University Library

MINERVA ACCESS

A gateway to Melbourne's research publications

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:
Porter, LH;Hashimoto, K;Lawrence, MG;Pezaro, C;Clouston, D;Wang, H;Papargiris, M;Thorne, H;Li, J;Ryan, A;Norden, S;Moon, D;Bolton, DM;Sengupta, S;Frydenberg, M;Murphy, DG;Risbridger, GP;Taylor, RA

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

Date:
2018-06-01

Citation:
Porter, L. H., Hashimoto, K., Lawrence, M. G., Pezaro, C., Clouston, D., Wang, H., Papargiris, M., Thorne, H., Li, J., Ryan, A., Norden, S., Moon, D., Bolton, D. M., Sengupta, S., Frydenberg, M., Murphy, D. G., Risbridger, G. P. & Taylor, R. A. (2018). Intraductal carcinoma of the prostate can evade androgen deprivation, with emergence of castrate-tolerant cells. BJU International, 121 (6), pp.971-978. https://doi.org/10.1111/bju.14043.

Persistent Link:
https://hdl.handle.net/11343/293774

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

Laura H. Porter[a,b*], Kohei Hashimoto[a,c,*], Mitchell G. Lawrence[a,b*], Carmel Pezaro[a,d], David Clouston[e], Hong Wang[a], Melissa Papargiris[a,f], Heather Thorne[g,h], Jason Li[i], kConFab[g,h], Andrew Ryan[e], Sam Norden[e], Daniel Moon[j,k], Damien M. Bolton[l,m], Shomik Sengupta[l,m], Mark Frydenberg[a,n], Declan G. Murphy[h,o], Gail P. Risbridger[a,b*], Renea A. Taylor[a,p*]

a) Department of Anatomy and Developmental Biology, Biomedicine Discovery Institute, Cancer Program, Monash University, Melbourne, Victoria, Australia, 3800
b) Prostate Cancer Research Program, Cancer Research Division, Peter MacCallum Cancer Centre, University of Melbourne, Melbourne, Victoria, Australia, 3000
c) Department of Urology, Sapporo Medical University School of Medicine, South 1, West 16, Chuo-ku, Sapporo, Hokkaido 060-8543 Japan
d) Eastern Health Clinical School, Monash University, Melbourne, Victoria, Australia, 3800
e) TissuPath, Mount Waverley, Victoria, Australia, 3149
f) Australian Prostate Cancer Bioresource, Victorian Node, Monash University, Melbourne, Victoria, Australia, 3800
g) kConFab, Research Department, Peter MacCallum Cancer Centre, Melbourne, Victoria, Australia, 3000
h) Sir Peter MacCallum Department of Oncology, University of Melbourne, Victoria, Australia, 3000
i) Bioinformatics Core, Peter MacCallum Cancer Centre, University of Melbourne, Victoria, Australia, 3000
j) Epworth Healthcare, Richmond, Victoria, Australia, 3121
k) Central Clinical School, Monash University, Melbourne, Victoria, Australia, 3800
l) Department of Urology, Austin Hospital, Melbourne Heidelberg, Victoria, Australia, 3084
m) Department of Surgery, University of Melbourne, Melbourne, Victoria, Australia, 3000
n) Department of Surgery, Monash University, Melbourne, Victoria, Australia, 3800
o) Division of Cancer Surgery, Peter MacCallum Cancer Centre, University of Melbourne, Victoria, Australia, 3000

Author Manuscript

This is the author manuscript accepted for publication and has undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/bju.14043

This article is protected by copyright. All rights reserved

p) Department of Physiology, Biomedicine Discovery Institute, Cancer Program, Monash University, Melbourne, Victoria, Australia 3800

**Authors contributed equally to this work*

Address for correspondence:

Dr. Renea Taylor, PhD

Monash Biomedicine Discovery Institute, Department of Physiology

26 Innovation Way, Clayton VIC Australia 3800

Phone: (03) 9902 9287; Fax: +61 3 9902 9223

Email: renea.taylor@monash.edu

Author Manuscript

This article is protected by copyright. All rights reserved

MR. DANIEL A MOON (Orcid ID : 0000-0002-1557-2584)
PROF. DAMIEN MICHAEL BOLTON (Orcid ID : 0000-0002-5145-6783)
PROF. SHOMIK SENGUPTA (Orcid ID : 0000-0003-3357-1216)
DR. DECLAN MURPHY (Orcid ID : 0000-0002-7500-5899)
DR. RENEA TAYLOR (Orcid ID : 0000-0003-2609-2380)

