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DNA Damage Repair Alterations are Frequent in Prostatic Adenocarcinomas with Focal Pleomorphic Giant Cell Features

DR Tamara L. Lotan^{1,2,3}, Harsimar B. Kaur¹, Abdullah M. Alharbi¹, Colin C. Pritchard^{#4}, and DR Jonathan I. Epstein^{#1,2,3}

¹Departments of Pathology, Johns Hopkins University School of Medicine, Baltimore, MD, United States

²Departments of Oncology, Johns Hopkins University School of Medicine, Baltimore, MD, United States

³Departments of Urology, Johns Hopkins University School of Medicine, Baltimore, MD, United States

⁴Department of Laboratory Medicine, University of Washington, Seattle, WA, United States.

These authors contributed equally to this work.

Abstract

Aims: Prostatic adenocarcinomas with focal pleomorphic giant cell features are a rare tumour subtype with abysmal clinical outcomes. More than one third of cases with this histology die within a year of the initial diagnosis of prostate cancer. Potential targeted therapies are desperately needed, however the molecular features of these tumours remain unknown.

Materials and Methods: Here, we performed next generation sequencing using a highly validated targeted panel (UW-Oncoplex) on somatic tumour DNA extracted from 8 cases of prostatic adenocarcinomas with focal pleomorphic giant cell features, including cases with and without prior treatment for prostate cancer.

Results: We find that DNA damage repair mutations are common in this rare subset of prostate tumours, with 2 of 8 having bi-allelic pathogenic mutations in homologous DNA repair genes (including *BRCA2* and *NBN*) and 2 of 8 having bi-allelic pathogenic mutations in mismatch repair genes (including *MSH2* and *MLH1*).

Conclusion: These data are consistent with emerging data that DNA repair alterations are enriched among castration resistant prostate cancer and aggressive subsets of primary tumours. Given that these patients are potential candidates for PARP inhibitor and/or immune checkpoint blockade and have poor prognosis with standard therapy, we recommend tumour and germline DNA sequencing with or without mismatch repair protein immunohistochemistry be considered for all prostatic adenocarcinomas with focal pleomorphic giant cell features.

Keywords

Prostatic adenocarcinoma; Pleomorphic giant cell adenocarcinoma; DNA damage repair; homologous recombination; mismatch repair; hypermutation; microsatellite instability

Introduction

Prostatic adenocarcinoma with focal pleomorphic giant cell features is a rare subtype of prostate cancer containing a subpopulation of large, anaplastic and cytologically bizarre nuclei typically within a Grade Group 5 (Gleason score 9–10) usual-type adenocarcinoma background^{1–4}. These tumours may develop *de novo* at initial prostate cancer diagnosis or may present after radiation and/or hormonal therapy during tumour progression^{1,2}, similar to other aggressive variants of prostate cancer, such as small cell carcinoma⁵. Recognized in the 2016 WHO Classification of Tumours of the Urinary System and Male Genital Organs⁶, pleomorphic giant cell adenocarcinomas differ both morphologically and clinically from high grade usual-type prostate carcinomas, reinforcing the importance of an accurate diagnosis. At a morphologic level, even very high grade usual-type adenocarcinomas generally have homogeneous cytological features without pleomorphism. Clinically, in our recently published case series of prostatic adenocarcinoma with focal pleomorphic giant cell features, prognosis was extremely poor in this subgroup, with 37% (7/19) of *de novo* cases and 57% (4/7) of treatment-related cases dying of disease, with a median of 7–8 months survival². This is strikingly different from even Grade Group 5 (Gleason score 9–10) usual-type adenocarcinoma, where there is <10% mortality 4 years after diagnosis and treatment⁷.

