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TITLE: Epigenetic Changes and Clinicopathological Characterization of Prostate Cancer from Patients of African Ancestry

PRINCIPAL INVESTIGATOR: Rhie, Suhn Kyong

CONTRACTING ORGANIZATION: University of Southern California, Los Angeles, CA

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Rhie, Suhn Kyong
E-Mail: rhie@usc.edu

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University of Southern California
1450 Biggy St NRT 3504
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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Prostate cancer is the most common non-skin cancer and the second leading cause of cancer death amongst men in United States. Prostate cancer heath disparities are largely coming from ethnic differences. In particular, men of African ancestry have higher risk of prostate cancer and progression is more aggressive than men of European ancestry. However, the factors and underlying mechanisms that lead to those ethnicity-related disparities are not yet fully understood. Prostate tumor molecular subtypes are defined by genetic alterations and it is reported that prostate molecular subtypes are associated with disease prognosis. However, molecular mechanisms, which are related to aggressiveness of prostate tumors in each prostate tumor molecular subtype, are not clear. Epigenetics enable us to unravel the hidden molecular mechanisms of gene regulation, which can give rise to heterogeneous tumor phenotypes that include aggressiveness of prostate cancer. The proposed study will investigate epigenetic changes linked to aggressive prostate tumors from men of African ancestry by generating new genome-wide epigenome datasets from metastatic and primary prostate tumors and performing multivariate statistical analyses. Moreover, integrating multi-omic datasets, this study will identify key epigenetic changes that are found in aggressive prostate tumors of each prostate tumor molecular subtype. Novel findings and newly uncovered biology of aggressive prostate cancer from the proposed study will facilitate developing biomarkers and treatments for aggressive prostate tumors from men of African ancestry. Moreover, the proposed study will accelerate the development of improved targeted therapeutic tools in the field and further providing benefits to prostate cancer patients.

15. SUBJECT TERMS
None listed.

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1. INTRODUCTION

Prostate cancer heath disparities are largely coming from ethnic differences. In particular, men of African ancestry have higher risk of prostate cancer and progression is more aggressive than men of European ancestry. However, the factors and underlying mechanisms that lead to those ethnicity-related disparities are not yet fully understood. Studies on identifying epigenetic alterations in aggressive prostate cancer from men of African ancestry are lacking. As far as we know, all epigenetic studies on prostate tumors from men of African ancestry are promoter-centric. However, activities of distal regulatory elements such as enhancers are altered in prostate cancer and their activities are more dynamic across individuals than promoters. Therefore, studies on epigenetic changes of enhancers are greatly needed to understand aggressive prostate cancers from men of African ancestry. In this study, we proposed to profile global DNA methylation including both promoters and enhancers in 50 metastatic and 50 primary prostate tumors from men of African ancestry to identify DNA methylation sites that are linked to aggressive prostate tumors from men of African ancestry (Aim 1). Moreover, integrating multi-omic datasets of prostate tumors, we proposed to identify and characterize key epigenetic alterations associated with aggressive prostate tumor molecular subtypes (Aim 2). By performing cutting-edge bioinformatic and statistical analyses using the datasets, we have been characterizing key epigenetic alterations linked to aggressive prostate tumor from men of African ancestry. Successful completion of this study will identify key epigenetic alterations linked to aggressive prostate molecular subtypes and characterize prostate tumor molecular subtypes, enlightening novel molecular mechanisms of aggressive prostate tumors.

2. KEYWORDS

Epigenetic alterations
DNA methylation
Aggressive prostate cancer
African ancestry
Racial Disparities
Bioinformatics
Molecular Biology

3. ACCOMPLISHMENTS

What were the major goals of the project?