Article type : Original Article

Article Category: Translational Science

ABSTRACT

Objectives: To determine the relevance of intraductal carcinoma of the prostate (IDC-P) in advanced prostate cancer, we first examined whether IDC-P was originally present in patients who later developed advanced prostate cancer and then used 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 men 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: 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: IDC-P is prevalent in aggressive prostate cancer and contains cells that can withstand androgen deprivation. Thus, IDC-P appears functionally relevant in advanced

This article is protected by copyright. All rights reserved

Author Manuscript

prostate cancer. The presence of IDC-P may be a trigger to develop innovative clinical management plans.

KEYWORDS

Prostate cancer, pathology, intraductal carcinoma of the prostate, androgen deprivation therapy, patient-derived xenografts, *BRCA*

MAIN TEXT

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 due to its low incidence in unselected biopsy specimens (6). However, 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 localised sporadic and *BRCA2*-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 localised prostate cancers following 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

This article is protected by copyright. All rights reserved

Author Manuscript

extent of IDC-P is, however, difficult to assess in matched pre- and post-treatment specimens due to difficulties in precisely resampling the same tumour region. Thus, it is unknown whether existing IDC-P lesions persist following treatment or are selected by treatment. Furthermore, the biological response of IDC-P to therapy compared to adenocarcinoma remains unknown. Therefore, we aimed to investigate whether IDC-P is a prominent pathological feature in patients who later failed treatment and to further understand the biological behavior 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-naïve localised prostate cancers to androgen deprivation and identified a subpopulation of 'castrate-tolerant' adenocarcinoma cells that can persist in an androgen-depleted environment (17). Herein, we use 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 (author DC) 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.

This article is protected by copyright. All rights reserved

Author Manuscript

Fresh patient specimens

Human ethics approval was obtained for the collection of fresh prostate tumour tissue through the following Human Research Ethics Committees: Cabrini Institute (07-07-04-14); Epworth Hospital (53611); Monash University Human Research Ethics Committees (RMO 2006/6108 – 2004000145); and Peter MacCallum Cancer Centre (97_27). Localised 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). Following 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 antimycin and 100 µg/ml gentamycin. To prepare the specimens for xenografting, tumour tissue was dissected into approximately 40 pieces per patient, each piece approximately 4 mm^3. 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 (NSG) mice, which were housed in a 12-hour light-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.

This article is protected by copyright. All rights reserved

Author Manuscript

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 following castration, testosterone levels were restored in a subset of castrated mice by implanting a 5 mm testosterone pellet subcutaneously. Grafts were harvested 4-8 weeks after castration or 4 weeks after testosterone restoration. Grafts from castrate hosts and castrate hosts supplemented with testosterone were compared to control grafts from non-castrated hosts.

Analysis of grafts

At collection, grafts were fixed in formalin before being embedded in paraffin. To assess the tumour content of each graft, the entire graft was sectioned into 5 μm sections and every 20th section was stained with haematoxylin and eosin. Regions of IDC-P were identified using the criteria defined by Guo and Epstein (1). The presence of IDC-P was confirmed by dual immunohistochemical staining for the malignant luminal cell marker alpha methyl coA racemase (AMACR) and the basal cell marker p63. Staining was also conducted for the proliferative marker Ki67, androgen receptor (AR), prostate-specific antigen (PSA), ERG and androgen receptor splice variant-7 (AR-V7). 22Rv1 cells were used as a positive control for AR-V7 staining. All immunohistochemistry was performed using the Leica BOND-MAX-TM automated system (Leica MicroSystems, Victoria, Australia). Primary and secondary antibody details and staining conditions are outlined in **Supplementary Methods**.

Adenocarcinoma and IDC-P were frequently present in the same xenografts. To determine the volume of adenocarcinoma and IDC-P within xenografts from control (non-castrated) host mice, slides were imaged using the Aperio ScanScope AT Turbo slide scanner (Leica Microsystems GmbH, Hesse, Germany). The area of adenocarcinoma and IDC-P on every 20th section per xenograft was then determined using ImageScope analysis software (Aperio, version 12.2). Volume was derived by multiplying the area by 100 to take into account the number of sections analysed (every 20th) and the thickness of each section (5 μm).

