Kevin Stachelek

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Education

PhD.	2018-present	Cancer Biology and Genomics, University of Southern California
M.S.	2016-2018	Translational Biotechnology, University of Southern California
B.A.	2009-2013	Biological Sciences, University of Southern California

Personal Statement

I am a student in the USC PIBBS Cancer Biology and genomics program mentored in the lab of Dr. David Cobrinik. My career aspiration is to lead a bioinformatics research group in industry or academia facilitating genomic analysis for research and clinical applications.

My recent work has focused on understanding the process of retinoblastoma tumor progression. As a master's and then PhD student I carried out a project analyzing retinoblastoma tumor and cell line exome sequencing data. Despite limited prior experience in bioinformatics, I developed skills working in the unix shell, Python and R for building biological pipelines, scripting, and visualization. I developed a pipeline with preliminary data on single nucleotide variants and somatic copy number alterations in retinoblastoma tumors and cell lines. I identified several newly recurrent and novel single nucleotide polymorphisms that may act as driver mutations in retinoblastoma and the resulting submission is now under review.

As Dr. Cobrinik's lab has grown, I have also developed skills working with single-cell sequencing data. I have incorporated unsupervised learning methods including clustering and dimensionality reduction and applied these skills using R packages such as Seurat and Monocle for trajectory inference and velocyto for RNA velocity determination. I have also developed complementary skills in python using scanpy and SCENIC. Single cell technologies are undergoing rapid development and it is challenging and exciting to work with these new methods.

I have also contributed to a project to define biomarkers of the pediatric bone cancer osteosarcoma. Patients with retinoblastoma have an increased risk of osteosarcoma later in life. To develop a non-invasive biomarker assay for osteosarcoma, we collected serum exosomes from patients and controls followed by RNA sequencing. I processed this data and performed differential expression to determine transcripts associated with the presence of osteosarcoma. I identified a set of repetitive nucleotide sequences that are strongly predictive of osteosarcoma incidence.

These projects have spurred my interest in statistical methods to draw knowledge from sequence data; however, I want to move from implementation of established methods to development of bioinformatics applications for genomic analyses.

B. Positions and Honors

Positions and Employment

2018-Present	Graduate Student Research Assistant, Program in Cancer Biology and Genomics, University of Southern California
2013-2018	Research Technician, Children's Hospital Los Angeles
2012-2013	Undergraduate Research Assistant, University of Southern California

Honors

2019	Poster Presenter, Keystone Symposia Single Cell Biology
2013	Undergraduate Research SOAR grant
2009-2013	Scholarship, National Merit Scholarship Program

C. Contributions to Science

1. **Graduate Research**:

Retinoblastoma progression. The overall objective of my dissertation research is to reveal the genomic changes and associated cell signaling changes that drive retinoblastoma progression. I have pursued this interest through single cell studies of the effects of somatic copy number alterations (SCNA) in retinoblastoma. I began this work through adaptation of a novel single cell isolation method, Genome Transcriptome One-tube (scGTO) developed by Naishitha Anaparthy working with Dr. James Hicks. In this approach, copy number profiles and transcriptomes may be obtained for the same cell. I performed extensive studies of scGTO during the first two years of my PhD research. I sought to compare the transcriptomes of tumor subclones with and without a copy number and identified both single cell copy number profiles and transcriptomes in a preliminary study. I presented this work as a poster at a Keystone Symposium[1]. I then adapted my project to a higher-throughput, droplet-based approach. My studies of tumor SCNAs complement my earlier research into patterns of mutation secondary to *RB1* loss during tumor progression. I performed exome sequencing of nucleotide variants and identified recurrently altered biological functions in primary tumors and tumor-derived cell lines. I developed a novel computational pipeline to call somatic variants from retinoblastoma tumors with matched cell line samples. After integrating these results with existing retinoblastoma whole exome and whole genome sequencing datasets, I found several new recurrently mutated genes and enrichment of variant genes related to specific biological process ontologies with significant contributions of synonymous and noncoding variants. I then characterized effects of such mutations reflecting a spectrum of biological processes using luciferase reporter assays and CRISPR-mediated base editing. These findings indicated that synonymous and noncoding variants affecting mitotic, epigenetic, cell adhesion, and RNA processing machineries contribute to retinoblastoma progression and are fully reported in a recently submitted manuscript [2].