Our major goals of the project for this funded period (Year 1: 9/30/2021 - 9/29/2022) were to profile global DNA methylation in metastatic and primary prostate tumors from men of African ancestry and to identify genes that are linked to aggressive prostate tumor molecular subtypes that are defined by genetic alterations, using matched DNA-seq and RNA-seq datasets obtained from the Cancer Genome Atlas (TCGA) and the Oncology Research Information Exchange Network (ORIEN). As we outlined in the statement of work (SOW) form, we performed the major task 1 - subtasks 1, 2, 3, and 4 for specific aim 1 and the major task 1 - subtask 1 for specific aim 2 during this funded period. There was a delay of collecting prostate tumor tissues (specific aim 1, major task 1, subtask 1), which resulted in not being able to put the efforts as we originally planned for the major task 1 - subtasks 2, 3, and 4 for specific aim 1. To compensate, we also put efforts to perform major task 1 - subtask 2 for specific aim 2 during this funded period. Major task 2 - subtask 1, 2, and 3 for specific aim 1, major task 1 - subtasks 2 and 3 for specific aim 2, and major task 2 - subtasks 1 and 2 for specific aim 2 were proposed to start from Year 2. Please see the below table that includes all of tasks proposed and target completion, completion dates and % of completion.

Specific Aim 1: Identify DNA methylation sites that are linked to aggressive prostate tumors from men of African ancestry			
Major Task 1: Profile global DNA methylation in 50 metastatic and 50 primary prostate tumors from men of African ancestry	**Target Completion Date**	**Completion Date**	**% Completion**
Subtask 1: Obtain IRB/HRPO approval to use prostate tumor tissues*	12/31/21	10/6/22	100%
Subtask 2: Collect and assess prostate tumor tissues	12/31/22	N/A	5%
Subtask 3: Obtain clinicopathological datasets	12/31/22	N/A	5%
Subtask 4: Profile global DNA methylation from prostate tumor tissues	12/31/23	N/A	5%
Major Task 2: Identify and characterize DNA methylation sites that are differentially methylated between metastatic and primary prostate tumors from men of African ancestry	**Target Completion Date**	**Completion Date**	**% Completion**
Subtask 1: Identify differentially methylated sites between metastatic and primary tumor tissues, building multivariate statistical regression models, associating clinicopathological datasets with DNA methylation datasets	3/31/24	N/A	N/A
Subtask 2: Process functional genomic datasets to annotate identified differentially methylated sites	3/31/24	N/A	N/A
Subtask 3: Identify and characterize DNA methylation sites that are linked to aggressive prostate tumors in men of African ancestry	9/30/24	N/A	N/A

Specific Aim 2: Identify key epigenetic alterations associated with aggressive prostate tumor molecular subtypes, integrating multi-omic datasets			
Major Task 1: identify genes that are linked to aggressive prostate tumor molecular subtypes that are defined by genetic alterations, using matched DNA-seq and RNA-seq datasets obtained from TCGA and ORIEN	**Target Completion Date**	**Completion Date**	**% Completion**
Subtask 1: Process matched DNA-seq and RNA-seq datasets	11/30/22	N/A	80%

Subtask 2: Identify prostate tumor molecular subtypes of each dataset	5/31/23	N/A	50%
Subtask 3: Using clinical information of de-identified prostate tumor samples, Perform multivariate statistical analyses to identify genes linked to aggressive prostate tumors of each molecular subtype	3/31/24	N/A	N/A
Major Task 2: Identify key epigenetic alterations that control the expression of genes linked to aggressive prostate tumor molecular subtypes	**Target Completion Date**	**Completion Date**	**% Completion**
Subtask 1: Process multi-platform epigenomic datasets	9/30/23	N/A	N/A
Subtask 2: Perform integrative analyses to identify key epigenetic alterations linked to prostate tumor molecular subtypes	9/30/24	N/A	N/A

What was accomplished under these goals?

Specific Aim 1. We assessed the quality of prostate tumor tissue samples (Figure 1) and realized that the condition of tissues, which were archived a long time ago (e.g. >20 years ago), was not optimum (Figure 2). To obtain additional prostate tumor tissue samples, we contacted clinicians in the USC Institute of Urology. The USC Institute of Urology has been continuously collecting prostate tumor tissue samples to the Urology database till now. We successfully amended our IRB to use tissue samples from the Urology database, and the amended IRB is recently approved.

