Identifying novel regulators of lineage plasticity in an organoid model of transdifferentiation.

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Resistance to anti-androgen therapy is the major clinical problem in prostate cancer treatment. There are three general mechanisms of resistance in prostate cancer: 1) activating mutations resulting in the restoration of AR signaling, 2) activation of bypass signaling, where activation of other nuclear receptors like the glucocorticoid receptor (GR), which can compensate for loss of AR signaling, and 3) lineage plasticity, where tumor cells acquire resistance by switching lineages from a cell type that is dependent on the drug target to a different cell type that is not. P53 and Rb co-mutation is strongly associated with transdifferentiation to a castration resistant prostate cancer with neuroendocrine phenotypes. We established an organoid model with inducible p53 and Rb deletion, which upon deletion progress spontaneously to neuroendocrine disease within 3 months. The *p53$_{\Delta/\Delta}$Rb1$_{\Delta/\Delta}$*-Organoids uniquely allow for the identification of factors involved in transdifferentiation. RNA sequencing of *p53$_{\Delta/\Delta}$Rb1$_{\Delta/\Delta}$*-Organoids after 8 weeks revealed a strong activation of an epithelial-to-mesenchymal transition program being turned on. ChIP sequencing correlated with activating histone marks at EMT associated genes and repressive Histonemarks at epithelial genes. Moreover treatment with enzalutamide enhanced the transdifferentiation process both in organoid culture and in xenografts. Using single cell sequencing we have identified two distinct cellular phenotypes undergoing EMT. Using CRISPR we are assessing whether EMT transcription factors are essential for transdifferentiation.

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Competing interests: C.L.S serves on the board of directors of Novartis; is a cofounder of ORIC Pharmaceuticals and coinventor of enzalutamide and apalutamide; is a science advisor to Agios, Beigene, Blueprint, Column Group, Foghorn, Housey Pharma, Nextech, KSQ, Petra, and PMV; and is a cofounder of Seragon, purchased by Genentech/Roche in 2014. W.R.K. is a patent holder and coinventor of organoid technology.