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Loss of *SNAI2* in prostate cancer determines clinical response to androgen deprivation therapy

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INTRODUCTION

- Androgen Deprivation Therapy (ADT) is the backbone of treatment for advanced prostate cancer.
- The duration and extent of response to ADT is variable[1-3].
- Although the molecular mechanism that meditate castration resistant escape are well characterized, what determines initial response to ADT is less clear[4].
- To investigate this, we conducted a study of profound androgen suppression.

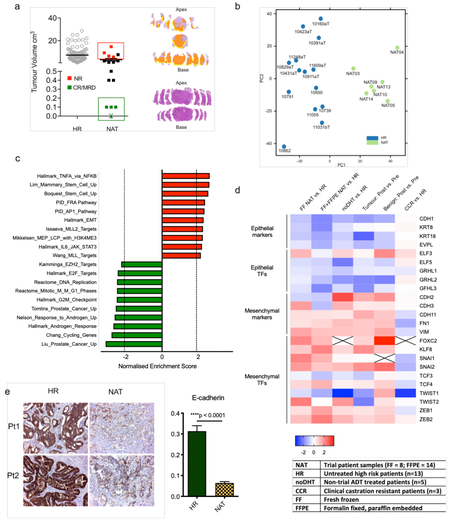
MATERIALS & METHODS

- **Study Overview**

Screening	→	Treatment	→	Surgery	→	Response
Dx High Risk CaP						
PSA > 20 OR		Degarelix 240/80mg SC q1/12 & Abiraterone 1000mg OD PO & Prednisolone 5mg BD PO & Bicalutamide 50mg OD PO		Radical Prostatectomy		CR: No viable tumour (pT0)
>/= cT2c OR				&/- Bilateral PLND		PR: Treatment effect
GS 7 (4+3) OR						NR: No treatment effect
GS 8-10 AND						
WBBS Negative		x 6/12				

- **RNA-Seq**: Libraries were created from poly-A selected (fresh) or ribosomal-RNA depleted (FFPE) RNA using the NEBNext Ultra RNA Library Prep Kit and sequenced on the Illumina HiSeq2500 using paired end chemistry at 150 base pairs/50 million reads per sample. Differential expression was undertaken using edgeR with TMM normalisation, fitted to a GLM-model. Gene set enrichment analysis was performed using the Broad Institute's GSEA module. RNA-based fusions were called using the JAFFA fusion caller.
- **WGS**: DNA libraries were prepared using the TruSeq DNA sample prep kit and sequenced to an average 37.8x depth for tumours and 20.1x depth for germline samples using a HiSeq X Ten sequencer (Illumina). Reads were aligned to hg19/GRCh37 using Genome Analysis Toolkit's (GATK) best practices protocol. MuTect (v1.1.7), Strelka (v1.0.14) and MultiSNV (v1.5) were run on tumour-normal pairs with default parameters to identify SNVs. Somatic copy-number aberrations were identified using ASCAT-NGS (v1.5.2) and Battenberg (v1.5.0) with default parameters. Structural variants (SVs) were called using the Socrates SV caller .
- **Clonality Analysis**: SNVs called using MuTect and one other caller were used for clonal inference. Any variants ≤0.07 VAF were removed. Only Battenberg copy-number states with a fraction of at least 0.9 were used to match copy-number states to SNV loci. SNVs in regions of significant subclonal copy-number (fraction <0.9) were removed. Battenberg's purity estimates were used per sample. PyClone (v1.3.0) was used to cluster samples containing both pre- and post-treatment samples using the beta-binomial clustering mode and parental copy-number prior (10,000 iterations with 5,000 burn-in). All samples from the same patient were clustered together.
- **FISH**: FISH method was performed according to the ZytoLight ® FISH-Tissue Implementation Kit instructions. Tumour regions were marked using an adjacent H&E stained slide as a guide and SNAI2 and Ch8 centromere control probes (Empire Genomics, USA) were applied and slides counterstained with DAPI.

RESULTS



NAT	Trial patient samples (FF = 8; FFPE = 14)
HR	Untreated high risk patients (n=13)
noDHT	Non-trial ADT treated patients (n=5)
CCR	Clinical castration resistant patients (n=3)
FF	Fresh frozen
FFPE	Formalin fixed, paraffin embedded

Figure 1. Response to profound androgen suppression is highly variable, with persistence characterised by global transcriptional reprogramming and phenotype switching. (a) Tumour volume in treated patients (termed NAT for Neo-Adjuvant Therapy, n=16) compared with a historical untreated cohort with similar pre-treatment characteristics (termed HR for High Risk, n=101). Solid bar = median volume. NR = no response. CR/MRD = complete response/minimal residual disease. Corresponding whole mount section map of prostate from a patient with no response (upper panel) and complete response (bottom panel). Tumour is marked in orange. (b) Principal component analysis of global transcriptional profile reveals distinct clustering of NAT patients (n=7) compared to HR control (n=13). (c) Normalised enrichment scores of selected gene-sets up- or down-regulated with neoadjuvant treatment from gene set enrichment analysis (FWER < 0.001). (d) Heatmap demonstrating relative logfold change of selected EMT related genes in various ADT treated specimens compared to untreated controls. X indicates no transcript detected. (e) Immunohistochemical analysis of E-cadherin expression in persistent tumours NAT and untreated HR controls. Upper panel shows examples of staining from two patients in each group. Low panel summarises expression in HR controls (n=5) and persistent NAT tumours (n=12) as determined by digital

