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Assessing the Advantages, Limitations and Potential of Human Primary Prostate Epithelial Cells as a Pre-Clinical Model for Prostate Cancer Research

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Keywords

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Cellular Heterogeneity

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Quantitative Phase Imaging

Patient tissue

ABSTRACT

Choosing an appropriate cell model(s) is the first decision to be made before starting a new project or programme of study. Here, we address the rationale that can be behind this decision and we summarise the current cell models that are used to study prostate cancer. Researchers face the challenge of choosing a model that recapitulates the complexity and heterogeneity of prostate cancer. The use of primary prostate epithelial cells cultured from patient tissue is discussed, and the necessity for close clinical-academic collaboration in order to do this is highlighted. Finally, a novel quantitative phase imaging technique is described, along with the potential for cell characterisation to not only include gene expression and protein markers but also morphological features, cell behaviour and kinetic activity.

INTRODUCTION

The foundation of all studies, whether to test novel therapeutic agents or to dissect molecular signaling pathways, is the cellular model that we choose (Figure 1). Ultimately, every model has its advantages and limitations but all too often perhaps a model is chosen because of cost, convenience and accessibility first, with biological relevance coming lower down the list. Despite several drugs showing promise after pre-clinical testing, many clinical trials fail (*1, 2*), and this is after testing in cell models as well as in xenografts (*3, 4*). Thus, this would argue that there is a need for more effective pre-clinical models to give greater chance of success, which would in turn mean improved patient benefit and reduction of wasted funds. Over the years several researchers have met the challenge to generate better and more relevant cellular models for prostate cancer (*5-8*). However, with prostate cancer, as with most cancers, a single model cannot be used to answer all questions.

Current Models for Prostate Cancer Research

Prostate cancer research has relied heavily on a few cell lines. A quick Pubmed search shows >8000 references using LNCaP cells, ~4000-5000 for PC3 and DU145 cells and several others (22RV1, RWPE-1, VCaP) coming in at a few hundred references or fewer. There is a perception in the field that we need more cell lines for prostate cancer but the concern is that new cell lines may not get disseminated globally and picked up by the prostate community and therefore their potential is not realized; this may already be the case for existing ones. Cell lines can be divided into four main groups, cells that are – (i) immortalized by viral

oncogenes (e.g. HPV and SV40) (*5, 9-11*), (ii) cultured from xenograft tumours (*5, 9, 10*), (iii) derived from metastatic lesions (e.g. ascites, lymph nodes, bone) (*5, 9, 10*) and (iv) immortalized using hTERT (*12-15*). The latter is an important group because the cell phenotype is retained and there are no oncogenic changes associated with this method of immortalization (*16*). Also, this method has produced pairs of cell lines (normal and cancer), which are useful for comparison studies (*12, 14*).

The popularity of LNCaPs is obvious since for decades the design of new therapeutics has been focused on improving androgen deprivation drugs. However, it has been known from the beginning that androgen deprivation was never going to be the whole answer to prostate cancer treatment (*17*). Castration-resistant prostate cancer and metastatic prostate cancer remain stubborn foes. More recently, with the advent of ever more sophisticated cell separation and genomic techniques, there have been leaps in understanding of the complexity of prostate cancer and its evolutionary progression (*18-24*).

Addressing Prostate Cancer Heterogeneity

The challenge with prostate cancer is to have cell line models that represent normal prostate, benign prostatic hyperplasia (BPH), Prostatic Intraepithelial Neoplasia (PIN), localized cancers of varying Gleason grades, aggressive and invasive cancers from within the prostate, metastatic cancers, hormone-responsive cancers, castration-resistant cancers and neuroendocrine cancers. And importantly there is also a need to have cell lines representing different races due to the higher incidence of prostate cancer in African American men (*25*). In

addition to the different disease states, severity and locations, there is further heterogeneity to take into account. Prostate cancer is a multifocal disease so there may be more than one tumour in each patient (*26*) and there is inter-patient variability. Along with this there is cellular heterogeneity within each tumour, multiple gene mutations, gene fusions and epigenetic changes (*27*). Finding a cellular model(s) to address all of these parameters is challenging. However, understanding and acknowledging heterogeneity is critical in terms of addressing diagnosis, treatment, resistance and recurrence (*28-30*).

