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TITLE: Defining over-expression of MYBL2 as a driver of lethal prostate cancer

PRINCIPAL INVESTIGATOR: Leigh Ellis

CONTRACTING ORGANIZATION: Cedars-Sinai Medical Center, Los Angeles, CA

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14. ABSTRACT
Prostate cancer (PCa) is the second most frequently diagnosed cancer type among men in America. Currently, the cumulative five-year survival rate for patients with prostate cancer is over 98%. Unfortunately, the average survival for men with metastatic or recurrent prostate cancer drops considerably. Second-generation androgen deprivation therapies (ADT) have provided significant life-extending therapies for recurrent, or metastatic castration resistant prostate cancer (mCRPC) patients. A particular resistant, aggressive subset of these mCRPC tumors is independent of AR activity (CRPC-AI). CRPC-AI primarily adapt to ADT via lineage plasticity rather than a result of resistant mutations, adopting a phenotype no longer reliant on AR expression and signaling. These tumors may display neuroendocrine features, a stem or basal cell-like phenotype, altered kinase signaling, and characteristic epigenetic alterations, including upregulation of EZH2. Currently there is no therapeutic option to provide long term durable response to CRPC-AI patients. Therefore, a deeper knowledge of molecular mechanisms driving CRPC-AI will significantly move the field forward to provide discoveries for therapeutic vulnerabilities to successfully inhibit progression or treat CRPC-AI. From this, we have a novel candidate, MYBL2, that may act as a master-regulator transcription factor that upon RB1 LOF drives linage plasticity and resistance to ADT.

15. SUBJECT TERMS
MYBL2, prostate cancer, stemness, lineage plasticity, castration-resistant prostate cancer, androgen-indifferent, mouse models, organoids, targeted therapy

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INTRODUCTION

Prostate cancer (PCa) is the second most frequently diagnosed cancer type among men in America. Currently, the cumulative five-year survival rate for patients with prostate cancer is over 98%. Unfortunately, the average survival for men with metastatic or recurrent prostate cancer drops considerably. Second-generation androgen deprivation therapies (ADT) have provided significant life-extending therapies for recurrent, or metastatic castration resistant prostate cancer (mCRPC) patients. A particular resistant, aggressive subset of these mCRPC tumors is independent of AR activity (CRPC-AI). CRPC-AI is associated with genetic aberrations including amplification of *MYCN* and *AURKA*, and concurrent loss-of-function mutations in *PTEN*, *TP53*, and *RB1* tumor suppressor genes, though primarily adapt to ADT via lineage plasticity rather than a result of resistant mutations, adopting a phenotype no longer reliant on AR expression and signaling. These tumors may display neuroendocrine features, a stem or basal cell-like phenotype, altered kinase signaling, and characteristic epigenetic alterations, including upregulation of EZH2. Currently there is no option to provide long term durable response to these patients. Therefore, a deeper knowledge of molecular mechanisms driving CRPC-AI will significantly move the field forward to provide discoveries for therapeutic avenues to successfully inhibit progression or treat CRPC-AI. From this, we have a novel candidate, MYBL2, that may act as a master-regulator transcription factor that upon RB1 LOF drives linage plasticity and resistance to ADT. In addition, MYBL2 or its activity (denoted by a gene signature score) may serve as a prognostic factor and predictive biomarker of response to ADT.

KEYWORDS

MYBL2, prostate cancer, stemness, lineage plasticity, castration-resistant prostate cancer, androgen-indifferent, mouse models, organoids, targeted therapy.

ACCOMPLISHMENTS

What were the major goals of the project?

For Year 1 of this application, the major goals were:

Major Task #1

1. To obtain Institute IRB and IACUC approval and submit for HARPO/ACCURO approval.
2. Breed Mybl2 floxed alleles into DKO mice to generate models of heterozygous (DKO+Mybl2F/+) and homozygous (DKO+Mybl2F/F) Mybl2 loss.

Major Task #2

1. Validate increased expression of MYBL2 in our nominated models by qRT-PCR and western blot analysis.
2. Perform in vitro drug screening.

Major Task #3

1. Conduct gene-expression profiling in collaboration with Genome-Dx.

What was the major accomplishments under these goals?

