* fusion between 35% and 50% when fluorescence in situ assay (FISH) was used to detect the rearrangement.[20–25] This was confirmed in the Cancer Genome Atlas (TCGA) study[1] (Figure 1), which also includes the *TMPRSS2-FLI1* fusion that had been predicted due to the similarity to ERG (i.e., they both belong to the same clade of ETS transcription factors). The exact frequency of rarer fusions may be below 1% but could also be more common in ethnic groups.

The ETS gene fusions are widely believed to have an important oncogenic role, but *ERG* or *ETV1* overexpression alone is not sufficient to lead to PCa. Some evidence suggests that ETV1 has a stronger phenotype than ERG in PCa disease progression.[26] There is mounting molecular data for an important concomitant role of *TMPRSS2-ERG* and Pten/PI3K/ATK pathway activation in PCa oncogenesis. Carver et al.[27] and King et al.[28] identified the co-occurrence of *TMPRSS2-ERG* and *PTEN* loss. Mouse studies by Carver et al.[27] suggested that the oncogenic role of *TMPRSS2-ERG* fusion is in tumor cell migration that is enhanced by the proliferative effects of Pten/PIK3/Akt pathway activation.

In summary, ETS fusions are the most 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 PSA screened PCa. It is worth noting that a number of cohorts that report very low TMRPSS2-ERG fusions may be due to the inability of the test to detect the fusion and may not represent the actual frequency.

2.4 | MYC

c-Myc, the protein encoded by the *MYC* oncogene on 8q24 is a transcription factor with a wide range of functions, including modulation of protein synthesis, cell cycle, and metabolism. Overexpression of *MYC* at the transcript level was observed by Fleming et al. in 1986 in human primary PCa using Northern blot technology.[29] In 1997, Jenkins et al. conducted the first extensive study using FISH at 8q24 to demonstrate gene amplification of *MYC*.[30] This amplification was observed in 25% of the clinically localized PCa tumors, but in 46% of the advanced PCa samples, 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. Further studies have confirmed that *MYC* is one of the genes that appear to be significantly altered in CRPC versus primary PCa.[31]

The importance of co-occurring molecular alterations is well-illustrated by the amplification of *MYC* together with activation of the PI3K-pathway. Clegg et al.[32] observed that there is a statistically significant association between PI3K-pathway alterations (i.e., *PTEN*, *PIK3CA*, *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 (GEMMs) to explore the relationship between the individual and co-occurring alterations. Using mice with either *PTEN* loss or AKT overexpression and crossing them respectively with high *MYC* overexpressing mice[33] in a prostate conditional context, they demonstrated that the addition of c-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 GEMMs, RAD001 did not abrogate the development of PIN in mice expressing both AKT and c-Myc. This suggests that c-Myc acts in a manner that is independent from

mTORC1 (mammalian target of rapamycin) 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.

3 | LATE SOMATIC PCa MUTATIONS

Somatic alterations in PCa also appear to become enriched with disease progression. Tumor cell selection may also occur through treatment with AR signaling inhibitors (ARSi) or other therapies that enrich certain somatic alterations. There are three key somatic alterations that go from relatively rare in untreated, clinically localized PCa and common in advanced ADT treated PCa: *TP53*, *RB1*, and *AR*.

3.1 | TP53 and RB1

Enrichment for *TP53* mutations with PCa disease progression has been confirmed in numerous studies as a consistent event. Robinson et al. first reported 53% mutations in a cohort of 150 metastatic PCa patients,[34] which was reduced to 40% when the cohort was extended to 429 patients[35] (Figure 2). Early studies suggested *RB1* loss ranging from around 30-60% using RFLP analysis for the 13q *RB1* locus.[37–39] In Robinson et al. *RB1* is reported lost in 21% of cases,[34] which was reduced to 13% in the larger cohort[35] (Figure 2).

