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TITLE: Chromatin Accessibility and the Convergent Oncogenic Pathways of Angiosarcomas

PRINCIPAL INVESTIGATOR: Jong Hyuk Kim

CONTRACTING ORGANIZATION: University of Florida, Gainesville, FL

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Angiosarcoma is a rare type of soft tissue sarcoma, with a prevalence of fewer than 300 cases in the US annually. Our understanding of the oncogenic mechanisms of aggressive angiosarcomas is rudimentary, and our ultimate goal is to develop appropriate and effective treatment options and protocols for patients with this disease. Angiosarcoma are genomically complex; however, they share a histological morphology that consists of disorganized, malignant vessel-forming cells. Our hypothesis is that chromatin accessibility is necessary to establish the mutational landscape, which consequently activates convergent signaling pathways that contribute to angiosarcoma development. In this report period, we established chromatin accessibility and the transcriptomic landscape in TP53 mutant hemangioblast cells differentiated from human induced pluripotent stem cells. This approach allows us to develop *in vitro* tumor models to define molecular mechanisms that regulate convergent oncogenic pathways in angiosarcomas. We will determine if p53 deficiency in hemangioblasts contributes to angiosarcoma development. This project will impact our understanding of aggressive angiosarcomas and specifically enhance our basic knowledge of how morphologic convergence with genetic chaos arises and contributes to angiosarcoma development. This career development award has also supported the PI, Dr. Kim in achieving his career goal of developing an independent research program to advance our understanding of aggressive sarcomas. Dr. Kim started a new position at the University of Florida as a tenure-track faculty in the previous period. Following the completion of the award transfer during this period, he has continued with the project.

15. SUBJECT TERMS
None listed.

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
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Unclassified	Unclassified	Unclassified	Unclassified	18	19b. TELEPHONE NUMBER *(include area code)*

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1. INTRODUCTION:

Angiosarcoma is a rare type of soft tissue sarcoma, with a prevalence of fewer than 300 cases in the US each year. These tumors are highly aggressive and metastatic, and more than half of the patients with this disease die within the first year of diagnosis. Angiosarcomas are genomically complex; however, they share a histological morphology that consists of disorganized, malignant vessel-forming cells. Our objective is to establish chromatin accessibility and the mutational landscape, which activate convergent signaling pathways contributing to angiosarcoma development. Specifically, we develop tumor models to define molecular mechanisms that regulate convergent oncogenic pathways in angiosarcomas using induced pluripotent stem cells and genome engineering. From this approach, this project tests a new concept that could change the paradigms for addressing the fundamental oncogenic mechanisms of angiosarcomas.

2. KEYWORDS:

Angiosarcoma; rare cancer; induced pluripotent stem cell; CRISPR/Cas9; ATAC-seq; RNA-seq; chromatin accessibility; transcriptomics; hemangioblast; sarcoma modeling; single cell genomics

3. ACCOMPLISHMENTS:

What were the major goals of the project?

The project has three specific aims. In this Year-3 report, we list specific aims 1 and 2 along with their pertinent major tasks and subtasks below. This report especially includes the inactive project period, which occurred due to the grant transfer (from 7/1/2022 to 3/31/2023). Therefore, the Year-3 report only covers research activities for 3 months after the award transfer.

Specific Aim 1: To establish chromatin accessibility and the transcriptomic landscape in angiosarcomas.

Major Task 1: Generation of next generation sequencing data from human angiosarcomas project

Subtask 1: Submit HRPO and ACURO documentation to DoD
- Completed on Apr 4th, 2023 at the University of Florida, PI's new institution

Subtask 2: Sample preparation of primary tissue samples for ATAC-Seq and RNA-Seq generation
- Partially completed (50% of completion) and deferred

Subtask 3: Quality control analysis of samples and sequencing run
- Partially completed (50% of completion) and deferred

Subtask 4: Initial bioinformatic analysis of sequenced data
- Partially completed (50% of completion) and ongoing

Subtask 5: Advanced bioinformatic analysis of ATAC-Seq and RNA-Seq
- Partially completed (50% of completion) and ongoing

Subtask 6: Project meetings
- Completed for this period

Subtask 7: Career development for Dr. Kim (learning ATAC-Seq analysis and developing the application)

- Partially completed (90% of completion) and ongoing

Major Task 2: Establishment of angiosarcoma xenografts and generation of single cell-ATAC-Seq and -RNA-Seq

Subtask 1: Ordering and housing mice in animal facilities
- Deferred

Subtask 2: Culture and expand angiosarcoma (AS5 and ISO-HAS) cells
- Partially completed (50% of completion) and ongoing