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

This article is protected by copyright. All rights reserved

Author Manuscript

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 difference between groups was determined using a paired t-test or a 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. Statistical significance was set at $P < 0.05$.

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; 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 (3.2 years) and without IDC-P (1.8; age-adjusted COXPH HR: 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 localised prostate cancers that later progress to CRPC. Given previous reports of IDC-P persisting in localised prostate cancer following therapy (13-15), this prompted us to examine the response of IDC-P to ADT.

To investigate the biological behavior of IDC-P *in vivo*, we established PDXs from seven patients with localised 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 (**Figure 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

This article is protected by copyright. All rights reserved

Author Manuscript

pathology of the original tissue (**Figure 1; Table S2**). Thorough examination of each PDX graft showed that IDC-P was present in PDXs from all seven cases, with 34% (28 of 85) of grafts containing IDC-P and 48% (38 of 85) of grafts containing adenocarcinoma from the seven patients (**Table S2**). Notably, IDC-P appeared to be a more prominent feature of PDXs from *BRCA*-mutant prostate cancers, present in 49% (17 of 37) of grafts derived from the three *BRCA*-mutant specimens compared to 22% (11 of 48) of grafts derived from the sporadic specimens (**Table S2**).

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 (**Figure 2A**). Similar to adenocarcinoma, IDC-P also showed PSA and nuclear AR expression and was negative for AR-V7 expression (**Figure 2A**). Notably, the average volume of IDC-P in the PDXs ($3.3 \times 10^7 \pm 1.8 \times 10^7$ μm^3) was similar to that of adenocarcinoma ($3.4 \times 10^7 \pm 2.6 \times 10^7$ μm^3; $P = 0.98$; **Figure 2B**), as was the percent of proliferating tumour cells, marked by Ki67 expression (15.6 ± 3.3% vs 19.0 ± 4.6%; $P = 0.29$; **Figure 2C**). No difference was observed in Ki67 expression and tumour volume between PDXs of sporadic and *BRCA*-mutant prostate cancers (**Figure 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 to patient-matched control PDXs from non-castrated hosts (**Figure 3A**). Notably, residual IDC-P lesions were identified in PDXs from castrated mice for 5 of the 7 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 localised to the cytoplasm instead of the nucleus, consistent with the acute decrease in systemic androgens (**Figure 3B**). The percentage of proliferating Ki67-positive tumour cells was also significantly reduced in IDC-P following castration (2.2 ± 1.0%) compared to control PDXs (15.6 ± 3.3%; $P < 0.05$; **Figure 3C**). This was consistent between PDXs of sporadic and *BRCA2*-mutant prostate cancers (**Figure 3C**). To determine whether the residual IDC-P lesions have regenerative potential, testosterone was re-administered to castrate hosts (**Figure 3A**). Testosterone re-

This article is protected by copyright. All rights reserved

Author Manuscript

administration led to larger lesions, nuclear localisation of the AR and restoration of PSA and Ki67 expression in 5 of the 7 patients (**Figure 3B, C; Table S2**). As previously shown, adenocarcinoma also persisted in PDXs after castration and regenerated after restoration of testosterone levels (**Table S2; Figure 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 following castration or testosterone-restoration (**Figure 3B, 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 demonstrates that IDC-P is common in patients who develop advanced prostate cancer and can persist following androgen deprivation. This extends our recent identification of 'castrate-tolerant' cells within hormone-naïve localised adenocarcinoma (17). We have now demonstrated the usefulness of PDXs for studying the functional behavior of IDC-P *in vivo* and confirm that a similar population of castrate-tolerant cells also reside in IDC-P foci. Importantly, 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 following 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 systematic 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 demonstrated 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

This article is protected by copyright. All rights reserved

Author Manuscript

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 cases, 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 *BRCA*2-mutant prostate cancer (5), IDC-P was a common feature of PDXs from *BRCA*-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 to adjacent adenocarcinoma demonstrated that these two pathologies arise from a common tumour clone, with no evidence of multiple independent tumours (9). IDC-P and adenocarcinoma shared the majority of the mutational profile, diverging later in tumour evolution (9). This supports previous studies suggesting that IDC-P arises due to 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 following androgen deprivation is consistent with data from patient specimens (13, 14). However, IDC-P was not more resistant to treatment than

This article is protected by copyright. All rights reserved

Author Manuscript

adenocarcinoma in our PDX model. It is unlikely that the previously described association between IDC-P and poor outcome is due 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 metastasise. 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 as 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 localised 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.