Given the dismal prognosis, molecular characterization of these tumours could be useful to help identify potentially efficacious therapies for these patients. In our recent case series, we used immunohistochemistry to molecularly subtype 10 cases of prostatic adenocarcinoma with focal pleomorphic giant cell features, examining both the pleomorphic and usual-type adenocarcinoma components². Strikingly, we found that none of the cases exhibited *ERG* gene rearrangement based on lack of ERG protein expression in either component, a surprising result given that up to one third of Gleason 9–10 cases express ERG⁸. Androgen signaling appeared to be at least partially active in many tumours based on expression of the androgen receptor (AR) in the majority, as well as expression of prostate-specific marker NKX3.1, though PSA was notably negative in a subset of tumours. Given that ERG is a key driver for usual-type adenocarcinomas⁹, the lack of apparent *ERG* rearrangements in this tumour type suggested the possibility that these tumours have alternative molecular driver alterations. Here, we performed next generation sequencing on 8 tumours with focal pleomorphic giant cell features and identified frequent mutations of DNA damage repair genes in this rare subtype of prostate cancer.

Methods

Patients and tissue samples:

With Johns Hopkins institutional review board approval (IRB# NA_00091198, in accordance with the Declaration of Helsinki), 32 cases of prostatic adenocarcinoma with

focal pleomorphic giant cell features were identified from 2005 to 2018, including 30 cases in a previous report² and two new cases. Adequate tumour tissue was available for sequencing in only 8 cases, representing a clinical cross-section of the cases from the prior report (Table 1). All cases represented primary tumours, and were sampled on transurethral resection of the prostate (TURP, n=6), transurethral resection of the bladder (TURB, n=1) or at radical prostatectomy (n=1). All cases contained 5% pleomorphic giant cells as previously described (Figure 1)².

Immunohistochemistry:

Six of the 8 cases had immunohistochemistry for PTEN and p53 performed and reported in our prior clinical-pathologic study of prostatic adenocarcinoma with focal pleomorphic giant cell features². The other two cases were immunostained using the same protocol for the current study. Briefly, PTEN immunohistochemistry was performed in a CLIA-accredited laboratory using a genetically validated protocol developed by our group¹⁰ for the Ventana autostaining system (Ventana/Roche Medican Systems, Tucson, AZ) using a rabbit monoclonal antibody (D4.3, Cell Signaling Technologies, Danvers, MA). P53 immunohistochemistry was performed in a CLIA-accredited laboratory using a genetically validated protocol developed by our group¹¹ using a mouse monoclonal antibody (BP53–11, Ventana/Roche Medican Systems, Tucson, AZ). Scoring for both assays was performed using a dichotomous system as previously described^{10–12}.

Mismatch repair protein immunohistochemistry was performed on the Ventana system (Ventana/Roche Medican Systems, Tucson, AZ) in a CLIA-accredited laboratory as described previously¹³. Briefly, we used a mouse monoclonal antibody for MSH2 (clone G219–1129), a mouse monoclonal antibody for MSH6 (clone 44), a mouse monoclonal antibody for MLH-1 (clone M1) and a rabbit monoclonal antibody for PMS2 (clone EPR3947). Tumours were visually dichotomously scored as described previously¹³.

Next-Generation Sequencing:

All available tissue was formalin-fixed and paraffin-embedded (FFPE). Hematoxylin and eosin stained sections were used to guide macrodissection of all tumour containing areas (both areas with pleomorphic giant cells and usual-type adenocarcinoma) from ten-micron tissue sections of tumour samples. DNA was extracted from macrodissected FFPE samples using the QiAamp DNA FFPE Tissue Kit (Qiagen, Germantown, MD) and sequenced using the targeted next-generation sequencing (NGS) platform UW-OncoPlex, as previously described¹⁴. Data was created by the UW NGS Laboratory and Analytics group. Reported alterations were limited to those deemed pathogenic or likely pathogenic (e.g. loss of function mutations in tumour suppressors, activating mutations in genes involved in oncogenic signaling pathways). Germline mutations were inferred by expert molecular pathologist review through cross-referencing against the ClinVar database and by multivariate analysis of the variant allele fraction in the context of tumour content, ploidy, and loss of heterozygosity status^{15, 16}. Two cases had limited studies due to low tumour purity such that false negative results could not be excluded (Table 1).

