Characterisation of primary cilia in prostate cancer as a new mechanism of drug resistance

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Introduction

Prostate cancer (PCa) is one of the most common cancers in men, with the median age of diagnosis being 67 years and incidence rates increasing in men over 50 (1). Although treatments like radical prostatectomy, external or internal radiotherapy with or without anti-androgen hormone therapy are effective in most cases of localised prostate cancer, it can become resistant to them and metastasise as a result (2). In cases where the cancer becomes metastatic, treatments include chemotherapy and/or hormone therapy with drugs such as enzalutamide, darolutamide, apalutamide (androgen receptor inhibitors), and abiraterone (enzyme CYP17A1 inhibitor) (3).

This study focuses on investigating primary cilia; small, antenna-like structures present on most cells that have a role in mediating signaling pathways that regulate cell growth and differentiation. Research has shown that dysfunction or loss of these cilia is linked to the development of prostate cancer. The mechanism behind this is that primary cilia regulate important signaling pathways, like Wnt and Hedgehog, which once dysregulated lead to uncontrolled proliferation that is associated with tumour progression (4).

Studying the appearance and functions of primary cilia is crucial to develop an understanding of how they regulate cell growth and play a role in different types of cancer. Targeting pathways associated with cilia dysfunction or restoring cilia growth and length could sensitise them to existing treatments, potentially offering new therapeutic strategies to treat more aggressive forms of cancer where other treatments are not effective.

Methods

Cell Line	Description	Aggressiveness	Androgen Sensitivity	Genotype
PNT2C2	Non-cancerous prostate epithelial cell line	Non-cancerous	Not applicable	Normal prostate epithelial cells
LNCap	Androgen-sensitive prostate cancer cell line derived from lymph node metastasis	Moderate	Sensitive	Mutations in AR (androgen receptor), PTEN loss
CWR22-Rv1	Prostate cancer cell line derived from a xenograft, slightly less aggressive than PC3	Moderate-High	Insensitive	Mutations in AR, PTEN loss, TP53 mutations
PC3	Aggressive prostate cancer cell line derived from bone metastasis	High	Insensitive	Loss of AR, PTEN loss, TP53 mutations, RB1 loss

Figure 1 - Descriptions of cell lines used in the study

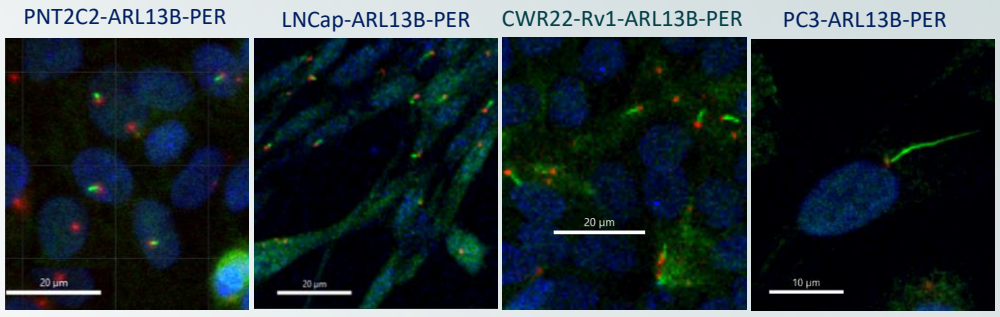
Cells were grown in a controlled environment using a nutrient-rich solution called RPMI-1640 culture medium and monitored over a month. Once the cells reached a sufficient number, they were transferred onto coverslips and stained. To visualize specific parts of the cells, 3 different stains were applied: DAPI, which highlights the cell nucleus blue, as well as ARL13B that highlights the primary cilia green, and pericentrin that highlights the cell's centrosomes red.

After staining, the coverslips were mounted onto glass slides for examination using confocal microscopy to visualise cells in 3D.

Microscopic images were analyzed using the Imaris software, allowing automation of counting cells and cilia. Primary cilia counts were verified manually, by detecting co-localization of green and red spots. Lengths of the cilia were calculated using a combination of the bounding box analysis protocol and addition of several measuring points placed across the length of the cilia in the software Imaris.

Graphs plotted show the mean percentage of cells with cilia per cell line, as well as the mean cilia length per cell line. Data were presented as mean ± SD, *P<0.05, **P<0.01, ***P<0.005. T tests and one-way ANOVA were run for both data sets.

Results



Images - Confocal microscopy images of the 4 cell lines stained with DAPI, pericentrin, ARL13B.

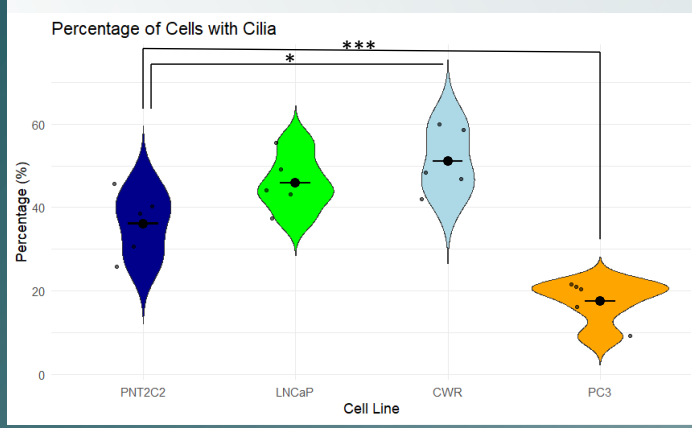


Figure 2
Violin plot showing mean number of cells with cilia per cell line. The percentages of cells with cilia were calculated using 5 confocal microscopy images, each image containing at least 100 cells, per cell line. Results of the ANOVA test are as follows:
Combined P-value = 0.00000608, indicating a high significance level between the group means.

