Intraductal carcinoma of the prostate can evade androgen deprivation, with emergence of castrate-tolerant cells

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Objective

To determine the relevance of intraductal carcinoma of the prostate (IDC-P) in advanced prostate cancer by first examining whether IDC-P was originally present in patients who later developed advanced prostate cancer and then using patient-derived xenografts (PDXs) to investigate the response of IDC-P to androgen deprivation therapy (ADT).

Materials and Methods

We conducted a retrospective pathology review of IDC-P in primary prostate biopsy or surgery specimens from 38 men who subsequently developed advanced prostate cancer. Overall survival was calculated using the Kaplan–Meier method. To demonstrate the response of IDC-P to ADT, we established PDXs from seven patients with familial and/or high-risk sporadic prostate cancer. After castration and testosterone restoration of host mice, we measured the volume and proliferation of IDC-P within PDX grafts.

Results

We found that IDC-P was a prominent feature in the primary prostate specimens, present in 63% of specimens and often co-existing with poorly differentiated adenocarcinoma. Overall survival was similar in patients with or without IDC-P. In the PDXs from all seven patients, IDC-P was identified and present at a similar volume to adenocarcinoma. Residual IDC-P lesions persisted after host castration and, similar to castrate-tolerant adenocarcinoma, testosterone restoration led to tumour regeneration.

Conclusion

The study showed that IDC-P is prevalent in aggressive prostate cancer and contains cells that can withstand androgen deprivation. Thus, IDC-P appears functionally relevant in advanced prostate cancer. The presence of IDC-P may be a trigger to develop innovative clinical management plans.

Keywords

pathology, intraductal carcinoma of the prostate, androgen deprivation therapy, patient-derived xenografts, BRCA, #PCSM, #ProstateCancer
Introduction
In prostate cancer, intraductal carcinoma of the prostate (IDC-P) is a distinct growth pattern where malignant cells grow in pre-existing prostatic ducts and acini [1]. IDC-P is an adverse pathological feature that is typically associated with high-grade disease and poor clinical outcomes [2–4]. This includes patients with germline BRCA2 mutations, where IDC-P is independently associated with decreased progression-free and overall survival [5]. Despite its association with aggressive disease, IDC-P has been overlooked as a rare pathology because of its low incidence in unselected biopsy specimens [6]. Yet, our recent systematic review showed that although IDC-P is rare in low-risk prostate cancer, its prevalence significantly increases in high-risk disease [7]. Thus, IDC-P may be relevant in more patients than previously appreciated.

Molecular evidence supports an association between IDC-P and aggressive disease [8–10]. Common TMPRSS2-ERG genomic breakpoints between adenocarcinoma and IDC-P suggest that IDC-P arises from the same tumour clone as adenocarcinoma [11]. Recent whole-genome sequencing of localized sporadic and BRCA-mutant prostate cancers supports the common origin of IDC-P and adenocarcinoma, with genomic divergence during tumour evolution [9]. This suggests that IDC-P is a morphological manifestation of underlying aggressive disease and is consistent with earlier reports of extensive allelic imbalance and loss of heterozygosity of RB1 and TP53 in IDC-P [8,12]. This genomic profile may underpin the aggressive clinical progression of tumours with IDC-P; however, the functional role of IDC-P in disease progression has not been elucidated.

Pathology studies have shown that IDC-P persists in localized prostate cancers after androgen deprivation therapy (ADT) and/or chemotherapy, maintaining its characteristic morphological features and, in some cases, increasing in prevalence [13–15]. This has led to speculation that IDC-P may be inherently resistant to current therapies. The incidence and extent of IDC-P is, however, difficult to assess in matched pre- and post-treatment specimens because of difficulties in precisely resampling the same tumour region. Thus, it is unknown whether existing IDC-P lesions persist after treatment or are selected by treatment. Furthermore, the biological response of IDC-P to therapy compared with adenocarcinoma remains unknown. The aim of the present study, therefore, was to investigate whether IDC-P is a prominent pathological feature in patients who later failed treatment and to further understand the biological behaviour of IDC-P during androgen deprivation.

