Identification and characterization of retinoblastoma tumor progression-related somatic copy number alteration (SCNA) driver genes.

Kevin Stachelek[1,2], Jinlun Bai[1] James Hicks[5,6] and David Cobrinik[1,3,4,5]
[1]The Vision Center and Saban Research Institute, Children's Hospital Los Angeles, Los Angeles, CA ;[2]Cancer Biology and Genomics Program, Keck School of Medicine, University of Southern California, Los Angeles, CA; [3]Department of Ophthalmology and Roski Eye Institute, Keck School of Medicine, University of Southern California, Los Angeles, CA; [4]Department of Biochemistry & Molecular Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA; [5]Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, CA; [6]Department of Biological Sciences, USC Dornsife College of Letters, Arts, and Sciences, University of Southern California, Los Angeles

Children's Hospital LOS ANGELES
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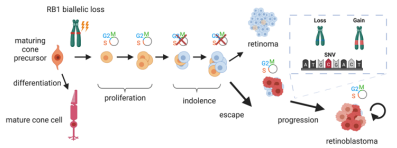
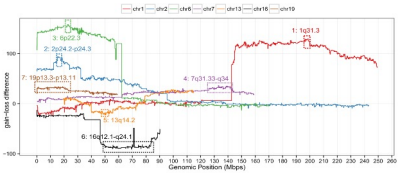
Background

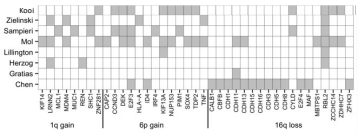
Tumors initiate by biallelic RB1 loss and progress with accumulation of Somatic Copy Number Alterations (SCNAs)



Somatic copy number alterations (SCNA) are highly recurrent in retinoblastoma



Candidate SCNA drivers from prior studies are not definitive

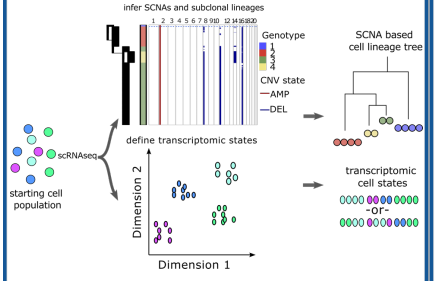


Aims

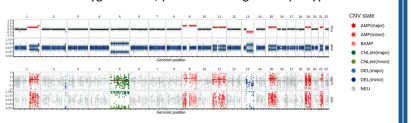
1. Define transcriptomic changes of recurrent SCNAs in heterogeneous retinoblastoma tumors.
   i. Identify retinoblastoma tumors with subclonal heterogeneity for SCNAs
   ii. Capture scRNAseq information
   iii. Infer SCNA subclones and identify SCNA-related gene expression
2. Define effects of candidate 16q- driver genes in a retinal organoid model of retinoblastoma progression.
   i. Identify candidate driver genes with downregulation in 16q- retinoblastoma tumors
   ii. Create heterozygous knockout of candidate 16q- drivers by CRISPR inactivation of GFP-marked RB-/- organoid cone precursors.
   iii. Define effects of hemizygous knockout by measuring proliferation and apoptosis through live cell imaging and immunofluorescence staining

Aim 1 Approach

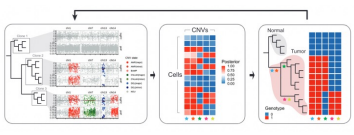
Define transcriptomic changes of recurrent SCNAs in heterogeneous retinoblastoma tumors.



Numbat infers SCNAS from gene expression, allelic imbalance in heterozygous SNPs, prior knowledge of haplotypes

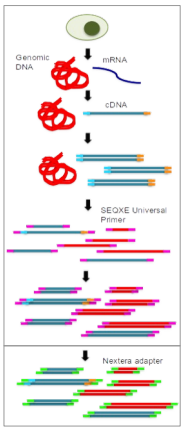


Quantify subclonal phylogeny and single cell SCNA profiles

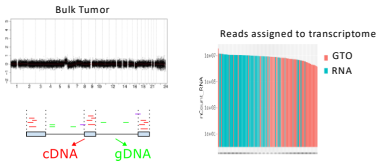


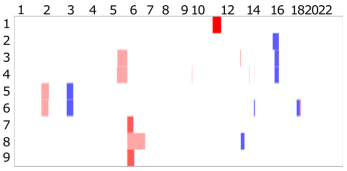
Gao et al. 2022 *Nature Biotechnology*

Aim 1 Preliminary Studies

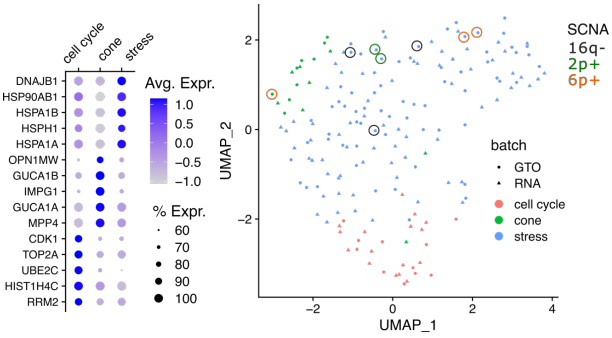


GTO can be used to simultaneously capture DNA and RNA from single cells



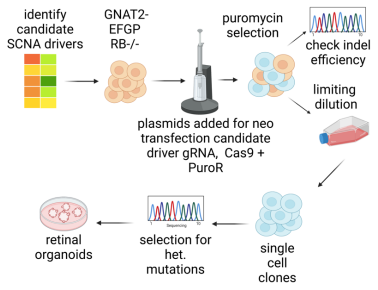


SCNA transcriptomic effects are overlaid on heterogeneous tumor cell states

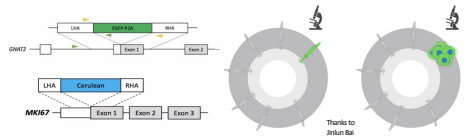


Aim 2 Approach

Define effects of candidate 16q- driver genes by CRISPR-mediated heterozygous knockout in a retinal organoid model

