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A synthetic lethal CRISPR/Cas9 screen for castration resistant prostate cancer

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INTRODUCTION

Prostate Cancer (PCa) is initially treated with Androgen Deprivation Therapy (ADT), however, a subset of patients progress on ADT to an aggressive form of PCa, namely Castration Resistance Prostate Cancer (CRPC), which is highly metastatic and frequently lethal. Importantly, the Androgen receptor (AR) is expressed at high levels in CRPC, as are multiple AR-regulated genes, indicating that AR transcriptional activity is at least partially reactivated. Treatment of CRPC with new generation antiandrogen treatment Abiraterone Acetate or Enzalutamide has improved survival outcomes. However, these drugs only retard disease progression, and resistance almost invariably arises. Several mechanisms have been postulated to explain how androgen-sensitive prostate cancer cells acquire resistance to hormone deprivation, yet actionable key drivers of CRPC remain elusive, complicating the development of effective therapies. Identifying synthetic lethal interactions with current anti-androgen therapies will lead to novel combinations therapies.

OBJECTIVES

We propose that these actionable mechanisms can be identified through a synthetic lethal CRISPRi/dCas9 screen with antiandrogens in an established cell line from NPp53 Genetically Engineered Mouse Model (GEMM), carrying the combined inactivation of *p53* and *Pten*, which are frequently mutated in humans and have been shown to progress to CRPC and androgen independent neuroendocrine phenotypes. Unveiling the vulnerabilities that arise as a consequence of the acquisition of the resistance to antiandrogenic therapy will contribute to novel prostate cancer treatments and guide future clinical trials.

METHODOLOGY

Cas9 endonuclease can be used for loss of function studies, as it can be converted into an RNA-guided transcription inhibitor via inactivation of its two catalytic domains (dCas9) and fusion to transcription repressive domains (CRISPR interference or CRISPRi). We are using a mouse genome-wide CRISPRi library for synthetic lethal interactions with Abiraterone Acetate.

Top hits from CRISPRi screening will be functionally validated (i) for their ability of decreasing AR activity; (ii) for their role in the acquisition of the stem-like plasticity characteristic of CRPC.

RESULTS

First, NPp53 cell lines have been engineered to stably express a dCas9 and efficiently silence target genes with specific sgRNAs against the promoter regions. Second, dose response curves and AR transcriptional target downregulation have been optimize for Abiraterone at IC80 so that

the remaining cells in the screen survive treatment and allow assessing the sgRNA content. Third, a genome wide library containing 5 sgRNAs/gene and covering 20,000 mouse genes (100.000 individual sgRNAs) has been amplified and sequenced and the lentiviral production of the pool library carried out.

We are now ready to perform the screen and to move to validation stages of the potential synthetic lethal interactions with Abiraterone.

CONCLUSIONS

- CRISPRi/dCas9 is an efficient and versatile system for gene expression inhibition that can be used for synthetic lethal screens with antiandrogenic drugs.

- Abiraterone is an antiandrogen drug with a notable effect on cell viability and AR transcriptional activity in our NPp53 cell line, which comes from GEMM with CRPC tumors.

- The optimization of the screening conditions with abiraterone will allow us to obtain soon the first results, which will be validated as actionable mechanisms against resistance to antiandrogenic therapies *in vitro* and *in vivo*.

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