Results

DNA was isolated from bulk tumour in 8 cases of prostatic adenocarcinoma with focal pleomorphic giant cell features. Given the low frequency of pleomorphic giant cells in most tumours (<5%)², it was not feasible to sequence only this cell population, however given how intermingled these cells are within the high grade usual-type adenocarcinoma background, it seems quite likely that the two components are clonally related. Of the 8 cases with adequate tumour tissue available for sequencing, patients ranged in age from 43 to 90 years at the time of diagnosis. Three of 8 cases had a known history of prostatic adenocarcinoma with prior treatment at the time of diagnosis (#16, #17, #18) including 2 of 8 cases with a documented prior history of androgen deprivation therapy (#17 and 18). Two patients (New#1 and New#2) had an unknown prior medical history, however it is possible that these had a prior history of androgen deprivation therapy given the finding of definite or possible androgen receptor gene (*AR*) copy number gain (Table 1).

Overall, 25% of cases (2/8) had bi-allelic pathogenic mutations in homologous DNA damage repair genes, including one case (#18) with a *BRCA2* nonsense mutation (p.L557*) and associated loss of heterozygosity (LOH), and one case (new #1) with a *BRCA2* frameshift mutation (p.N1666Ifs*4) and associated LOH in addition to bi-allelic loss of function mutations in *NBN* (p.P198Kfs*30 and LOH) (Table 1). *BRCA2* has a well-characterized role in DNA repair by homologous recombination and *NBN*, encoding the protein Nibrin, is a key constituent of DNA repair complexes. Only one of the two cases was suspicious for having a germline alteration based on allele fraction (*BRCA2* p.L557*, present in 234/263 reads or 0.89). Interestingly, this patient was only 43 years of age at the time of diagnosis and had a history of mycosis fungoides, which has been associated with *BRCA2* alterations in a prior report¹⁷.

Another 25% (2/8) of cases had bi-allelic pathogenic mutations in mismatch repair genes, including one case with bi-allelic *MSH2* mutations (frameshift: p.Q397* with a second splice site alteration on the second allele: c.1276+2T>A and *MLH1*) and one case with homozygous *MLH1* copy number loss. This case also had a pathogenic *BRCA2* deletion (exon 9 and part of exon 10) without evidence of LOH or bi-allelic loss. As expected, both cases showed elevated mutation burden (62 and 9 mutations/Mb, respectively) and both showed evidence of microsatellite instability on next generation sequencing of microsatellite loci (34/65 and 23/65 microsatellite loci unstable, respectively)^{18, 19}. In the case with bi-allelic *MSH2* mutations, immunohistochemistry for mismatch repair proteins showed loss of *MSH2* and *MSH6* nuclear protein expression in tumour cells, with intact staining in the tumour microenvironment as expected (Figure 1). In the case with homozygous loss of *MLH1*, *MLH-1* and *PMS2* protein expression was completely lost in the majority of tumour cells (Figure 1), though there was expression in a separate smaller nodule of tumour in the same sample suggestive of a second tumour clone.

The most common recurrently mutated gene across all 8 cases was *TP53*, which showed pathogenic mutations or homozygous copy loss in 88% (7/8) of cases. p53 nuclear accumulation by immunohistochemistry indicating underlying *TP53* missense mutation¹¹ was present in 6 of 8 cases, four of which had p53 immunohistochemistry results included in

our previous report ². Of these 6 cases, 5 had underlying *TP53* missense mutations and one had an in-frame deletion (Table 1). Of the two cases without p53 nuclear accumulation, one had an underlying homozygous *TP53* deletion and another case did not have a detectable *TP53* alteration. Though PTEN protein loss by immunohistochemistry was common in the current tumours as we previously reported (7/8 cases or 88%, 6 of which were included in our previous study ²), *PTEN* mutations were only identified in three cases (Table 1). Copy number alterations affecting chromosome 10 were common, possibly representing underlying *PTEN* gene deletions which are markedly more frequent than mutations ²⁰. Interestingly, one case with mismatch repair gene loss (*MSH2*) and PTEN protein loss also had a *PI3KCA* activating mutation (p.Y1021C) and one had an in-frame deletion of uncertain significance involving the gene encoding the p85 subunit of PI3K, *PIK3RI*. The case with the *PIK3CA* activating mutation (p.Y1021C) also had a *BRAF* activating mutation (p. K601E), which has been reported at a low frequency in prostate cancer ²¹, as well as mismatch repair deficiency which explains the high number of alterations in this tumour.