Major Task #1

1. *To obtain Institute IRB and IACUC approval and submit for HARPO/ACCURO approval.*

We had successfully accomplished both IRB, IACUC, HARPO and ACURRO approval for relevant studies specific for this application at Dana-Farber Cancer Institute (DFCI). In September 2020, Dr. Ellis relocated to Los Angeles after being recruited to Cedars-Sinai Medical Center. This has generated some obvious delays with the overall progression of the project. Currently at Cedars-Sinai we have gained IACUC approval and currently obtaining ACCURO approval. All IRB/HARPO approvals remained at DFCI with Dr. Christopher Sweeney.

Major Task #1

2. *Breed Mybl2 floxed alleles into DKO mice to generate models of heterozygous (DKO+Mybl2F/+) and homozygous (DKO+Mybl2F/F) Mybl2 loss.*

We have been successful at generating our desired genotypes and conducted a 30-week aging study as outlined in specific aim 1 of the application (Figure 1). We have collected tissue for organoid generation, histopathology and sequencing analysis. This work is ongoing.

Major Task #2

1. *Validate increased expression of MYBL2 in our nominated models by qRT-PCR and western blot analysis.*

We have currently validated MYBL2 expression in our DKO mouse model, and human prostate samples and cancer cells line – LNCaP/MYBL2^{oe} (Figure 3 and 4).

Major Task #2

2. *Perform in vitro drug screening.*

This task has not been started due to the move of my lab from Boston to LA. My new postdoc that will be performing this work is currently preparing to carry out these assays.

Major Task #3

1. *Conduct gene-expression profiling in collaboration with Genome-Dx.*

Dr. Chris Sweeney at DFCI is overseeing this work which is ongoing. We have generated Kaplan-Meier curves from CHAARTED, TCGA and SU2C prostate cancer patient cohorts to demonstrate that over-expression of MYBL2 or a MYBL2 gene signature (indicating MYBL2 transcriptional activity) selects for patients with more aggressive disease and shorter time to resistance when treated with androgen deprivation therapy (Figure 2).

What opportunities for training and professional development has the project provided?
Nothing to report.

How were the results disseminated to communities of interest?
Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?
We will continue to analyze our in vivo mouse samples by histopathology and next generation sequencing techniques. In addition, we will carry out our in vitro drug screening and complete our gene-expression profiling with data from Genome-Dx. This is complete our major accomplishments from year one. Concurrently, we will use our in vitro drug screening data to begin our in vivo preclinical therapeutic experiments, as well as gene-expression profiling from human samples based on MYBL2 quartile analysis.

IMPACT
What was the impact on the development of the principal discipline(s) of the project?
Nothing to report.

What was the impact on other disciplines?
Nothing to report.

What was the impact on technology transfer?
Nothing to report.

What was the impact on society beyond science and technology?
Nothing to report.

CHANGES/PROBLEMS
Changes in approach and reason for change.
Nothing to report.

Actual or anticipated problems or delays and actions or plans or plans to resolve them.
Nothing to report.

Changes that had significant impact on expenditures.
Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents.
Nothing to report.

PRODUCTS
Publications, conference papers, and presentations

Journal publications
Nothing to report.

Books or other non-periodical, one-time publications
Nothing to report.

Other publications, conference papers, and presentations
Nothing to report.

Website(s) or other internet site(s)
Nothing to report.

Technologies or techniques
Nothing to report.

Inventions, patent applications, and/or licenses
Nothing to report.

Other products
Nothing to report.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS
What individuals have worked on the project?

Name:	Dr. Leigh Ellis
Project Role:	Co-PI
Researcher Identifier:	0000-0003-4739-5049
Nearest person month worked:	3
Contribution to project:	Co-PI
Funding Support:	NCI, DOD

Name:	Dr. Christopher Sweeney
Project Role:	Co-PI
Researcher Identifier:	
Nearest person month worked:	3
Contribution to project:	Co-PI
Funding Support:	NCI, DOD

Name:	Dr. Deborah Burkhart
Project Role:	Scientist
Researcher Identifier:	
Nearest person month worked:	6
Contribution to project:	Scientist
Funding Support:	

Name:	Dr. Rodolfo Serafirm
Project Role:	Post-doc
Researcher Identifier:	
Nearest person month worked:	6
Contribution to project:	Post-doc
Funding Support:	

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
Dr. Leigh Ellis has relocated his research lab to Cedars-Sinai Medical Center. Dr. Ellis has hired a post-doc with prostate cancer research to take over the experiments outlined on the SOW.