3.2 | AR

Mutations have been long known to exist in AR. AR mutations occur and result in a germline disorder called androgen insensitivity syndrome (AIS), an X chromosome-linked inherited disorder (reviewed in Hughes et al.[40] and Shukla et al.[41]). Mutations in the ligand-binding domain of the AR receptor were first observed in an androgen-responsive PCa cell line, LNCaP.[42] Newmark et al. reported the first AR mutations associated with primary PCa.[43] Frequent AR mutations were observed in CRPC (50%) demonstrating for the first time that AR resistance via mutation occurs with AR targeted therapy.[44] Taplin et al. stated, "Our results suggest that mutant androgen-receptor genes in androgen-independent PCa could be useful targets of new drugs for the treatment of PCa." Another mechanism for AR resistance can be explained by AR gene amplification. Array CGH and FISH technology helped define 4- to over 20-fold AR amplification in hormone-treated PCa patients but not in untreated hormone-naïve PCa.[45] 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. queried the AR status of 371 PCa samples by FISH.[46] In this study, AR was determined to be amplified in 23% of the 47 CRPC cases in contrast to 2 of 205 (1%) of the primary hormone-naïve PCa cases. In more recent studies using NGS, the frequency of AR aberration shows the same patterns. In the TCGA study of 333 hormones naïve PCa, no AR mutations were detected.[1] However, in studies where tumors were evaluated after androgen deprivation therapy (ADT), AR mutations and amplification frequencies were in the range of the initial reports.[34,47–50] Other mechanisms of AR resistance have been proposed including AR-V7 splice variants[51] and lineage plasticity to AR indifferent CRPC.[47,52]

4 | NGS REVEALS DISTINCT SUBCLASSES OF PCa; RECURRENT SPOP MUTATIONS PROVIDE A GOOD EXAMPLE WITH POTENTIAL CLINICAL IMPLICATIONS

NGS has made a major impact on our understanding of the types and frequency of somatic mutations in PCa.[13,49,53–61] Many of the NGS genomic studies have confirmed genomic events in PCa such as *PTEN* loss, *TP53* mutation/loss, and *ETS* gene fusions. One important observation is that some events such as *ETS* gene fusions and *SPOP* mutation (the most common point mutation in primary PCA) are mutually exclusive, leading to the view that PCa represents a collection of potentially definable molecular subclasses.[13,60,61] Such subtyping is largely based on the presence or absence of recurrent gene fusions. Comprehensive copy number profiling, whole exome sequencing (WES) and whole genome sequencing (WGS) 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; i.e., 8p loss and 8q gain), but relatively few focal

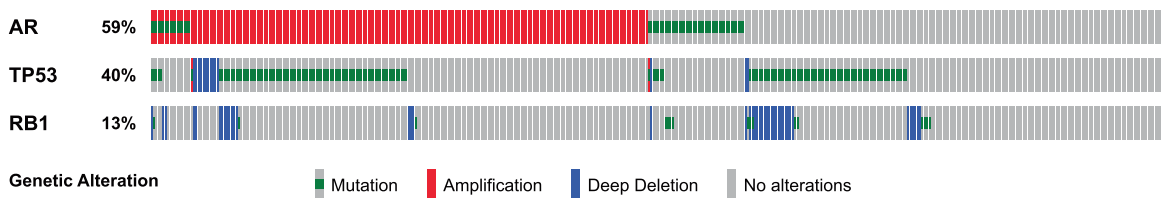

FIGURE 2 The landscape of advanced castration-resistant prostate cancer (CRPC) from the CRPC500 SU2C PCF study.[36] After AR signaling inhibitors therapy, there is a dramatic increase in AR alterations particularly gene amplification and somatic mutations. TP53 and RB1 loss are also commonly seen in CRPC. These alterations have been more frequently detected with the advent of next-generation sequencing, metastatic biopsies, and cfDNA analysis (oncoprint generated from cBioportal)

and/or high-level CNAs (usually focal *PTEN*, *TP53*, and *RB1* losses). The TCGA publication of 333 PCa genomes, transcriptomes, and methylomes solidify the idea of PCa molecular subclasses.[62]

Recurrent missense mutations in *SPOP* are the most common point mutations in primary PCa, occurring in about 10% of clinically localized and metastatic CRPC.[1,34,54,63,64] *Hotspot SPOP* mutations occur at F133, Y87, F102, and W131. Exploring cBioportal[65] for *SPOP* mutations in over 8521 PCa samples, 9.7% of cases demonstrate *SPOP* mutations with the most frequent mutations at F133 (F133L/F133V/F133I/F133S/F133C/Phe133Leu) (Figure 3).

