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Research Article

Histopathologic and Molecular Characterization of *IDH*-Mutant Prostatic Adenocarcinoma

Benzion Samueli, Hikmat Al-Ahmadie, Ying-Bei Chen, Anuradha Gopalan, Judy Sarungbam, Satish K. Tickoo, Victor E. Reuter, Samson W. Fine, Jie-Fu Chen*

Department of Pathology and Laboratory Medicine, Memorial Sloan Kettering Cancer Center, New York, New York

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ABSTRACT

Gain-of-function isocitrate dehydrogenase (*IDH*) mutations are pathogenically significant in many tumor types and are actionable in cholangiocarcinoma, low-grade glioma, and acute myeloid leukemia. Rare *IDH* mutations have been described in prostatic adenocarcinoma (PCa). Recent publications have suggested that psammomatous calcifications in PCa are associated with *IDH1* mutations. In this retrospective study, we queried our institutional clinical sequencing database (cohort 1), and previously published PCa data sets in cBioPortal (cohort 2). Samples were stratified based on oncogenic hotspot *IDH* mutations at *IDH1* R132 and *IDH2* R140/R172, and other nonhotspot *IDH* mutations. Seventeen (0.4%) cases were identified from 4033 PCa cases in cohort 1 harboring mutually exclusive oncogenic hotspot *IDH1* (N = 15, 1 of which was subclonal) or *IDH2* (N = 2) mutations, and 20 (0.5%) cases had nonhotspot *IDH1/2* mutations. A histologic review of 13 cases with *IDH1* hotspot mutations and available material showed grade group 3 or higher disease. Immunohistochemistry was performed on cases with *IDH1* hotspot mutations when possible and showed AR, PSA, PSMA, and NKX3.1 positive in all the 4 cases stained. In cohort 2, 9 cases (0.3%) harboring *IDH1* hotspot mutations were identified from 2749 patients, and 9 cases carried nonhotspot *IDH1/2* mutations. The combined cohorts of 23 PCa cases with clonal *IDH1* hotspot mutations had no ETS fusions, *SPOP* hotspot mutations, and somatic or germline alterations in *BRCA1/2, ATM, RB1,* or *AR*; 19 cases with successful microsatellite instability testing were all microsatellite stable. Conversely, among 29 cases with nonhotspot *IDH* mutations, there were 4 with *TMPRSS2::ERG* fusions, 6 with *SPOP* hotspot mutations, and 10 with *AR* amplifications/hotspot mutations; 8 were microsatellite instability high. Notably, two cases with *IDH1* hotspot mutations had psammomatous calcifications. Our findings provide evidence that *IDH1* hotspot mutations serve as driver alterations in this rare yet distinct molecular subset of PCa. Further studies are warranted to correlate response to androgen deprivation and IDH inhibitors.

Introduction

Isocitrate dehydrogenase (IDH) functions in the Krebs cycle by catalyzing the oxidative decarboxylation of isocitrate to α-ketoglutarate.[1,2] Oncogenic hotspot *IDH1* R132 and *IDH2* R140/R172 mutations are most frequently detected in central nervous system gliomas and acute myelogenous leukemia, in which they have clear treatment implications.[1-6] The Food and Drug Administration has approved IDH inhibitors for malignancies based on their *IDH* gene mutation status, including the IDH1 inhibitor for cholangiocarcinoma as well as IDH1 and IDH2 inhibitors for low-

* Corresponding author.
E-mail address: chenJ14@mskcc.org (J.-F. Chen).

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grade glioma, acute myelogenous leukemia, and refractory myelodysplastic syndrome. *IDH* mutations have been described in a minority of prostatic adenocarcinoma (PCa) cases,[7-9] the clinical relevance of which remains largely underexplored. However, it has been suggested that *IDH*-mutant PCa may be a unique cohort both in terms of the overall genetic profile and clinical behavior.[7,10]

Two recent reports[11,12] have identified 6 high-grade PCa cases from routine clinical practice (5 with grade group (GG) $\geq$ 3 and 1 with grade group 2 with tertiary pattern 5) and an additional 4 cases from The Cancer Genome Atlas Prostate Adenocarcinoma (TCGA-PRAD) cohort with psammomatous calcifications. This is a notable finding given that calcifications are most often identified in benign prostatic conditions.[13,14] Nine of these 10 cases harbored oncogenic hotspot mutations in the *IDH1* R132 locus. Although the sample size in these studies was small, the authors suggest that psammomatous calcifications are highly associated with and may be used to identify PCa with *IDH1* mutations.

In the present study, we aimed to further elucidate the histologic and molecular features of PCa with *IDH1/2* oncogenic hotspot mutations. We present the largest study to date on *IDH1/2* mutations in PCa, detected using comprehensive genomic profiling. We further examined the sensitivity of psammomatous calcifications as a marker for *IDH* mutations.