Patient-derived xenografts (PDXs) are invaluable models for studying prostate tumour biology as they retain the histopathology and molecular profile of the original specimens [5,16]. Previously, we used PDXs to investigate the response of hormone-naive localized prostate cancers to androgen deprivation and identified a subpopulation of ‘castrate-tolerant’ adenocarcinoma cells that can persist in an androgen-depleted environment [17]. In the present study, we used the same approach to investigate the presence of castrate-tolerant cells in IDC-P.

Materials and Methods
Retrospective Pathology Review
A retrospective pathology review was conducted with human ethics approval (Eastern Health Human Research Ethics Committee approval number LR89/2015). A contemporaneous cohort of patients receiving treatment at Eastern Health for advanced prostate cancer were screened and those who underwent prostate biopsy or surgery at Eastern Health prior to the commencement of ADT were selected. The patients’ diagnostic biopsy, radical prostatectomy and/or TURP specimens were retrieved from pathology for further review. Clinical follow-up data were collected using patient medical records.

The histopathology of archival tissue was reviewed by a single pathologist (D.C.) to assess Gleason grade, the presence or absence of IDC-P, high-grade prostatic intraepithelial neoplasia and perineural or lymphovascular invasion. Gleason grade group was reported according to the revised Gleason grading system [18]. IDC-P was scored using the diagnostic criteria defined by Guo and Epstein [1]. These diagnostic criteria require that prostatic ducts have at least 50% filling of malignant cells with a partial or fully conserved basal cell layer. The lesions must display either a solid or dense cribriform architecture or a loose or micropapillary architecture with central comedonecrosis and/or nuclear atypia.

Fresh Patient Specimens
Human ethics approval was obtained for the collection of fresh prostate tumour tissue through the following Human Research Ethics Committees: the Cabrini Institute (07-07-04-14); Epworth Hospital (53611); Monash University Human Research Ethics Committees (RMO 2006/6108 – 200400145); and Peter MacCallum Cancer Centre (97_27). Localized prostate cancer specimens were obtained from seven patients at the time of radical prostatectomy with informed, written consent. Specimens were obtained from patients with high-risk sporadic prostate cancer or patients with a germline BRCA mutation, as these patients are more likely to have IDC-P within their primary tumour [5,7]. Patient specimens with germline BRCA mutations were obtained through the Kathleen Cuningham Consortium for Research into Familial Beast Cancer (kConFab). After surgery, pathologists dissected
a region of tumour tissue from each patient specimen, which was transported to the laboratory on ice in RPMI 1640, supplemented with 10% fetal calf serum, 1% penicillin-streptomycin, 0.5 µg/mL amphotericin B antymycin and 100 µg/mL gentamycin. To prepare the specimens for xenografting, tumour tissue was dissected into ~40 pieces per patient, each piece ~4 mm³. At least five pieces of tissue were immediately fixed in formalin to assess the histology of ungrafted tissue. The remaining tissue pieces were recombined with neonatal seminal vesicle mesenchyme, obtained from C57BL/6 or Balb/c mice (Monash Animal Research Platform Ethics Approval Number MARP/2012/158), and embedded in collagen gel, as previously described [16].

Patient-derived Xenografts

All experimental procedures were approved by the Monash Animal Research Platform Animal Ethics Committee (MARP/2012/158). Xenografts were established from tumour tissue as previously described [16]. In brief, grafts were established in 6–8-week-old male non-obese diabetic severe-combined immunodeficient (NOD-SCID) or NOD-SCID gamma mice, which were housed in a 12-h light:12 h dark cycle and allowed to access food and water ad libitum. Two to three grafts were implanted under the renal capsule per kidney. At the time of engraftment, a 5-mm testosterone pellet was implanted subcutaneously to supplement host testosterone levels. Grafts were allowed to grow under the renal capsule for 6–14 weeks before being collected and analysed.

To assess the response of tumour tissue to androgen deprivation, grafts were established for 6 weeks before host mice were castrated by surgical removal of the testes and testosterone pellets. Four weeks after castration, testosterone levels were restored in a subset of castrated mice by implanting a 5-mm testosterone pellet subcutaneously to supplement host testosterone levels. Grafts were allowed to grow under the renal capsule for 6–14 weeks before being collected and analysed.

To determine the number of proliferating tumour cells within IDC-P lesions, Ki67 immunohistochemical staining was conducted on three representative sections per xenograft. Slides were imaged using the Aperio ScanScope AT Turbo slide scanner and the number of Ki67-positive tumour cells was counted using ImageScope analysis software. Data are expressed as a percentage of the total number of cells counted.