SPOP mutations often co-occur with specific genomic features including deletions at 5q21, 6q15, and 2q21.[63,66] Molecularly, human PCa can be classified into those harboring rearrangements in *ETS* transcription factors (e.g., *TMPRSS2-ERG*) with co-occurring *PTEN* loss and those lacking *ETS* rearrangements. *SPOP* mutant PCa also defines characteristic genomic rearrangements, gene expression profiles, and methylation patterns.[1,63,64,66] *SPOP* mutations occur early in the natural history of PCa solely as heterozygous missense mutations with dominant-negative, selective loss of function towards the remaining wild-type allele.[8,53,66,67]

SPOP encodes the substrate recognition component of a CUL3-based E3 ubiquitin ligase, and PCa derived *SPOP* mutants appear to act as dominant-negative with selective loss of function.[66] 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,[67] the oncogenic co-activator TRIM24,[67,68] and AR itself.[69,70] There may also be phenocopies of SPOPmut PCa. For example, Mukhopadhyay et al. recently proposed that G3BP1 is an interactor of SPOP and functions as a competitive inhibitor of Cul3SPOP.[71] Their study supports the role of G3BP1 in disabling the tumor-suppressive Cul3SPOP, thus defining a PCa cohort independent of *SPOP* mutation.

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.[67,70,72–74] Blattner et al.[75] reported the development of the first conditional mouse model showing that *SPOP* mutation drives prostate tumorigenesis in vivo. Mice conditionally expressing mutant SPOP in the prostate have the minimal histologic phenotype, but show focal areas of cytologic atypia. In contrast, mutant SPOP results in early high-grade PIN with striking nuclear atypia in the setting of heterozygous *Pten* loss, and invasive, poorly differentiated carcinoma with homozygous *Pten* loss. *PTEN* deletions and mutations, while rare in the early phases of *SPOP* mutant human PCa,[1,63] become more frequent in CRPC [34,76] suggesting that *PTEN* deletion may contribute to the progression of *SPOP* mutant PCa.[77,78] 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.

As noted previously, *SPOP* and *TMRPSS2-ERG* mutant tumors are mutually exclusive. Bernasocchi et al. recently asked if this mutual exclusivity could provide important insights into the relationship of these two-common early PCa mutations.[79] They found that ERG upregulates wild-type SPOP to dampen AR signaling and sustain ERG activity through degradation of the bromodomain histone reader ZMYND11. Conversely, SPOP-mutant tumors stabilize ZMYND11 to repress ERG function and enable oncogenic AR signaling. This study proposes that SPOPmut renders tumor cells susceptible to ADT and ERG promotes sensitivity to high-dose androgen therapy and pharmacological inhibition of wild-type SPOP. This study designates SPOP and ERG mutations as distinct class of antagonistic cancer drivers and represents an opportunity to exploit this therapeutic vulnerability.[79] The relationship to AR signaling seen in in vitro and model systems also has strong correlates in human disease.

There are two intriguing clinical observations associated with SPOPmut PCa. SPOPmut PCa tends to have a higher level of PSA and AR signaling before therapy[1,80] and the frequency of SPOPmut cancers appear to be depleted after therapy.[31,34] The TCGA PCa study demonstrated higher AR signaling in localized SPOPmut PCa as compared to non-SPOP mutant PCa.[1] To extend these observations into significantly larger patient populations, Liu et al. developed a surrogate gene signature of SPOPmut PCa that allowed multiple

