A detailed analysis of gene expression in human basal, luminal, and stromal cell populations from benign prostatic hyperplasia tissues and comparisons with cultured basal cells.

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The understanding of common prostatic disorders has been restricted by both cellular heterogeneity and the scarcity of established cell lines, although organoid technology, based on primary cultures, promises much for the future. In particular, little is known about the aetiology of benign prostatic hyperplasia (BPH), where culture of single cell types might not accurately not reflect the stromal and epithelial overgrowths observed in tissues [1]. To address the applicability of primary cell culture models of prostate disease, we compared cell type-specific messenger RNA expression patterns, from BPH tissues, and primary basal cells cultured from the same transurethral biopsies. We resolved cellular heterogeneity by direct tissue fractionation into luminal cells ($Lin^-/CD31^-/EpCAM^+/CD24^+$), basal cells ($Lin^-/CD31^-/EpCAM^+/CD44^+$), and stromal cells ($Lin^-/CD31^-/EpCAM^-$) obtained from 3 patient samples, prior to analysis using Affymetrix GeneChip® Human Transcriptomic Arrays 2.0 [1, 2].

Unsupervised clustering and principal component analysis (Figure 1a) of the microarray data revealed subpopulations clustered together in a cell-type specific manner. However, even short-term culture in a defined medium induced significant transcriptomic changes in basal cells. This was confirmed by qRT-PCR analysis for representative genes (Figure 1b). We next sought to put the principal differences in gene expression between cell populations into a disease context, using gene set enrichment analysis. When non-malignant BPH basal cells were grown in a defined, serum-free culture medium previously shown to amplify skin epithelial stem cells, differential overexpression of gene sets associated with (i) stemness, (ii) aggressive breast and prostate cancers, and (iii) STAT5 signalling were observed. These results support the findings from our own and other laboratories i.e. human prostate stem cells most likely reside in the basal compartment, and recent data associating this stem-like phenotype with aggressive prostate cancer (Figure 1c) [3]. STAT5 signalling has been shown to promote pro-survival pathways in prostate epithelial cells [4]. Paradoxically, gene expression in BPH luminal cells matched well with several published prostate cancer profiles (Figure 1c), suggesting that many PCa ‘signatures’ are inevitably dominated by luminal gene

expression. We propose that filtering heterogeneous PCa tissue expression profiles for this normal (benign) luminal signature would yield better cancer biomarkers/targets.

When gene expression in primary cultures of basal cells was compared to tissue basal cells, (figure 1a) the cultured basal cells clustered distantly from the uncultured profile, and separately from both luminal and stromal cell profiles. It has been shown that prostate cancer cells (with a luminal phenotype) can be cultured in organoid cultures [5, 6], but we could not culture BPH-derived luminal cells in 2-D culture. In culture, more BPH basal cells were induced to cycle compared to tissues [7] (Figure 1d), whilst expressing both stem cell-associated genes (Figure 1e) and telomerase enzyme component (TERT) target genes (Figure 1f). TERT and telomerase are both critical for cell cycle control, and perhaps for stemness. The overexpression of (i) TERT mRNA, (ii) RNA component of telomerase (TERC), and (iii) longer telomere lengths (Figure 1g-j) revealed that telomerase biology could be significantly altered, even during short-term cell culture. Therefore, extra precautions must be taken when extrapolating studies from cultured cells (both primary cancer cells and cell lines) to a therapeutic relevance for human BPH, which nevertheless appears to be 'driven' by a replicative basal compartment in man [1,5].