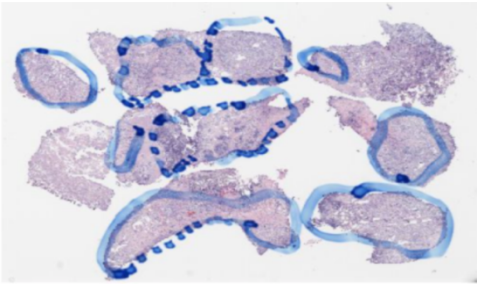

Figure 1. Example prostate tumor tissue hematoxylin and eosin (H&E) stained slide image. After obtaining prostate tumor blocks, we sliced the block to 5um sections to make slides. We stained one slide with hematoxylin and eosin (H&E) and marked prostate tumor regions. By overlying slides, we extracted DNA of tumor tissues from unstained slides to profile DNA methylation.

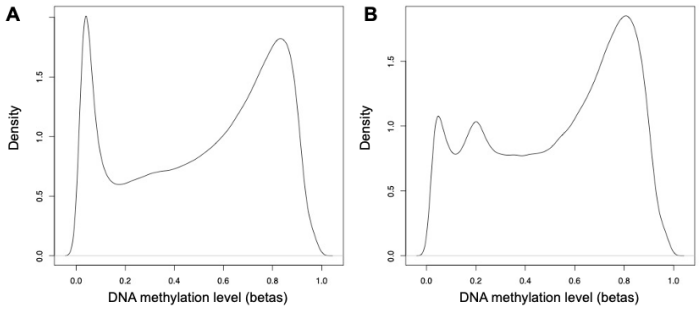

Figure 2. Quality check of DNA methylation datasets. After performing Illumina EPIC array of prostate tumor tissues to profile DNA methylation, we assessed the quality of our samples by plotting the distribution of DNA methylation levels. (A) A plot generated from a sample that passed QC (B) A plot generated from a sample that did not pass QC.

Specific Aim 2. We successfully updated bioinformatic pipelines for DNA-seq and RNA-seq data analysis. We obtained and processed hundreds of prostate cancer DNA-seq and RNA-seq datasets from the Cancer Genome Atlas (TCGA) and the Oncology Research Information Exchange Network (ORIEN) databases using the updated bioinformatic pipelines. For example, when we analyzed prostate tumor DNA-seq datasets from ORIEN, we found a subset of tumors harboring different mutations at genes including KMT2C, KMT2D, BRCA2, FOXA1, as well as SPOP (Figure 3). By performing integrative data analysis, we were able

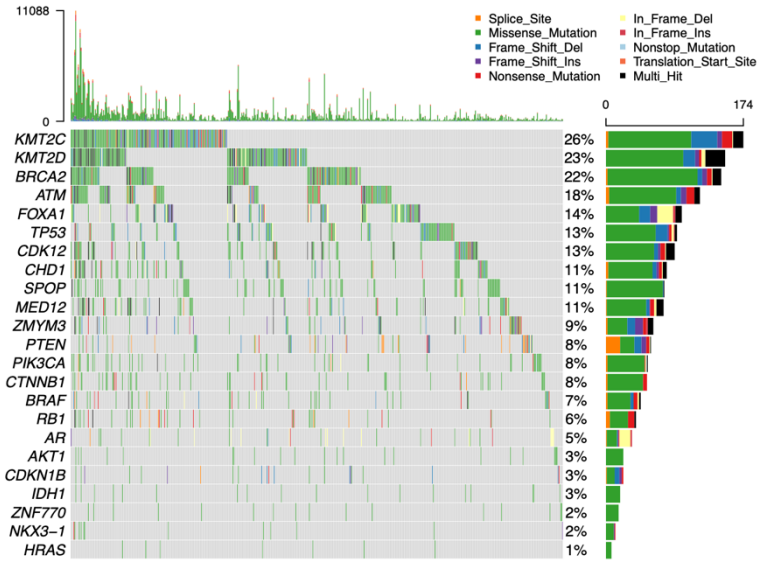

Figure 3. Somatic mutations found from ORIEN prostate cancer DNA-seq data. Using the updated in-house bioinformatic pipeline, we processed hundreds of ORIEN prostate cancer DNA-seq data. We were able to identify tumor samples that have mutations in different genes.