As is typical in prostate cancer, numerous copy number alterations were identified in most samples ²¹. The most notable was the presence of *AR* copy number gains in 2 of 8 tumours, which are highly suggestive of previous treatment with AR-directed or androgen deprivation therapies (ADT) ²². One of these patients did have a documented history of prior ADT (#18), while the other patient did not have available clinical history (New#1). All 4 cases with DNA repair alterations (homologous repair or mismatch repair) had an established prior history (or likely history based on *AR* gene status) of prior hormonal or, in some cases, radiation treatment for prostate cancer (Table 1). The three cases without any clinical or molecular evidence of prior therapy did not show DNA repair pathway alterations. Consistent with our prior immunohistochemistry study ², we did not find evidence of *ERG* gene rearrangements in any cases by sequencing.

Discussion

Emerging data suggest that the prevalence of DNA repair gene mutations is enriched in aggressive morphologic and clinical subsets of primary prostate cancer. For example, ductal and intraductal carcinoma have been reported to be associated with mutations in mismatch and homologous DNA repair genes ^{23–25}. Our group has previously published that mismatch repair mutations are substantially more common in primary prostate tumours with primary Gleason pattern 5 compared to all other Gleason groups ¹³. Similarly, castration resistant metastatic prostate carcinoma (CRPC) cases have somatic and/or germline mutations in DNA repair pathways in up to 25% of cases, compared to only 8–12% of primary localized cases based on recent large case series ^{21, 22, 26}. In the current study, we add to these data by molecularly-characterizing prostate cancers with focal pleomorphic giant cell features. Strikingly, we find that these unusual tumours have a very high rate of mutations in both homologous and mismatch DNA repair pathways, with half of all profiled cases having bi-allelic pathogenic alterations in one of these two pathways. Though this frequency must be interpreted with caution given our small cases series, it is markedly higher than that reported for any other histologic or clinical subset of prostate cancer to date.

Overall, we found that two of eight cases or 25% with focal pleomorphic giant cell features had bi-allelic pathogenic mutations in *BRCA2* and an additional case had a monoallelic alteration in this gene. One of these three patients had a likely underlying germline mutation. This frequency of *BRCA2* alteration is consistent with our previous report on the aggressive behavior of tumours with pleomorphic giant cells. *BRCA2* is the most commonly mutated DNA repair gene across primary and metastatic prostate cancer, with bi-allelic mutation reported in nearly 13% of metastatic CRPC cases (5% germline) compared to <3% of primary prostate cancers^{21, 22, 27}. Since *BRCA2* alterations are generally early and clonal events, the enrichment of *BRCA2* alterations among CRPC compared to primary tumours suggests that tumours with these mutations are frequently aggressive. This is concordant with a number of other studies showing that carriers of germline *BRCA2* mutations have higher grade and stage prostate cancer compared to non-carriers²⁸, and worse outcomes after treatment for local or locally advanced prostate cancer compared to non-carriers²⁹. Similarly, our group recently found that somatic DNA repair alterations in *BRCA2* or *ATM* are almost three times as common in primary prostate tumours with Grade Groups 3–5 (Gleason score 4+3=7, 8 and 9–10) compared to those in Grade Groups 1 and 2 (Gleason score 6 and 3+4=7)³⁰.