What other organizations were involved as partners?
Nothing to report.

SPECIAL REPORTING REQUIRMENTS
Nothing to report.

APPENDICES
- Figures 1-4

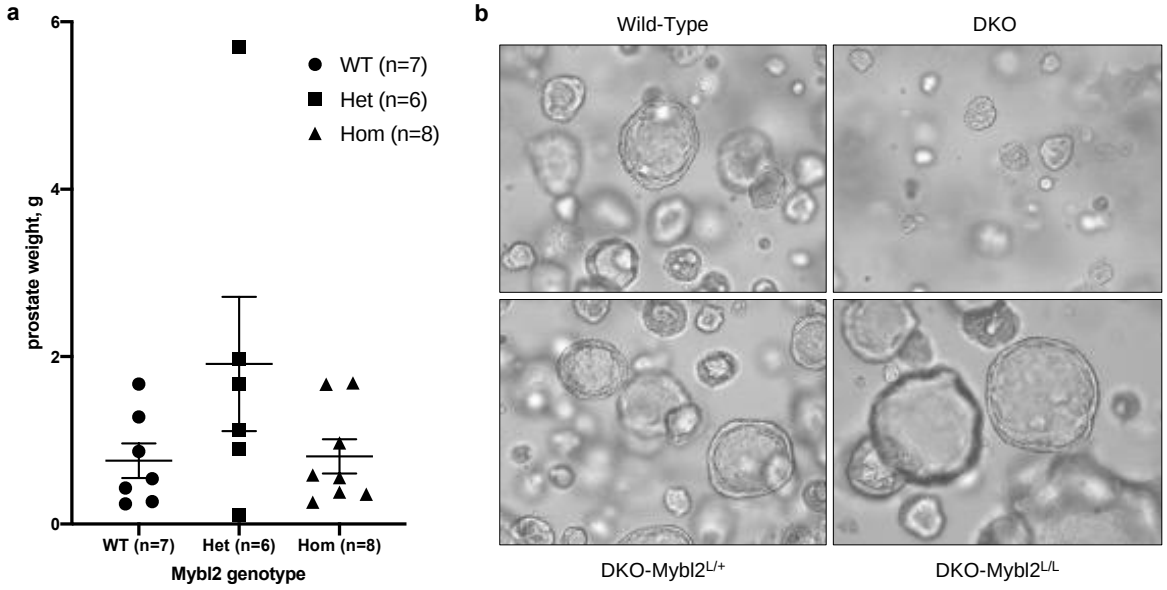

Fig 1: a) Genetically engineered mouse models presenting PbCre:Pten$^{L/L}$:Rb1$^{L/L}$ (DKO, WT for Mybl2), PbCre:Pten$^{L/L}$:Rb1$^{L/L}$:Mybl2$^{L/+}$ (Mybl2 heterozygous deletion) and PbCre:Pten$^{L/L}$:Rb1$^{L/L}$:Mybl2$^{L/L}$ (Mybl2 homozygous deletion) were aged to 30 weeks of were sacked and their genitourinary tracts weighed. Data indicates that loss of Mybl2 expression does not impede overall genitourinary size. b) 3-dimensional organoids generated from wild-type, DKO, DKO-Mybl2$^{L/+}$, and DKO- Mybl2$^{L/L}$. Representative pictures indicate that loss of Mybl2 alters the morphology of DKO organoids.