Using Primary Prostate Epithelial Cells as a Cell Model

Primary cell cultures derived from human prostate tumours have the potential to be excellent cellular models to study the disease. They are clinically relevant, representative of current disease and are not difficult to grow (*31-37*). However, one limitation is that they can usually only be obtained from the prostate and not metastatic sites. Using primary cells also opens up the possibility of testing multiple patients thereby taking into account patient variation. However, this also leads to the challenge of assessing and interpreting the variation in response that is observed. How do we decide how many patient samples is enough within a single study? In addition, it is advisable to only use them at low passages, which limits the number of assays that can be done on each sample.

A controversy with use of primary prostate patient cells is the potential for normal cells to overgrow cancer cells (*38, 39*). However, several studies have observed differences between normal and cancer primary prostate epithelial cells such as differential expression of matrix metalloproteinases, integrins, E-cadherin and

behaviour in collagen I gels (*32, 40-42*). When retrieving our samples, we have a dedicated tissue procurement officer who samples the prostate post-radical prostatectomy based on diagnostic MRI scans and trans-rectal ultrasound (TRUS) guided biopsies, taking a needle core from a palpable tumour. Our own studies have shown that cancer cultures are more invasive than benign cultures (*43*), are positive for the TMPRSS2:ERG fusion at low passages (*44*) and samples from high Gleason grades respond differently to drug than benign and low Gleason grade samples (*45*).

One major issue within the field is the lack of standardization in terms of culture media between laboratories. There are differences in terms of the supplements used, some being chosen for their ability to boost cell growth (*32, 33*) and others to maintain a stem/progenitor population within the culture (*31, 43, 46*). This makes comparisons between studies more challenging.

Undertaking a continual assessment and more complete characterization of primary epithelial cells in culture is needed. The advent of new techniques means that it is both possible and necessary to re-visit the characterization of these models. For this reason we are currently collecting both transit amplifying (progenitor) and committed basal cells (more differentiated) cultured from 6 samples each of Normal, BPH, Gleason 6, Gleason 7(3+4), Gleason 7(4+3), Gleason 8(4+4) and Gleason 9 tissue for RNA sequencing. This should hopefully identify new markers related to disease status.

Using patient cells for a variety of future studies will ultimately determine the

utility of these cell cultures and provide support for strengthening clinical-laboratory relations as a research strategy. However, if more labs are to access primary patient material there has to be the will, the funds and the continuity to make a network of relationships work (Figure 2).

Using Quantitative Phase Imaging (QPI) to Characterize a Cell Model and Address Heterogeneity

Cell characterization usually relates to gene expression patterns, protein markers and cell behavior such as invasive potential. However, in order to also take into account cell heterogeneity when considering the effect of a drug or radiation on primary cultures there are two ways to assess their response; the first is to treat the whole population of cells and then separate out different cell types, and the second is to label cells with a fluorescent marker to be able to identify cells within the heterogeneous mixture. Both of these methods have their challenges; cell separation is a laborious procedure and it could be argued that cells change their behaviour when separate relative to when they are a mixture, and fluorescent labeling by whatever means always has the potential to change the cell behaviour. A new technique now available is trying to overcome both these challenges by using quantitative phase imaging (QPI) on heterogeneous cultures to observe individual cell response to drugs in real-time. The Livecyte™ is a microscope that uses the principle of ptychography to generate highly contrasted images such that several parameters can be measured for each cell. The measurements can be used to assess cell morphology (e.g. area, thickness sphericity), cell kinetics (e.g. velocity, meandering index) and population dynamics. For more detailed studies using this technology see these references (*47-49*). The potential advantages of

this technique are to establish cell signatures for different cell types and to identify rare outlier cells. Our previous studies using this technique showed that prostate cell lines and primary prostate epithelial cells differ in their size, speed and growth rates. We also showed that the transit amplifying cells (TA) and committed basal cells (CB) found within primary cultures have different cell signatures, with the TA cells being smaller, thicker and faster than the CB cells (*27*).

Typical drug treatment assays look at the average population, whether there is reduction in cell viability, death by apoptosis or effect on colony forming ability. The rare cells that show resistance to therapeutic agent are likely to be the ones that don't fit in with the average and are therefore masked by their response. By treating every cell as a data-point it is hoped that another layer can be peeled away and more information can be garnered that will contribute to the understanding of therapy resistance, tumour recurrence and identification of resistant cell features.