Similar to the frequency of homologous DNA repair alterations, we found that 25% of tumours with pleomorphic giant cells had pathogenic alterations mismatch repair genes, accompanied by evidence of microsatellite instability by sequencing. Previous reports have suggested that as many as 12% of advanced and metastatic prostate tumours have alterations in mismatch repair genes and microsatellite instability, however the rate in primary prostate tumours is likely less than a few percent³¹. This enrichment in CRPC also suggests that tumours with mismatch repair alterations may behave more aggressively. Consistent with this, we recently validated an assay to detect protein loss of the most commonly altered mismatch repair gene in prostate cancer, *MSH2*, and showed that the frequency of *MSH2* protein loss and pathogenic gene alterations are also strikingly associated with Grade Group/Gleason score¹³. While 8% of localized tumours with primary Gleason pattern 5 had *MSH2* loss and hypermutation, less than 1% of tumours from all other Gleason score groups had *MSH2* loss. Like *BRCA2* alterations, *MSH2* loss was likely an early and clonal event, present in all tumour cells within the primary dominant tumour nodule¹³.

As suggested by the immunohistochemistry results reported in the prior study², *TP53* mutation and PTEN loss were also very common among the cases we sequenced of prostatic adenocarcinoma with focal pleomorphic giant cell features. Loss of both of these tumour suppressors is enriched in aggressive subsets of prostate cancer as we and others have previously described^{10–12}, thus the high rate of loss in tumours with pleomorphic giant cells is not surprising. Overall, 7 of 8 of the current cases had inactivating mutations in *TP53*. This represents an enrichment above the rate of *TP53* mutations in Grade Group 5 (Gleason score 9–10) primary prostate tumours, such as those profiled in the TCGA, where 1/21 showed *TP53* mutations²¹. However, given that some of the cases in our current series were previously treated with androgen deprivation therapy, the more relevant comparison may be to castration resistant disease, where as many as half of tumours have inactivating *TP53* mutations or deletions²².

Some limitations of the current study warrant discussion. First, due to the extreme rarity of the diagnosis of prostatic adenocarcinoma with focal pleomorphic giant cell features, this was a relatively small study and the findings herein require validation in independent cohorts. Given that we only had adequate tumour to sequence a subset of our previously characterized cohort of these cases, it is possible that this subset is not representative of the underlying larger group of tumours, however their clinical-pathologic characteristics are generally in line with the larger group. Second, the tumours in this study represent a mixture of both treated and apparently de novo cases of prostatic adenocarcinoma with focal pleomorphic giant cell features.

Though all tumour material sequenced was from the primary tumour, there are certainly molecular differences between tumours that have progressed after hormonal therapy and de novo cases. Interestingly, we found that all cases with DNA repair alterations had clinical or molecular evidence (*AR* copy gain) of prior hormonal (or in one case, radiation therapy), making them more comparable to metastatic CRPC cohorts than to untreated primary disease. This raises the question of whether the finding of pleomorphic giant cells is most directly associated with castration resistant disease, which is itself associated with deficient DNA repair. Nonetheless, the frequency of DNA repair alterations noted in this study exceeds that reported in CRPC as well as that reported in primary disease, suggesting that these tumours may be a unique entity. We applied a targeted sequencing method rather than whole exome sequencing in this study, however the targeted panel we utilized has been highly validated¹⁴, captures gene rearrangements which are common causes for DNA repair gene alterations in prostate cancer^{26, 31} and covers all relevant DNA repair genes described as mutated in the disease²⁶. Thus, we feel comfortable that it is reasonably sensitive for these alterations.

Though we utilized radical prostatectomy or TURP samples for the DNA isolation in these cases, targeted sequencing panels such as UW-Oncoseq or similar panels can be applied to prostate needle core biopsies with a high tumour content. Finally, we did not have available germline DNA for sequencing in this study, thus only tumour somatic mutations were ascertained while germline alterations were only inferred. Future studies with available germline DNA will be better suited to determine the frequency of germline alterations in DNA repair genes in prostatic adenocarcinoma with focal pleomorphic giant cell features.

Overall, our study suggests that prostate tumours with focal pleomorphic giant cell features may benefit from both somatic and germline DNA sequencing with or without mismatch repair protein immunohistochemistry to rule out the presence of an underlying pathogenic DNA repair defect. Given their very poor oncologic outcomes, it is likely that the majority of these tumours will require multi-modal and in most cases systemic therapy. Recent clinical trial data has underscored the potential sensitivity of tumours with *BRCA2* and other homologous repair gene alterations to PARP inhibitor therapy^{32, 33}. Similarly, the recent FDA approval of immune checkpoint blockade with pembrolizumab for all tumours with mismatch repair defects, regardless of histologic subtype, has made recognition of prostate tumours with these alterations increasingly important. Given the high incidence of prostate cancer, it is not practically or economically feasible to perform sequencing or

immunohistochemistry on all newly diagnosed cases, however some histologic subsets such as those with pleomorphic giant cells, may be particularly high yield in this regard.