to start determining molecular subtypes (e.g. TMPRSS2-ERG fusion, SPOP mutation status) of prostate tumor samples.

Recent studies indicate that self-reporting ethnicity information may not be best, and heterogeneous molecular signature patterns were seen among prostate tumors obtained from African Americans. To compare self-reporting ethnicity information with ancestry information, we called allele information of genetic variants from DNA-seq datasets. We also collected over two hundred genetic variants associated with prostate cancer risk. Integrating genetic variant datasets with clinicopathological datasets, we are currently in the process of determining aggressive prostate cancer cases from African ancestry.

We were able to search and read over twenty published papers, which profiled DNA, RNA, and DNA methylation of prostate tumors from African ancestry. We organized datasets and made a list of mutations, copy number variations, fusions, genes, CpG sites that were dysregulated in prostate tumors from African ancestry (Figure 4). Moreover, we started to download additional publicly available DNA-seq and RNA-seq datasets of prostate tumors obtained from African Americans. We also identified DNA methylation regions and genes that were differentially activated between prostate tumors from African ancestry and prostate tumors from European ancestry. We wrote a review paper that includes key molecular signatures linked to aggressive prostate cancer patients from African ancestry and submitted it to the Frontiers in Oncology journal. Our paper is currently under review.

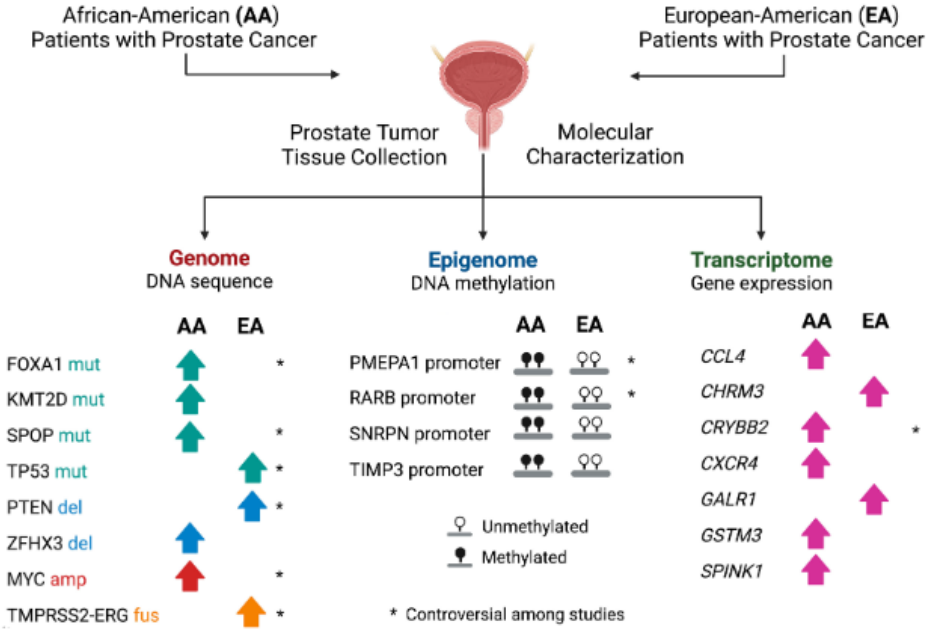

Figure 4. Molecular genetic signatures in prostate tumor tissue samples obtained from African American (AA) and European American patients (EA). We reviewed over twenty published papers and identified key genetic alterations, epigenetic alterations, and dysregulated genes reported to be linked to racial disparity.

