

A new bladder cancer model based on tissue reprogramming and gene targeting

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With recent advances in cellular reprogramming and gene editing it became possible to envision new approaches for tissue modeling in normal and disease contexts. Specifically, we propose to use transdifferentiation and gene targeting to generate a novel genetically-engineered model system for studies of human cancer. We recently developed a highly innovative methodology for generating fully functional prostate tissue in renal grafts based on a computational system approach that identifies synergistic specification genes (Talos et al., Nature Commun, 2017). We propose here to apply and expand these methods for modeling bladder cancer by combining lineage conversion of fibroblasts with tissue recombination assays, advanced computational systems biology algorithms and CRISPR/Cas9-mediated gene targeting of clinically-relevant mutations. In our preliminary studies, we have shown that fibroblasts can be directly converted into epithelial cells following transient expression of the pluripotency factors in pro-epithelial culture conditions. Moreover, these induced epithelial cells are amenable to further terminal differentiation into bladder tissue in tissue recombination assays in vivo under the inductive force of bladder specific mesenchyme. We have also employed computational algorithms to infer master regulators of urothelium development. Based on these preliminary data, we hypothesize that the inherent plasticity of readily-accessible fibroblasts can be exploited to generate bladder epithelia through a combination of key bladder specification genes, reprogramming techniques and tissue recombination assays. Moreover, we hypothesize that the reprogrammed bladder tissue is amenable to malignant transformation through CRISPR-mediated gene targeting.

To test this hypothesis and generate a new model of human cancer, we propose to (1) Convert human fibroblasts to bladder epithelium by activating the developmental regulatory genes and (2) Model bladder cancer by CRISPR-mediated gene targeting in the reprogrammed tissue of tumor suppressors and oncogenes relevant for human disease. Our studies will provide novel insights into the mechanisms underlying bladder tumorigenesis and a novel platform for drug screening and for discovery of patient-specific early prognostic biomarkers.