Materials and Methods

Patient Selection

We retrospectively studied patients with PCa from 2 cohorts. Cohort 1 included patients who had pathology review and genomic profiling of their PCa tumors at Memorial Sloan Kettering Cancer Center (MSKCC). Cohort 2 comprises previously published PCa data sets[7-9,15-18] with the *IDH1/2* mutation status available in cBioPortal,[19,20] including The Cancer Genome Atlas (TCGA) Pan-Cancer Analysis Project,[21] as well as the 4 *IDH1*-mutant PCa cases from the TCGA-PRAD cohort reported by Mehra et al.[7,11] The duplicated entries from additional TCGA-based studies and published cases contributed by MSKCC were excluded from cohort 2 upon manual review. Confirmed small cell carcinoma or prostatic neuroendocrine carcinoma cases were also excluded from analysis. The study was approved and is under the oversight of the MSKCC Institutional Review Board.

For cohort 1, all *IDH*-mutant cases with slides available were reviewed by experienced genitourinary pathologists to identify morphologic features potentially associated with *IDH* mutations, including psammomatous calcifications. For patients whose genomic profiling was performed on a needle biopsy (NB) specimen and a subsequent radical prostatectomy (RP) specimen was available, the latter was also reviewed to rule out false negatives owing to sampling errors on NB. Metastatic specimens were reviewed for calcifications only and not for morphologic growth patterns.

From both cohorts, the rate of *IDH* mutations in prostate cancer was assessed. Patients were stratified based on oncogenic hotspot *IDH1* R132 or *IDH2* R140 or R172 mutations vs all other nonhotspot *IDH* alterations. Patients were then stratified based on *IDH* mutation status for comparison of the molecular characteristics.

Immunohistochemical Stains

The following immunohistochemistry (IHC) stains were used to analyze protein expression for cohort 1 cases with available material: IDH1 with R132H mutations (IDH1 R132H, clone H09; Biozol), as well as PCa-associated markers, including AR (SP107; Abcam), PSA (polyclonal/760-2506; Ventana), PSMA (3E6; Agilent/Dako), NKX3.1 (EP356; Cell Marque), and ERG (EPR3864; Vantana).

Comprehensive Genomic Profiling and Microsatellite Status

Genomic profiling was performed on the genomic DNA from tumor tissue and matched normal peripheral blood samples using the Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) panel as described previously.[22,23] Briefly, MSK-IMPACT is a hybrid capture-based next-generation sequencing assay, which covers the exons and selected intronic regions of 341 to 505 genes to detect somatic single-nucleotide variants (including small insertions/deletions), copy number alterations, and select structural variants from tumor tissue. Variants of germline or clonal hematopoiesis origin are filtered during bioinformatic analysis via comparison with the patient's matched normal samples. The oncogenicity of detected variants was classified based on OncoKB database (oncokb.org).[24] Information regarding germline variants and their pathogenicity was obtained from the reports of patients who underwent germline genetic testing.

For cases in cohort 1, tumor mutation burden was defined as the total number of nonsynonymous mutations (single-nucleotide variants) in the targeted coding region. Microsatellite instability (MSI) status in the tumor was analyzed with MSIsensor using previously validated cutoffs, as follows: an MSIsensor score of $\geq$10 was defined as MSI high (MSI-H), whereas an MSIsensor score of <3 was defined as microsatellite stable (MSS); MSIsensor scores between the 2 cutoffs ($\geq$3 and <10) were classified as MSI indeterminate, and the MSI status was further clarified using IHC for DNA mismatch repair (MMR) proteins (MLH1, PMS2, MSH2, and MSH6).[25,26] For selected cases in cohort 2, MSI status was evaluated using MANTIS, and scores $\geq$ 0.4 were recommended for MSI-H.[27]

Results

From the MSKCC cohort (cohort 1), 4033 patients with PCa with MSK-IMPACT sequencing data were identified. Seventeen (0.4%) patients had mutually exclusive oncogenic hotspot *IDH1* (N = 15) or *IDH2* (N = 2) mutations (Table 1); 16 of 17 were clonal (case numbers 1-16 in Table 1) and 1 *IDH1* mutation was subclonal (case number 17 in Table 1). An additional 20 patients (0.5%) had nonhotspot *IDH1* and/or *IDH2* mutations. Among the 16 cases with clonal hotspot mutations, molecular results were obtained from 5 RP specimens, 7 NB specimens, and 4 metastases. The age range at primary diagnosis (time of first NB) was 48 to 84 years (median = 65 years).

