**Differentiation and subpopulation dynamics of treatment-emergent neuroendocrine prostate cancer in patient-derived organoids.**

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**Background:** Treatment-induced transdifferentiation of metastatic castration-resistant adenocarcinoma prostate cancer towards a neuroendocrine phenotype (t-NEPC) is increasingly common since the introduction of enzalutamide and abiraterone. The mechanisms that drive this phenomenon are not well understood, and drugs that effectively target this subtype are not known. This is in part due to a dearth of representative, preclinical models of prostate cancer in general, and treatment-emergent subtypes specifically, to study mechanisms of lineage-plasticity and drug-responses. **Methods:** Whole genome and whole exome sequencing of patient-derived t-NEPC organoids and primary tumor-derived tissue to determine the clonal evolution. Single-cell RNA-seq (scRNA-seq) analysis to identify and characterize lineage-distinct subpopulations. EdU pulse chase assays combined with immunofluorescence to quantify the rates of proliferation of lineage-defined subpopulations. **Results:** We have established mCRPC organoid models derived directly from patients, and from the LuCaP series of patient-derived xenografts. These include a unique set of patient-derived t-NEPC models designated NCI-PC35-1, and -2 that capture the subpopulation heterogeneity and multiple states of differentiation observed in the patient's tumor. Notably, NCI-PC35 represents an example of t-NEPC from the ~50% of cases that are not deficient for pRB and p53. It does however have mutations in the *ARID1A* and *ARID1B* genes encoding two core subunits of the BAF (mSWI/SNF) chromatin remodeling complex that is the most frequently mutated chromatin-regulatory complex in human cancer. One known function of BAF is to act in direct opposition to EZH2. Phenotypic and genotypic analysis of NCI-PC35-1 and 2 reveals a heterogeneous mix of subpopulations in various states of differentiation that is not explained by heterogeneous mutations. We performed scRNA-seq on NCI-PC35-1 and -2 where we identified six distinct subpopulations representing a self-renewing state defined by expression of cell cycle genes and reduced/absent lineage marker expression; an ACPC state defined by luminal prostate differentiation marker expression; multiple neuroendocrine (NE) states defined by NE markers; and two states of undefined lineage that may be transitional or perhaps dead ends. This heterogeneity is maintained over time (>2 yrs.) and generations (>20), despite quantifiable differences in the rate of proliferation between subpopulations. These observations suggest a population in a continuous state of differentiation that may be explained by the presence of a self-renewing, stem-like subpopulation that maintains the heterogeneity of the total population through ongoing asymmetrical division and differentiation. **Conclusions:** These organoid models are genetically stable with respect to driver mutations; but, contain multiple lineage-distinct subpopulations that exist in a dynamic state of balance, pointing to an epigenetic component driving differentiation. They maintain multiple lineage-distinct phenotypes over time that proliferate at different rates suggesting the presence of a stem-like progenitor population.

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