We carried out a study using primary prostate epithelial cells treated with docetaxel, a standard of care chemotherapy treatment for prostate cancer. Five concentrations were chosen to take a closer look at cell behavior (Figure 3). When looking at overall metrics it became clear that as drug concentration increases, cell motility decreases, measured as cell velocity and meandering index. In addition, the sphericity of the cells increases with cells entering mitosis and not being able to divide due to the effect of the docetaxel (*50*); this can clearly be seen in the video captured by the Livecyte™. Interestingly, when looking at the whole population, particularly at something like sphericity, a bi-modal or tri-modal response can be

observed. Thus, it is necessary to closely observe the videos to identify unusual behaviour. One such cell is represented in Figure 4. In comparison to the healthy cell that divides, and a cell that responds to drug in a typical way (failing to divide, spreading out and halting movement), we also show the outlier cell that is more erratic and sequentially rounds up, fails to divide and yet keeps moving. Only further analysis of outlier cells towards their ultimate fate will determine if the power of this new technique can be harnessed to identify resistant cell populations.

DISCUSSION

Going forward, relationships between scientists, clinicians and patients are critical for the progression of prostate cancer research. When addressing treatment response and tumour recurrence, there are several levels of heterogeneity to take into account, which can only be done through use of patient material. Thus, the feasibility of having primary cell cultures, which crucially represent modern disease, as a critical step in the lab to clinic pipeline, has to be explored. One successful example is the use of primary cells to develop an oncolytic adenovirus that is now in clinical trials (*51, 52*). In order for this to become a broad reality, consistency, reproducibility, standardization and practicality are key. The method of conditional reprogramming (CR) (using feeder cells and ROCK inhibitor) has been used by researchers in other cancer research fields and also by some researchers in the prostate field (*53-55*). This is another method that requires further investigation and could be explored alongside the questioning of other fundamentals of tissue culture such as oxygen concentration; several studies have shown that cells can grow indefinitely in physiological oxygen concentrations of

2% and not the standard 20% (*56-58*).

The desire to have more consistent use of primary cells does not negate the need for useful cell lines but it does highlight the need to explore the cell lines that already exist because there may already be the correct model to answer critical research questions. We also have to embrace new technology, to examine cell behaviour at another level of complexity. Combined with traditional markers these technologies could help to give a more complete idea of individual cell signatures (Table 1).

Currently there are many methods that can translate patient prostate tissue to 2D cell culture, co-cultures with patient stroma and 3D models such as spheroids and organoids (Figure 5). However, the range of media, matrices and apparatus that are used are vast (*40, 59-67*). There is much work to be done if there is to be standardization across the prostate community globally, starting with the will to make it so.

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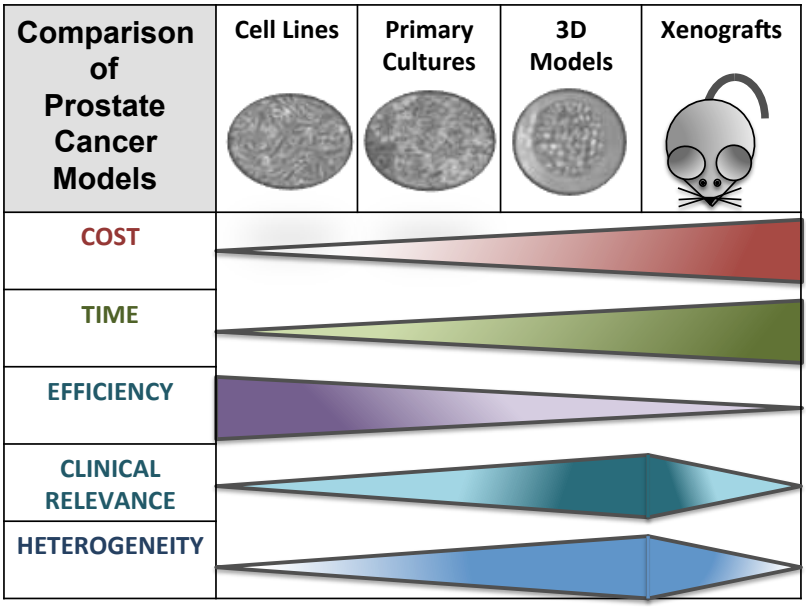
Table 1