ACKNOWLEDGEMENTS

We thank the Australian Prostate Cancer BioResource for specimen collection and the patients who donated their tissue. We also thank Stephen Plymate for providing the AR-V7 antibody. This work was supported by funding from the National Health and Medical Research Council of Australia (Fellowship to MGL 1035721, Fellowship to GPR 1102752, Project Grant 1077799), the Victorian Cancer Agency (Fellowship to RAT MCRF15023, CAPTIV Program), the EJ Whitten Foundation, the Peter and Lyndy White Foundation and TissuPath Pathology.

CONFLICTS OF INTEREST

None

REFERENCES

This article is protected by copyright. All rights reserved

Author Manuscript

1. Guo CC, Epstein JI. Intraductal carcinoma of the prostate on needle biopsy: histologic features and clinical significance. Mod Pathol. 2006;19(12):1528-35.

2. Van Der Kwast T, Al Daoud N, Collette L, Sykes J, Thoms J, Milosevic M, et al. Biopsy diagnosis of intraductal carcinoma is prognostic in intermediate and high risk prostate cancer patients treated by radiotherapy. Eur J Cancer. 2012;48(9):1318-25.

3. Kato M, Tsuzuki T, Kimura K, Hirakawa A, Kinoshita F, Sassa N, et al. The presence of intraductal carcinoma of the prostate in needle biopsy is a significant prognostic factor for prostate cancer patients with distant metastasis at initial presentation. Mod Pathol. 2016;29(2):166-73.

4. Kimura K, Tsuzuki T, Kato M, Saito AM, Sassa N, Ishida R, et al. Prognostic value of intraductal carcinoma of the prostate in radical prostatectomy specimens. Prostate. 2014;74(6):680-7.

5. Risbridger GP, Taylor RA, Clouston D, Sliwinski A, Thorne H, Hunter S, 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.

6. Watts K, Li J, Magi-Galluzzi C, Zhou M. Incidence and clinicopathological characteristics of intraductal carcinoma detected in prostate biopsies: A prospective cohort study. Histopathology. 2013;63(4):574-9.

7. Porter LH, Lawrence MG, Ilic D, Clouston D, Bolton DM, Frydenberg M, et al. Systematic review links prevalence of intraductal carcinoma of the prostate to prostate cancer risk categories. Eur Urol. 2017;In press.

8. Dawkins HJ, Sellner LN, Turbett GR, Thompson CA, Redmond SL, McNeal JE, et al. Distinction between intraductal carcinoma of the prostate (IDC-P), high-grade dysplasia (PIN), and invasive prostatic adenocarcinoma, using molecular markers of cancer progression. Prostate. 2000;44(4):265-70.

9. Taylor RA, Fraser M, Livingstone J, Espiritu SMG, Thorne H, Huang V, et al. Germline *BRCA2* mutations drive prostate cancers with distinct evolutionary trajectories. Nat Commun. 2017;8:13671.

10. Chua MLK, Lo W, Pintilie M, Murgic J, Lalonde E, Bhandari V, et al. A Prostate Cancer "Nimbosus": Genomic Instability and SChLAP1 Dysregulation Underpin Aggression of Intraductal and Cribriform Subpathologies. Eur Urol. 2017;In press.

This article is protected by copyright. All rights reserved

Author Manuscript

11. Haffner MC, Weier C, Xu MM, Vaghasia A, Gurel B, Gumuskaya B, et al. Molecular evidence that invasive adenocarcinoma can mimic prostatic intraepithelial neoplasia (PIN) and intraductal carcinoma through retrograde glandular colonization. J Pathol. 2016;238(1):31-41.

12. Bettendorf O, Schmidt H, Staebler A, Grobholz R, Heinecke A, Boecker W, et al. Chromosomal imbalances, loss of heterozygosity, and immunohistochemical expression of TP53, RB1, and PTEN in intraductal cancer, intraepithelial neoplasia, and invasive adenocarcinoma of the prostate. Genes Chromosomes and Cancer. 2008;47(7):565-72.

