**Development of tools to define single-cell evolution of prostate cancer metastasis under therapeutic pressure**

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The severity of prostate cancer is mainly due to its ability to spread and resist treatment. While androgen deprivation therapy (ADT) initially slows tumor growth, it inevitably leads to castration-resistant prostate cancer (CRPC), an aggressive and heterogeneous form of the disease. This project aims to elucidate the impact of ADT on prostate cancer metastasis, and the emergence of therapeutic resistance and clonal diversity using *EvoCaP*, a somatically engineered mouse model recently developed in our lab.

*EvoCaP* is a lentiviral-based Cre-inducible system that initiates focal disease and faithfully recapitulates the genetic and phenotypic landscape of human metastatic prostate cancer. Cre expression also activates Firefly-Luciferase and Cas9-eGFP cassettes, for longitudinal monitoring and post-mortem analysis of organs, respectively. In addition, *EvoCaP* also enables inference of clonal architecture and metastatic seeding patterns using a DNA recordable and Cas9-editable barcode. The development of robust, physiologically relevant platform like *EvoCaP*, is essential to advance our understanding of metastatic progression and improve patient care.

Our objective is to improve our *EvoCaP* system and apply it to therapeutic pressure, to decipher the particularities of the rare subclones that emerge as resistant, metastatic populations, culminating in incurable disease.

Our preliminary data show that Pten/Trp53-loss *EvoCaP* is responsive to castration therapy, and rapidly turns resistant, with metastatic dissemination, confirming a clinically relevant model. In parallel, we are refining our current lineage-tracing model by developing a transcribable version of the evolving barcode, enabling integrated transcriptomic and clonal evolution analyses at single-cell resolution with (sc)*EvoCaP*. Our results show the development of an optimal lineage tracing barcode within a modular plasmid system. This system enables testing of various element combinations to enhance long-term expression efficiency of the transcribable barcode (TBC). To anticipate *in vivo* application, Cre is driven by probasin promoter to ensure epithelial cell-specific targeting. The construct includes a red fluorescent (RFP) reporter linked to TBC expression. We showed that RFP expression correlates with eGFP and increases over time. The barcode is positioned in the 3'LTR region of the lentivirus, immediately upstream of the polyadenylation signal. cDNA amplification confirms TBC transcription, and sequencing reveals high clonal diversity of the transcribed barcode compared to the genomic DNA. Importantly, our preliminary *in vivo* data show that we can detect double $eGFP^+/RFP^+$ cells in the prostate at early timepoint.

In summary, we developed a robust *in vivo* system, mimicking prostate cancer progression to metastasis. Currently, our platform is optimized for single-cell approach, to describe evolution of clonal lineage upon castration resistance. We will provide unprecedented insights into the complexity of metastatic and therapy-resistant subclones and the influence of ADT on cancer cell migration and evolution. Ultimately, we aim to identify alternative therapeutic targets to counteract resistance and prevent relapse and subsequent incurable metastasis.

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