

Using *EvoCaP* Model To Reveal Therapy-Driven Metastatic Dissemination in Prostate Cancer

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Metastatic prostate cancer almost universally develops resistance to androgen-deprivation therapy (ADT), progressing to metastatic castration-resistant prostate cancer (mCRPC). To investigate how therapeutic pressure shapes tumor evolution, metastatic dissemination, and clonal dynamics, we established and characterized *EvoCaP*, a *Pten*; *Trp53*-loss somatically engineered mouse lineage-tracing platform built around a Cas9-editable barcode.

Castration induced an initial therapeutic response in most tumors, but this response was heterogeneous, with clear responder and non-responder phenotypes. Despite reduced prostate size after castration, tumor volume increased, indicating persistent primary tumor burden under ADT. Metastatic dissemination was quantitatively comparable between SHAM (control) and castrated cohorts, but castration shifted metastatic tropism toward increased bone involvement and reduced visceral and lymph-node metastases. We are now using the Cas9-editable barcode to reconstruct clonal phylogenies and metastatic routes, resolving the clonal architectures and seeding patterns underlying this shift in tropism.

To investigate transcriptional and evolutionary features that enable metastatic competence, we are developing *scEvoCaP*, a lineage-resolved platform that couples single-cell RNA-seq with paired barcode sequencing to jointly infer clonal identity, transcriptional state, and migration topology. Early in vitro studies demonstrate efficient barcode transcription, Cas9 editing, and recovery of diverse amplicon sequence variants (ASVs) from both genomic DNA and complementary DNA. Initial in vivo experiments further suggest that *scEvoCaP* is feasible and will enable integrated clonal and transcriptional tracking during metastatic dissemination under therapy pressure.

Together, these data establish *EvoCaP* and *scEvoCaP* as platforms for dissecting therapy-driven evolutionary dynamics in prostate cancer.

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