Fifteen of these 16 patients had slides from the primary tumor for review, 2 of which had additional samples from metastases and 3 had both NB and RP specimens available. Among the primary prostatic specimens were 8 RP specimens and 10 NB specimens. Five of the 8 RP specimens had slides for the entire prostate or entire tumor nodule, 3 of which had tumors that extended from anterior-to-posterior aspects, whereas 2 were located at anterior prostate. The other 3 RP specimens only had selected slides available for review; 1 tumor showed extensive involvement from anterior-to-posterior, whereas 1 tumor each was predominantly anterior or posterior based on the available slides and report. All

Table 1
Clinical characteristics of PCa with *IDH* oncogenic hotspot mutations in cohort 1

Case no.	Age at earliest diagnosis (NB)	Material for molecular testing	*IDH* mutation	GG (NB)	GG (RP)	Slides reviewed	Pertinent histologic findings
1	64	RP	*IDH1* R132H	3	3 With TP	RP	EPE
2	49	RP	*IDH1* R132H	5	5	NB/RP	EPE, BNI, margin+, psammomatous calcification
3	59	NB	*IDH1* R132H	4	3	NB/RP	EPE, SVI, margin+
4	63	NB	*IDH1* R132H	5		NB	
5	65	RP	*IDH1* R132H	5	3 With TP5	RP	
6	68	NB	*IDH1* R132H	5		NB	
7	64	LN metastasis	*IDH1* R132H	3		N/A	
8	70	NB	*IDH1* R132H	4		NB	
9	67	NB	*IDH1* R132H	5		NB	
10	56	LN metastasis	*IDH1* R132C	1	5	LN metastasis/RP	EPE, LVI, margin+
11	72	RP	*IDH1* R132C	2	3	NB/RP	EPE, psammomatous calcification (single focus)
12	N/A	LN metastasis	*IDH1* R132C	N/A	5	LN metastasis/RP	EPE, LVI
13	70	NB	*IDH1* R132C	5		NB	
14	84	NB	*IDH1* R132C	5		NB	EPE, diffuse ERG immunoreactivity
15	48	RP	*IDH2* R172S	N/A	5	RP	EPE, margin+
16	81	Mesorectal cytology	*IDH2* R172S	4		NB	
17	46	RP	*IDH1* R132H (subclonal)	3	3	RP	

BNI, bladder neck invasion; EPE, extraprostatic extension; GG, grade group; LN, lymph node; LVI, lymphovascular invasion; N/A, not available; NB, needle biopsy; PCa, prostatic adenocarcinoma; RP, radical prostatectomy; SVI, seminal vesicle invasion; TP5, tertiary pattern 5.

cases were high grade with GG3 4/15 (including 2 RP with tertiary pattern 5), GG4 2/15, and GG5 9/15. Tumors displayed poorly formed (9/15), fused (10/15), and cribriform (6/15) glands and single cells (7/15). None of the tumors in our study displayed atrophic appearance (Fig. 1). One case showed prominent vacuolization; no ductal features or necrosis were identified. Only 2 cases displayed psammomatous calcifications (case numbers 2 and 11 in Table 1). These findings were observed in the RP specimens performed at MSKCC. One patient (case number 2) had previous NB showing 7 of 12 cores with PCa (highest GG5), and the other (case number 11) had 10 of 11 cores with PCa (highest GG2), both lacking psammomatous calcifications in the NB cores available for review. The above assessment includes the 2 cases with oncogenic hotspot *IDH2* mutations (1 GG4 and 1 GG5), both of which had cribriform glands and 1 of which displayed single cells.

From public data sets available in cBioPortal (cohort 2), 9 of 2749 (0.3%) patients with sequencing data had oncogenic hotspot *IDH1* mutations, and 9 (0.3%) had nonhotspot *IDH1/2* mutations.