Subtask 3: Mice xenograft experiment by transplantation of angiosarcoma cells
- Deferred

Subtask 4: Sacrifice mice, generation of histological samples, and harvest xenograft tumors to prepare single cells of tumors
- Deferred

Subtask 5: Generation of single cell-sequencing (scATAC-Seq and scRNA-Seq) data libraries
- Partially completed (50% of completion) and ongoing

Subtask 6: Quality control analysis of samples and sequencing run
- Partially completed (75% of completion) and ongoing

Subtask 7: Initial bioinformatic analysis of sequenced data
- Partially completed (75% of completion) and ongoing

Subtask 8: Project meetings
- Completed for this period

Subtask 9: Career development for Dr. Kim (learning scATAC-Seq and scRNA-Seq)
- Partially completed (75% of completion) and ongoing

Specific Aim 2: To develop in vitro tumor models to define molecular mechanisms that regulate convergent oncogenic pathways in angiosarcomas
Major Task 1: Gene engineering and differentiation in iPSCs

Subtask 1: Generation of iPSCs and preparation of reagents; commercially available iPSC cell lines (iPS12-10 and BYS-0110)
- Partially completed (75% of completion) and ongoing

Subtask 2: Engineering gene mutation (*TP53*, *PIK3CA*, *TP53*/*PIK3CA*) in iPSCs
- Partially completed (40% of completion) and deferred

Subtask 3: Differentiation of hemangioblasts from engineered iPSCs
- Partially completed (50% of completion) and deferred

Subtask 4: Functional validation of engineered cells
- Partially completed (50% of completion) and deferred

Subtask 5: Cell line authentication and Mycoplasma screening (iPSCs-derived cells, HUVEC, fibroblasts, AS5, ISO-HAS)
- Completed for this period

Subtask 6: Project meetings
- Completed for this period

Subtask 7: Career development for Dr. Kim (acquisition of new experimental skills for iPSCs generation and genome engineering)
- Partially completed (50% of completion) and ongoing

Subtask 8: Career development for Dr. Kim (starting development of strategies to secure funding)
- Partially completed (75% of completion) and ongoing

What was accomplished under these goals?
This report includes the inactive project period, which occurred due to the grant transfer (from 7/1/2022 to 3/31/2023). Therefore, the Year-3 report only covers research activities for 3 months after the award transfer.

Specific Aim 1: To establish chromatin accessibility and the transcriptomic landscape in angiosarcomas.

Subtask 3: Quality control analysis of samples and sequencing run
- Completed
In the previous report, we generated ATAC-seq and RNA-seq from iPSC-derived cells and performed initial quality control analysis. In this report, we have conducted additional quality control analysis.

ATAC-seq data: Previously, we sequenced a total of 28 human samples obtained at each differential stage, including replicates. During this period, our work primarily focused on comparing quality control data of *TP53* mutant and WT cells.