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References:

1. Parwani AV, Herawi M, Epstein JI. Pleomorphic giant cell adenocarcinoma of the prostate: Report of 6 cases. *Am J Surg Pathol* 2006;30:1254–1259. [PubMed: 17001156]
2. Alharbi AM, De Marzo AM, Hicks JL, Lotan TL, Epstein JI. Prostatic adenocarcinoma with focal pleomorphic giant cell features: A series of 30 cases. *Am J Surg Pathol* 2018;42:1286–1296. [PubMed: 29944471]
3. Lopez-Beltran A, Eble JN, Bostwick DG. Pleomorphic giant cell carcinoma of the prostate. *Arch Pathol Lab Med* 2005;129:683–685. [PubMed: 15859643]
4. Larnaudie L, Comperat E, Conort P, Varinot J. Hoxb13 a useful marker in pleomorphic giant cell adenocarcinoma of the prostate: A case report and review of the literature. *Virchows Arch* 2017;471:133–136. [PubMed: 28484843]
5. Epstein JI, Amin MB, Beltran H et al. Proposed morphologic classification of prostate cancer with neuroendocrine differentiation. *Am J Surg Pathol* 2014;38:756–767. [PubMed: 24705311]
6. Moch H, Humphrey PA, Ulbright TM, Reuter V. Who classification of tumours of the urinary system and male genital organs Lyon: IARC, 2016.
7. Leapman MS, Cowan JE, Simko J et al. Application of a prognostic gleason grade grouping system to assess distant prostate cancer outcomes. *Eur Urol* 2017;71:750–759. [PubMed: 27940155]
8. Fine SW, Gopalan A, Leversha MA et al. Tmprss2-erg gene fusion is associated with low gleason scores and not with high-grade morphological features. *Mod Pathol* 2010;23:1325–1333. [PubMed: 20562851]
9. Tomlins SA, Rhodes DR, Perner S et al. Recurrent fusion of tmprss2 and ets transcription factor genes in prostate cancer. *Science* 2005;310:644–648. [PubMed: 16254181]
10. Ahearn TU, Pettersson A, Ebot EM et al. A prospective investigation of pten loss and erg expression in lethal prostate cancer. *J Natl Cancer Inst* 2016;108.
11. Guedes LB, Almutairi F, Haffner MC et al. Analytic, preanalytic, and clinical validation of p53 ihc for detection of tp53 missense mutation in prostate cancer. *Clin Cancer Res* 2017;23:4693–4703. [PubMed: 28446506]
12. Maughan BL, Guedes LB, Boucher K et al. P53 status in the primary tumor predicts efficacy of subsequent abiraterone and enzalutamide in castration-resistant prostate cancer. *Prostate Cancer Prostatic Dis* 2018;21:260–268. [PubMed: 29302046]
13. Guedes LB, Antonarakis ES, Schweizer MT et al. Msh2 loss in primary prostate cancer. *Clin Cancer Res* 2017;23:6863–6874. [PubMed: 28790115]
14. Pritchard CC, Salipante SJ, Koehler K et al. Validation and implementation of targeted capture and sequencing for the detection of actionable mutation, copy number variation, and gene rearrangement in clinical cancer specimens. *J Mol Diagn* 2014;16:56–67. [PubMed: 24189654]
15. Li MM, Datto M, Duncavage EJ et al. Standards and guidelines for the interpretation and reporting of sequence variants in cancer: A joint consensus recommendation of the association for molecular

pathology, american society of clinical oncology, and college of american pathologists. J Mol Diagn 2017;19;4–23. [PubMed: 27993330]