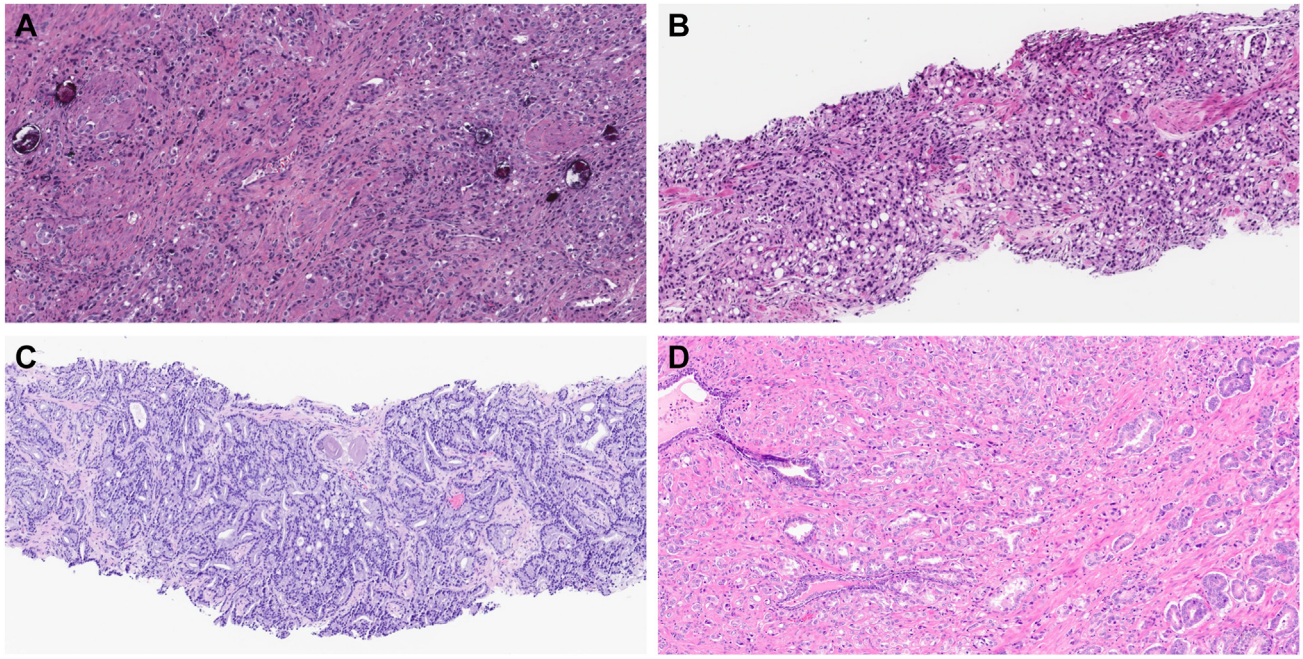

Figure 1.
Histologic features of *IDH*-mutant prostate cancer. (A) A low-power representative of a case with high-grade prostatic adenocarcinoma (Gleason score 5 + 5 = 10, grade group 5) and an *IDH1* R132H mutation exhibiting psammomatous calcification on RP. (B) Prominent vacuolization was observed in 1 case with an *IDH1* R132H mutation; no psammomatous calcifications were identified (representative image from NB). (C, D) Additional cases of *IDH1*-mutant prostatic adenocarcinoma without psammomatous calcification on NB and RP, respectively. IDH, isocitrate dehydrogenase; NB, needle biopsy; RP, radical prostatectomy.