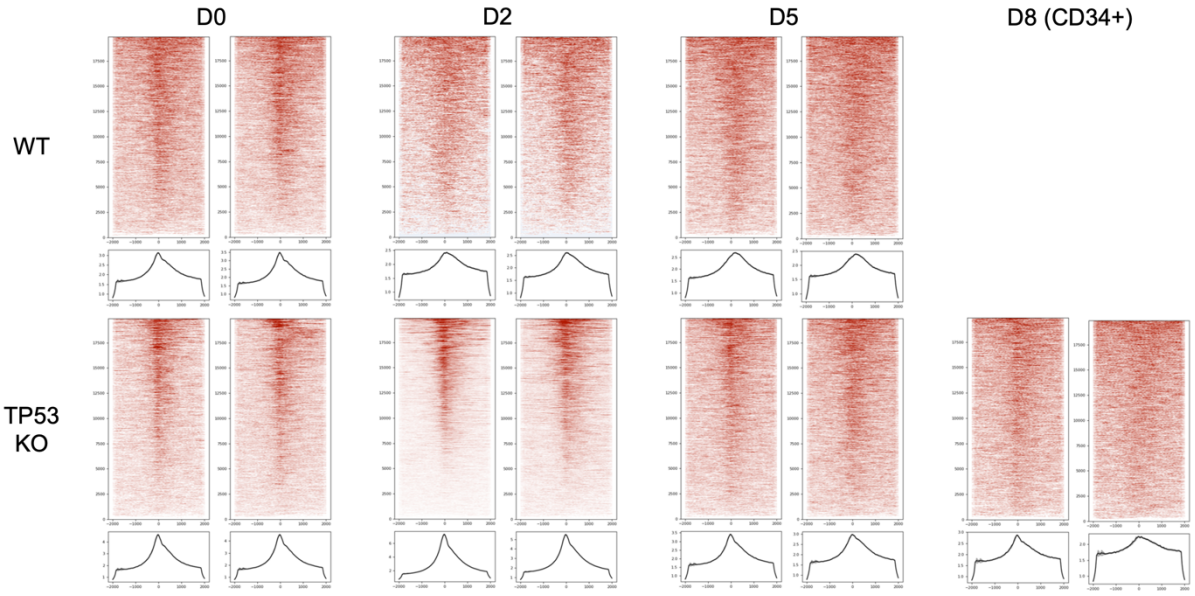

Figure 1. Transcription Start Site (TSS) enrichment analysis between WT and *TP53* mutant cells. Representative heatmap plots visualize Transcription Start Site (TSS) enrichment data changed at multiple differentiation time points: day 0 (D), day 2 (D2), day 5 (D5), and day 8 (D8).

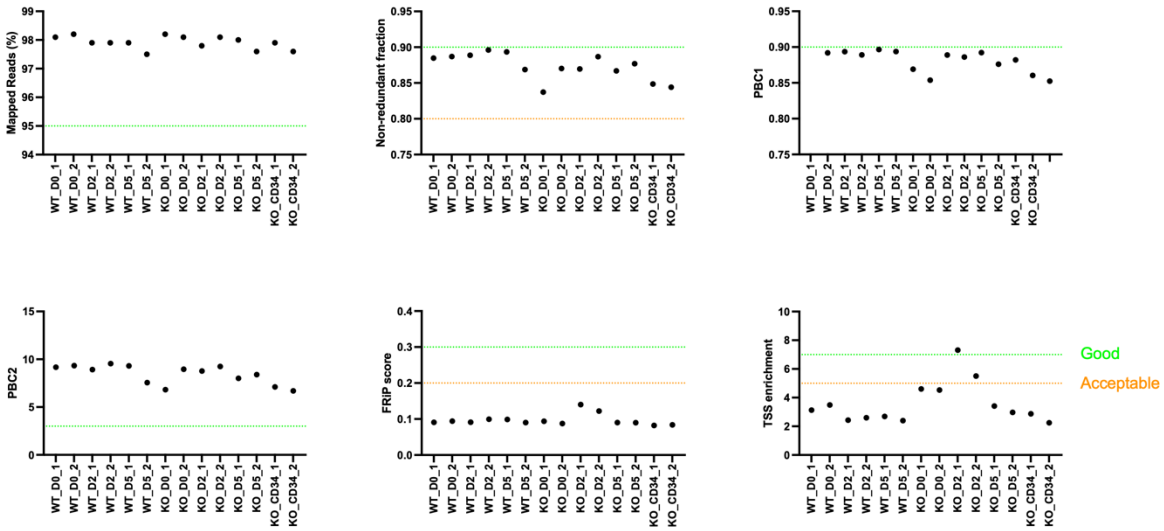

Figure 2. Quality control analysis of ATAC-seq data. Dot plots display quality control parameters: Mapped Reads; Non-redundant fraction; PCR Bottlenecking Coefficient 1 (PBC1); PBC2; Fraction of Reads in Peak (FRiP) score; TSS enrichment.

RNA-seq data: We completed the quality control analysis in the previous report. We will perform in-depth analysis in the next perioda.

Subtask 4: Initial bioinformatic analysis of sequenced data

- Completed

The sequenced read data as FASTQ format were transferred to the University of Florida (UF) HiPerGator supercomputer following the PI's transition. In previous report, we performed the

initial bioinformatic analysis. In this report, we ran additional ATAC-seq and RNA-seq analysis pipelines after configuring the UF supercomputer.

Subtask 5: Advanced bioinformatic analysis of ATAC-Seq and RNA-Seq
- Partially completed (75% of completion) and ongoing

ATAC-seq analysis: We completed running two ATAC-seq pipelines, ENCODE [Ref #1] and PEPATAC [Ref #2]. We will compare output data from the two tools to perform downstream analysis including combined analysis of ATAC-seq and RNA-seq data.

[1] https://github.com/ENCODE-DCC/atac-seq-pipeline

[2] http://pepatac.databio.org/: Smith JP, Corces MR, Xu J, Reuter VP, Chang HY, Sheffield NC. PEPATAC: an optimized pipeline for ATAC-seq data analysis with serial alignments. NAR Genom Bioinform. 2021 Nov 23;3(4):lqab101. doi: 10.1093/nargab/lqab101. PMID: 34859208

RNA-seq analysis: We adapted new pipelines for comprehensive RNA-seq data analysis using nf-Core [Ref #3]. We established new pipeline for comprehensive RNA-seq data analysis including gene expression profiling, fusion gene detection, and variant calling at the UF.

