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# Patient-derived Models Reveal Impact of the Tumor Microenvironment on Therapeutic Response

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**Abstract**

***Background:*** Androgen deprivation therapy is a first-line treatment for disseminated prostate cancer (PCa). However, virtually all tumors become resistant and recur as castration-resistant PCa, which has no durable cure. One major hurdle in the development of more effective therapies is the lack of preclinical models that adequately recapitulate the heterogeneity of PCa, significantly hindering the ability to accurately predict therapeutic response.

***Objective:*** To leverage the ex vivo culture method termed *patient-derived explant* (PDE) to examine the impact of PCa therapeutics on a patient-by-patient basis.

***Design, setting, and participants:*** Fresh PCa tissue from patients who underwent radical prostatectomy was cultured as PDEs to examine therapeutic response.

***Outcome measurements and statistical analysis:*** The impact of genomic and chemical perturbations in PDEs was assessed using various parameters (eg, AR levels, Ki67 staining, and desmoplastic indices).

***Results and limitations:*** PDE maintained the integrity of the native tumor microenvironment (TME), tumor tissue morphology, viability, and endogenous hormone signaling. Tumor cells in this model system exhibited de novo proliferative capacity. Examination of the native TME in the PDE revealed a first-in-field insight into patient-specific desmoplastic stromal indices and predicted responsiveness to AR-directed therapeutics.

***Conclusions:*** The PDE model allows for a comprehensive evaluation of individual tumors in their native TME to ultimately develop more effective therapeutic regimens tailored to individuals. Discernment of novel stromal markers may provide a basis for applying precision medicine in treating advanced PCa, which would have a transformative effect on patient outcomes.

***Patient summary:*** In this study, an innovative model system was used to more effectively mimic human disease. The patient-derived explant (PDE) system can be used to predict therapeutic response and identify novel targets in advanced disease. Thus, the PDE will be an asset for the development of novel metrics for the implementation of precision medicine in prostate cancer.



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## 1. Introduction

Prostatic adenocarcinoma (PCa) is the most frequently diagnosed noncutaneous malignancy and the second leading cause of cancer-related death in American men [1,2]. As prostate tumor cells are reliant on the androgen receptor (AR), first-line therapy for disseminated disease involves targeting the AR signaling axis via androgen deprivation therapy (ADT), often coupled with antiandrogens [3,4]. Although initially effective, these patients develop resistance and experience relapse within a median of 3–4 yr on the development of castration-resistant PCa (CRPC) [5]. Intriguingly, CRPC remains largely AR-dependent due to aberrant reactivation of AR through multiple distinct mechanisms that promote cell survival and proliferation [1,6]. Currently, metastatic CRPC remains universally fatal with no effective cure, highlighting the need to further define mechanisms that control AR activity and thus develop novel means to target recurrent AR activity.

The lack of adequate preclinical models is a major hurdle in discerning innovative methods to target AR and effectively combat PCa. Many promising new therapies that perform well in preclinical evaluations ultimately fail in the clinic, which may reflect the fact that conventional cell lines lack predictive value for drug treatments in clinical disease [7–11]. Studies suggest that the generation of cell lines results in major genetic alterations, loss of tumor heterogeneity, and alterations in growth and invasion properties. Furthermore, culturing cell lines alone deprives them of microenvironmental signal reciprocity. With such key limitations, cell lines cultured as two-dimensional monolayers are not the ideal preclinical platform for studying personalized medicine. To overcome these limitations, patient-derived organoids have recently been developed as three-dimensional culture methods to better recapitulate the biological characteristics of the original tumor [12–14]. However, this technique still fails to effectively mimic the intricate microenvironmental influences contributing to human disease. Thus, there remains a need for improved systems that retain patient-specific genetic alterations and tumor microenvironment (TME) signaling to more accurately nominate effective therapies.

One exciting advance for personalized medicine is the use of patient-derived xenograft (PDX) models, which are increasingly used in translational cancer approaches for drug screening, biomarker development, and preclinical evaluation of therapies through the use of murine “avatars” in co-clinical trials [15]. However, the time to engraftment is a major limitation for the PDX model since it can require between 4 and 8 mo to develop “avatars”. Moreover, although PDXs are effective for many types of cancer, they typically have poor engraftment rates for genitourinary cancers, including PCa, and fail to recapitulate cancer heterogeneity and TME characteristics [16–19]. The TME regulates cellular responses to hormones, growth factors, and therapeutic agents, and is thus a major determinant of carcinogenesis and response to therapeutic intervention. Thus, models that preserve the native human TME are vital for effective evaluation of drug responses.

Building on models previously described [9], we used an ex vivo explant model termed *patient-derived explant* (PDE) to assess drug responses in clinical PCa specimens. The PDE system involves a more simplified and rapid approach to culturing of patient tissue immediately after radical prostatectomy than has been used in current patient-derived models. Importantly, the PDE maintains native TME integrity, tumor tissue morphology, viability, and endogenous AR signaling. Tumor cells in this model system exhibited de novo proliferative capacity, which was an asset when investigating the impact of PDE use for predicting the efficacy of selected therapies. Lastly, examination of the native TME in the PDE revealed first-in-field insight into patient-specific desmoplastic stromal indices that predicted responsiveness to AR-directed therapeutics. In summary, the PDE model more closely mimics TME-inclusive human disease and can facilitate the development of novel metrics for the implementation of precision medicine in PCa.

## 2. Patients and methods

### 2.1. Patient selection

We used material from patients undergoing radical prostatectomy at Thomas Jefferson University (TJU; Philadelphia, PA, USA) and the Royal Adelaide Hospital (Adelaide, Australia). Matched non-neoplastic and tumor tissues were obtained from each patient’s prostate after radical prostatectomy. Patient demographics are described in Supplementary Table 1.

### 2.2. Explant establishment

Patient de-identified prostate tissues were established as ex vivo explant cultures as previously described [9,20,21]. The institutional review board of TJU and the human research ethics committee of the University of Adelaide reviewed the study protocol and deemed the research to be in compliance with federal regulations [45 CFR 46.102(f)].

### 2.3. Immunohistochemistry

For histological analysis, formalin-fixed, paraffin-embedded (FFPE) sections were stained with hematoxylin and eosin (H&E) using standard techniques. For Ki67 analysis, tissues were stained as previously described [22] and scored by a board-certified clinical pathologist using an Aperio microscope and software. The following antibodies were used in the Ki67 protocol at 1:50 dilution for immunohistochemistry (IHC) staining of tissue: HIF1α (Novus Biologicals, Littleton, CO, USA), AR (in house), prostate-specific antigen (PSA; Abcam, Cambridge, UK) and 5-bromo-2-deoxyuridine (BrdU; BD Biosciences, Franklin Lakes, NJ, USA). Clinical grade IHC staining was performed by the clinical pathology department at TJU using a cocktail of antibodies to α-methyl-coA racemase (AMCAR) and the basal cell markers p63 and HMW keratin. Alkaline phosphatase was used to detect racemase expression, while 3,3′-diaminobenzidine was used to detect basal cells.

### 2.4. Enzyme-linked immunosorbent assay

PSA in PDE conditioned culture media diluted 1:50 was detected using a total PSA enzyme-linked immunosrbent assay (ELISA) kit (ALPCO, Salem, NH, USA).

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