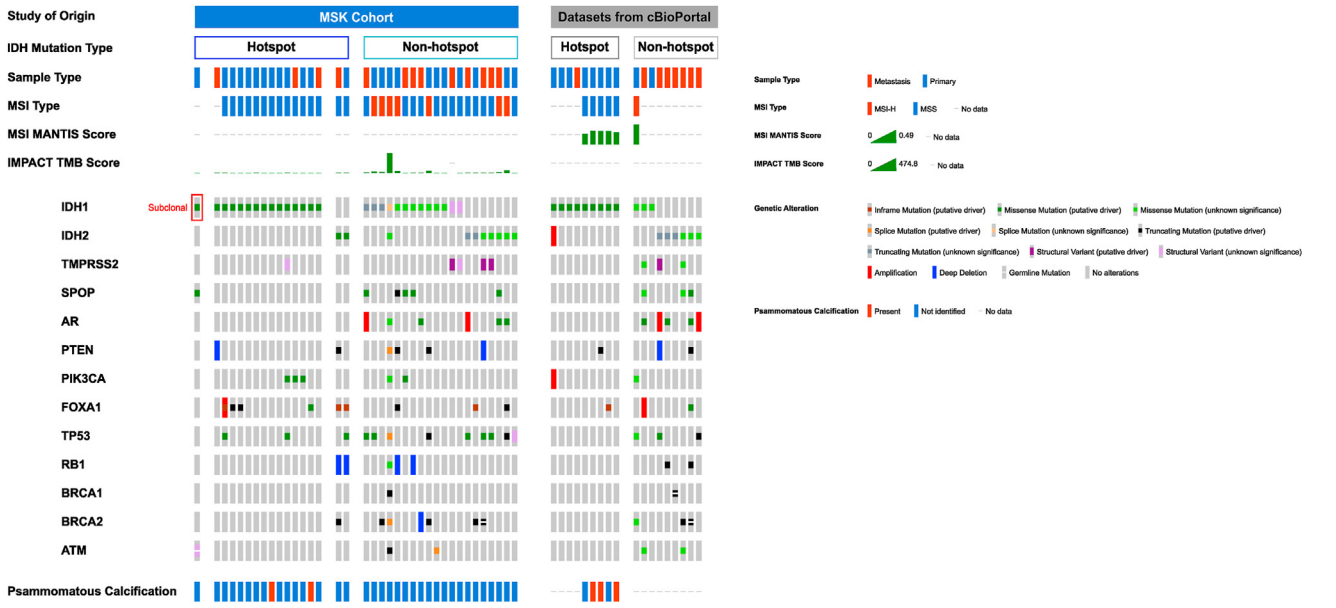

Figure 2.
Molecular characteristics of *IDH*-mutant prostate cancer. Oncoprint summarizes the molecular alterations of *IDH*-mutant prostatic adenocarcinoma cases from our institutional clinical sequencing database (N = 37; cohort 1) and previously published prostatic adenocarcinoma data sets in cBioPortal (N = 18; cohort 2). Samples were stratified based on oncogenic hotspot *IDH* mutations at *IDH1* R132 and *IDH2* R140/R172, and other nonhotspot *IDH* mutations. IDH, isocitrate dehydrogenase; IMPACT, -Integrated Mutation Profiling of Actionable Cancer Targets; MSI, microsatellite instability; MSI-H, MSI high; MSK, Memorial Sloan Kettering; MSS, microsatellite stable; TMB, tumor mutation burden.

Thus, the combined total incorporating both cohorts represent 6929 patients, of which 25 had clonal, mutually exclusive *IDH1* or *IDH2* hotspot mutations, 1 had subclonal *IDH1* hotspot mutations, and 29 had nonhotspot *IDH* mutations (Fig. 2).

Among the 23 cases with clonal *IDH1* hotspot mutations, *IDH1* R132H mutations comprised 65% (n = 9 [cohort 1] and n = 6 [cohort 2], respectively); the remainder were *IDH1* R132C mutations, with a single *IDH1* R132G mutation in cohort 2. None of these cases had classic PCa driver alterations such as *ETS* gene fusions or *SPOP* hotspot mutations. Additional pertinent somatic or germline alterations in *BRCA1/2*, *ATM*, *RB1*, and *AR* were also not observed. Conversely, somatic alterations in *PTEN*, *PIK3CA*, *TP53*, and *FOXA1* were detected in a subset of cases with *IDH1* hotspot mutations.

We also observed 2 cases with *IDH2* R172S hotspot mutations. These 2 cases did not harbor ETS gene fusions or *SPOP* hotspot mutations. However, both cases had an *RB1* deletion and a *FOXA1* in-frame deletion; 1 case also had a somatic *BRCA2* mutation. Among the patients with *IDH1/2* hotspot mutations (n = 26), germline sequencing data were available from 14 patients in cohort 1, as well as from 4 patients in cohort 2. Only 1 patient with a clonal *IDH1* R132H mutation carried a heterozygous germline pathogenic *APC* mutation c.3920T>A (p.I1307K). The case with a subclonal *IDH1* mutation carried a germline pathogenic *ATM* intragenic deletion and a somatic clonal *SPOP* F102V mutation.

Conversely, among 29 cases with nonhotspot *IDH* mutations (20 from cohort 1 and 9 from cohort 2), 4 had *TMPRSS2::ERG* fusions and 6 had *SPOP* hotspot mutations. Four cases had *AR* amplifications, and 6 had *AR* mutations including hotspots L702H, T878A, and H875Y, which have been associated with castration resistance.[28-30] Additional pertinent findings included truncating mutations and deletions in *PTEN* and *RB1*, as well as mutations in *TP53*, *PIK3CA*, and *FOXA1*. *BRCA2*, *ATM*, and *BRCA1* were mutated in 8, 4, and 2 cases, respectively. Three cases with nonhotspot *IDH* mutations from cohort 1 carried pathogenic germline variants, including 1 case in *BRCA2*, 1 case in *APC*, and the third case in both *CHEK2* and *MSH2* genes.

Microsatellite status was evaluated for all cases in cohort 1 using MSIsensor/MMR IHC, and for 5 cases from cohort 2 using MANTIS. Of the 17 cases with oncogenic hotspot *IDH* mutations in cohort 1, 15 had successful MSI status assessment and were all classified as MSS; 14 of 15 were classified as MSS using MSIsensor, and 1 had an MSIsensor score at an indeterminate range but subsequent IHC showed retained MMR protein expression in the tumor. Similarly, the 4 cases with *IDH1* hotspot mutations in cohort 2 showed low MANTIS scores, consistent with MSS. In contrast, we observed an enrichment of MSI-H tumors among cases with nonhotspot *IDH* mutations, with 6 classified as MSI-H using MSIsensor and 1 using IHC in cohort 1, as well as an additional case in cohort 2 with a MANTIS score at the MSI-H range. The *IDH* mutations in these MSI-H tumors were more commonly substitutions (n = 7) as C>T or C>A than small insertions/deletions (n = 2).