[3] https://nf-co.re/: Ewels PA, Peltzer A, Fillinger S, Patel H, Alneberg J, Wilm A, Garcia MU, Di Tommaso P, Nahnsen S. The nf-core framework for community-curated bioinformatics pipelines. Nat Biotechnol. 2020 Mar;38(3):276-278. doi: 10.1038/s41587-020-0439-x. PMID: 32055031.

Subtask 6: Project meetings
- Completed for this period

Subtask 7: Career development for Dr. Kim (learning ATAC-Seq analysis and developing the application)
- Completed (90% of completion) and ongoing
After the award transfer, Dr. Kim, PI resumed the project by further enhancing his ability to obtain bioinformatic and computational skills for ATAC-seq analysis in the new supercomputing system. He is continuing the work as AI Initiative faculty at the University of Florida. He became a member of the UF Intelligent Critical Care Center and the Cancer AI Working Group in the UF Health Cancer Center. Dr. Kim is continuing to develop comprehensive data analytics by integrating AI and machine learning applications.

Major Task 2: Establishment of angiosarcoma xenografts and generation of single cell-ATAC-Seq and -RNA-Seq

Subtask 1: Ordering and housing mice in animal facilities
- Deferred

Subtask 2: Culture and expand angiosarcoma (AS5 and ISO-HAS) cells
- Partially completed (50% of completion) and deferred
In previous report, we used alternative cell lines (two canine hemangiosarcoma cell lines, DHSA-1426 and COSB) due to authentication issue in ISO-HAS cells. We are working on the

material transfer of new batch of ISO-HAS cells. After authentication in new batched cells, we will perform tumor xenograft experiments in the next project period.

Subtask 3: Mice xenograft experiment by transplantation of angiosarcoma cells
- Deferred

Subtask 4: Sacrifice mice, generation of histological samples, and harvest xenograft tumors to prepare single cells of tumors
- Deferred

Subtask 5: Generation of single cell-sequencing (scATAC-Seq and scRNA-Seq) data libraries
- Partially completed (50% of completion) and ongoing
The generation of single-cell sequencing data was initially planned from a mouse xenograft experiment of human angiosarcoma cells. In the previous report, we opted for scRNA-seq data generation from canine hemangiosarcoma cells as alternative. During this period, we transferred the data to the UF for downstream analysis.

Subtask 6: Quality control analysis of samples and sequencing run
- Partially completed (75% of completion) and ongoing
The previous report presented the quality control results of the single-cell sequencing data. In this report, we re-ran the bioinformatic pipelines using the updated canine reference genome (CanFam 6) on the new supercomputer. We also re-evaluated the quality control data to assess reproducibility.

Subtask 7: Initial bioinformatic analysis of sequenced data
- Partially completed (75% of completion) and ongoing
We first analyzed bulk RNA-seq data to establish gene expression profiling data in *PIK3CA* mutant hemangiosarcoma cells at the whole-cell population level. Principal component analysis (PCA) revealed that WT and mutant (C8 and C35) cells exhibited distinct global gene expression patterns (**Figure 3A**). Similarly, single cell data showed distinct cell clusters between WT and the mutant cells as depicted by t-distributed Stochastic Neighbor Embedding (t-SNE) plot (**Figure 3B**).