Statistical Analysis

Significant differences between groups were determined using a paired t-test or one-way ANOVA with a Dunnett’s post hoc test. Overall survival from the diagnosis of castrate-resistant prostate cancer was determined using the Kaplan–Meier method. P values <0.05 were taken to indicate statistical significance.

Results

We performed a retrospective pathology review of primary prostate specimens from 38 men who developed metastatic prostate cancer to determine the prevalence of IDC-P. Using Guo and Epstein’s diagnostic criteria [1], IDC-P was observed in 24 patient specimens (63%); 18 of which (47% of patients) had moderate-extensive IDC-P (Table 1 and Table S1). IDC-P was often, but not exclusively, identified in conjunction with poorly differentiated adenocarcinoma, perineural invasion and high-grade prostatic intraepithelial neoplasia (Table S1). There was no difference in median overall survival in this series of patients with vs without IDC-P (3.2 vs 1.8 years; age-adjusted Cox proportional hazard ratio 0.75, P = 0.47 [Table 1]), with a median follow-up of 4.9 years from diagnosis of castrate-resistant prostate cancer (CRPC). These data confirm that IDC-P is a common feature of
hormone-naïve localized prostate cancers that later progress to CRPC. Given previous reports of IDC-P persisting in localized prostate cancer after therapy [13–15], this prompted us to examine the response of IDC-P to ADT.

To investigate the biological behaviour of IDC-P in vivo, we established PDXs from seven patients with localized high-risk and/or familial prostate cancer. The cohort included four sporadic patients with Gleason grade group 5 tumours, two BRCA2 carriers, and one patient with a family history of cancer, but no identified BRCA mutation (designated BRCAX [Table 2]). Tumour tissue was dissected from radical prostatectomy specimens and cut into multiple pieces that were implanted under the renal capsule of immunocompromised host mice (Fig. 1). Host mice were supplemented with testosterone to model androgen-replete conditions. PDXs were established in host mice for up to 14 weeks before grafts were collected and analysed. PDXs contained both adenocarcinoma and IDC-P, reflecting the mixed pathology of the original tissue (Fig. 1 and Table S2). Thorough examination of each PDX graft showed that IDC-P was present in PDXs from all seven patients (Table S2). Notably, IDC-P appeared to be a more prominent feature of PDXs from BRCAX-Mutant prostate cancers, present in 49% of grafts (17/37) derived from the three BRCAX-Mutant specimens compared with 22% of grafts (11/48) derived from the sporadic specimens (Table S2).

The IDC-P retained its characteristic morphological features in the PDXs, including a cribriform or solid architecture with AMACR- and ERG-positive luminal cells and peripheral p63-positive basal cells (Fig. 2A). Similar to adenocarcinoma, IDC-P also showed PSA and nuclear AR expression and was negative for AR-V7 expression (Fig. 2A). Notably, the average volume of IDC-P in the PDXs (3.3 \( \pm 1.8 \times 10^7 \) mm\(^3\)) was similar to that of adenocarcinoma (3.4 \( \pm 2.6 \times 10^7 \) mm\(^3\); \( P = 0.98 \) [Fig. 2B]), as was the percent of proliferating tumour cells, marked by Ki67 expression (15.6 \( \pm 3.3 \% \) vs 19.0 \( \pm 4.6 \% \); \( P = 0.29 \) [Fig. 2C]). No difference was observed in Ki67 expression and tumour volume between PDXs of sporadic and BRCAX-Mutant prostate cancers (Fig. 2B,C). This demonstrates that IDC-P comprises a significant proportion of the cancer burden in PDXs of high-risk prostate cancer.

