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SHORT COMMUNICATION

Polypoidal giant cancer cells in metastatic castration-resistant prostate cancer: observations from the Michigan Legacy Tissue Program

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Abstract

Despite early diagnosis and established protocols, a subset of prostate cancer patients will eventually be categorized as castration-resistant prostate cancer. Recently, it has been reported that these multi-modal therapy cases may harbor a special subset of cancer cells termed as polypoidal giant cancer cells (PGCC). These cells are phenotypically described either as possessing highly irregular polylobated nuclei or multiple pleomorphic nuclei. To identify and characterize the distribution of these cells, we created a cohort of 5 randomly selected cases of multi-modal therapy failure prostate cancer (16 selected non-osseous and osseous tumor sites) enrolled in Michigan Legacy Tissue Program. In all cases, specific "regions of interest" or "hot spots" within tumor areas showing an increased proportion of these multi-nucleated/polylobated cells under light microscopy were labeled as PGCC-rich area. On microscopic evaluation, overall mean count of PGCC was 42.4 ± 3.91 with case 2 in the study cohort with the highest number of average PGCC count of 17 ± 4.04. Site wise analysis showed retroperitoneal lymph node as the tissue with highest number of average PGCC number/site (5.0 ± 0.32). On correlating the average number of PGCC recorded with the time elapsed from last dose of chemotherapy administered to autopsy, the spearman correlation value (R) was 0.67, but the result was not statistically significant ($p = 0.22$). A systematic assessment of PGCC in a large stratified cohort of prostate cancer patients integrated with various histopathological and clinical parameters along with discovery of specific biomarkers for PGCC are the future studies suggested.

Keywords Castration resistant · Polypoidal giant cancer cells · Prostate cancer · Rapid autopsy

Introduction

Prostate cancer continues to be a major cause of morbidity and mortality across the world and is a common cancer in American men with 174,650 new cases and 31,620 deaths reported in 2019 [1]. Although many prostate cancer patients are diagnosed early with good survival outcomes from treatment with prostatectomy and/or radiation therapy, a subset

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of these cases become metastatic. Androgen deprivation therapy (ADT) is started, but resistance in inevitable and the patients may transition from hormone-sensitive to the therapy-resistant and lethal phase of the disease, castration-resistant prostate cancer (CRPC). Recent studies suggest that the therapy-resistant tumors may be enriched with particular type of cancer cells called polypoidal giant cancer cells (PGCC) [2]. PGCC are defined as cancer cells with morphological appearance of highly irregular polylobated nuclei or multiple pleomorphic nuclei. It was reported that the presence of PGCC in prostate cancer may be associated with disease progression and metastasis along with resistance to therapy [2–6]. Based on this data, we investigated a cohort of 5 patients with CRPC enrolled in our Michigan Legacy Tissue Program (MLTP) at Michigan Medicine. We characterized the PGCC and their distribution in 16 selected non-osseous and osseous tumor sites from patient specimens and evaluated the association between PGCC frequency, the sites of metastasis, and clinico-pathological parameters.

Materials and methods

Case selection

The rapid autopsy program at Michigan Medicine or Michigan Legacy Tissue Program (MLTP) was approved by the Institutional Review Board of University of Michigan and is supported by Specialized Program of Research Excellence (SPORE) in Prostate Cancer (National Cancer Institute grant 2P50CA186786-06). The goal of MLTP has been to maximize the sample number and diversity of metastatic prostate cancer tissue available for research purposes. Patients were identified with CRPC by the Medical Oncology Service of the Comprehensive Cancer Center at the University of Michigan Hospitals as described previously [7, 8]. Autopsies were performed on patients with CRPC as part of the MLTP program; these autopsies have been referred to as "rapid" or "warm" because of the short time interval (average 3 h) between patient death and starting the autopsy.

We randomly selected 5 CRPC patients enrolled in MLTP for this study who were treated with multimodality therapies (various combination of radical prostatectomy, radiation, chemotherapy, and anti-androgen agents). The formalin-fixed paraffin-embedded (FFPE) blocks representing procured autopsy tissue were retrieved from the MLTP archival tissue core ($n = 16$) and selected blocks were sectioned at 5-micron thickness and stained with hematoxylin and eosin (H&E) for histologic evaluation. A minimum of 3 different metastatic sites from each patient were selected for assessment that included both non-osseous (liver, lung, pancreas, retroperitoneal lymph nodes, urinary bladder, perivesical region, and dura) and osseous sites (femur, rib, and thoracic vertebra) (Table 1). Tumor within the prostate was evaluated when available (i.e., in the absence of previous prostatectomy).

