Prostate cancer affects one in eight men in the developed world, and now accounts for more cancer related deaths in men than females dying of breast cancer. Despite improvement in patient survival with novel androgen receptor (AR) inhibitors and taxane chemotherapy, a significant proportion of patients with advanced disease still die within five years of diagnosis.

Our research aims to highlight novel molecular drivers of treatment resistance, thus identifying new therapeutic strategies for future development. In this report, we describe our recent findings from a novel panel of preclinical models that mimic treatment resistant prostate cancer - specifically castration resistance - and highlight molecular players that allow tumours to evade androgen deprivation therapy.

Proteomic analysis of 2- and 3-dimensional in vitro cultures of treatment resistant human prostate cancer cell lines

Despite the clinical success of AR-targeted therapies, reactivation of AR signalling remains the main driver of castration-resistant prostate cancer (CRPC) progression. Human androgen receptor expressing and hormone-dependent LNCaP prostate cancer cells were chronically exposed to androgen-receptor inhibitors (namely bicalutamide, nilutamide and enzalutamide) to generate CRPC cell models.

Combined proteomics and metabolomics analyses revealed a shared adaptive metabolic phenotype among the treatment resistant cells with prominent alterations in fatty acid metabolism. In an AR-dependent manner, the observed metabolic rewiring ultimately led to profound reorganisation of the cellular lipidome of resistant cells, marked by an accumulation of multiple classes of sphingolipids and polyunsaturated triglycerides.

To exploit this phenotype, we delineated a subset of proteins consistently associated with resistance to AR pathway inhibitors, and highlighted mitochondrial 2,4-dienoyl-CoA reductase (DECR1), an auxiliary enzyme of beta-oxidation, as a clinically relevant biomarker for CRPC. Mechanistically, we found that DECR1 participated in redox homeostasis to maintain a balance between saturated and unsaturated phospholipids. DECR1 knockdown induced ER stress and sensitised CRPC cells to ferroptosis. In vivo, DECR1 deletion impaired lipid metabolism and reduced CRPC tumour growth, suggesting a potential value of exploring DECR1 as a target to overcome treatment resistance towards androgen receptor inhibitors.

Proteomics analysis of matched isogenic hormone-naive and castration-resistant prostate cancer orthotopic xenografts (orthografts)

To study androgen deprivation resistance, we developed three sets of CRPC orthograft models by injecting matched pairs of hormone-naive and castration-resistant prostate cancer cells into the prostates of immunodeficient mice. The isogenic pairs of hormone-naive and castration-resistant cell lines were LNCaP/LNCaP AI and CWR22v1/CWR22v1 respectively, while VCaP cells were able to grow as orthografts in both androgen-sufficient and -deprived conditions.

To achieve patient-like CRPC conditions, orthotopic injections of prostate cancer cells were immediately followed by orchidectomy to mimic androgen deprivation therapy (Figure 1).

In-depth quantitative SILAC-based proteomic analysis revealed a complex response to hormone deprivation, characterised by distinct molecular mechanisms across different castration-resistant prostate cancer models. Comparing all three sets of hormone-naive and castration-resistant cell lines, we noticed a shared theme of altered metabolism. Of note, differences in the underlying metabolic pathways involved signified the molecular heterogeneity among the three models of CRPC. Nonetheless, we identified Schlafen family member 5 (SLFN5) as a common target; its expression was consistently upregulated in all three CRPC prostate orthografts. In clinical tumours, SLFN5 expression was elevated in treatment resistant prostate biopsies, while SLFN5 deletion dramatically impaired the growth of CRPC tumours in vivo. Mechanistically, our data highlighted direct interaction between SLFN5 and the transcription factor ATF4, and that SLFN5 modulated the expression of several ATF4-target genes, including the amino acid transporter LAT1 (Large Amino Acid Transporter 1). Furthermore, we demonstrated that SLFN5 knockout in CRPC cells could alter amino acid metabolism through altering intracellular levels of amino acid substrates of LAT1, ultimately resulting in suppressed MTORC1 signalling in a LAT1-dependent manner. Our data, therefore, highlighted the AR/SLFN5/MTORC1 pathway as a potential therapeutic target.

In a more focused analysis of the isogenic pair of hormone-naive CWR22 and derived castration-resistant 22rv1 orthografts, we identified upregulated JMJD6 (Jumonji domain containing 6) expression in treatment resistant 22rv1 tumours (Figure 2). THEM6 proteins (classified as Type II Acyl-CoA thioesterases) are characterised by their presence of an evolutionarily conserved "Hotdog" domain, which gives rise to their thioesterase enzymatic activity to deactivate fatty acyl-CoA thioesters and generate free fatty acids and CoA.

In patients, THEM6 expression correlated with progressive disease and was associated with poor patient survival. THEM6 deletion reduced in vivo tumour growth and re-sensitised castration-resistant orthograft to androgen deprivation therapy by means of surgical castration. THEM6 was located at the endoplasmic reticulum membrane and mechanistically, controlled lipid homeostasis by regulating intracellular levels of either lipids. We found that THEM6 loss in CRPC cells significantly altered endoplasmic reticulum lipid function, preventing lipid-mediated induction of ATF4 and reducing de novo sterol biosynthesis. Interestingly, THEM6 and cMYC were often co-amplified in advanced prostate cancer. Our data suggested that THEM6 was required for the establishment of the MYC-induced stress response. Beyond prostate cancer, our research highlighted that loss of THEM6 expression significantly impaired tumorigenesis in the MYC-dependent subtype of triple negative breast cancer. Altogether, our results highlighted THEM6 as a novel component of the treatment-induced stress response and a promising target for the treatment of CRPC and MYC-driven cancer.

Transcriptomic gene regulation network analysis of human prostate orthografts

We exploited a graph-based enrichment score to integrate transcriptomic data from gene regulatory network and differently expressed genes in clinical reseted prostate tumours. We tested whether a network of genes similarly regulated by transcription factors (gene products that control the expression of target genes) were associated with patient outcome. We identified regulons (networks of genes similarly regulated) within our preclinical prostate cancer models and further evaluated the top ranked JMJD6 gene related regulated network in three independent clinical patient cohorts.

JMU6 belongs to the Jumonji (JUM) domain containing family of proteins. JMU6 is thought to function mainly as a lys1-5-hydroxylase. Its ability to regulate the transcriptional activity of p53 through hydroxylation of a lysine in the p53 C-terminus is highly relevant in cancer biology. Upregulated JMU6 expression has been implicated in tumour growth, tumour metastasis and high tumour pathological grades. Our data from transcriptomic network analysis highlighted the value of future studies on JMU6 mediated function in prostate cancer biology.

Concluding comment

In summary, the use of novel preclinical models that mimic clinically relevant treatment resistance in prostate cancer has identified multiple actionable genes as potential targets for therapy in castration-resistant prostate cancer.

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Figure 1 Schematic representation of the three sets of isogenic hormone-naive and castration-resistant prostate cancer orthograft models

Figure 2 Volcano plot of the differentially modulated proteins in 22rv1 castration-resistant prostate cancer (CWR22) versus CWR22v1 hormone-naive (HN) tumours. Red and blue dots represent the proteins that are significantly up- and down-regulated, respectively (p-value < 0.05, FC = 1.5). THEM6 protein is highlighted in green.