13. Chen Z, Chen N, Shen P, Gong J, Li X, Zhao T, et al. The presence and clinical implication of intraductal carcinoma of prostate in metastatic castration resistant prostate cancer. Prostate. 2015;75(12):1247-54.

14. Efstathiou E, Abrahams NA, Tibbs RF, Wang X, Pettaway CA, Pisters LL, et al. Morphologic characterization of preoperatively treated prostate cancer: toward a post-therapy histologic classification. Eur Urol. 2010;57(6):1030-8.

15. O'Brien C, True LD, Higano CS, Rademacher BLS, Garzotto M, Beer TM. Histologic changes associated with neoadjuvant chemotherapy are predictive of nodal metastases in patients with high-risk prostate cancer. Am J Clin Pathol. 2010;133(4):654-61.

16. Lawrence MG, Taylor RA, Toivanen R, Pedersen J, Norden S, Pook DW, et al. A preclinical xenograft model of prostate cancer using human tumors. Nat Protoc. 2013;8(5):836-48.

17. Toivanen R, Frydenberg M, Murphy D, Pedersen J, Ryan A, Pook D, et al. A preclinical xenograft model identifies castration-tolerant cancer-repopulating cells in localized prostate tumors. Sci Transl Med. 2013;5(187):187ra71.

18. Delahunt B, Egevad L, Grignon DJ, Srigley JR, Samaratunga H. Prostate cancer grading: recent developments and future directions. BJU Int. 2016;117 Suppl 4:7-8.

19. Robinson D, Van Allen EM, Wu YM, Schultz N, Lonigro RJ, Mosquera JM, et al. Integrative clinical genomics of advanced prostate cancer. Cell. 2015;161(5):1215-28.

20. Zhao H, Nolley R, Chen Z, Peehl DM. Tissue slice grafts: an in vivo model of human prostate androgen signaling. Am J Pathol. 2010;177(1):229-39.

21. Zhao H, Thong A, Nolley R, Reese SW, Santos J, Ingels A, et al. Patient-derived tissue slice grafts accurately depict response of high-risk primary prostate cancer to androgen deprivation therapy. J Transl Med. 2013;11:199.

This article is protected by copyright. All rights reserved

Author Manuscript

22. Risbridger G, Taylor R. To target or not to target the enemy within localized prostate cancer. Cell Cycle. 2013;12(21):3349-50.
23. Zhao T, Liao B, Yao J, Liu J, Huang R, Shen P, et al. Is There any prognostic impact of intraductal carcinoma of prostate in initial diagnosed aggressively metastatic prostate cancer? Prostate. 2015;75(3):225-32.
24. Wang Y, Revelo M, Sudilovsky D, Cao M, Chen W, Goetz L, et al. Development and characterization of efficient xenograft models for benign and malignant human prostate tissue. The Prostate. 2005;64(2):149-59.
25. Thorne H, Willems AJ, Niedermayr E, Hoh IM, Li J, Clouston D, et al. Decreased prostate cancer-specific survival of men with BRCA2 mutations from multiple breast cancer families. Cancer Prev Res (Phila). 2011;4(7):1002-10.
26. Montironi R, Cheng L, Lopez-Beltran A, Scarpelli M, Montorsi F. A better understating of the morphological features and molecular characteristics of intraductal carcinoma helps clinicians further explain prostate cancer aggressiveness. Eur Urol. 2015;67(3):504-7.
27. Robinson BD, Epstein JI. Intraductal carcinoma of the prostate without invasive carcinoma on needle biopsy: emphasis on radical prostatectomy findings. J Urol. 2010;184(4):1328-33.
28. Miyai K, Divatia MK, Shen SS, Miles BJ, Ayala AG, Ro JY. Heterogeneous clinicopathological features of intraductal carcinoma of the prostate: A comparison between 'precursor-like' and 'regular type' lesions. Int J Clin Exp Pathol. 2014;7(5):2518-26.
29. Khani F, Epstein JI. In response to 'a plea for greater standardization' in intraductal carcinoma of the prostate: Greater standardization requires greater evidence. Histopathology. 2016;Dec 10:10.1111/his.13147.
30. Lindberg J, Kristiansen A, Wiklund P, Grönberg H, Egevad L. Tracking the origin of metastatic prostate cancer. Eur Urol. 2015;67(5):819-22.
31. Corfield J, Crozier J, Joshua AM, Bolton D, Lawrentschuk N. Understanding the role of new systemic agents in the treatment of prostate cancer. BJU Int. 2016;118 Suppl 3:8-13.