Histopathological evaluation and inclusion criteria

Polypoidal giant cancer cells (PGCC) may present as either multi-nucleated/polylobated cancer cells or as relatively larger cells characterized by a "giant" nucleus that is three times larger than that of the neighboring diploid cancer cell [2–5]. For the purpose of this study, only multi-nucleated/polylobated cancer cells were classified as PGCC; the larger cells were not included in the analysis to maintain a high degree of diagnostic and recognition specificity for PGCC. We identified specific "regions of interest" or "hot spots" within tumor areas showing an increased proportion of these multi-nucleated/polylobated cells under light microscopy at ×200 magnification and labeled these as PGCC-rich areas. PGCC were assessed in 5 consecutive PGCC-rich areas by two pathologists including a genitourinary pathologist (RM and RM) at intermediate-power (200x) and high-power magnification/field (×400 or HPF).

Since the main focus of our study was to interrogate the presence and characterization of PGCC from a qualitative/quantitative perspective, we counted and recorded the total number of PGCC per metastatic site from 5 topographically separate PGCC-rich areas ("hot spots") utilizing high power evaluation (400x) in each case of our cohort. In total, we assessed 16 different anatomical sites with each site sampled through 5 PGCC-rich areas ("hot spots") thereby leading to final assessment of 80 PGCC-rich areas of this cohort (Supplementary Table 1).

For statistical purposes, an average number of PGCC per each of the 5 cases (Table 2) and average number of PGCC per site in each individual case were recorded (Supplementary Table 1). We also calculated the overall total average PGCC number (calculated by summing up the individual PGCC recorded in all 80 PGCC-rich fields in 16 metastatic sites of the 5 cases in the study); this was performed to compare and assess features with the overall PGCC recorded in some non-prostate cancer studies published in the literature.

Results

Clinico-pathological parameters

Most patients in our cohort had high-grade disease with Grade Group 5 and an average Gleason score of $4 + 5 = 9$ or $5 + 5 = 10$ (Table 1). Autopsy findings in the patients recapitulated the known phenomenon of pro-skeletal disease progression of metastatic prostate cancer. Skeletal metastasis, especially in lower extremities, vertebra, and

Table 1 Clinico-pathological parameters of the selected cohort

Case no	Age at death (years)	Disease extent	Gleason score at initial diagnosis	Directed therapy	Systemic therapy	Duration between last chemotherapy and autopsy (days)
1	55	Urinary bladder, seminal vesicle, lung (Lt), iliac soft tissue, liver (multifocal), adrenal (Lt), pancreas, peri-tracheal and peri-aortic soft tissue, pituitary, both femur and humerus, and thoracic and lumbar vertebra	4 + 5	Adjunct RT	ADT and CT	44
2	69	Dura, sternum, ribs, liver, retroperitoneum, humerus (Rt), femur, retroperitoneal and mediastinal lymph nodes, lumbar and thoracic vertebra	5 + 5	NA	ADT and CT	932
3	76	Skull base, vertebra, ribs, extremities, pelvis, cerebellum, liver, lungs, diaphragm, spleen and pancreas	5 + 5	Transurethral prostatic resection	CT	170
4	60	Sternum, ribs, femur, humerus, lumbar vertebra, thoracic vertebra, humerus, mediastinal/retroperitoneal/supraclavicular lymph nodes, liver, spleen, dural and leptomeningeal	5 + 5	Adjunct RT	ADT and CT	569
5	65	Bilateral femur, liver, lung, thoracic lymph nodes, ribs, vertebral column	5 + 5	Radical prostatectomy	CT	413

ADT androgen deprivation therapy, *CT* chemotherapy, *RT* radiotherapy, *Lt* left, *Rt* right

Table 2 Comparison of overall and site wise average number of PGCC with maximum and minimum PGCC noted in CRPC cohort

Case #	No of sites assessed/case	Total PGCC-rich areas assessed/Case	Total no of PGCC/case	Average PGCC/case	Site with maximum PGCC no	Site with minimum PGCC no
1	4	20	51	12.7 ± 1.11	Peri-vesical region	Urinary bladder
2	3	15	51	17.0 ± 4.04	Retroperitoneal lymph node	Liver site 2
3	3	15	34	11.3 ± 0.33	Prostate	Liver and pancreas
4	3	15	43	14.33 ± 1.76	Dura	Lung
5	3	15	33	11 ± 0.57	Rib	Thoracic vertebra

ribs were observed in all cases. In the non-osseous sites, liver was the most common site for metastasis (seen in all 5 cases), followed by lung and lymph nodes (seen in 3/5 cases). In case 3, tumor within prostate was available for evaluation, while the remainder of the sites comprised of metastatic tumor (Supplementary Table 1).