Four cases from cohort 1 had sufficient material available for additional IHC workup, including 3 cases with clonal *IDH1* R132C mutations and 1 case with clonal *IDH1* R132H mutations. Although the case with the R132H mutation was strongly and diffusely positive for IDH1 R132H IHC, 1 case with a *IDH1* R132C mutation was negative whereas the other 2 showed weak-to-moderate labeling. All the 4 tumors displayed diffuse, strong staining for AR, NKX3.1, and PSMA, as well as weak staining for PSA. The only case with positive ERG staining (case number 14 in Table 1) had an *IDH1* R132C mutation and a translocation of unknown significance involving *TMPRSS2* exons 1 to 2 and *GPHN* exons 2 to 23 (on chromosome 14). Although the possibility of a more complex rearrangement involving *ERG* cannot be completely excluded, further investigation was not possible owing to the lack of material.

Discussion

IDH1/2 mutations in central nervous system, hematologic, and biliary malignancies have therapeutic significance, and it is biologically plausible that the same could be true for PCa. Not only are

Table 2
Previous studies of prostate cancer with *IDH* mutations[a]

Reference	Tumor type	Specimen type	Method of detection	No. of cases tested for *IDH1* mutations	Rate of *IDH1* mutations, N (%)	No. of cases tested for *IDH2* mutations
Yan et al[31]	Not specified	Not specified	PCR and Sanger sequencing	7	0 (0)	7
Kang et al[4]	Not specified	Not specified	PCR-SSCP and Sanger sequencing	75	2 (2.7)	0
Bleeker et al[3]	Not specified	Not specified	PCR and Sanger sequencing	4	0 (0)	0
Park et al[32]	Not specified	Not specified	PCR-SSCP and Sanger sequencing	0	N/A	123
Ghiam et al[33]	Not specified	Not specified	SNP array and Sanger sequencing	158	2 (1.2)	158
Mauzo et al[34]	Not specified	Not specified	IHC	118	3 (2.5)	No
Hovelson et al[35]	Primary	Prostatectomy	Targeted NGS	116	1 (0.8)	Not specified
Cancer Genome Atlas Research Network[7]	Primary	Prostatectomy	NGS (WES) and methylation analysis	333	3 (0.9)	333
Palapattu et al[36]	Primary	Biopsy	Targeted NGS	13	1 (7.7)	Not specified
Armenia et al[9]	Primary and metastasis	Not specified	NGS (WES)	1013	9 (0.9) with 8 at *IDH1* R132	1013
Zhang et al[37]	Primary	Prostatectomy	IHC; selected cases confirmed by Sanger sequencing	336	2 (0.6)	0
Hinsch et al[10]	Primary	Prostatectomy	IHC	15,531	42 (0.3)	0
Zhao et al[38]	Metastasis	Biopsy	NGS (WGS) and methylation analysis	100	1 (1)	100
Mehra et al[11]	Primary	Prostatectomy	IHC and targeted NGS	4	4 (N/A)	Not specified
Montero-Ovalle et al[39]	Primary	Prostatectomy	PCR and Sanger sequencing	112[b]	1 (0.9)	0
Galea et al[12]	Primary	Prostatectomy	IHC and targeted NGS	2	2 (N/A)	Not specified

IHC, immunohistochemistry; MSK-IMPACT, Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets; N/A, not applicable; NGS, next-generation sequencing; SNP, single-nucleotide polymorphism; SSCP, single-strand conformation polymorphism; WES, whole exome sequencing; WGS, whole genome sequencing.

[a] This table excludes prior studies that were based on the MSK-IMPACT clinical sequencing data set.

[b] In the study by Montero-Ovalle,[39] only the 41 samples demonstrated to be negative for the *TMPRSS2-ERG* fusion were directly tested for *IDH1* mutations on the presumption that the 71 fusion-positive patients would be *IDH1* wild type.

IDH isoforms druggable targets, but also the molecular mechanisms by which *IDH* mutations may contribute to oncogenesis are distinct from other common prostate cancer alterations. In this study, we describe the largest cohort of PCa with oncogenic hotspot *IDH1/2* mutations, including 14 clonal *IDH1*-mutated cases from MSKCC and 9 *IDH1*-mutated cases from public data sets, and the first 2 reported *IDH2*-mutated PCa. These cases represent a molecularly distinct cohort that lacks *ETS* gene fusions, *SPOP* hotspot mutations, *BRCA1/2* alterations, or *AR* alterations, and was classified as MSS. As in prior studies, we found that *IDH*-mutant cases were high grade (GG $\geq$ 3), although this may represent a selection bias in the samples submitted for genomic profiling. In contrast to previous studies, which demonstrated a strong relationship between the presence of psammomatous calcifications and *IDH1* hotspot mutations, we observed this finding in 2 of 13 *IDH1*-mutant cases from cohort 1.