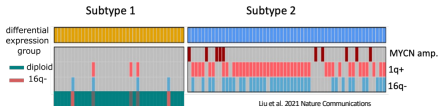


A novel chimeric stem cell-derived retinal organoid model with RB-/-, cone-specific GNAT2-GFP, and Cerulean-Ki67 expression

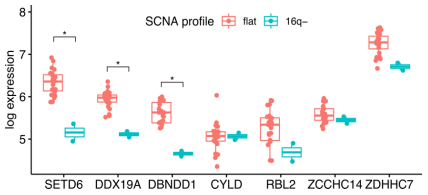


Aim 2 Preliminary Studies

Identification of 16q genes downregulated in Subtype 1 (early) retinoblastomas with limited copy number alterations.



Differential expression (16q- vs. diploid)

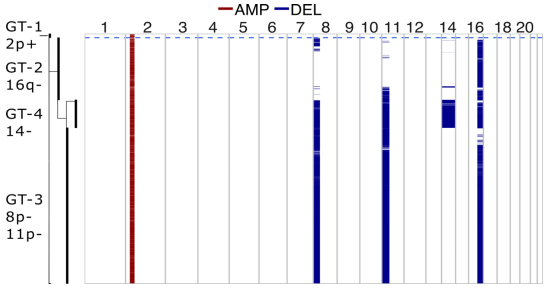


Potential tumor suppressive features of SETD6, DDX19A, and RBL2

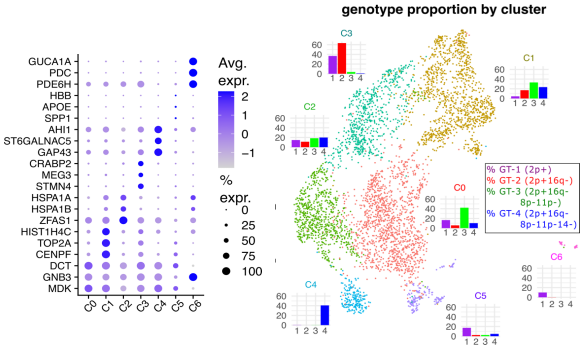
- SETD6: protein lysine methyltransferase that methylates transcriptional coactivator BRD4 to negatively regulate genes involved in translation and inhibit total mRNA translation13.
- DDX19A: DEAD-box ATPase that remodels mRNPs at the nuclear pore complex to terminate mRNP export.
- RBL2: pRB-related; combined RB1 and RBL2 KD leads to more rapid retinoblastoma tumors
- These activities are highly relevant to retinoblastoma where activation of translation related genes by MYCN is implicated in tumor progression.

SCNA inference reveals clonal dynamics in retinoblastoma

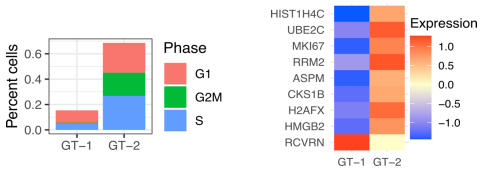
Numbat analysis of existing scRNAseq reveals SCNA subclones



SCNA subclone proportions vary according to cell states



SCNA subclone proportions vary according cell cycle and proliferation state



Conclusions

Aim 1.

- scGTO was used to concurrently define single cell SCNAs and transcriptomes.
- scGTO-derived transcriptomes formed the same transcriptomic groups as standard Smart-seq based scRNA-seq.
- Retinoblastoma cells with recurrent SCNAs contributed to the same transcriptomic groups as cells with no SCNAs, implying that differential expression should be performed between cells with and without SCNAs within each transcriptomic group.
- Application of Numbat to a public retinoblastoma scRNA-seq dataset revealed patterns of SCNA subclone distribution according to gene expression states.

Aim 2.

- Identified candidate 16q- driver genes in a publicly available retinoblastoma gene expression dataset.
- CRISPR-based indel production of SETD6, DDX19A, and RBL2 is in progress

Dominic Shayler
Sunhye Lee
Jennifer Aparicio
Jinlun Bai
Narine Harutyunyan
Andrew Salas
Kayla Stepanian
Liya Xu
Miller Huang
Rishvanth Prabakar
Naishitha Anaparthy

Acknowledgements

Thesis Committee
David Craig
David Cobrinik
James Hicks
Suhn Rhie

Funding Support
- Nautica Malibu Triathlon produced by MSP Inc
- an unrestricted grant to the USC Department of Ophthalmology from Research to Prevent Blindness
- NIH K08CA232344 (JLB)
- the Larry and Celia Moh Foundation
- the Neonatal Blindness Research Fund
- the St. Baldrick's Foundation
- NIH R01CA137124 (DC).