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.

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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.

Conflict of Interest: none to report.

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