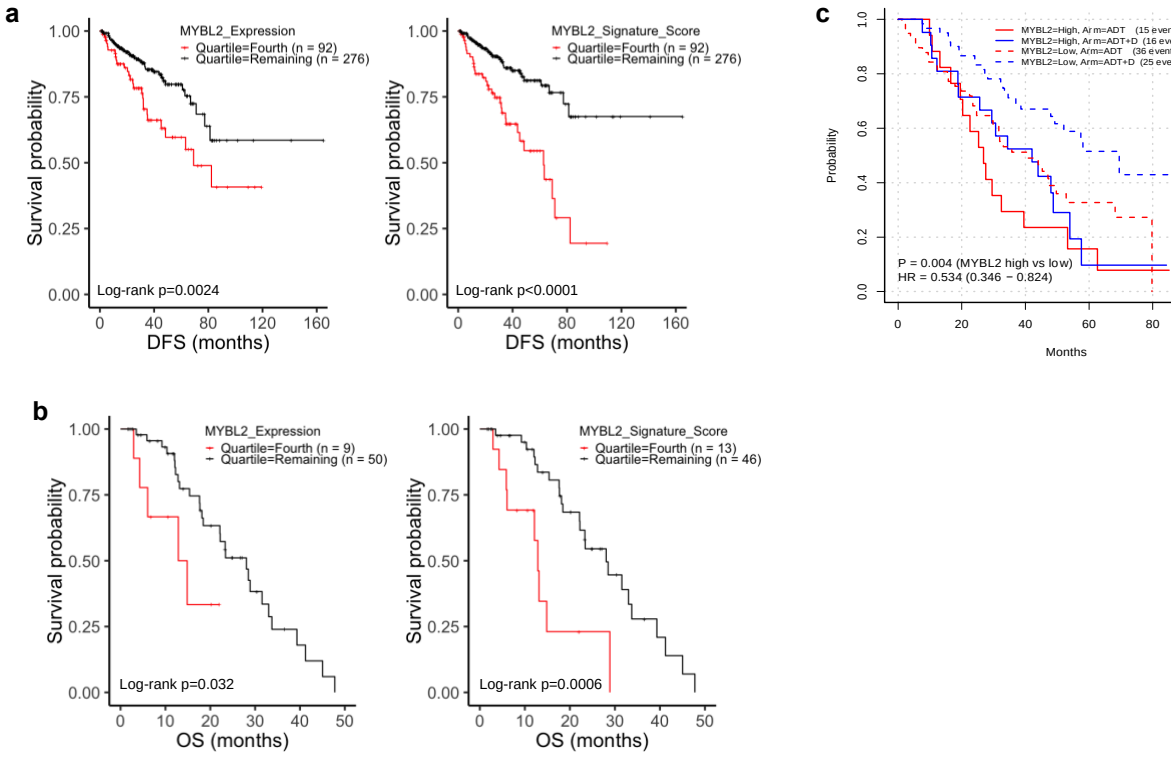

Fig 2: a) Kaplan-Meier survival analysis indicating TCGA primary prostate cancer with increased MYBL2 expression/activity have significantly shorter overall survival. b) Kaplan-Meier survival analysis indicating SU2C CRPC with increased MYBL2 expression/activity have significantly shorter disease-free survival. c) Kaplan-Meier analysis from CHAARTED patients indicating high MYBL2 expression indicates decreased overall survival.