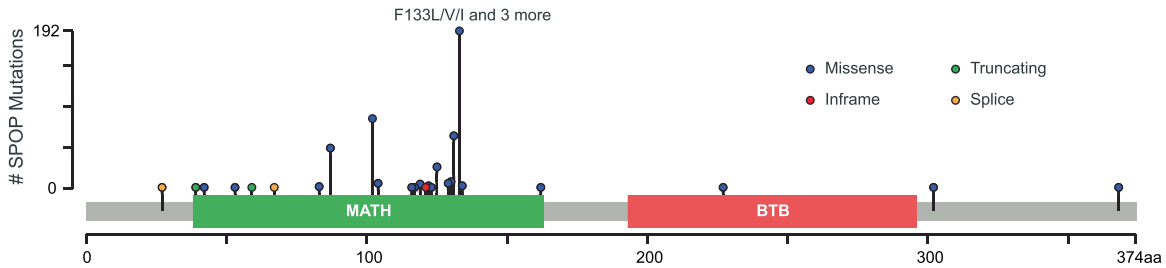

FIGURE 3 Distribution of SPOP mutations in the MATH domain. Data from cBioportal on 8521 prostate cancer (PCa) samples from 22 studies of localized and advanced PCa demonstrate a mutation frequency of 9.7%. The most common mutations are seen at F133, while other recurrent mutations occur at Y87, F102, and W131 (generated from cBioportal query December 10, 2021)

transcriptomic studies to also be included yielding a cohort of 8158 PCa patients.[80] This large study clearly demonstrated that presurgical PSA levels were higher in SPOPmut PCa as compared to *TMPRSS2-ERG* fusions PCa. Higher PSA levels in SPOPmut PCa were confirmed in four validation cohorts (Figure 4 from Liu et al.[80]). As previously reported in a PSA screening cohort, PCa exhibiting *TMPRSS2-ERG* fusions has lower PSA levels as compared to nonfusion PCa.[81] This finding was subsequently confirmed in a validation study using patient data from the GRID cohort.[82] Liu et al. also found significant associations with more favorable pathological and clinical outcomes.[80] SPOPmut PCa had the highest biochemical-free, metastasis-free, and lowest PCa-specific mortality compared with ERG-positive, ETS-positive, and other subtypes in the retrospective GRID cohort. One possible interpretation is related to AR signaling being highest in the SPOPmut subclass of PCa as compared to ERG, ETS, and other subclasses leading to earlier detection via PSA screening techniques. One would then predict that SPOP tumors would be smaller on clinical detection given the same PSA level. The opposite was observed for *TMPRSS2-ERG* fusion PCa, which present with lower PSA levels but larger tumors at the time of diagnosis compared to non-ERG tumors. The higher AR signaling observed in SPOPmut PCa also has implications for advanced PCa.

In early studies comparing the frequency of mutations between clinically localized untreated PCa and advanced, treated PCa there