Please see the below table that includes the summary of experiments results for each aim/task/subtask. Major task 2 - subtask 1, 2, and 3 for specific aim 1, major task 1 - subtasks 2 and 3 for specific aim 2, and major task 2 - subtasks 1 and 2 for specific aim 2 were proposed to start from Year 2. Therefore, we indicated N/A for those aims except the major task 1 - subtask 2 for specific aim 2, which we were able to start performing during this funded period.

Specific Aim 1: Identify DNA methylation sites that are linked to aggressive prostate tumors from men of African ancestry	
Major Task 1: Profile global DNA methylation in 50 metastatic and 50 primary prostate tumors from men of African ancestry	**Summary of experiments results**
Subtask 1: Obtain IRB/HRPO approval to use prostate tumor tissues*	IRB is finally approved.
Subtask 2: Collect and assess prostate tumor tissues	We assessed the quality of prostate tumor tissue samples and realized that the condition of tissues which were archived a long time ago (e.g. >20 years ago) was not optimum. We contacted clinicians in the USC Institute of Urology and obtained additional prostate tumor tissue samples that include ones from patients who visited the clinic more recently.
Subtask 3: Obtain clinicopathological datasets	To obtain tissues and clinicopathological datasets from the USC Institute of Urology,

	we amended our IRB and have started receiving the data.
Subtask 4: Profile global DNA methylation from prostate tumor tissues	We started profiling samples to measure DNA methylation levels. We established the workflow to generate high quality data.
Major Task 2: Identify and characterize DNA methylation sites that are differentially methylated between metastatic and primary prostate tumors from men of African ancestry	**Summary of experiments results**
Subtask 1: Identify differentially methylated sites between metastatic and primary tumor tissues, building multivariate statistical regression models, associating clinicopathological datasets with DNA methylation datasets	N/A for this funded period
Subtask 2: Process functional genomic datasets to annotate identified differentially methylated sites	N/A for this funded period
Subtask 3: Identify and characterize DNA methylation sites that are linked to aggressive prostate tumors in men of African ancestry	N/A for this funded period

Specific Aim 2: Identify key epigenetic alterations associated with aggressive prostate tumor molecular subtypes, integrating multi-omic datasets	
Major Task 1: identify genes that are linked to aggressive prostate tumor molecular subtypes that are defined by genetic alterations, using matched DNA-seq and RNA-seq datasets obtained from TCGA and ORIEN	**Summary of experiments results**
Subtask 1: Process matched DNA-seq and RNA-seq datasets	We successfully obtained hundreds of prostate cancer DNA-seq and RNA-seq datasets from the Cancer Genome Atlas (TCGA) and the Oncology Research Information Exchange Network (ORIEN) databases. We processed most of datasets except some which were either with high background noise or potential outliers. We wrote a review paper that summarizes key molecular signatures linked to aggressive prostate cancer patients from African ancestry in the process of collecting datasets and reading published manuscripts.
Subtask 2: Identify prostate tumor molecular subtypes of each dataset	We established the update bioinformatic pipelines to analyze DNA-seq and RNA-seq data for prostate tumor samples. By performing data analysis, we were able to determine molecular subtypes (e.g. TMPRSS2-ERG fusion, SPOP mutation status) of about half of prostate tumor samples of the aforementioned DNA-seq and RNA-seq datasets. To compare self-reporting ethnicity information with ancestry information, we called allele information of genetic variants including prostate cancer risk genetic variants from DNA-seq datasets.
Subtask 3: Using clinical information of de-identified prostate tumor samples, perform multivariate statistical analyses to identify genes linked to aggressive prostate tumors of each molecular subtype	N/A for this funded period

Major Task 2: Identify key epigenetic alterations that control the expression of genes linked to aggressive prostate tumor molecular subtypes	Summary of experiments results
Subtask 1: Process multi-platform epigenomic datasets	N/A for this funded period
Subtask 2: Perform integrative analyses to identify key epigenetic alterations linked to prostate tumor molecular subtypes	N/A for this funded period

What opportunities for training and professional development has the project provided?