16. Shirts BH, Casadei S, Jacobson AL et al. Improving performance of multigene panels for genomic analysis of cancer predisposition. Genetics in medicine : official journal of the American College of Medical Genetics 2016;18;974–981. [PubMed: 26845104]
17. Woollard WJ, Pullabhatla V, Lorenc A et al. Candidate driver genes involved in genome maintenance and DNA repair in sezary syndrome. Blood 2016;127;3387–3397. [PubMed: 27121473]
18. Hempelmann JA, Lockwood CM, Konnick EQ et al. Microsatellite instability in prostate cancer by pcr or next-generation sequencing. J Immunother Cancer 2018;6;29. [PubMed: 29665853]
19. Salipante SJ, Scroggins SM, Hampel HL, Turner EH, Pritchard CC. Microsatellite instability detection by next generation sequencing. Clinical chemistry 2014;60;1192–1199. [PubMed: 24987110]
20. Jamaspishvili T, Berman DM, Ross AE et al. Clinical implications of pten loss in prostate cancer. Nature reviews. Urology 2018;15;222–234. [PubMed: 29460925]
21. Cancer Genome Atlas Research N. The molecular taxonomy of primary prostate cancer. Cell 2015;163;1011–1025. [PubMed: 26544944]
22. Robinson D, Van Allen EM, Wu YM et al. Integrative clinical genomics of advanced prostate cancer. Cell 2015;161;1215–1228. [PubMed: 26000489]
23. Schweizer MT, Cheng HH, Tretiakova MS et al. Mismatch repair deficiency may be common in ductal adenocarcinoma of the prostate. Oncotarget 2016;7;82504–82510. [PubMed: 27756888]
24. Isaacsson Velho P, Silberstein JL, Markowski MC et al. Intraductal/ductal histology and lymphovascular invasion are associated with germline DNA-repair gene mutations in prostate cancer. Prostate 2018;78;401–407. [PubMed: 29368341]
25. Risbridger GP, Taylor RA, Clouston D et al. Patient-derived xenografts reveal that intraductal carcinoma of the prostate is a prominent pathology in brca2 mutation carriers with prostate cancer and correlates with poor prognosis. Eur Urol 2015;67;496–503. [PubMed: 25154392]
26. Pritchard CC, Offit K, Nelson PS, et al. DNA-repair gene mutations in metastatic prostate cancer. N Engl J Med 2016;375;1804–1805. [PubMed: 27806229]
27. Antonarakis ES, Lu C, Lubner B et al. Germline DNA-repair gene mutations and outcomes in men with metastatic castration-resistant prostate cancer receiving first-line abiraterone and enzalutamide. Eur Urol 2018;74;218–225. [PubMed: 29439820]
28. Castro E, Goh C, Olmos D et al. Germline brca mutations are associated with higher risk of nodal involvement, distant metastasis, and poor survival outcomes in prostate cancer. J Clin Oncol 2013;31;1748–1757. [PubMed: 23569316]
29. Castro E, Goh C, Leongamornlert D et al. Effect of brca mutations on metastatic relapse and cause-specific survival after radical treatment for localised prostate cancer. Eur Urol 2015;68;186–193. [PubMed: 25454609]
30. Marshall CH, Fu W, Wang H, Baras AS, Lotan TL, Antonarakis ES. Prevalence of DNA repair gene mutations in localized prostate cancer according to clinical and pathologic features: Association of gleason score and tumor stage. Prostate Cancer Prostatic Dis 2018.
31. Pritchard CC, Morrissey C, Kumar A et al. Complex msh2 and msh6 mutations in hypermutated microsatellite unstable advanced prostate cancer. Nat Commun 2014;5;4988. [PubMed: 25255306]
32. Mateo J, Carreira S, Sandhu S et al. DNA-repair defects and olaparib in metastatic prostate cancer. N Engl J Med 2015;373;1697–1708. [PubMed: 26510020]
33. Hussain M, Daignault-Newton S, Twardowski PW et al. Targeting androgen receptor and DNA repair in metastatic castration-resistant prostate cancer: Results from nci 9012. J Clin Oncol 2018;36;991–999. [PubMed: 29261439]

Figure 1: Mismatch repair protein immunohistochemistry in prostate adenocarcinoma with pleomorphic giant cells.