1. Stachelek, K., Lee, S., Xu, L., Anaparthy, N., Hicks, J. & Cobrinik, D. Transcriptomic effects of retinoblastoma somatic DNA copy number alterations deduced by single cell RNA and DNA sequencing. (2019). Keystone Meeting on Single Cell Biology, Keystone CO. (poster)
2. Stachelek, K., Harutyunyan, N., Lee, S., Harutyunyan, A., Kim, J., Xu, L., Berry, J. L., Nagiel, A., Reynolds, C. P., Murphree, A. L., Lee, T. C., Aparicio, J. G. & Cobrinik, D. Non-synonymous, synonymous, and non-coding nucleotide variants contribute to recurrently altered biological processes during retinoblastoma progression. *Submitted*.

Aqueous humor as a liquid biopsy of retinoblastoma. I contributed to studies from the laboratory of Dr. Jesse Berry that demonstrate the utility of aqueous humor as a liquid biopsy for detection of retinoblastoma SCNAs from cell free DNA. I provided bioinformatic analyses of aqueous humor SCNAs and assisted in processing of samples collected during clinical exams[3,4]. I have supported subsequent studies of methylation patterns in retinoblastoma aqueous humor and tumor samples that have found DNA methylation prognostic signatures for eye salvage versus treatment failure (enucleation) for retinoblastoma patients [5].

3. Berry, J. L., Xu, L., Murphree, A. L., Krishnan, S., Stachelek, K., Zolfaghari, E., McGovern, K., Lee, T. C., Carlsson, A., Kuhn, P., Kim, J. W., Cobrinik, D. & Hicks, J. Potential of aqueous humor as a surrogate tumor biopsy for retinoblastoma. *JAMA Ophthalmol.* **135**, 1221–1230 (2017).
4. Berry, J. L., Xu, L., Kooi, I., Murphree, A. L., Prabakar, R. K., Reid, M., Stachelek, K., Le, B. H. A., Welter, L., Reiser, B. J., Chévez-Barrios, P., Jubran, R., Lee, T. C., Kim, J. W., Kuhn, P., Cobrinik, D. & Hicks, J. Genomic cfDNA Analysis of aqueous humor in retinoblastoma predicts eye salvage: The surrogate tumor biopsy for retinoblastoma. *Mol. Cancer Res.* **16**, 1701–1712 (2018).
5. Li, H.-T., Xu, L., Weisenberger, D. J., Li, M., Zhou, W., Peng, C.-C., Stachelek, K., Cobrinik, D., Liang, G. & Berry, J. L. Characterizing DNA methylation signatures of retinoblastoma using aqueous humor liquid biopsy. Nat Commun 13, 5523 (2022).

Single cell and sequencing analysis. My ongoing predoctoral research includes studies of retinoblastoma transcriptomics by single cell analysis. As a PhD candidate I became skilled in R programming and in the use of popular R packages such as Seurat, Monocle, and Scanpy for processing, differential expression analysis, visualization and pseudotime inference. I applied these skills to provide primary bioinformatics support for several Cobrinik lab scRNA-seq projects. In one study, we used deep, full-length scRNA-seq to define developmental trajectories to infer the pseudotemporal progression of cone cell states during retinal development, using both human fetal retina and stem cell-derived retinal organoid models. Through RNA velocity and SCENIC regulon analysis we identified a potentially novel bifurcation of postmitotic photoreceptor precursors towards rod or cone cell fates[6]. We also applied these analyses to infer the cell proliferative states of pRB-depleted fetal cone and rod photoreceptor precursors — a model of retinoblastoma tumor initiation. Ordering

these states along a continuum allowed a trajectory to be determined amid an asynchronously responding pRB-deficient cell population that mimics the earliest stages of tumor formation [7,8].