Dr. Rhie has had numerous avenues of professional development as a result of this grant. Dr. Rhie took several trainings and workshops to advance her wet and dry lab skills. Dr. Rhie honed her presentation skills by presenting in several meetings, conferences, and classes. During this funded period, Dr. Rhie also served as a reviewer for Nature Communications, Cell Reports, and Frontiers in Oncology journals. Dr. Rhie also served as a grant reviewer for Wright foundation, Austrian Science Fund, and National Institute of Health. Dr. Rhie was able to mentor and train graduate, undergraduate, and postbac students (e.g. Lauren Han, Andrew Vu, Zexun Wu, Claire Stevens, Huan Cao, Alexandria Hightower, Alanna Brown). Her students have been developing their professional careers by attending seminars, workshops, and meetings and taking trainings.

How were the results disseminated to communities of interest?

Dr. Rhie attended the National Cancer Institute Center to Reduce Cancer Health Disparities meetings and participated in the mock grant reviews. Dr. Rhie attended American Association for Cancer Research (AACR) Cancer Health Disparities meeting and presented her research in the meeting. Dr. Rhie has been afforded several speaking engagements to disseminate research stemming from this grant, including the Chromatin-Conference: Epigenetic Mechanisms & Human Disease, USC Department of Biochemistry and Molecular Medicine, USC Chromatin Club, and USC Cancer Biology Genomics/Biochemistry 581 Cancer Bio and 'Omics meetings. Moreover, she actively participated in the USC Norris Comprehensive Cancer Center genomics and epigenomics program and Disease affinity group meetings. She was newly appointed as the USC Norris Comprehensive Cancer Center Diversity, Equity, and Inclusion Council Committee member and participated in various activities that promote diversity workforce.

What do you plan to do during the next reporting period to accomplish the goals?

Overall plans remain the same and on track. We will continue profiling global DNA methylation in metastatic and primary prostate tumors from men of African ancestry. We will continue identifying DNA methylation sites that are differentially methylated between metastatic and primary prostate tumors. We will continue identifying genes that are linked to aggressive prostate tumor molecular subtypes that are defined by genetic alterations using matched DNA-seq and RNA-seq datasets obtained from TCGA and ORIEN. We will put our efforts to identify key epigenetic alterations that control the expression of genes linked to aggressive prostate tumor molecular subtypes.

4. IMPACT

What was the impact on the development of the principal discipline(s) of the project?

During this funded project, Dr. Rhie, who became a tenure-track Assistant Professor in 2021, was able to establish the prostate cancer epigenomic lab in the USC Norris Comprehensive Cancer Center. Her team also established and updated bioinformatic pipelines to process multi-omic datasets that include DNA-seq, RNA-seq, ChIP-seq, Hi-C, and DNA methylation datasets. Using multi-omic datasets and performing cutting-edge bioinformatic and statistical analyses, we have been identifying key epigenetic alterations linked to aggressive prostate tumors from men of African ancestry. Moreover, we have been characterizing their functions, identifying candidate master transcription factors that make cells become metastatic. Due to COVID-19, obtaining clinical data and tissue samples from prostate cancer patients and generating DNA methylation datasets got delayed. However, as we updated IRB and arranged to obtain more samples and recruited talented graduate students in the lab, we will be able to generate datasets more efficiently in the near future. The newly datasets and analyses will be a useful resource to the prostate cancer, health disparity, and epigenetics research community. Identification of key epigenetic alterations linked to aggressive prostate molecular subtypes and characterization of prostate tumor molecular subtypes will also enlighten novel molecular mechanisms of aggressive prostate tumors. Our long-term goals are to understand the molecular mechanisms underlying aggressive prostate cancer from men of African ancestry. Identified key epigenetic alterations of aggressive prostate cancer will be potential biomarkers and therapeutic targets. The proposed study will accelerate the development of improved targeted therapeutic tools in the field and further providing benefits to prostate cancer patients.