In PCa, studies on recurrent *IDH* mutations have primarily focused on the *IDH1* R132 locus, with a frequency ranging from 0% to 7.7% (Table 2).[3,4,7,10-12,31-39] Some earlier studies used IHC specific to *IDH1* R132H mutations and may have missed mutations such as R132C and R132G.[10] In the current study, we aggregated 23 PCa cases with clonal *IDH1* hotspot mutations identified using next-generation sequencing–based genomic profiling from 6782 patients, 2 cases with *IDH2* hotspot mutations, as well as 29 PCa cases with nonhotspot *IDH* mutations, making it the largest examination of *IDH*-mutant PCa to date. Oncogenic hotspot *IDH1* mutations occurred in 0.3% of profiled patients with PCa, a rate similar to the earlier snapshot of the MSK sequencing cohort in 2020[40] and other large sequencing studies. This group of PCa appears to be molecularly distinct, lacking the classic PCa driver events as well as *BRCA1/2* and *ATM* alterations; similar findings were observed in prior larger sequencing studies.[7-9] It has been demonstrated that D-2HG (an "oncometabolite" of mutant IDH) affects demethylase activity and that markedly abnormal methylation patterns are seen in other tumors with *IDH* mutations.[1,2] Analysis on *IDH1*-mutant PCa revealed genome-wide hypermethylation.[7,38] Some have suggested that the CpG methylator phenotype in PCa generally[41,42] and the *IDH1*-mutated PCa specifically[10] are associated with worse clinical outcomes. Based on these observations, it is plausible that *IDH1*-mutated PCa is a unique type of *ETS*-negative prostate cancer and deserves to be investigated for its therapeutic and prognostic implications.[43]

The IDH pathway is only weakly connected to the AR pathway.[44-46] Our investigation on selected cases with *IDH1* R132C and R132H mutations showed similar staining patterns for AR, NKX3.1, PSA, and PSMA. Although data were limited, the findings suggest that the AR signaling pathway is still active in these *IDH1*-mutant PCa cases. In vitro studies suggest that *IDH* is a potential target in PCa in mutated[37] and wild-type[45,47] settings. Although selective IDH1 and IDH2 inhibitors are approved for several *IDH*-mutant neoplastic diseases, and numerous clinical trials are in progress, IDH targeted therapy in PCa is understudied. Identification of a cohort of patients with PCa with *IDH1* hotspot mutations that lack other common drivers, as in the current study, provides a rationale for clinical studies correlating *IDH* mutations with response to ADT and the potential inclusion of these patients in IDH1 inhibitor trials. The same may be true for *IDH2*-mutated cases, although identifying a larger number of such patients would be necessary.

We report the first 2 cases of PCa with *IDH2* R172 mutations and note their similarity in molecular profile to PCa cases with *IDH1* oncogenic hotspot mutations. Although it is biologically feasible that an *IDH2* R172 mutation produces the same biological

effect on PCa as an *IDH1* R132 mutation, there are conflicting data in the literature. For instance, in vitro silencing of *IDH2* in PCa cells alters cellular dynamics in a way that would favor malignant behavior,[48] yet other in vitro studies of human and murine PCa suggest that 90% of IDH activity in PCa is carried out by IDH1 rather than IDH2.[45] Additional functional studies are required to investigate whether *IDH2* hotspot mutations have the same biological effect on PCa as *IDH1* hotspot mutations.

Unlike PCa with oncogenic hotspot *IDH* mutations in our cohorts, PCa specimens with nonhotspot *IDH* mutations often cooccurred with classic PCa driver alterations such as *TMPRSS2::ERG* fusions and *SPOP* hotspot mutations. In cases without classic PCa drivers, other oncogenic events such as MSI were detected. Moreover, these tumors commonly had other actionable targets such as *BRCA1/2* and *ATM* alterations. Overall, our findings suggest that nonhotspot *IDH* mutations are more likely to occur as passenger events or as the result of other mutagenic processes.