FIGURE LEGENDS

Figure 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

This article is protected by copyright. All rights reserved

Author Manuscript

implanted under the renal capsule of immunocompromised host mice (dotted lines). (**C**) Six to fourteen 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).

Figure 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 alpha 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, prostate-specific antigen (PSA) and androgen receptor (AR) and are both negative for androgen receptor splice variant-7 (AR-V7). (**B**) Adenocarcinoma and IDCP 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 to 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. Abbreviations: AdCa, adenocarcinoma; AMACR, alpha methyl coenzyme-A racemase; AR, androgen receptor; AR-V7, androgen receptor splice variant-7; IDC-P, intraductal carcinoma of the prostate; PSA, prostate-specific antigen. Scale bars = 50 μm.

Figure 3. Intraductal carcinoma of the prostate (IDC-P) contains regenerative, castrate-tolerant cells. (**A**) Schematic representation of the experiment. (**B**) Immunohistochemical staining for alpha methyl coA racemase (AMACR; red) and p63 (brown), ERG, prostate-specific antigen (PSA), androgen receptor (AR) and androgen receptor splice variant-7 (AR-V7) PDXs following castration (Cx) and testosterone re-administration (Cx + T) from a representative patient. (**C**) The percentage of proliferating tumour cells, marked by Ki67 expression, in control (n = 7), Cx (n = 4) and Cx + T (n = 5) PDXs of high-risk sporadic (triangles) and *BRCA*-mutant (circles) prostate cancers. Proliferation was significantly decreased in IDC-P following castration and restored following testosterone re-administration (*P < 0.05; one-way ANOVA with post hoc Dunnett's test). All data are expressed as mean ± SEM. Abbreviations: AdCa, adenocarcinoma; AMACR, alpha methyl coenzyme-A racemase; AR, androgen receptor; AR-V7, androgen receptor splice variant-7; Cx, PDXs growing in castrated host mice; Cx + T, PDXs

This article is protected by copyright. All rights reserved

Author Manuscript

growing in castrated host mice supplemented with testosterone; PSA, prostate-specific antigen; T, testosterone. Scale bars = 50 μm.

Author Manuscript

This article is protected by copyright. All rights reserved

Table 1. Summary of the metastatic prostate cancer patient cohort.

Patient characteristics	**Entire cohort**	**With IDC-P**	**Without IDC-P**
Number of patients	38	24 (63%)	14 (37%)
Age at diagnosis	65.6 (53.4-83.0)	65.7 (53.4-82.6)	65.1 (57.6-83.0)
Gleason grade group	5 (2-5)	5 (3-5)	3 (2-5)
Gleason score	9 (7-10)	9 (7-9)	8 (7-10)
Time to death from CRPC diagnosis (years)	3.0 (1.5-4.5)	3.2 (1.9-4.5)	1.8 (0.0-3.7)

The presence of IDC-P was reviewed in the patients' diagnostic biopsy and/or surgery specimens. Age, Gleason grade group and Gleason score are shown as median with range. Time to death from CRPC diagnosis is shown as median with 95% confidence intervals.

Abbreviations: CRPC, castrate-resistant prostate cancer; IDC-P, intraductal carcinoma of the prostate.

Author Manuscript

This article is protected by copyright. All rights reserved

Table 2. Clinicopathological features of patients used for patient-derived xenografts.