What was the impact on other disciplines?
Noting to Report

What was the impact on technology transfer?
Noting to Report

What was the impact on society beyond science and technology?
Nothing to Report

5. CHANGES/PROBLEMS TO REPORT

Due to COVID-19, obtaining tissue samples from prostate cancer patients has been challenging and performing wet lab experiments was very difficult due to the campus shut-down and capacity limit. We realized that the quality of prostate tumor tissue samples which were archived a long time ago was not optimum for DNA methylation assays. We contacted clinicians and arranged to obtain additional prostate tumor tissue samples from USC Institute of Urology. In order to do this, we had to amend our IRB, and the review process of IRB took longer than we anticipated. Fortunately, the amended IRB which includes the Urology database is now approved. Due to COVID-19, hiring of personnel (e.g. postdoc) has been very challenging. However, I was able to recruit several PhD students for rotation in 2022 fiscal year, and they joined the lab this summer. The campus is now fully open and hard-working graduate students joined the lab, so we anticipate that we can perform proposed experiments more efficiently.

No significant changes to Vertebrate Animal(s)

No significant changes to Human Subjects

No significant changes in use of biohazards and/or select agents

6. PRODUCTS

Presentations:

Using 3D epigenomic maps to understand gene regulation and human diseases Rhie SK *Chromatin-Conference: Epigenetic Mechanisms & Human Disease Meeting Organized by Active Motif,* October 26, 2021; San Diego, CA, USA through virtual meeting

Using high-resolution 3D epigenomic maps to understand gene regulation Rhie SK *University Southern California Cancer Biology Genomics/Biochemistry 581 Cancer Bio and 'Omics seminar*, December 1, 2021; Los Angeles, CA, USA

Using 3D epigenomic maps to understand gene regulation and human diseases Rhie SK University of Southern California Health Science Campus Chromatin Club, April 20, 2022; Los Angeles, CA

Characterizing DNA methylation signatures in prostate tumors to understand health disparities in Black and White men Rhie SK *American Association for Cancer Research (AACR) Cancer Health Disparities Conference Ancillary Event CaRE2 Partnership Meeting* September 16, 2022; Philadelphia, PA, USA

Investigating molecular and genetic contributions to racial disparities in prostate cancer by integrating multi-omic datasets available in public Hightower A, Stevens C, Buxbaum S, Falzarano S, Rhie SK *American Association for Cancer Research (AACR) Cancer Health Disparities Conference* September 18, 2022; Philadelphia, PA, USA

Using 3D epigenomic maps to understand gene regulation and human diseases Rhie SK *University Southern California Department of Biochemistry and Molecular Medicine Master Student Seminar Faculty Presentation,* September 27, 2022; Los Angeles, CA

Websites:

https://sites.usc.edu/rhielab/

No inventions, patents, or licenses

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Suhn K. Rhie, PhD
Project Role:	PI
Research Identifier (e.g. ORCID ID):	0000-0002-5522-5296
Nearest person month worked:	2
Contribution to Project:	Dr. Rhie has performed data analyses and supervised the study.
Funding Support:	N/A

Name:	Huan Cao, BS
Project Role:	Graduate Student
Research Identifier (e.g. ORCID ID):	0000-0003-0942-4769
Nearest person month worked:	2
Contribution to Project:	Ms. Cao has collected and assessed prostate tumor tissue samples and worked on wet lab experiments.
Funding Support:	N/A

Name:	Claire Stevens, BS
Project Role:	Graduate Student
Research Identifier (e.g. ORCID ID):	0000-0003-2695-0410
Nearest person month worked:	2
Contribution to Project:	Ms. Stevens has collected publicly available multi-omic datasets, performed data analyses, and organized studies on prostate cancer racial disparities.
Funding Support:	N/A

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to Report

What other organizations were involved as partners?

Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

Not Applicable