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Figure 1: Transcriptomic analyses of benign prostatic hyperplasia samples-derived cells

a. Principal component analysis (PCA) for transcriptomic expression of different cell sub-types in benign prostatic hyperplasia. Note that each sub-population clusters together. [n=3 basal, 3 luminal, 2 stromal, and 3 cultured samples, collected from 3 distinct patients)
b. qRT-PCR analysis for representative genes specifically characterizing uncultured basal, luminal, and stromal populations [n=3 for each population].
c. Gene set enrichment analysis (GSEA) demonstrating gene sets enriched for uncultured basal and uncultured luminal cells in comparison with each other, as demonstrated by significant FDR (False Discovery Rate) q-values. GSEA analysis was performed using GSEA desktop suite.
d. qRT-PCR analysis for genes associated with cell cycle [n=3 for each population]. Expression was normalized to the housekeeping gene RPLP0.
e. GSEA analysis showing enrichment of stem cell associated pathways in cultured basal cells compared to uncultured basal cells, FDR q value=0.00, suggesting significant enrichment of stem cell associated genes in cultured basal cells compared to uncultured basal cells.
f. GSEA analysis showing enrichment of TERT target genes in cultured basal cells compared to uncultured basal cells, FDR q value=0.00, suggesting significant enrichment of TERT target genes in cultured basal cells compared to uncultured basal cells.
g. qRT-PCR analysis of TERC RNA expression in uncultured (UC) and cultured (C) basal cells, TaqMan probe id: Hs03454202_s1, n=5 each. Expression was normalized to the housekeeping gene RPLP0.
h. qRT-PCR analysis of TERT RNA expression in uncultured (UC) and cultured (C) basal cells, TaqMan probe id: Hs00972650_m1, n=5 each. Expression was normalized to the housekeeping gene RPLP0.
i. qRT-PCR analysis for determination of telomere length in uncultured (UC) and cultured (C) basal cells, n=6 BPH. The telomere length for each sample was normalised to lymphocyte telomere length from the corresponding sample.

Data are expressed as mean ± s.d. *P < 0.05, **P < 0.01, ***P < 0.001 (Student's t-test).

References:

[1] Rane JK, Greener S, Frame FM, Mann VM, Simms MS, Collins AT, et al. Telomerase Activity and Telomere Length in Human Benign Prostatic Hyperplasia Stem-like Cells and Their Progeny Implies the Existence of Distinct Basal and Luminal Cell Lineages. European urology. 2016;69:551-4.

[2] Collins AT, Berry PA, Hyde C, Stower MJ, Maitland NJ. Prospective identification of tumorigenic prostate cancer stem cells. Cancer research. 2005;65:10946-51.
[3] Smith BA, Sokolov A, Uzunangelov V, Baertsch R, Newton Y, Graim K, et al. A basal stem cell signature identifies aggressive prostate cancer phenotypes. Proceedings of the National Academy of Sciences of the United States of America. 2015;112:E6544-52.
[4] Liao Z, Nevalainen MT. Targeting transcription factor Stat5a/b as a therapeutic strategy for prostate cancer. Am J Transl Res. 2011;3:133-8.
[5] Chua CW, Shibata M, Lei M, Toivanen R, Barlow LJ, Bergren SK, et al. Single luminal epithelial progenitors can generate prostate organoids in culture. Nature cell biology. 2014;16:951-61, 1-4.
[6] Gao D, Vela I, Sboner A, Iaquinta PJ, Karthaus WR, Gopalan A, et al. Organoid cultures derived from patients with advanced prostate cancer. Cell. 2014;159:176-87.
[7] Bonkhoff H, Stein U, Remberger K. The proliferative function of basal cells in the normal and hyperplastic human prostate. Prostate. 1994;24:114-8.

***Take Home Message**

Take home message: Rane, Droop and Maitland

Gene expression in human BPH-derived specific cell sub-populations was profiled, giving insights into BPH pathophysiology. Significant culture-induced perturbations were found in basal cells, which must be taken into account when testing new treatments or modelling disease.