(A) Hematoxylin and eosin staining of case #16 reveals rare bizarre pleomorphic cells, comprising <5% of total tumor cells (arrow). Consistent with bi-allelic *MSH2* gene loss in case #16, MSH2 nuclear immunostaining is absent in tumour cell nuclei (arrow) and present in infiltrating lymphocytes and overlying benign urothelial cells as an internal control (arrowhead). As expected, the MSH2 binding partner, MSH6, is also absent in nuclei in tumour cells (arrow) but present in overlying benign urothelium consistent with MSH2 loss.

(B) Hematoxylin and eosin staining of case New #2 reveals rare bizarre pleomorphic cells, comprising <5% of total tumor cells (arrow). Consistent with bi-allelic *MLH1* gene loss in new case #2, MLH-1 nuclear immunostaining is absent in tumour cell nuclei (arrow) and present in endothelial cells providing an internal control (arrowhead). As expected, the MLH-1 binding partner, PMS2, is also absent in nuclei in tumour cells (arrow) but not in endothelial cells (arrowhead).

Clinical-Pathologic and Genomic Features of Prostatic Adenocarcinoma with Focal Pleomorphic Giant Cell Features

Table 1:

ID (from reference 2)	Age	Gleason Grade (Grade Group [GG])	Specimen type	Prior treatment	MMR Mutation	MSI (mSINGS)	mutations/Mb	HRD Mutation(s)	Germline DDR Suspected?	Activating Driver Mutation(s)	TP53 alteration	p53 nuclear accumulation by IHC	PTEN IHC	AR alteration	Limited Study? *
18	43	4+5=9 (GG5)	RP	h/o PCa with RP, salvage RT and ADT	none	NEG	7	<i>BRCA2</i> (p.L557*) with A associated LOH	yes	None	p.M133_K139del + LOH	present	lost	Copy gain	no
15	86	5+5=10 (GG5)	TURB	none	none *	NEG	1	none *	no	none *	p.R273H	present	lost	none *	yes
20	87	5+5=10 (GG5)	TURP	none	none	NEG	4	none	no	None	homozygous copy loss	absent	lost	none	no
17	82	5+5=10 (GG5)	TURP	h/o PCa with ADT	none *	NEG	1	none *	no	none *	none *	absent	lost	none *	yes
16	73	5+5=10 (GG5)	TURP	h/o PCa with RT	<i>MSH2</i> (p.Q397*) + c.1276-2T>A)	POS	62	none	no	<i>BRCA1/p.K601E, PIK3CA p.Y1021C</i>	p.G245S	present	lost	none	no
29	39	5+5=10 (GG5)	RP	none	none	NEG	0	none	no	<i>PIK3R1</i> (p.P298_L303del)	p.R175H + LOH likely	present	lost	none	no
New #1	66	5+5=10 (GG5)	TURP	unknown; prior ADT likely based on AR copy gain	none	NEG	5	<i>BRCA2</i> (p.N1666IIs*) + LOH, <i>NRXN</i> (p.P198Kfs*) + LOH)	No (<i>BRCA2</i>), Maybe (<i>NRXN</i>)	none	p.A161T + LOH	present	intact	Copy gain	no
New #2	90	5+5=10 (GG5)	TURP	unknown	<i>MLH1</i> (homozygous copy loss)	POS	9	<i>BRCA2</i> (exon 9 + part of exon 10 del)	no	none	p.R248Q	present	lost	IND	no

TURP: transurethral resection of prostate; TURB: transurethral resection of bladder; RP: Radical prostatectomy; h/o: history of; ADT: Androgen deprivation therapy; PCa: prostate cancer; RT: radiation therapy; MMR: mismatch repair; Mb: megabase; HRD: homologous repair deficiency; DDR: DNA damage response; IHC: immunohistochemistry; IND: indeterminate;

* Limited study due to limited quantity and quality of tumour DNA, false negative results cannot be excluded