Whole genome gene-silencing screens in prostate cancer metastasis-derived cells identify a commonly inactivated tumor suppressor, ERF, and suggest rationale drug combination strategies for targeted therapies.

Rohit Bose¹, Wouter R. Karthaus¹, Joshua Armenia¹, Wassim Abida², Phillip J. Iaquinta¹, John Wongvipat¹, Zeda Zhang¹, Elizabeth V. Wasmuth¹, Neel Shah¹, Michael G. Doran¹, Ping Wang³, Patrick S. Sullivan⁴, Anna Patruno⁵, Young Sun Lee¹ International SU2C/PCF Prostate Cancer Dream Team⁶, Deyou Zheng², Nikolaus Schult¹ and Charles L. Sawyers¹,⁶*

¹Human Oncology and Pathogenesis Program, Memorial Sloan Kettering Cancer Center, 1275 York Ave, New York, NY 10065, USA
²Department of Medicine, Memorial Sloan Kettering Cancer Center, 1275 York Ave, New York, NY 10065, USA
³Department of Neurology, Albert Einstein College of Medicine, Bronx, NY 10461, USA
⁴Center for Molecular Oncology, Memorial Sloan Kettering Cancer Center, 1275 York Ave, New York, NY 10065, USA
⁵Departments of Genetics, and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461, USA
⁶Howard Hughes Medical Institute, Chevy Chase, Maryland 20185, USA

Background and Objectives: pooled gene-knockout and gene-silencing screens are efficient and valuable strategies to identify previously unknown biology and novel therapeutic approaches, particularly when integrated with patient-derived tumor profiling data. Here we describe two such conditional proliferative screens in metastatic prostate cancer cells, one interrogating a hypomorph of the ERG oncogene, and another interrogating cells’ ability to respond to the androgen-receptor antagonist enzalutamide. Methods: VCaP cells were infected with a whole genome lentiviral library containing 80,000 unique shRNAs to achieve 1 shRNA infected per cell, then passaged and manipulated as described above. Their genomes were subjected to deep sequencing at different time points and the relative abundance of lentiviral-integrated shRNA sequences was quantified, allowing one to infer the effect of each unique shRNA on cellular proliferation under the specified condition. Subtractive analysis between conditions enabled shRNAs targeting housekeeping genes to be eliminated from the analysis, and a focus to be placed on genes that when inhibited specifically affect the proliferation of the ERG hypomorph, or sensitize cells to enzalutamide. These potential hits were further filtered by overlaying human tumor profiling data. Results: from the ERG hypomorph screen, we identified ERF as a genetic suppressor of the ERG hypomorph phenotype. Upon shRNA and CRISPR validation in a variety of model systems including normal prostate organoids, followed by examination of human tumor data, we discovered that ERF is a prostate cancer tumor suppressor. From the enzalutamide sensitization screen, we have identified epigenetic targets that when inhibited, lead to increased enzalutamide responses. These are currently undergoing CRISPR validation. Conclusion: 1) ERF is a prostate cancer tumor suppressor. Occasionally, it is lost due to genomic alterations in tumors lacking ERG upregulation. However, more commonly it is functionally inactivated in tumor cells possessing ERG upregulation, which demonstrate a loss of ERF suppressor binding to chromatin. 2) The epigenetic state of prostate tumor cells likely controls their degree of sensitivity to androgen receptor antagonists. Implications: 1) ERF may be a biomarker
predictive of response to androgen axis therapy, and/or of prostate cancer prognosis, although this remains to be demonstrated. 2) Epigenetic targeted therapy may be effective to sensitize cells to simultaneous androgen receptor antagonism.

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