Patient no.	Tumour type	BRCA nomenclature	Age	PSA (ng/dL)	Gleason grade group	Gleason score	Pathological stage	IDC-P in RP tissue
1	Sporadic[#]	n/a	73.6	16.8	5	4+5 (9)	T3bN0	Yes
2	Sporadic[#]	n/a	61.4	6.2	5	4+5 (9)	T3bN1	Yes
3	Sporadic[#]	n/a	68.5	2.6	5	4+5 (9)	T3bN1	Yes
4	Sporadic[#]	n/a	70.5	7.0	5	4+5 (9)	T3bN1	Yes
5	*BRCA2*[*]	*BRCA2* 5507 C>G (S1760X)	54	5.8	2	3+4 (7)	T3aN0	Yes
6	BRCAX[*]	n/a	62	6.1	5	5+4 (9)	T3bN0	Yes
7	*BRCA2*	*BRCA2* 5301_5302 ins A (STOP 1694)	56	2.2	5	4+5 (9)	T3aN0	Yes

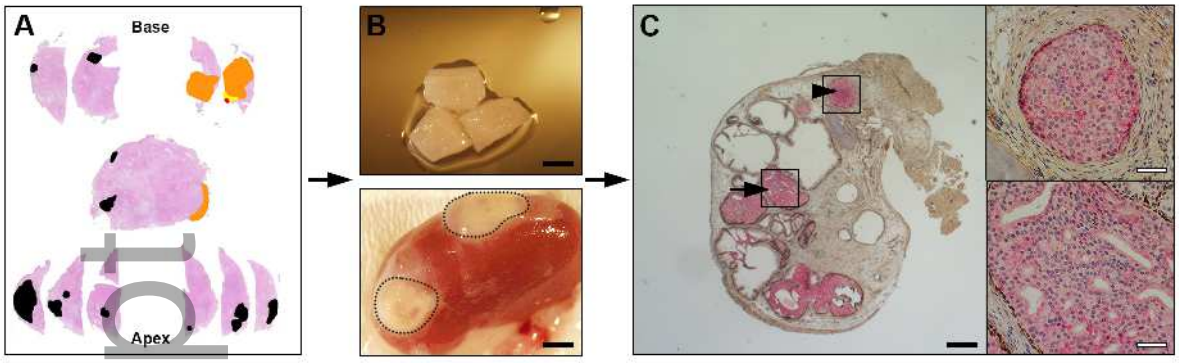
[#] Sporadic patients classified as high-risk according to D'Amico criteria.

[*] Patients included in Risbridger et al. 2015 [4].

Abbreviations: BRCAX, family history without an identified *BRCA* mutation; IDC-P, intraductal carcinoma of the prostate; n/a, not applicable; PSA, prostate-specific antigen; RP, radical prostatectomy.

Author Manuscript

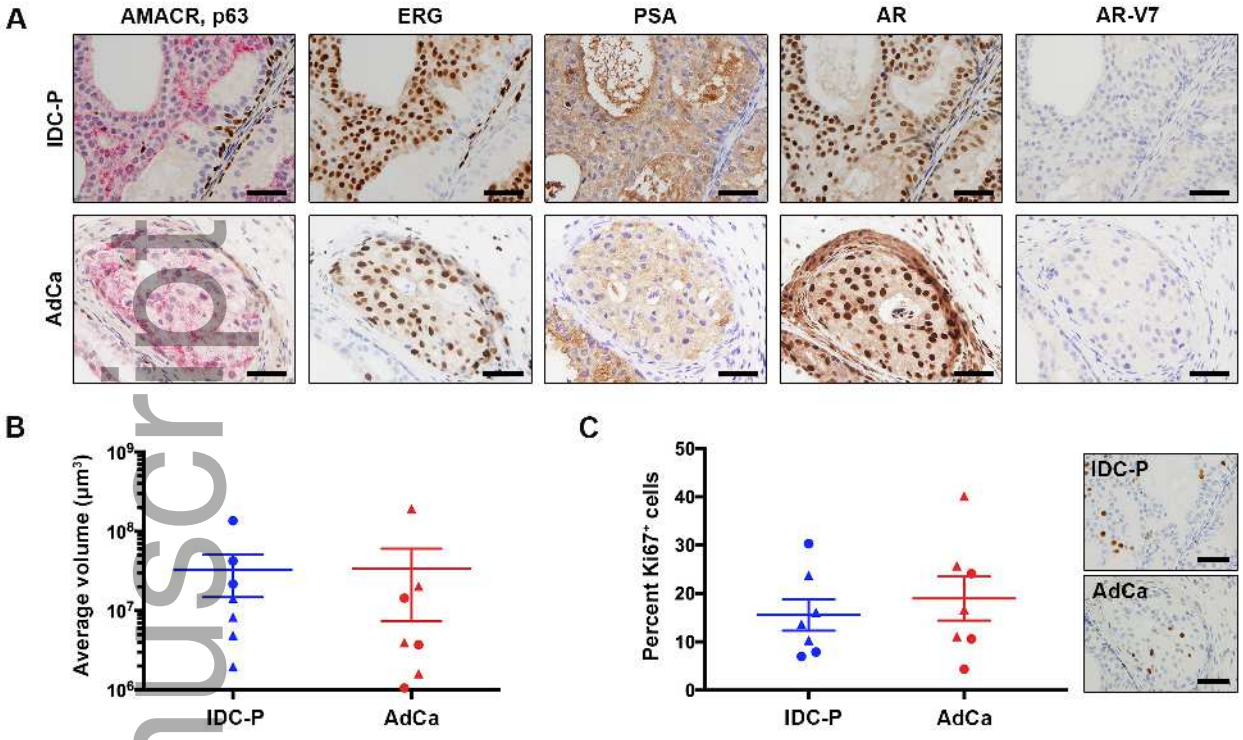
This article is protected by copyright. All rights reserved