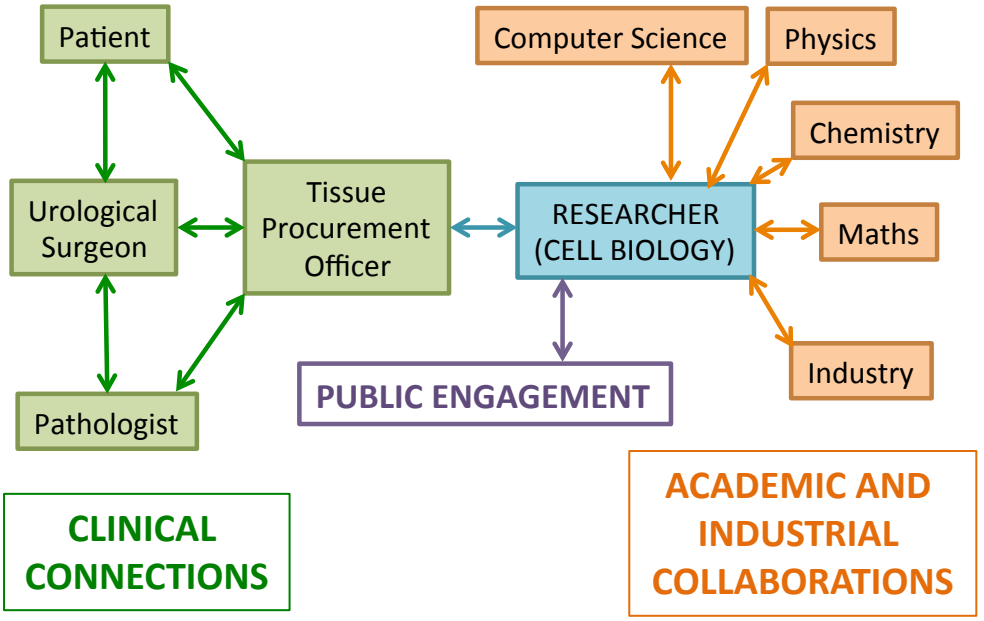
COMPREHENSIVE CELL SIGNATURE CHARACTERISTICS	COMPARISONS TO BE MADE		
Morphological Features	**Disease State**		
	Normal	v	Cancer
Cell Behaviour	**Epithelial Hierarchy**		
Kinetic Activity	Undifferentiated (progenitor) cells	v	Differentiated Cells
Gene Expression	**Response to Therapeutics**		
Protein Markers	Resistant Cells	v	Susceptible Cells

Figure 1



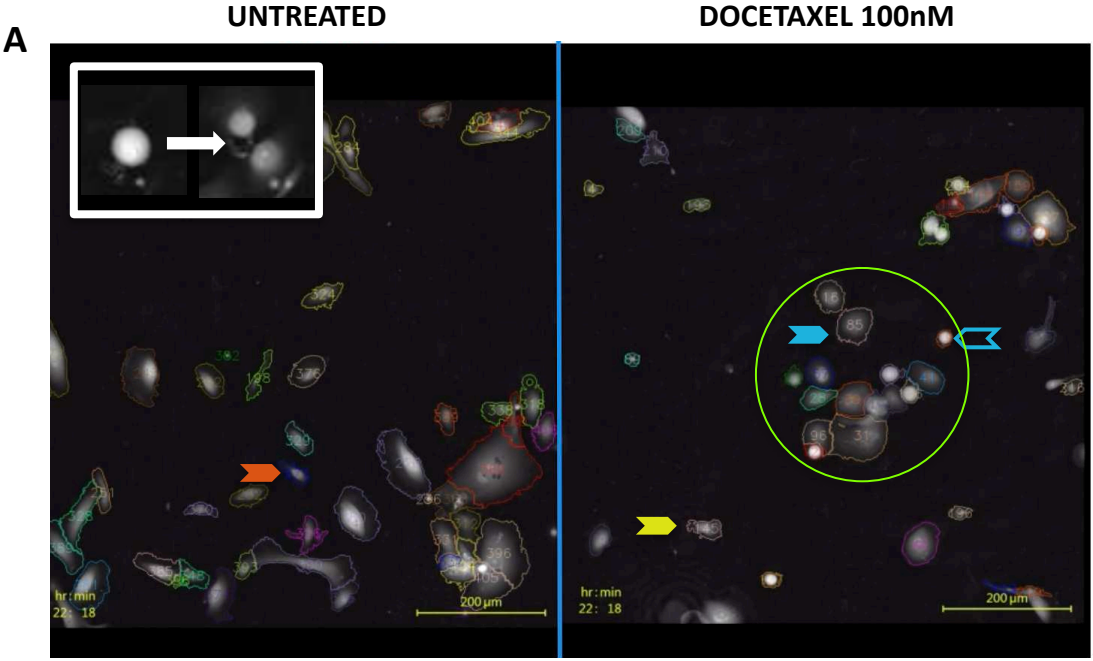
Prostate Cancer Models: Every model has advantages and limitations.