Figure 2 - Independent T-tests were conducted for the three cancerous groups compared to the PNT2C2 cell line. The results revealed no considerable difference between the percentages of cells with cilia in PNT2C2 and LNCaps, a substantial difference between PNT2C2 and CWR22Rv1, and the most significant difference between the control and PC3.

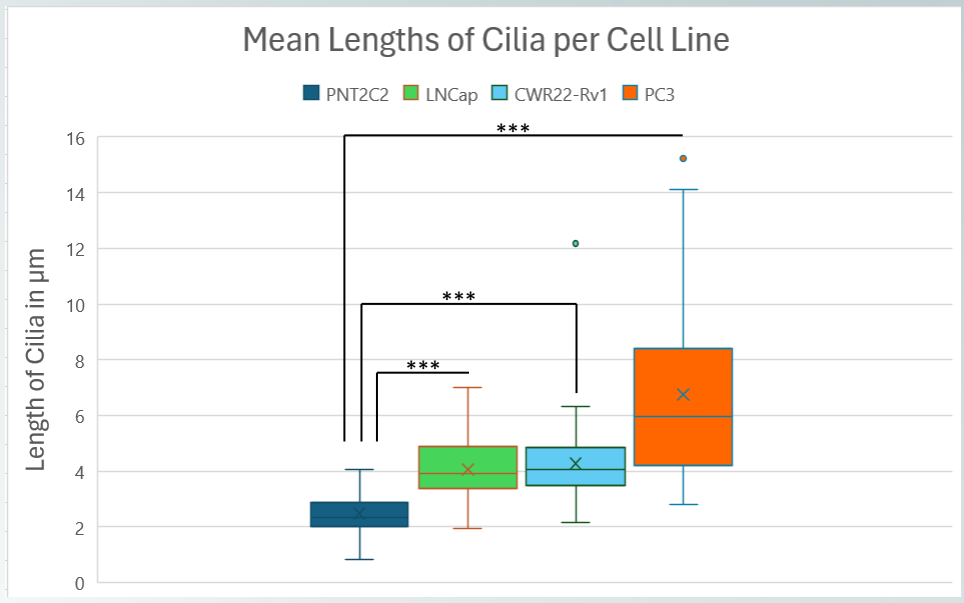


Figure 3
Cilia in all cancerous cell lines were significantly longer than in the control PNT2C2 cell line. There is also greater variability in length in the PC3 cell line, compared to the rest, in addition to a more evident increase in length. Additionally, the one-way ANOVA results were as follows:
Combined P value = 2.89*10^-16
This suggests that there are significant differences between the group means.
T-test was completed for each cancerous cell line compared with PNT2C2. P values were all below 0.005, indicating a high level of significance in all groups compared to the non-cancerous cell line. Both sets of data convey the drastic change that the most aggressive cell line, PC3, undergoes.

Discussion

The most aggressive PC3 cell line has fewer and longer primary cilia, with most variation in their length. This is consistent with evidence of disruptions in primary cilia growth seen in metastatic cancers in research (4). Primary cilia are involved in signal transduction pathways, which are often dysregulated in cancer. Mutations PTEN, TP53 and RB1 (see Figure 1) in PC3 cells reflect this dysregulation, so the abnormal length and reduced frequency of cilia may reflect the abnormal signalling. Research suggests that lower cilia numbers correlate to increased tumour size and Wnt signalling. Others have acknowledged the primary cilium's role as a tumour-suppressor that regulates cellular responses upon hormonal changes (5,6).

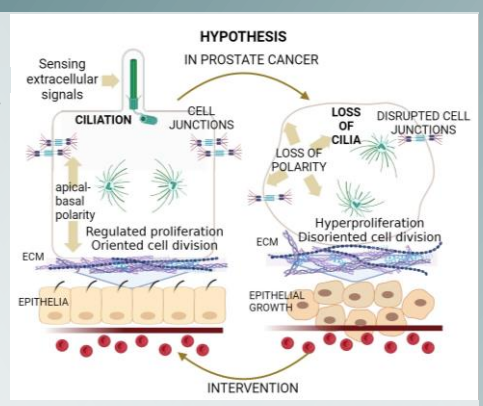


Figure 4: cell deciliation could a key driver of abnormal cell proliferation, loss of polarity control, and drug resistance; and modulators of ciliary growth restore primary cilia and re-sensitise cancer cells to hormone therapy

Restoration of cilia length and abundance may help re-sensitise cells to existing hormone therapies. It has been demonstrated that cilia can be restored through administration of mTOR inhibitors, which have been shown to increase cilia expression in PC3 cell line and cilia formation in DU145s (7). Supporting this, modulation of primary cilium assembly can lead to its restoration (8). This means that the events caused by loss of cilia, such as loss of polarity, resistance to hormone treatment and uncontrolled cell growth can all be reversed with administration of novel therapeutics like modulators of ciliary growth.

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