Morphological assessment

Histopathological evaluation by light microscopy revealed the presence of isolated to small groups of relatively larger and hyperchromatic appearing cells admixed within a population of tumor cells in PGCC-rich areas ("regions of

interest"/"hot spots"). When reviewed under higher magnification, these cells were proportionally much larger than the neighboring tumor cell population exhibiting two types of identifiable and reproducible morphology. The first morphologic phenotype comprised of cells with multi-nucleated, hyperchromatic nuclei showing irregular nuclear membrane, presence of multiple nucleoli, and minimal amount of cytoplasm with indistinct cytoplasmic membrane. The second phenotype we observed of PGCC was that of larger cells with high nuclear cytoplasmic ratio, hyperchromatic pleomorphic nuclei showing irregular nuclear membrane exhibiting indentations and polylobation, multiple nucleoli, and indistinct cytoplasmic membrane (Fig. 1).

Overall average PGCC count

Based on morphologic assessment, PGCC were identified in all the cases evaluated (5/5) and at all metastatic sites, characterized by the presence of large multi-nucleated/polylobated cancer cells as described above. Overall, the mean count of PGCC per case (calculated by summing up the individual PGCC recorded in all the five consecutive hot spot areas in 16 sites of all the 5 cases in the study) was 42.4 with standard deviation of ± 3.91.

Case wise average PGCC count

Sub-dividing the cases in the study cohort individually, the case 2 in the study cohort showed the highest number of average PGCC count of 17 ± 4.04 with a range of 2 to 6 PGCC/HPF at different anatomical sites, followed by case 4 with an average PGCC count of 14.33 ± 1.76 with a range of 2 to 4 PGCC/HPF at different representative sites. Case 5, which had only osseous metastatic disease with no non-osseous organ involvement by metastatic tumor, demonstrated the lowest recorded PGCC with an average count of 11 ± 0.57 with a range of 2–3 PGCC/HPF at various anatomical sites (Table 2, Fig. 2).

Site wise average PGCC count

Systematic site wise analysis showed that retroperitoneal lymph node metastasis in case 2 exhibited the highest average PGCC number/site (5.0 ± 0.32). Thoracic vertebral metastasis recorded the least number of average PGCC/site (2.0 ± 0.0). The detailed description of site wise ($n = 16$) and the corresponding field wise ($n = 80$) PGCC observed are provided in Supplementary Table 1.

Correlation of presence of PGCC with most recent chemotherapy administration

On correlating the average number of PGCC recorded with the time elapsed from last dose of chemotherapy administered to autopsy (Tables 1 and 2), the spearman correlation value (R) was 0.67, but the result was not statistically significant ($p = 0.22$) (Supplementary Fig. 1).