RESULTS

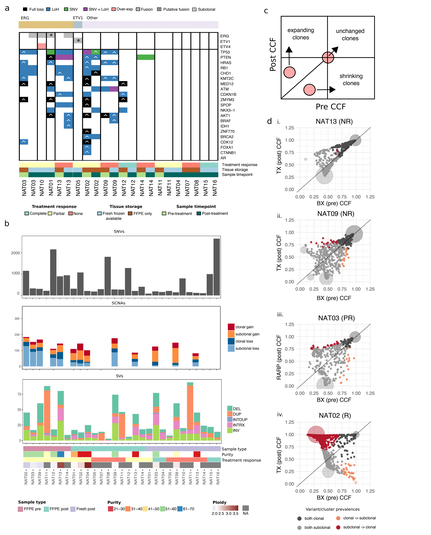


Figure 2. Persistence is not driven by somatic features or the evolution of a resistant clone. (a) Summary of somatic changes identified in neo-adjuvant trial samples. Tumours are classified according to the molecular subtype present in any one sample from a patient. Subclonal indicates >0.2 and <0.9 copy-number fraction from Battenberg. Subclonal copy-number losses at <0.2 CCF were only considered if the SCNA was present at >0.2 in at least one other sample from the patient. Only losses were considered; no amplifications (>4 allelic copies) affecting target genes were found. Samples with unclear purity/ploidy solutions. were not considered for copy-number losses. (b) Summary of SCNAs, SNVs and SVs of samples. SCNAs are not displayed for samples with unclear purity/ploidy solutions. Subclonal SCNAs were considered as present at <0.9 copy-number fraction from Battenberg. (c) 2D cluster plot of cancer cell fractions derived from PyClone. (d) Cancer cell fraction of SNV-derived clusters of pre- (BX) and post- (TX) treatment samples from one resistant patient (NAT09). (e) Cancer cell fraction of SNV-derived clusters of pre- (BX) and post- (TX) treatment samples from patients demonstrating varying levels of treatment response including no response (NR), partial response (PR) and almost complete response (R).

RESULTS

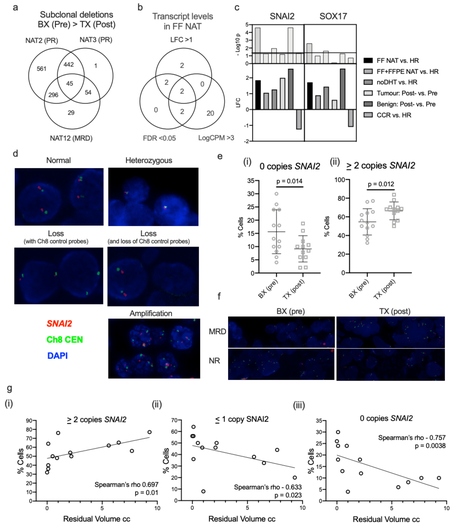


Figure 3. SNAI2 loss correlates with tumour response. (a) Venn diagram summarising overlapping gene deletions identified subclonally in pre-treatment samples but not in post-treatment specimens. (b) Prioritisation of overlapping genes from (a) based on level of expression in FF NAT vs. HR RNA-seq dataset. (c) Relative logfold change in *SNAI2* transcript measured by RNA-seq in various ADT treated specimens compared to untreated controls. Annotation as for Figure 1d. (d) Indicative FISH images (magnification 100x) of tumour samples following hybridisation with probes against *SNAI2* (red) and Ch8 centromere (green). (e) Mean ± SD of percentage of cells with deep deletion (i) or ≥ two copies (ii) of *SNAI2* in pre- and post-treatment specimens. (f) Representative images of changes in the prevalence of cells harbouring deep deletions of *SNAI2* in pre- and post-treatment specimens in patients with minimal residual disease (NAT16) and no response (NAT11). (g) Scatter plots (i-iii) demonstrating the relationship between the prevalence of cells with the indicated *SNAI2* copy number state pre-treatment and residual tumour volume.

CONCLUSIONS

- Acute resistance to profound androgen suppression is characterized by an intermediate EMT phenotype[5], associated with the upregulation of the key EMT regulator *SNAI2*.
- Clonality analysis suggests that treatment response is primarily determined by the loss of 'hormone-sensitive' clone, rather than the emergence of a resistant clone.
- Treatment is associated with the loss of cells deficient for *SNAI2*, and an enrichment cells harbouring 2 or more copies of the gene.
- Objective response as measured by residual tumour volume correlates strongly with the proportion of cells deficient in *SNAI2*.
- Taken together, these data suggest that *SNAI2* status is a key determinant of acute response to ADT.
- Further work is ongoing to:
  - Confirm these findings in a larger independent cohort
  - Explore alternative mechanisms of SNAI2 knockdown (eg. Inactivation by methylation)
  - Functionally validate the effect of SNAI2 knockdown on sensitivity to acute AR inhibition

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