6. Shayler, D., Stachelek, K., Lee, S., Bai, J., Aparicio, J. G., Kim, Y., Singh, M., Bay, M., Thornton, M., Grubbs, B., Bonaguidi, M., Singh & Cobrinik, D. High resolution single cell transcriptomics reveals novel photoreceptor trajectories and a cancer-predisposed developmental state. *In preparation*.
7. Lee, S., Stachelek, K., Fouladian, Z., Triska, M., Singh, H., Thornton, M., Grubbs, B. & Cobrinik, D. Rod precursor responses to pRB loss reveal a metabolic checkpoint for pRB-deficient cancers. *In preparation*.
8. Lee, S., Stachelek, K., Triska, M., Bay, M., Thornton, M., Grubbs, B., Bonaguidi, M. & Cobrinik, D. Proliferation and differentiation responses to pRB loss in the cone precursor origin of retinoblastoma tumors. *In preparation*.

I also applied supervised methods of statistical learning including differential expression and classification to identify novel biomarkers of osteosarcoma, a pediatric bone cancer for which retinoblastoma patients have an increased risk. To develop a non-invasive biomarker assay for osteosarcoma, we collected serum exosomes from patients and controls followed by combined RNA and DNA sequencing. I then performed differential expression to identify sequences associated with osteosarcoma. This led to the surprising finding that serum exosome-associated repetitive element DNA sequences are consistently increased in osteosarcoma patients. My contributions led to second authorship paper in Scientific Reports[9].

9. Cambier, L., Stachelek, K., Triska, M., Jubran, R., Huang, M., Li, W., Zhang, J., Li, J. & Cobrinik, D. Extracellular vesicle-associated repetitive element DNAs as candidate osteosarcoma biomarkers. *Sci. Rep.* **11**, 94 (2021).

Technical contributions. I have contributed technical expertise to several studies of retinoblastoma genetics using animal models and cultured cell lines. I provided support to a study of *Rb1*-mutant mouse models through mouse husbandry and live surgery [10]. As a master's student I helped to manage the Retinoblastoma Registry tissue bank, coordinating tumor sample collection and training staff to ensure management of scarce donor primary tissue samples. Through this effort I contributed to two manuscripts using retinoblastoma tumor and cell lines samples, an evaluation of a rabbit model of photodynamic therapy and a study of mitochondrial DNA variants in a panel of cancer cell lines [11,12].

10. Singh, H. P., Wang, S., Stachelek, K., Lee, S., Reid, M. W., Thornton, M. E., Craft, C. M., Grubbs, B. H. & Cobrinik, D. Developmental stage-specific proliferation and retinoblastoma genesis in RB-deficient human but not mouse cone precursors. *Proc. Natl. Acad. Sci.* **115**, E9391–E9400 (2018).
11. Kaneva, K., Merkurjev, D., Ostrow, D., Ryutov, A., Triska, P., Stachelek, K., Cobrinik, D., Biegel, J. A. & Gai, X. Detection of mitochondrial DNA variants at low level heteroplasmy in pediatric CNS and extra-CNS solid tumors with three different enrichment methods. *Mitochondrion* **51**, 97–103 (2020).
12. Kim, J. W., Jacobsen, B., Zolfaghari, E., Ferrario, A., Chevez-Barrios, P., Berry, J. L., Lee, D. K., Rico, G., Madi, I., Rao, N., Stachelek, K., Wang, L.-C. & Gomer, C. Rabbit model of ocular indirect photodynamic therapy using a retinoblastoma xenograft. *Graefes Arch. Clin. Exp. Ophthalmol.* **255**, (2017).