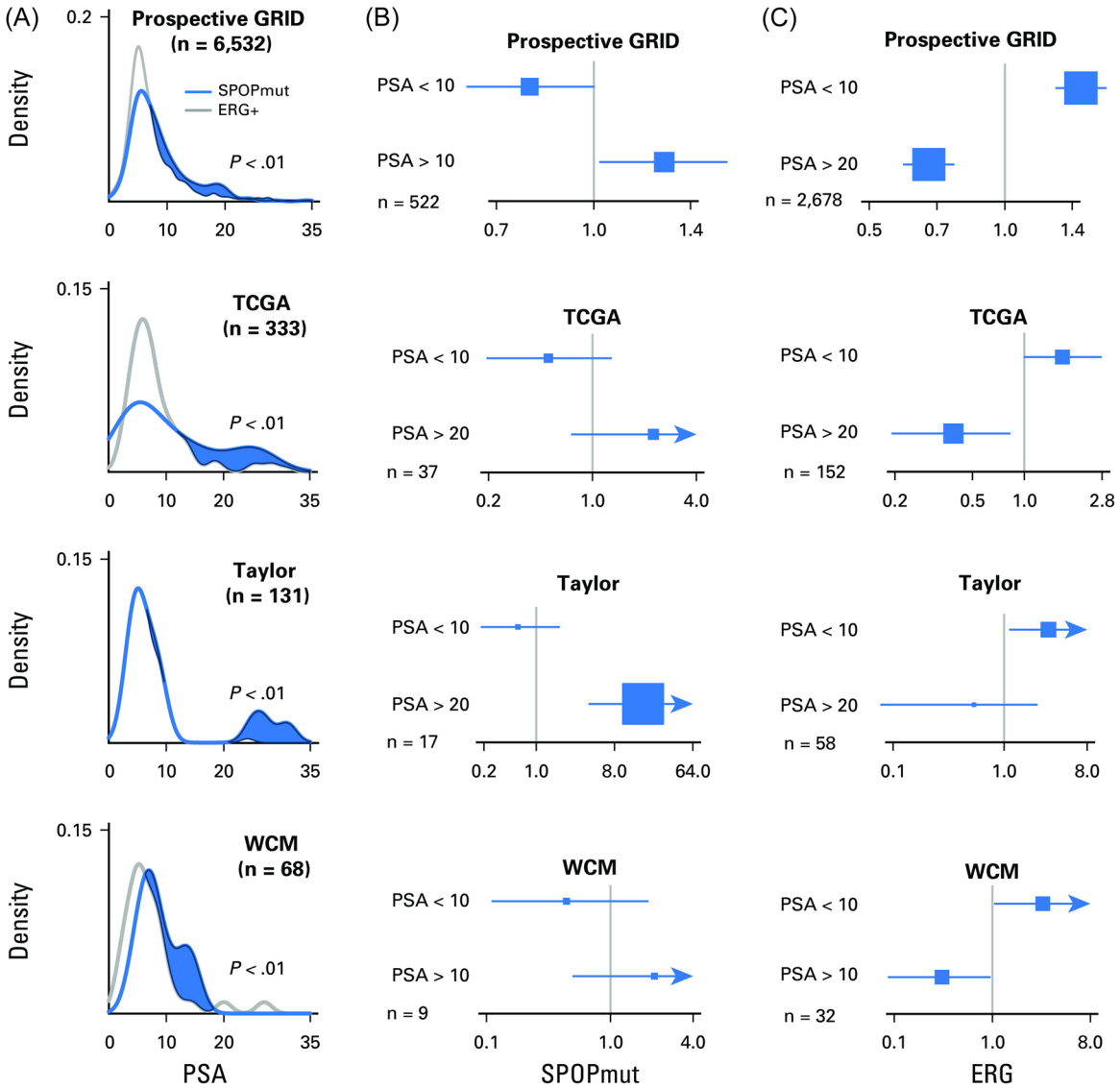

FIGURE 4 Association of SPOP mutant (SPOPmut) status and higher prostate-specific antigen (PSA) from four independent studies. (A) Enrichment of SPOPmut cases among higher PSA subgroups from prospective GRID, the Cancer Genome Atlas (TCGA), Taylor, and Weill Cornell Medicine (WCM) cohorts. *p* Value indicates the significant difference between SPOPmut and ERG-positive cases via the Kolmogorov–Smirnov test in each cohort. (B) Positive association between SPOPmut status and higher PSA via univariable analysis. The number of cases is shown in each cohort. (C) Positive association between ERG fusion status and lower PSA via univariable analysis. The number of cases is shown in each cohort. Reprinted from Liu et al.[80]

were some highly consistent observations.[31,34] AR mutations and amplifications while rare in localized PCa were common in metastatic advanced PCa (i.e., mCRPC). These findings also hold true for increased genomic loss of *TP53*, *RB1*, and *PTEN*. Each of these alterations is also associated with poorer outcome.[36] In contrast, these and other studies have now shown consistently a decrease in the frequency of SPOPmut PCa. One interpretation that is gaining more support is that SPOPmut PCa are more sensitive to AR targeted therapy and therefore are more responsive. This might account for why they are underrepresented in cohorts of advanced PCa.

Two studies examining men with advanced PCa treated with ARSi both suggested SPOPmut PCa have an increased sensitivity to ARSi.[83,84] However, both studies could be seen as hypothesis-generating given the small numbers[83] and mixed treatment populations.[84] Nakazawa et al. recently reported on the outcome of 72 consecutive SPOPmut PCa from a single institution.[84] The SPOPmut PCa came from patients who had durable responses to first-line ADT, but their cohort included a heterogeneous group of patients with metastatic and nonmetastatic hormone-sensitive disease, as well as patients receiving concurrent chemotherapy and/or ASRi (i.e., abiraterone, enzalutamide). Therefore, from these observations and the observations regarding the decreased frequency of SPOPmut in ARSi treated patients, one can hypothesize that increased sensitivity of SPOPmut tumors could have clinical implications, however, they will require larger prospective studies.