The co-existence of both adenocarcinoma and IDC-P in PDXs allowed us to compare the response of both pathologies to androgen deprivation. To mimic ADT in our PDX model, host mice were castrated. PDXs were collected 4–8 weeks after castration and compared with patient-matched control PDXs from non-castrated hosts (Fig. 3A). Notably, residual IDC-P lesions were identified in PDXs from castrated mice for five of the seven patients, demonstrating that a subpopulation of cells within IDC-P withstand castration (Table S2). Residual IDC-P lesions maintained AMACR and p63 expression; however, ERG staining was decreased, PSA expression markedly reduced or absent and AR was predominantly localized to the cytoplasm instead of the nucleus.
Fig. 1 Schematic overview of the patient-derived xenograft (PDX) protocol. (A) Radical prostatectomy specimens were obtained from patients with high-risk and/or BRCA-mutant prostate cancer. (B) Patient specimens were dissected into multiple small pieces and implanted under the renal capsule of immunocompromised host mice (dotted lines). (C) Six to 24 weeks after implantation, grafts were collected and their histology analysed. The PDXs contained both adenocarcinoma (arrowhead) and IDC-P (arrow). Scale bars = 2 mm (B), 200 µm (C; black) and 50 µm (C; white).

Fig. 2 Intraductal carcinoma of the prostate (IDC-P) is a prominent feature in patient-derived xenografts (PDXs) of high-risk prostate cancers. (A) PDX tumours from sporadic and BRCA-mutant prostate cancer contained both adenocarcinoma and IDC-P. Adenocarcinoma and IDC-P are both positive for the luminal cell marker α-methyl acyl coenzyme-A racemase (AMACR; red). IDC-P can be identified by the presence of p63-positive basal cells (brown) surrounding the periphery of the lesion. IDC-P and adenocarcinoma show similar expression of ERG, PSA and androgen receptor (AR) and are both negative for AR splice variant-7 (AR-V7). (B) Adenocarcinoma and IDC-P were present at a similar volume in PDXs of high-risk sporadic (triangles) and BRCA-mutant (circles) prostate cancers (n = 7; P = 0.98; paired t-test). (C) The percentage of proliferating cells, marked by Ki67, was similar in IDC-P compared with adenocarcinoma in PDXs from sporadic (triangles) and BRCA-mutant (circles) prostate cancers (n = 7; P = 0.29; paired t-test). All data are expressed as mean ± SEM. AdCa, adenocarcinoma. Scale bars = 50 µm.
nucleus, consistent with the acute decrease in systemic androgens (Fig. 3B). The percentage of proliferating Ki67-positive tumour cells was also significantly reduced in IDC-P after castration (2.2 ± 1.0%) compared with control PDXs (15.6 ± 3.3%; P < 0.05 [Fig. 3C]). This was consistent between PDXs of sporadic and BRCA-mutant prostate cancers (Fig. 3C). To determine whether the residual IDC-P lesions have regenerative potential, testosterone was re-administered to castrate hosts (Fig. 3A). Testosterone re-administration led to larger lesions, nuclear localization of the AR and restoration of PSA and Ki67 expression in five of the seven patients (Fig. 3B, C and Table S2). As previously shown, adenocarcinoma also persisted in PDXs after castration and regenerated after restoration of testosterone levels (Table S2 and Fig. S1) [17]. Increased expression of AR splice variants, including AR-V7, is a potential mechanism for AR transcriptional activity in CRPC [19]. With this in mind, we investigated the expression of AR-V7 across all PDXs. AR-V7 expression was not increased in IDC-P and adenocarcinoma after castration or testosterone restoration (Figs 3B and S1). Thus, IDC-P and adenocarcinoma exhibit a similar response to castration, both containing residual populations of castrate-tolerant, regenerative tumour cells that persist after androgen depletion.

**Discussion**

This study shows that IDC-P is common in patients who develop advanced prostate cancer and can persist after androgen deprivation. This extends our recent identification of ‘castrate-tolerant’ cells within hormone-naive localized adenocarcinoma [17]. We have now demonstrated the usefulness of PDXs for studying the functional behaviour of IDC-P in vivo and confirm that a similar population of castrate-tolerant cells also reside in IDC-P foci. Importantly, the castrate-tolerant cancer cells identified in IDC-P displayed regenerative potential; re-administration of testosterone resulted in the re-emergence of proliferating tumours, with pathology matching the original specimens. Adenocarcinoma also regressed after androgen deprivation in these PDXs. This finding is similar to previous studies, where castrate-tolerant cells have been identified in other cohorts of PDXs from moderate- to high-grade tumours [17,20,21]. The residual
tumour cells in IDC-P and adenocarcinoma are not yet castrate-resistant, as they do not proliferate autonomously in the absence of systemic androgens. Nevertheless, by surviving androgen deprivation, they may act as precursors to CRPC that can acquire additional mechanisms of castration resistance over time [22]. The retrospective study of prostate cancer cases showed that IDC-P is highly prevalent (63%) in patients destined to develop advanced prostate cancer. This is consistent with our recent systematic review, which showed that the prevalence of IDC-P increases from 2.1% in low-risk patient cohorts to 23.1%, 36.7% and 56.0% in large cohorts of moderate-risk, high-risk, and metastatic or recurrent disease categories, respectively [7]. Whilst IDC-P has been associated with poor clinical outcomes [3,4,10,23], our retrospective analysis did not show a significant difference in survival from development of CRPC in patients with or without IDC-P. Indeed, patients with or without IDC-P had a similar time to death from CRPC diagnosis; however, this was a retrospective study on a highly selected, small cohort of patients with aggressive clinical characteristics. Prospective reporting of IDC-P in patients with diverse clinical features is required to definitely establish the relationship between IDC-P and clinical outcomes.

