Keck School of Medicine of USC

Department of Translational Genomics - Virtual Distinguished Lecture Series

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"SINGLE-CELL RNA-SEQ PROFILING OF THE TRANSCRIPTOMIC RESPONSE TO RB LOSS IN THE RETINOBLASTOMA CELL OF ORIGIN"

Retinoblastoma is a rare childhood eye tumor initiated by biallelic inactivation of the *RB1* gene and loss of function of retinoblastoma (RB) protein. Although RB loss is a key-initiating event, the molecular mechanisms controlling malignant transformation remain unclear. In this project, we applied single cell RNA-sequencing (scRNA-seq) and *in vivo* live imaging to define transcriptomic and cell cycle changes that follow RB loss in prenatal cone photoreceptor precursors, the cell of origin of retinoblastoma. We transduced intact retina and isolated cone precursors with a cone-specific H2B-cerulean fluorescent protein (CFP) reporter vector, and transduced prospectively isolated cones with YFP-marked lentivirus carrying either of two *RB1* shRNAs or a *SCR* control shRNA. CFP+YFP+ cells were re-isolated by FACS for scRNA-seq at multiple time points over 15 days, and single cell transcriptomes were pseudotemporally ordered. For RB-depleted cone precursors, we defined two pseudotemporal trajectories (PT1 and PT2), of which PT2 was strongly associated with cell cycle related gene ontology terms and PT1 was associated with cone photoreceptor differentiation, and we identified differentially expressed genes that may mediate the proliferation (PT2) vs differentiation (PT1) decision. Surprisingly, few PT2 cells were found after 9 days post-RB knockdown, suggesting there was an abrupt change in phenotype or cell loss following S phase entry. Concordantly, live imaging of intact retinae revealed numerous RB-depleted cone precursors with prolonged and failed metaphases suggestive of a mitotic cell cycle checkpoint, as well as rare successful mitoses. Taken together, these findings suggest that RB-depleted cone precursors encounter novel and unexpected barriers to tumorigenesis, including accelerated photoreceptor differentiation and a mitotic checkpoint-mediated block, that rare cells must surpass in order to form retinoblastoma tumors. Understanding transcriptional events that underlie the proliferation vs differentiation decision as well as subsequent tumorigenesis barriers may provide clues to prevent RB-deficient cone precursor proliferation and retinoblastoma genesis in genetically predisposed children.

Tuesday, November 17, 2020 11:00am – 12:00pm **Register Here**