Finally, *SPOP* mutations have been observed in other cancers at the varying frequency (Figure 5A). In this study of over 10 K tumors from MSKCC,[85] in addition to PCa, endometrial, adrenocortical, and cancers of unknown origin also harbored *SPOP* mutations. However, as in the example of cancers of unknown origin, the mutations occur outside the MATH domain and with endometrial cancer, they occur in the MATH domain but at sites not seen in PCa. In the former case, the mutations are probably passenger mutations. In the latter case, the mutations may serve a different biologic function.

In summary, SPOP mutations are common and define a discrete subclass of PCa, which may have therapeutic implications.

5 | 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.[86,87] Multifocal PCA represent clones of independent origin, as supported by numerous

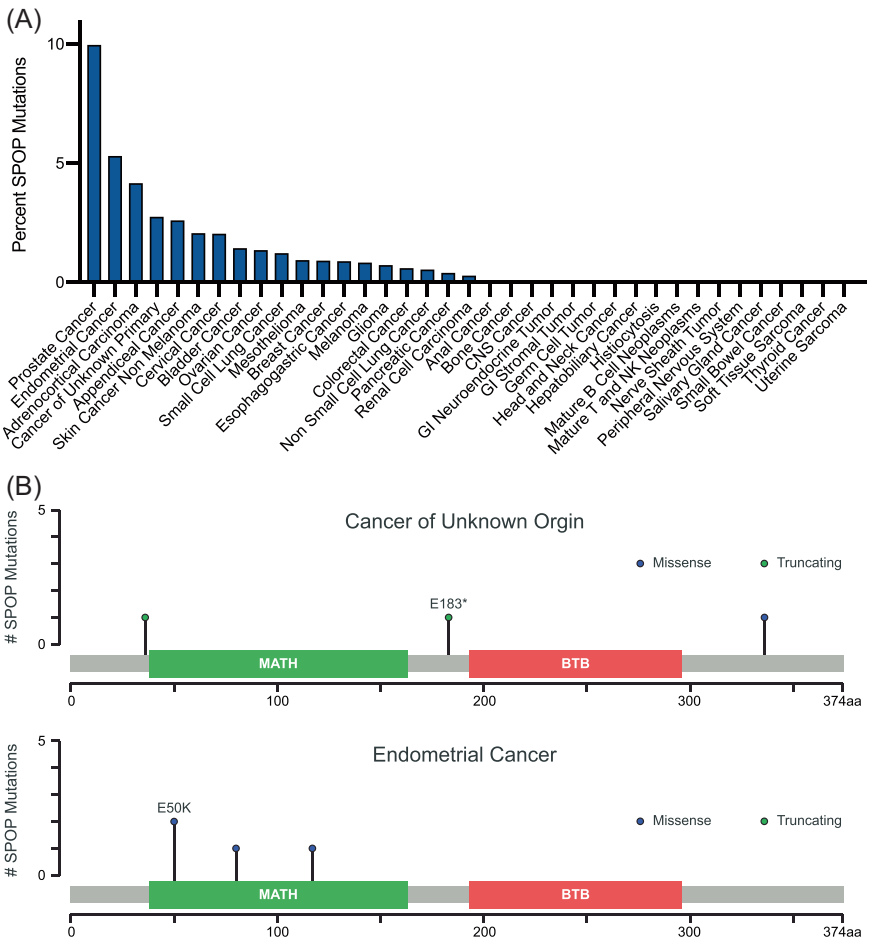

FIGURE 5 Frequency of SPOP mutations in over 10 K tumors from MSKCC.[85]
(A) Besides prostate cancer, a number of other cancers demonstrate SPOP mutations with the frequencies of mutations shown for tumor subsets with greater than 20 samples.
(B) However, the types of mutations are different. For example, tumors of unknown origin show mutations outside of the MATH domain, and endometrial cancer demonstrates mutations in the MATH domain but at sites not typical for prostate cancer

approaches, including *ERG* rearrangement status (by FISH or Immunohistochemistry (IHC))[55,88–99]; in contrast, lethal, metastatic CRPC appears uniformly *ERG* rearrangement positive or negative in all sites in a given patient, consistent with the clonal origin, although the extensive subclonal structure is present.[49,100–105]

