Molecular Profiling and Identification of Novel Targets for the Treatment of Metastatic Castration-resistant Prostate Cancer

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1 Summary

While androgen ablation through surgical or chemical castration has been a mainstay therapy since its first description, it frequently fails after its initial use and can lead to the development of aggressive, often lethal, prostate cancer (PCa) known as metastatic castration-resistant prostate cancer (mCRPC)[1,2]. Although prostate-confined tumours are often treatable by surgery, distal metastatic disease remains incurable[3,4]. Clinically, there is a **critical need to develop additional targeted, therapeutic strategies for patients with mCRPC**.

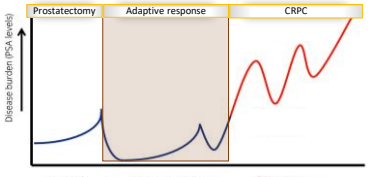


Figure 1. Adaptive response of PCa to androgen targeted therapies resulting in CRPC

Aims

1. To use two models of advanced PCa to unveil new gene targets for therapeutic targeting of this progression
2. Uncover underlying biology processed that may be crucial to this progression

3 Deriving Castration Associated Transcripts (CATs)

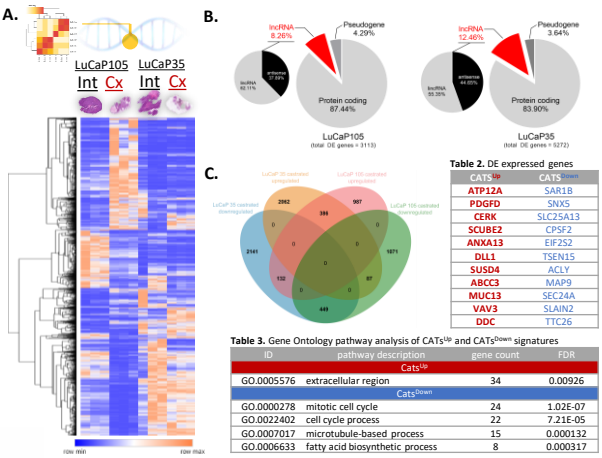


Table 2. DE expressed genes

CATs^{Up}	CATs^{Down}
ATP12A	SAR1B
PDGFD	SNX5
CERK	SLC25A13
SCUBE2	CPSF2
ANXA13	EIF2S2
DLL1	TSEN15
SUSD4	ACLY
ABCC3	MAP9
MUC13	SEC24A
VAV3	SLAIN2
DDC	TTC26

Table 3. Gene Ontology pathway analysis of CATs^{Up} and CATs^{Down} signatures

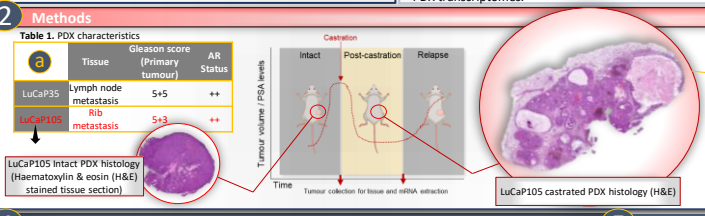
ID	pathway description	gene count	FDR
CATs^{Up}			
GO.0005576	extracellular region	34	0.00926
CATs^{Down}			
GO.0000278	mitotic cell cycle	24	1.02E-07
GO.0022402	cell cycle process	22	7.21E-05
GO.0007017	microtubule-based process	15	0.000132
GO.0006633	fatty acid biosynthetic process	8	0.000317

Figure 2. (A) Heatmap representation of significant differentially regulated genes in LuCaP105^{Cx} & LuCaP35^{Cx} PDXs compared to intact (Int) PDX cohort (n=3/group). **(B)** Pie chart visualisation of significantly differentially expressed (DE) genes (protein coding *vs* lncRNA *vs* pseudogenes) determined from RNA-seq data. **(C)** Venn-diagram shows commonly induced and repressed genes between LuCaP105^{Cx} and LuCaP35^{Cx} PDXs and intact PDX transcriptomes.

2 Methods

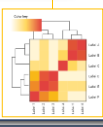
Table 1. PDX characteristics

	Tissue	Gleason score (Primary tumour)	AR Status
LuCaP35	Lymph node metastasis	5+5	++
LuCaP105	Rib metastasis	5+3	++



c RNA-seq performed (40 million reads) on a BGISEQ-500 with 100bp paired-end reads

b RNA extracted from PDX lines (pre- and post-castration). RNA integrity was assessed using an Agilent 2100 Bioanalyzer



4 *MUC13* is upregulated by castration in PCa PDXs

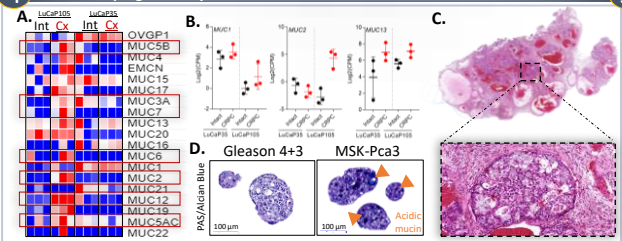


Figure 3. (A) Heatmap representation of mucin genes in LuCaP105^{Cx} & LuCaP35^{Cx} PDXs compared to intact (Int) PDX cohort. **(B)** Scatter plot visualisation of Mucin 1, cell surface associated (*MUC1*), Mucin 2, oligomeric mucus gel-forming (*MUC2*) & Mucin 13 (*MUC13*) expression. **(C)** H&E of LuCaP105^{Cx} showing histological architecture. Inset image shows representative mucinous area only present in Cx samples. **(D)** PAS/AB glycoprotein stain of G7 PCa & MSK-Pca3 (mucinous prostate adenocarcinoma cell line) tumouroids showing neutral & acidic mucins.

5 Expression of novel lncRNAs following castration

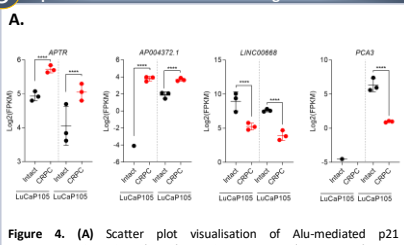


Figure 4. (A) Scatter plot visualisation of Alu-mediated p21 transcriptional regulator (*APTR*), GPN-loop GTPase 1 (*AP004372.1*), Long Intergenic Non-Protein Coding RNA 668 (*LINC00668*) & Prostate cancer antigen 3 (*PCA3*) lncRNA expression between LuCaP105^{Cx} & LuCaP35^{Cx} CRPC PDXs compared to intact PDX cohort. Represented as log2-transformed FPKM. n=3/group, mean ± s.e.m.

6 Conclusions

Using unique models of mCRPC, our transcriptomic analyses show:

- Novel genes upregulated following castration were shared between two high-grade prostate cancer PDX models - termed the *Castration Associated Transcriptional Signature* (CATS^{up})
- A subset of the differentially expressed genes may represent novel therapeutic targets or biomarkers that are upregulated during the adaptive response of prostate tumours as it develops to mCRPC

Future work will explore the potential of these genes as putative therapeutic targets to prevent the progression of prostate cancer into aggressive, metastatic prostate cancer

7 References & Acknowledgements

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