bju_14043_f1.tif

Author Manuscript

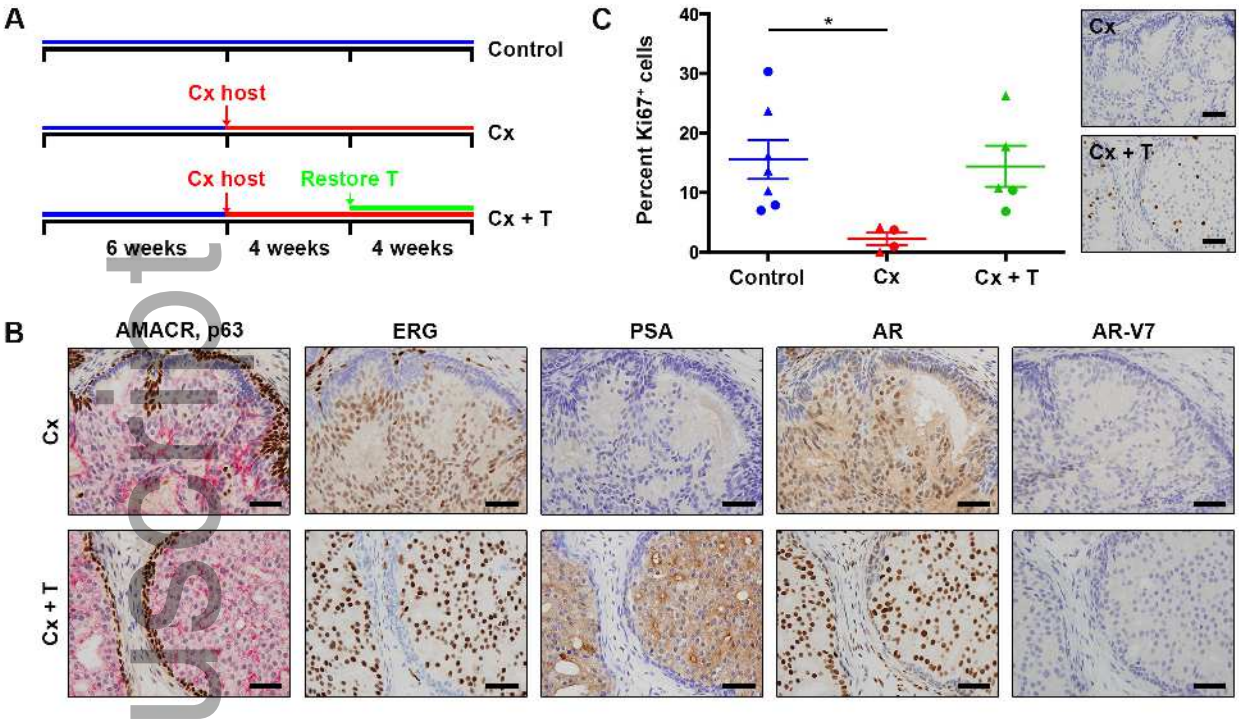
This article is protected by copyright. All rights reserved



bju_14043_f2.tif

Author Manuscript

This article is protected by copyright. All rights reserved



bju_14043_f3.tif

Author Manuscript

This article is protected by copyright. All rights reserved