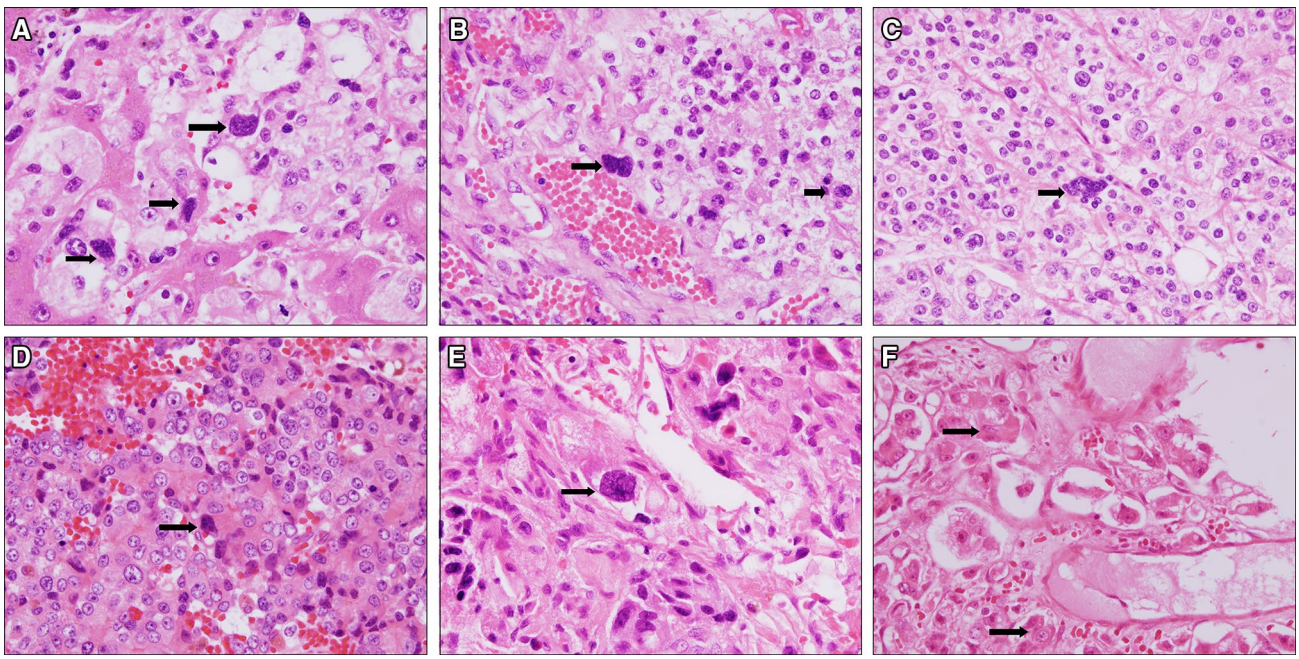

Fig. 1 Polypoidal giant cancer cells (PGCC, indicated by arrows) in **a** prostate and in various representative metastatic sites: **b** liver; **c** dura; **d** peri-vesical tissue; **e** femur; and **f** thoracic vertebra

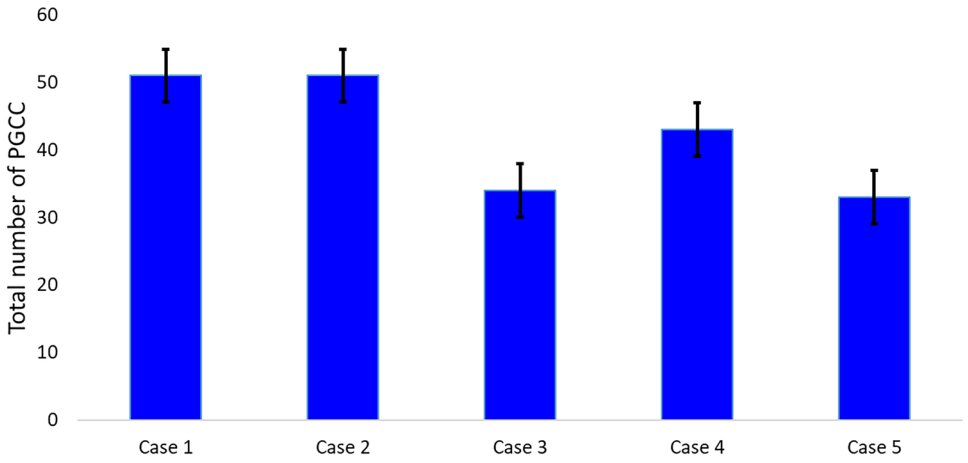

Fig. 2 Case wise distribution of PGCC in the study cohort

Discussion

The development of therapy-resistant metastatic prostate cancer accounts for the majority of deaths related to prostate cancer. Our group has previously investigated castration resistance mechanisms, *ERG* gene rearrangement, ERG protein expression discordance, and other clinico-pathological and genomic sequencing-based studies in patients with CRPC [7–9]. A recent and interesting report/review by Amend et al. has described the presence of polypoidal giant cells (PGCC) in tumor from patients who failed multi-modal therapies and proposed these as the "keystone species" in the prostate cancer ecosystem [2]. Citing multiple pertinent studies available in the published literature the authors have also proposed that these rare subpopulation of cells may be able to survive harsh tumor ecosystem of low oxygen tension, low pH, and low nutrients and therefore drive metastasis and therapeutic resistance [2]. Interestingly, it has been reported and shown that both therapeutic intervention (chemotherapy and radiation) and experimental induction of hypoxia reportedly simulate the tumor microenvironment and may result in the generation of PGCC [10–12].

