

# N-Myc-mediated epigenetic reprogramming drives lineage plasticity in advanced prostate cancer

Adeline Berger<sup>1,8</sup>, Nicholas J. Brady<sup>1,8</sup>, Etienne Dardenne<sup>1</sup>, Rohan Bareja<sup>2</sup>, Brian Robinson<sup>1,2</sup>, Vincenza Conteduca<sup>3</sup>, Michael A. Augello<sup>4</sup>, Adnan Ahmed<sup>5</sup>, Inah Hwang<sup>1</sup>, Alyssa M. Bagadion<sup>1</sup>, Loredana Puca<sup>3</sup>, Andrea Sboner<sup>1,2,6,7</sup>, Olivier Elemento<sup>2,6,7</sup>, Jihye Paik<sup>1,7</sup>, Christopher E. Barbieri<sup>2,4,7</sup>, Noah Dephore<sup>5,7</sup>, Himisha Beltran<sup>2,3,7</sup> and **David S. Rickman<sup>1,2,7,\*</sup>**

<sup>1</sup>Department of Pathology and Laboratory Medicine, <sup>2</sup>Caryl and Israel Englander Institute for Precision Medicine, New York-Presbyterian Hospital, <sup>3</sup>Department of Medicine, <sup>4</sup>Department of Urology, <sup>5</sup>Department of Biochemistry, <sup>6</sup>Department of Physiology and Biophysics, Institute for Computational Biomedicine, <sup>7</sup>Meyer Cancer Center, Weill Cornell Medicine, New York, NY 10065, USA, <sup>8</sup>These authors contributed equally

## Abstract

Despite recent advances in the development of highly effective androgen receptor (AR)-directed therapies for the treatment of prostate cancer, nearly 37% of patients develop resistance. A further third of these patients progress to develop aggressive neuroendocrine prostate cancer (NEPC) for which no effective therapies exist. Lineage plasticity, a process by which differentiated cells lose their identity and acquire an alternative lineage phenotype, has recently been proposed as a mechanism of resistance to targeted therapies in several cancer types, such as leukemias and epithelial tumors (including prostate cancer). However, the molecular programs underlying this lineage plasticity are poorly understood. Previously, we observed that the majority of NEPC and 20% of castration-resistant prostate cancer (CRPC) aberrantly overexpress the transcription factor *MYCN* (N-Myc). In spite of this frequent occurrence, the role of N-Myc in driving lineage plasticity and the epigenetic mechanisms which regulate disease progression remain to be elucidated. To address this, we analyzed overall survival and whole transcriptome data from a cohort of over 200 prostate cancer patients, including the largest-to-date population of NEPC patients. We also analyzed epigenetic modifications along with the N-Myc transcriptome, cistrome and chromatin-bound interactome by performing ChIP-seq, RNA-seq and RIME in a combination of mouse models, human prostate cancer cell lines, and NEPC patient-derived organoids. We found that the expression of *MYCN* in CRPC and NEPC patients correlates with reduced overall survival. NEPC tumors are significantly enriched for stem cell genes associated with normal neuroendocrine cell precursors and embryonic stem cells as well as for neural lineage-defining genes from activated neural stem cells. The integration of next-generation sequencing data revealed that the N-Myc cistrome is androgen-dependent and drives a transcriptional program leading to epithelial plasticity and the acquisition of clinically relevant neuronal lineage markers. Interestingly, histone marks specifically associated with lineage-defining genes are epigenetically reprogrammed by N-Myc. Finally, we demonstrated that N-Myc-induced gene expression and epigenetic changes can accurately classify our patient cohort. In summary, here we describe a novel role for N-Myc in prostate cancer, characterized by changes in the N-Myc cistrome and interacting co-factors, as well as reprogramming of the epigenome in an androgen context-dependent manner. This reprogramming is associated with an induction of a lineage plastic state and a switch towards a neural identity that favors the development of AR independence and NEPC.

## Conflicts of Interests

The authors declare no conflicts of interests.

## Funding

This work was supported in part by USA Department of Defense Early Investigator Research Award (A.B., E.D., M.A.A., L.P.), Department of Defense Impact Award PC160264 (H.B, D.S.R.), National Cancer Institute (T32CA203702 (N.J.B.), SPORE Grant P50CA211024 (B.R., O.E., C.E.B., H.B., D.S.R.), K08CA187417 (C.E.B.), R01CA215040 (C.E.B.)), Urology Care Foundation Rising Star in Urology Research Award (C.E.B.), Damon Runyon Cancer Research Foundation MetLife Foundation Family Clinical Investigator Award (C.E.B.), National Institute on Aging Grant R01AG048284 (J.P.), Prostate Cancer Foundation Young Investigator Award (M.A.A., L.P.) and Prostate Cancer Foundation Challenge Award (O.E., H.B., D.S.R.).