Two exceptional cases were identified in cohort 1 of our study—1 with an *SPOP* F102V hotspot mutation and a subclonal *IDH1* R132H hotspot mutation (case number 17 in Table 1), and the other with an *IDH1* R132C hotspot mutation and a *TMPRSS2* structural variant, as well as ERG expression based on IHC (case number 14 in Table 1). In the former case, histologic review did not identify apparent separate tumor nodules in the block for genomic sequencing. The findings may represent a subset of tumor cells acquiring *IDH1* R132H mutations during tumor evolution or 2 intimately admixed PCa clones that independently arose from *SPOP* and *IDH1* mutations. An in situ analysis with mutation-specific probes or antibodies targeting *SPOP* F102V and *IDH1* R132H mutations may help further characterize the findings; however, we were not able to perform additional analysis owing to the lack of material. In the latter case, genomic sequencing identified *IDH1* R132C mutations and a translocation event that involved *TMPRSS2* exons 1 and 2 and *GPHN* exons 2 to 23. Rare fusion events involving the *GPHN* gene have been described in PCa; however, *GPHN* has not been associated with *TMPRSS2*, and its role in PCa pathogenesis is unclear.[49] Further review of the molecular data did not reveal any significant alterations involving the *ERG* gene. Although the possibility of complex genomic rearrangements or other forms of alterations that led to the activation of *ERG* cannot be completely excluded, further characterization such as targeted RNA sequencing for fusion detection was prohibited by the lack of material. A further study on a larger *IDH*-mutant PCa cohort is required to elucidate the biology in these unusual cases.

In contrast to previous publications,[11,12] which showed a striking association between *IDH1* mutations and psammomatous calcifications, we identified this finding in only 2 of 13 primary *IDH1*-mutant PCa cases. We note that these calcifications were only seen in RP specimens from these patients; review on the prior NB cores did not identify psammomatous calcifications. Previous reports on this morphologic phenomenon have likewise come from RP specimens.[11,12] Mehra et al[11] reviewed molecular data and frozen section images from the TCGA Pan-Cancer Atlas[21] and the TCGA study in 2015,[7] finding an additional 5 samples with *IDH1* R132 mutations, 3 of which displayed psammomatous calcifications, implying a sensitivity of 60%. Conversely, they found a total of 4 tumors with psammomatous calcifications on the histologic examination of those cohorts, of which 3 harbored *IDH1* R132 mutations, implying a specificity of 75%. The distribution of the psammomatous calcification may be highly variable; we only detected a single psammoma body in 1 of our cases as well as in 1 of the previously described cases (TCGA-EJ-7125) classified as *IDH1*-mutant PCa. These findings suggest that searching for psammomatous calcifications as a screening tool for *IDH1* mutations may be an insensitive test. The previous reports also highlight an association between *IDH1*-mutant PCa and anterior prostatic tumors in RP specimens. We were not able to validate this, in part owing to many of our cases coming from NB specimens.

The study has several limitations. First, although this study is similar to others that find an association between *IDH* mutations and a high tumor grade, our observation may be confounded by a selection bias in which high-grade tumors were prioritized for genomic profiling. As it is unclear how previous reports selected cases, the association between histologic grade and *IDH* mutations in PCa remains to be verified in an unbiased larger cohort. Similarly, the selection bias toward high-grade or metastatic disease (particularly in cohort 2) may contribute to an overall higher rate of genomic alterations associated with advanced/aggressive disease, such as *AR* mutations/amplifications and DNA damage repair pathway alterations. In addition, our analysis using IHC on IDH1 R132H and PCa-related markers was limited to a small number of cases with available material. The study was not designed to investigate the clinical follow-up and treatment-specific outcomes of *IDH*-mutant PCa. These aspects remain for future study and will need to be addressed to optimize management for these patients.

In conclusion, we present a rare but distinct cohort of *IDH*-mutant PCa, including 16 previously unpublished cases with clonal *IDH1* R132 oncogenic hotspot mutations, with only 2 new cases showing psammomatous calcifications. We also report 2 novel PCa cases with *IDH2* R172 hotspot mutations. Our data support *IDH* hotspot mutations as driver alterations in PCa. Owing to the biological plausibility that these mutations are driving tumorigenesis, further studies are warranted to correlate clinical outcomes in this cohort, including response to ADT and, potentially, IDH inhibitors.

Author Contributions

B.S., S.W.F., and J.-F.C. were responsible for study concept, design, data interpretation, and manuscript preparation. B.S. and J.-F.C. were additionally responsible for data acquisition and analysis. S.W.F. and J.-F.C. provided funding support. All authors performed critical review of study results and approved the final version of the manuscript.

Data Availability

The data sets used and/or analyzed for the current study are available from the corresponding author on reasonable request.

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Declaration of Competing Interest

B.S. is a recipient of a Fellowship Grant from the American Physicians Fellowship for Medicine in Israel. All other authors have no financial interests to declare.

Ethics Approval and Consent to Participate

The study was approved and is under the oversight of the Memorial Sloan Kettering Cancer Center Institutional Review Board. This study was performed in accordance with the Declaration of Helsinki.

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