Here, we interrogated the presence of PGCC in a small cohort of CRPC patients (representing a lethal phenotype of prostate cancer) enrolled in MLTP. Our observations support the identification of sparse and focal topographical presence of these cells within the tumor landscape of prostate cancer, similar to previous findings from Amend et al.[2]. The cells exhibited two types of morphology on light microscopy—multi-nucleated and polylobated— thus also corroborating earlier work in non-prostate malignancies such as ovarian, breast, melanoma, lung, pancreas, urinary bladder, kidney, and thyroid, amongst others [3–6]. Recently, a study by Alharbi et al. noted the presence of pleomorphic giant cells (possibly akin to PGCC) in their selected cohort of 30 cases of prostate cancer with pleomorphic giant cells visualized in less than 5% of the tumor area [13]. This is relatively consistent with our observations that PGCC are localized in a few PGCC-rich areas or hot spots and are not present diffusely within the tumor. We did observe that every tumor we examined though had PGCC within them, supporting their potential role in metastasis formation or maintenance. Also, the microenvironment of the resident tissue for a specific metastasis may interact with PGCC development or maintenance, indicated by the variable amounts we see between tissues/tumor sites. Comparing the mean count of PGCC in the present study of 42.4 ± 3.91 to the other studies in different non-prostate malignancies, we noted a wide range reported in the literature as 22.61 ± 1.15 in breast cancer [5], 18.12 ± 8.70 in serous epithelial ovarian tumors [6], and 32.75 ± 22.21 in anorectal malignant melanoma [14].

Various studies of PGCC in non-prostate tumors have also attempted to correlate PGCC with clinical and histopathological variables, vasculogenic mimicry, and poor survival [14–17]. Such a parametric description and systematic analysis in context of standardized clinico-pathological evaluation regarding PGCC in prostate cancer is currently lacking [2, 13]. The samples included in the present study were tissues harvested from the patients who died due to the complications of metastatic CRPC. In this cohort of patients with highly aggressive disease showing wide spread multiple non-osseous and osseous metastasis, high Gleason scores (9 and 10), and high PSA levels, PGCC could be identified in all the investigated cases and sites. Similarly, other cancer sub-types have showed an increase in the number of PGCC at metastatic sites and in higher tumor-grade areas [6, 14, 16]. We hypothesized that chemotherapy may maintain PGCC number, thus comparing time from last chemotherapy to the amount of PGCC observed at autopsy. The correlation value of $R = 0.67$ is intriguing, but clearly not statistically significant possibly due to small cohort size or a long mean time from chemotherapy of 426 days.

The current challenges to studying PGCC include the lack of a universally acceptable and reproducible definition of

PGCC and the lack of specific biomarkers and approaches to harvest a significant population of PGCC from both solid tumor tissue and liquid biopsies [2, 18]. Tissue banks from rapid autopsy programs such as MLTP with access to specimens from metastatic and lethal prostate cancers may provide an opportunity to further study and assess PGCC, from a pathology, functional, and mechanistic standpoint. Since 1996, MLTP at Michigan Medicine has conducted systematic studies of morphology, immunophenotype, genomic alterations, transcriptome profiling, and clinical outcomes through a large number of rapid autopsies performed on men who died from complications of metastatic CRPC [8, 19, 20]. Like other rapid autopsy programs, this has aided in elucidating the molecular mechanisms underlying hormone resistance as well as the rare presenting phenotypic/genotypic prostate cancer sub-types [7, 9, 21]. It has also allowed us to obtain biospecimens from inaccessible sites that could aid in the isolation of pure PGCC population and discovery of specific biomarkers for PGCC, which we consider as aims for future projects.

The present morphology-driven study to ascertain PGCC on routine H&E stained sections was aimed at identifying the cells of interest (PGCC) in castration-resistant prostate cancer. Despite utilizing strict morphological criteria, assessing PGCC on histological sections is associated with challenges; the low percentage of PGCC observed in each 'hot spot' further demands a rigorous microscopic evaluation. The cell membranes in H&E stained sections are often if not frequently indistinct thereby making a comprehensive distinction of a true PGCC from group of cells overriding and overlapping and pseudo multi-nucleation (due to artifact of sectioning) difficult at times. Companion immunohistochemical membranous markers such as EpCAM and E-Cadherin may be of assistance in the identification and confirmation of PGCC, which would be aims for future studies.

Although the molecular, cellular, and genetic biology behind pathogenesis of PGCC have yet to be fully elucidated, recording the histopathological observations in prostate cancer could have future clinical significance when integrated with other laboratory and clinical data. Finally, it is conceivable that the unique biology of PGCC may make them susceptible to novel pharmacological agents [2].

Summary

The study documents the presence of PGCC in a small cohort of prostate cancer patients with a lethal clinical phenotype and enrolled in a rapid autopsy program (MLTP). Future studies aimed at systematic assessment of PGCC in a large stratified cohort of prostate cancer patients integrated with various histopathological parameters along with other clinical and radiological features of disease could reveal associative information between PGCC and prostate cancer progression and therapy resistance.

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

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