Using the PDX model, we were able to study the functional features of IDC-P in vivo. During the first generation of grafting, primary PDXs retain the existing architecture of the original patient tissue [16,17,24]. PDXs were established from high-risk prostate cancer based on Gleason grade group or the presence of a germline BRCA mutation, because IDC-P is more prevalent in these patient cohorts [5,7]. In all seven patients, IDC-P was present in the original radical prostatectomy specimen and its growth pattern was maintained in the PDXs. Consistent with our previous work demonstrating a high prevalence of IDC-P in BRCA2-mutant prostate cancer [5], IDC-P was a common feature of PDXs from BRCA2-mutant specimens. Whilst prostate cancers from germline BRCA2 carriers show aggressive clinical progression and contain de novo genomic aberrations usually associated with metastatic disease [9,25], the response of IDC-P to androgen deprivation was indistinguishable between sporadic and familial cases.

IDC-P typically co-exists with adenocarcinoma [26]. This was observed in our PDX model, as both growth patterns were present within the same xenografts. Recent genomic profiling of IDC-P compared with adjacent adenocarcinoma showed that these two pathologies arise from a common tumour clone, with no evidence of multiple independent tumours [9]. IDC-P and adenocarcinoma share the majority of the mutational profile, diverging later in tumour evolution [9]. This supports previous studies suggesting that IDC-P arises as a result of the retrograde movement of tumour cells back into pre-existing prostatic ducts [11]; however, in rare cases, IDC-P has been observed in isolation where it may act as a precursor lesion [27–29].

The persistence of IDC-P in PDXs after androgen deprivation is consistent with data from patient specimens [13,14]; however, IDC-P was not more resistant to treatment than adenocarcinoma in our PDX model. It is unlikely that the previously described association between IDC-P and poor outcome is attributable to inherent therapy resistance. Rather, the co-existence of distinct tumour clones from IDC-P and adenocarcinoma may increase the risk that at least one clone will metastasize. Indeed, there is evidence to suggest that IDC-P does have metastatic seeding potential [30]. Alternatively, IDC-P may simply be a common feature of aggressive tumours. Recent studies have shown that tumours with IDC-P are enriched for adverse prognostic features, including genomic aberrations, genomic instability and hypoxia [9,10], which may collectively result in aggressive tumours with increased metastatic potential. Further work is still required to understand the significance of IDC-P in disease progression.

In summary, we have shown that IDC-P is relevant in patients with aggressive prostate cancer and contains castrate-tolerant cells that withstand androgen deprivation. Castrate-tolerant cells in both IDC-P and adenocarcinoma warrant further investigation because targeting these cells may delay biochemical and clinical treatment failure. Indeed, it will be important to examine the response of castrate-tolerant cells to systemic therapies that are currently used for advanced prostate cancer [31], given the increasing interest in using these compounds in the setting of high-risk localized prostate cancer. PDXs provide an opportunity to further address these questions and implement new strategies to improve our understanding of the biological significance of IDC-P.

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Conflicts of Interest

None declared.
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Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Fig. S1.** Adenocarcinoma contains regenerative castrate-tolerant cells.

**Table S1.** Clinical and pathological features of metastatic patient cohort.

**Table S2.** Different pathology types identified in individual patient-derived xenografts from seven patients with high-risk prostate cancer.

**Appendix S1.** Antibody details and staining conditions for immunohistochemistry.