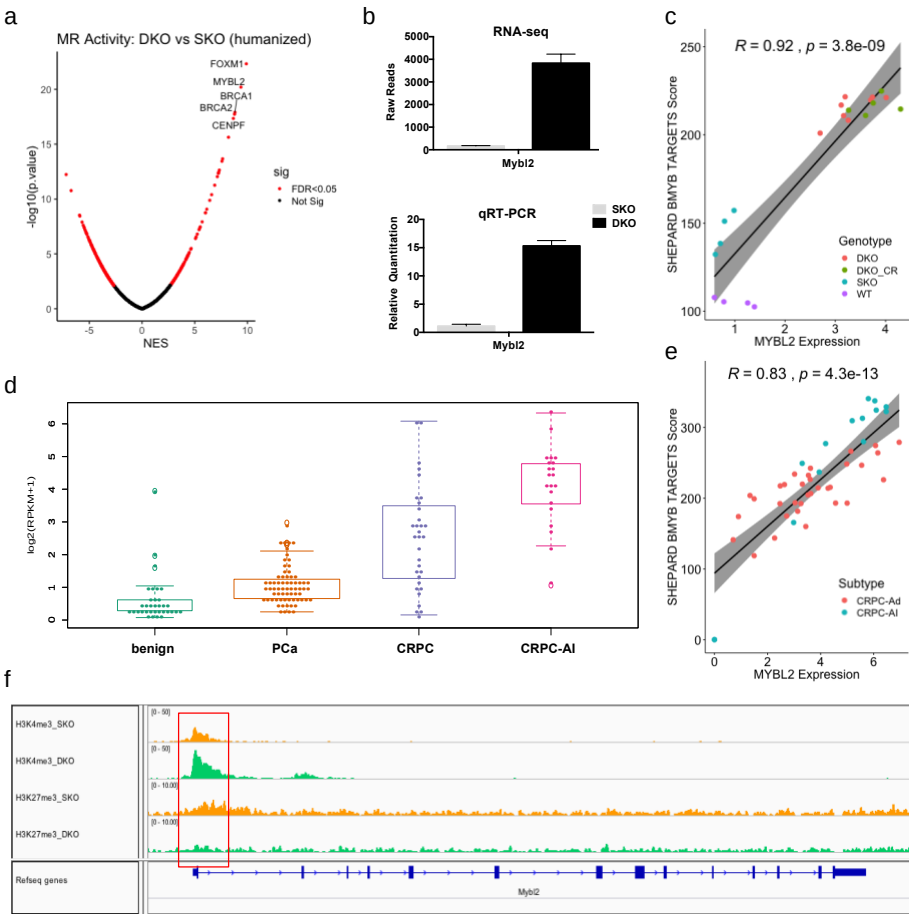

Fig 3: a) Mybl2 is identified as a potential master regulator of CRPC-AI by master regulator analysis of this DKO vs SKO RNA-seq data. b) Mybl2 mRNA upregulation is observed by RNA-seq and qRT-PCR analysis in DKO GEMMs compared to SKO GEMMs. c) MYBL2 expression and function are upregulated in DKO tumors. d) RNA-Seq analysis also indicates MYBL2 upregulation in CRPC-AI patient tumors. e) Both MYBL2 expression and function are upregulated in human CRPC-AI tumors. f) Visualization of ChIPSeq data shows increased H3K4me3 and decreased H3K27me3 in the Mybl2 promoter region in DKO tumors.

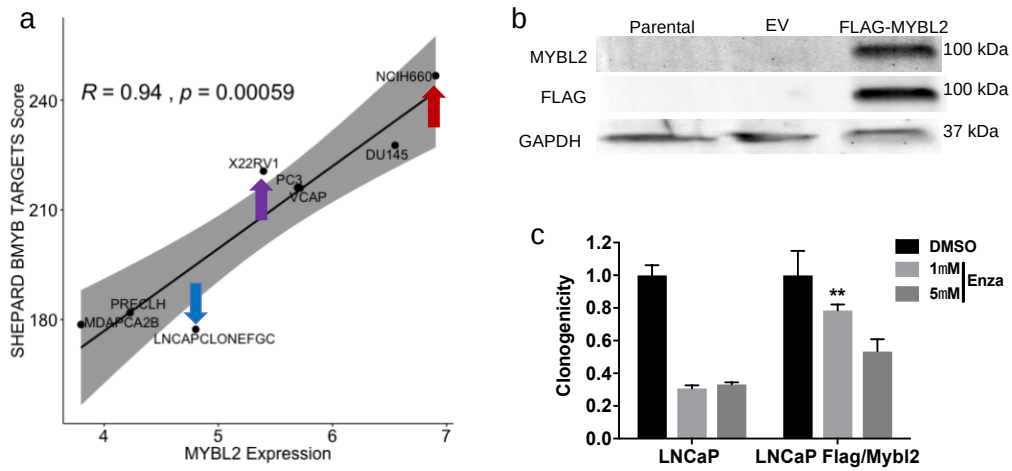

Fig 4: a) MYBL2 expression and function (downstream factor expression) are upregulated in H660 cell line (indicated by red arrow) compared to the LNCaP cell line (blue arrow). Intermediate levels are observed in the 22RV1 cell (purple arrow). (b) Upregulation of MYBL2 is achieved in LNCaP cell lines by stable expression of FLAG-MYBL2. c) Upregulation of MYBL2 in the LNCaP cell line results in decreased sensitivity to enzalutamide.