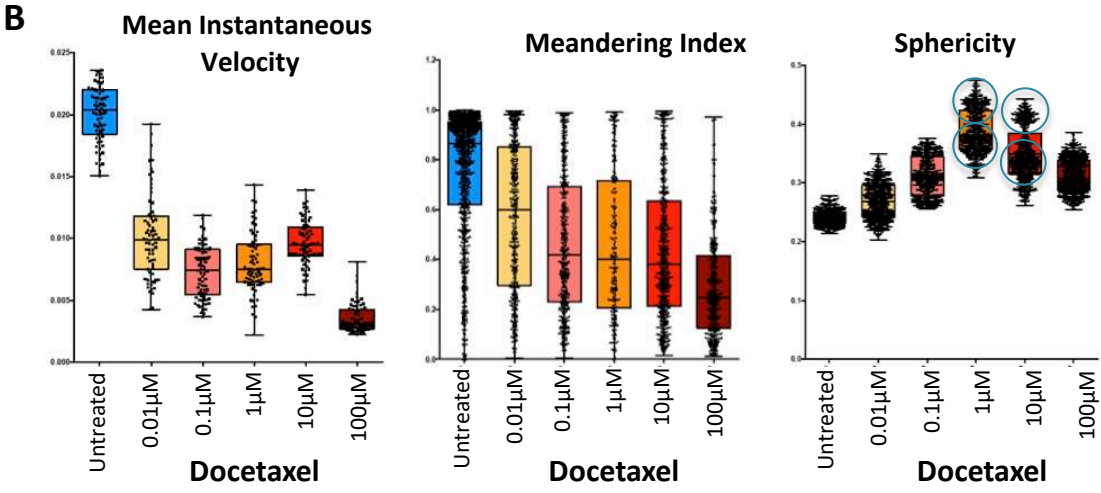
Figure 2



Collaborative network: In order to establish clinical collaborations, several parties have to be invested and committed. There also has to be long-term continuity within the laboratory to maintain the links and the knowledge. Once the clinical connections are established, the tissue and cell resource attracts collaboration from other parties including academic departments and industry. The importance of the resource and the nature of the work can also be disseminated through public engagement.