ERG rearrangement status (ERG^+ or ERG^-) is a useful clonal marker to demonstrate spatially distant multifocal tumors.[89,91,94,95,98,99,106] Several anecdotal NGS studies (*n*'s ≤ 5–10) have added complexity to tracking the eventual CRPC clone through identifying intrafocal heterogeneity at RP.[100,104,105,107–110] These series of locally advanced PCa vary from reporting little divergence to a complete lack of shared alterations between the index focus and lymph node metastases and/or CRPC. Haffner et al. tracked the lethal clone in a single patient.[78] 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. explored PCa clonal evolution in 10 men with heavily treated CRPC at rapid autopsy.[104] 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 (e.g., 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.

In a recent pilot study, we collected 12 RP specimens from men who subsequently developed metastatic mCRPC.[111] Based on combined pathology and molecular analysis, seven (58%) RP specimens harbored monoclonal and topographically continuous disease, albeit with some degree of intratumor heterogeneity; four (33%) specimens showed true multifocal disease, and one displayed monoclonal disease with discontinuous topography. Early (truncal) events in primary PCa included *SPOP* p.F133V (one patient), *BRAF* p.K601E (one patient), and *TMPRSS2-ERG* rearrangements (nine patients). Activating *AR* alterations were seen in nine (75%) mCRPC patients, but not in matched primary PCa. Hotspot *TP53* mutations, found in metastases from three patients, were readily present in matched primary samples. Alterations in genes encoding epigenetic modifiers were observed in several patients (either shared between primary foci and metastases or in metastatic samples only). WES-based phylogenetic reconstruction and/or clonality scores were consistent with the index focus designated by pathology review in six out of nine (67%) cases. The three instances of discordance pertained to monoclonal, topographically continuous tumors, which would have been considered unique diseases in routine practice. Overall, this pilot study emphasizes pathologic and molecular heterogeneity of primary PCa, and suggests that comprehensive IHC-assisted pathology review and genomic analysis are highly concordant in nominating the "index" primary PCa area.

In the near future, more and more studies will use single-cell sequencing approaches to gain an understanding of cancer heterogeneity during progression as has been recently demonstrated.[112–114] Key questions will be to determine which mutations preexist at low numbers and which evolve during therapy.

6 | THE DYNAMIC NATURE OF NGS DATA CAPTURED BY CBIOPORTAL

The availability of genomic data on public portals and repositories is greater than ever. One resource that stands out and is highly valuable for PCa researchers is called cBioportal[65] (available at https://www.cbioportal.org/). As of December 15, 2021, there are 22 nonredundant PCa studies listed on cBioportal. These data contain 8360 PCa samples. The majority of samples are from localized PCa but there are an increasing number of studies that have advanced PCa samples including mCRPC and neuroendocrine PCa (NEPC) samples. Therefore, any general query of the entire 8,360 will provide information such as mutation frequencies for a variety of PCa tumor stages and treatment modalities. This is important to note when use such data for studies.

The other significant variable is that each study was performed with some specific genomic platform. Some studies are whole genome, some whole exome, and some utilize targeted exome panels focused on known and predefined mutations. Therefore, the platform may also impact the frequency of a given mutation. For example, if you query the entire cohort for *TMPRSS2-ERG* gene fusions (using the query tool only allows for ERG queries) gives the following result. As demonstrated by Figure 6, three studies report a frequency of around 47% but the remaining studies show significantly lower levels. In this example, the reality is that the assays are not all designed to capture structural variations around *TMPRSS2-ERG* and, therefore, underestimate this common gene fusion unless they also combine an RNA-based assay. A further example is *SPOP* mutations. These are the most common recurrent gene mutations (see above), however, many of the earlier studies do not cover the *SPOP* gene mutations because they were not included in the design of the targeted NGS panels. Therefore one needs to exclude studies that do not cover your genes of interest, and this is one of the features that can improve the accuracy of a cBioportal query.