Disclosure for Rane, Droop and Maitland

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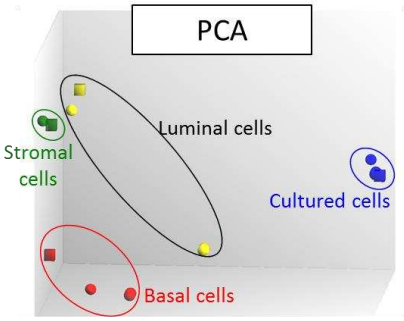
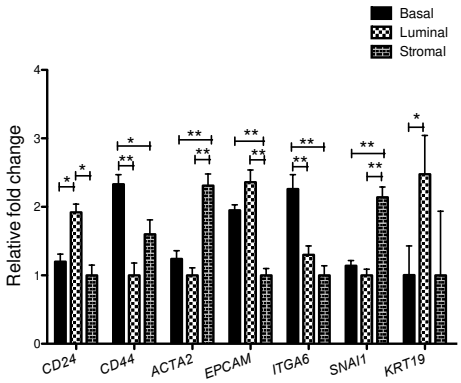
Illustration

(a)



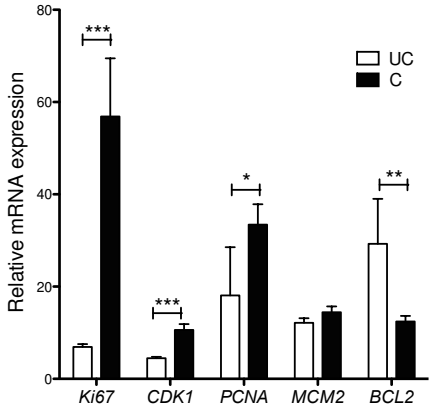
(b)



(c)

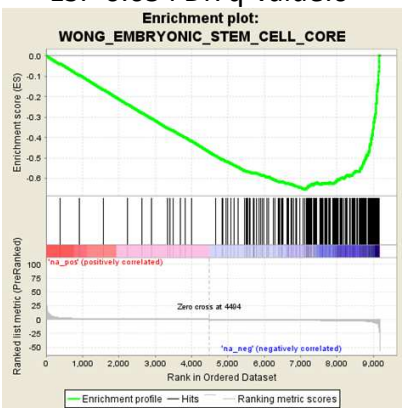
Enriched pathway	FDR q-value
Basal	
WALLACE_PROSTATE_CANCER_RACE_UP	0
POOLA_INVASIVE_BREAST_CANCER_UP	0
JAATINEN_HEMATOPOIETIC_STEM_CELL_UP	0.001
WIERENGA_STAT5A_TARGETS_UP	0.009
Luminal	
LU_EZH2_TARGETS_UP	0
LIM_MAMMARY_STEM_CELL_DN	0
YEGNASUBRAMANIAN_PROSTATE_CANCER	0
LIU_PROSTATE_CANCER_UP	0.002
HWANG_PROSTATE_CANCER_MARKERS	0.0011
YAMASHITA_METHYLATED_IN_PROSTATE_CANCER	0.05

(d)



(e)

ES: -0.65 FDR q-value:0



(f)

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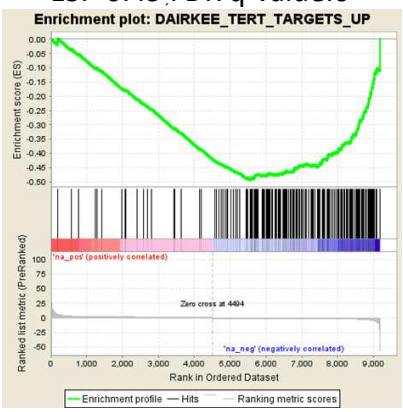
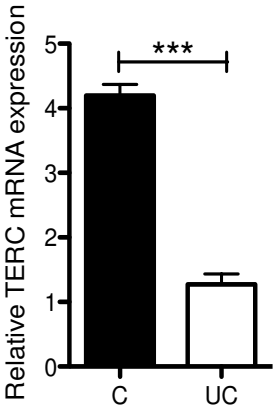
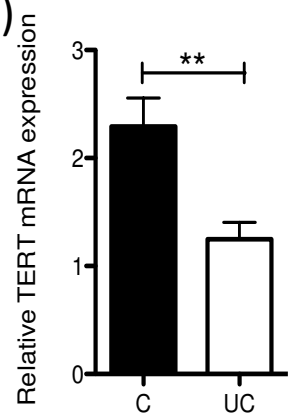


(g)



(h)



(i)