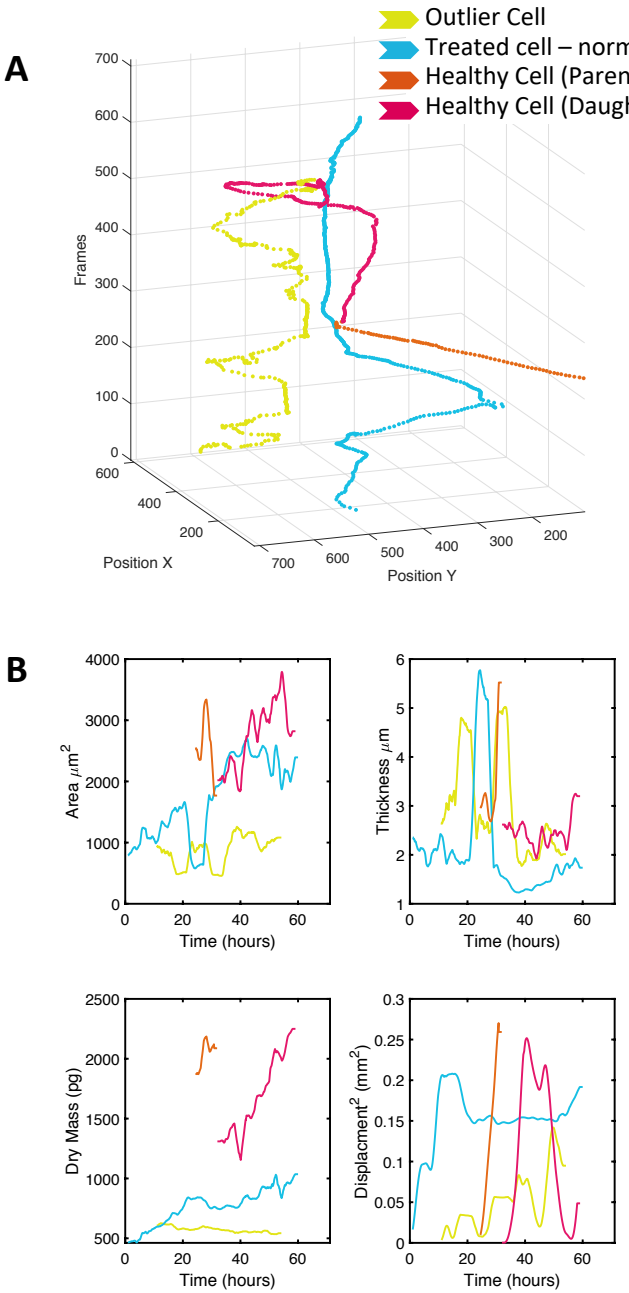
Figure 3





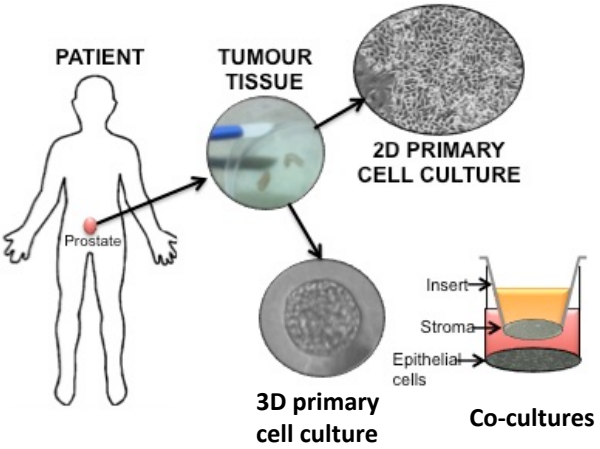
Response of Primary Cells to Docetaxel: (**A**) A time-lapse movie was generated over 72 hours with images captured at 6 minute intervals to observe the real-time response of primary prostate cells to docetaxel. Untreated cells could be seen moving and dividing in a continuous motion (red arrow). Dividing cell shown in white box insert. Treated cells predominantly responded by entering mitosis (cells rounding up – blue outline arrow) and when mitosis failed, due to the effect of docetaxel, the cells spread out and stopped moving (blue arrow). An outlier cell was observed that continuously rounded up to try to divide but upon failure continued moving around in an erratic fashion (yellow arrow). (**B**) Kinetic and morphological features can be extracted from the data. Each cell is measured and patterns of response recorded. Bimodal responses are observed with some parameters that can be related to the cell behaviour (turquoise circles). (Images were captured on a Livectye™ and data was analysed using the Cell Analysis Toolbox (CAT) software: www.phasefocus.com)

Figure 4



Tracking Individual Cell Behaviour: (**A**) Individual cells were tracked over time following response to docetaxel treatment (100nM). Untreated (Healthy) cell has single direction of movement then divides and the daughter cell has meandering directionality. Typical cell response to docetaxel is to round up and enter mitosis then following incomplete mitosis the cell stops moving. The outlier (putative resistant cell) is much more erratic in its behaviour and is very motile. Following attempted divisions the cell maintains its highly motile behaviour. (**B**) Individual parameters also indicate the change in cell behaviour. Thickness clearly indicates the points at when the cell rounds up to enter mitosis.

Figure 5



Patient Samples: Primary prostate epithelial cells can be cultured from patient tissue. These cells can be grown in 2D, in co-culture systems and in 3D culture.