There are other useful websites to discover the frequency and association of mutations in prostate and other cancers. For example, the international cancer genome consortium (https://dcc.icgc.org/) is another publicly available website to query genomic data across

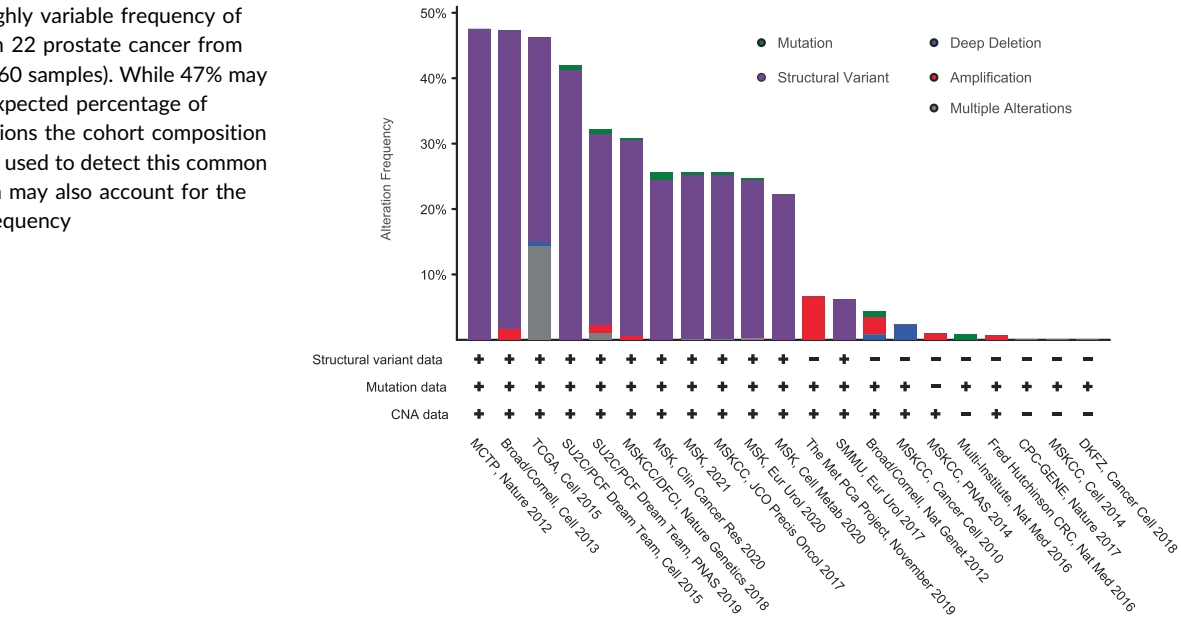

FIGURE 6 Highly variable frequency of ERG alterations in 22 prostate cancer from cBioportal (n = 8360 samples). While 47% may be close to the expected percentage of TMRPSS-ERG fusions the cohort composition and the platforms used to detect this common somatic alteration may also account for the highly variable frequency

many cancer cohorts. There are six PCa data sets available on this website. The tool provides some additional whole genome data sets that are not available on cBioportal. AACR Genie website (https://genie.cbioportal.org) also provides data on many tens of thousands of annotated cancer and uses the cBioportal as a query dashboard.

In summary, the heterogeneity of PCa is mimicked by the heterogeneous type of genomic data available from multiple cohorts. Understanding the background and types of cases collected for each study is essential for using these valuable tools to develop hypotheses.

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CONFLICTS OF INTEREST

MAR is a coinventor on issued US Patents in the field of diagnosis and therapeutics for *ETS* gene fusion prostate cancer (the University of Michigan and Harvard Medical School) and SPOP (Cornell University). He is on the SAB of Neogenomics, Inc. His laboratory receives active support from Roche and Novartis.

DATA AVAILABILITY STATEMENT

Data sharing not applicable—no new data generated

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