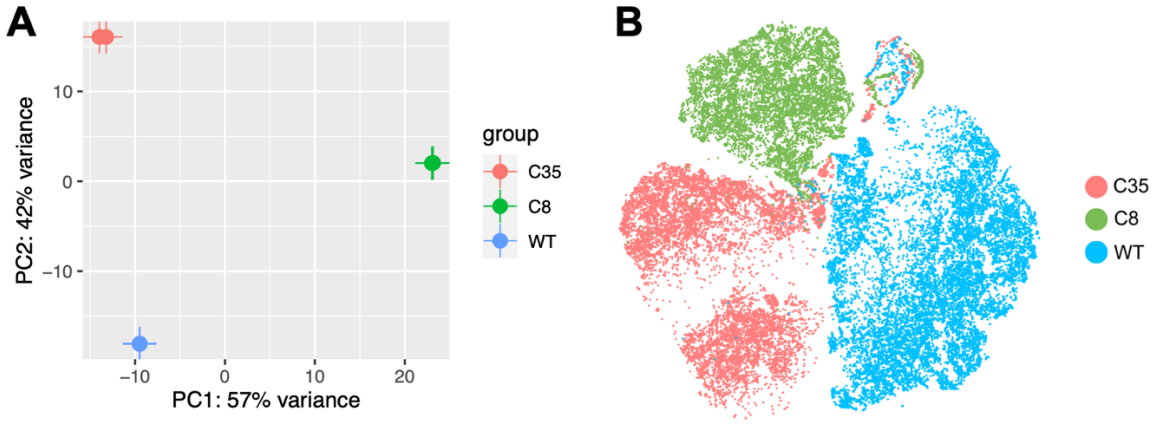

Figure 3. Gene expression profiles of *PIK3CA* WT and mutant canine hemangiosarcoma cells at bulk cell population and at single cell level. **(A)** PCA revealed that WT and mutant (C8 and C35) cells establish distinct global gene expression patterns. (**B**) t-SNE plot presented distinct single cell clusters of WT and mutant cells re-mapped with new canine reference genome (CanFam6).

Then, our data showed 619 differential expressed genes (DEGs; 489 up-regulated; 130 down-regulated) in the mutant C8 and C35 cells compared WT cells (adjusted p <0.01; log2 fold change > |1.5|) (**Figure 4A**). Gene set enrichment analysis (GSEA) showed that genes associated with cytokine/chemokine signaling pathways, angiogenesis, and extracellular matrix regulation were enriched in the *PIK3CA* mutant cells (**Figure 4B).**

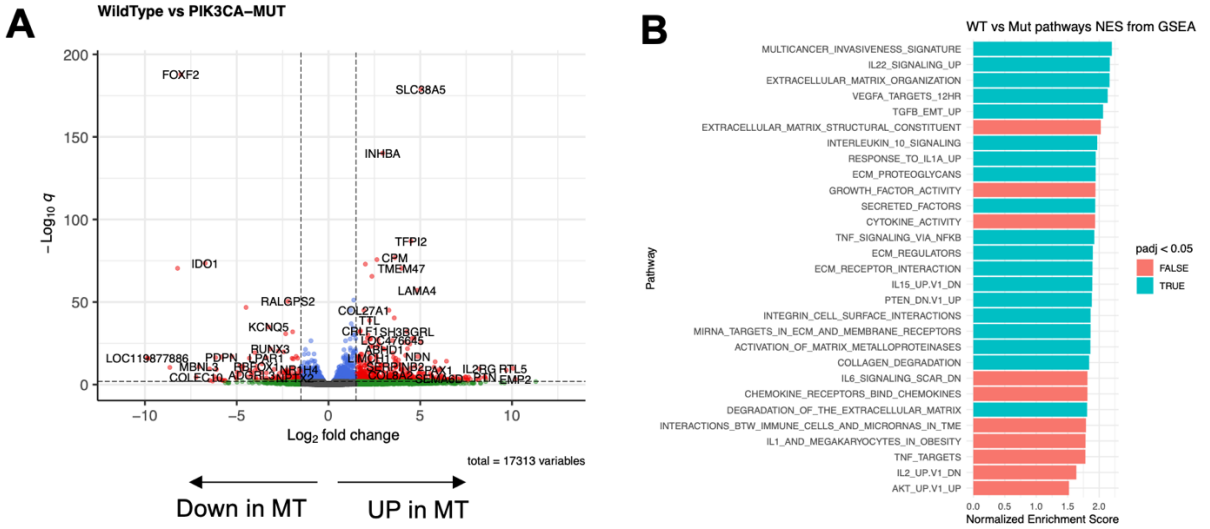

Figure 4. Gene signatures enriched in *PIK3CA* mutant hemangiosarcoma cells. (A) Volcano plot depicts DEGs between mutant and WT cells. **(B)** GSEA identified significant gene pathways associated with the DEGs.

Since different gene expression patterns were observed between the two mutant cells, C8 and C35, we further examined their distinct gene signatures (**Figure 5**). We identified a total of 1,222 DEGs, with 608 genes up-regulated and 614 genes down-regulated in the mutant C8 cells compared to the C35 cells (adjusted p < 0.01; log2 fold change > |1.5|) (**Figure 5A, B**). GSEA data presented gene enrichment of the hypoxia-inducible factor (HIF)-1 pathway and glycolysis pathway in the mutant C8 cells compared to C35 cells (**Figure 5C, D**).

Subtask 8: Project meetings
- Completed for this period

Subtask 9: Career development for Dr. Kim (learning scATAC-Seq and scRNA-Seq)
- Partially completed (75% of completion) and ongoing
Dr. Kim continued to enhance his bioinformatics and data analysis skills for single-cell data, specifically by adapting those skills to the new supercomputer and conducting data analysis using the Seurat R package.

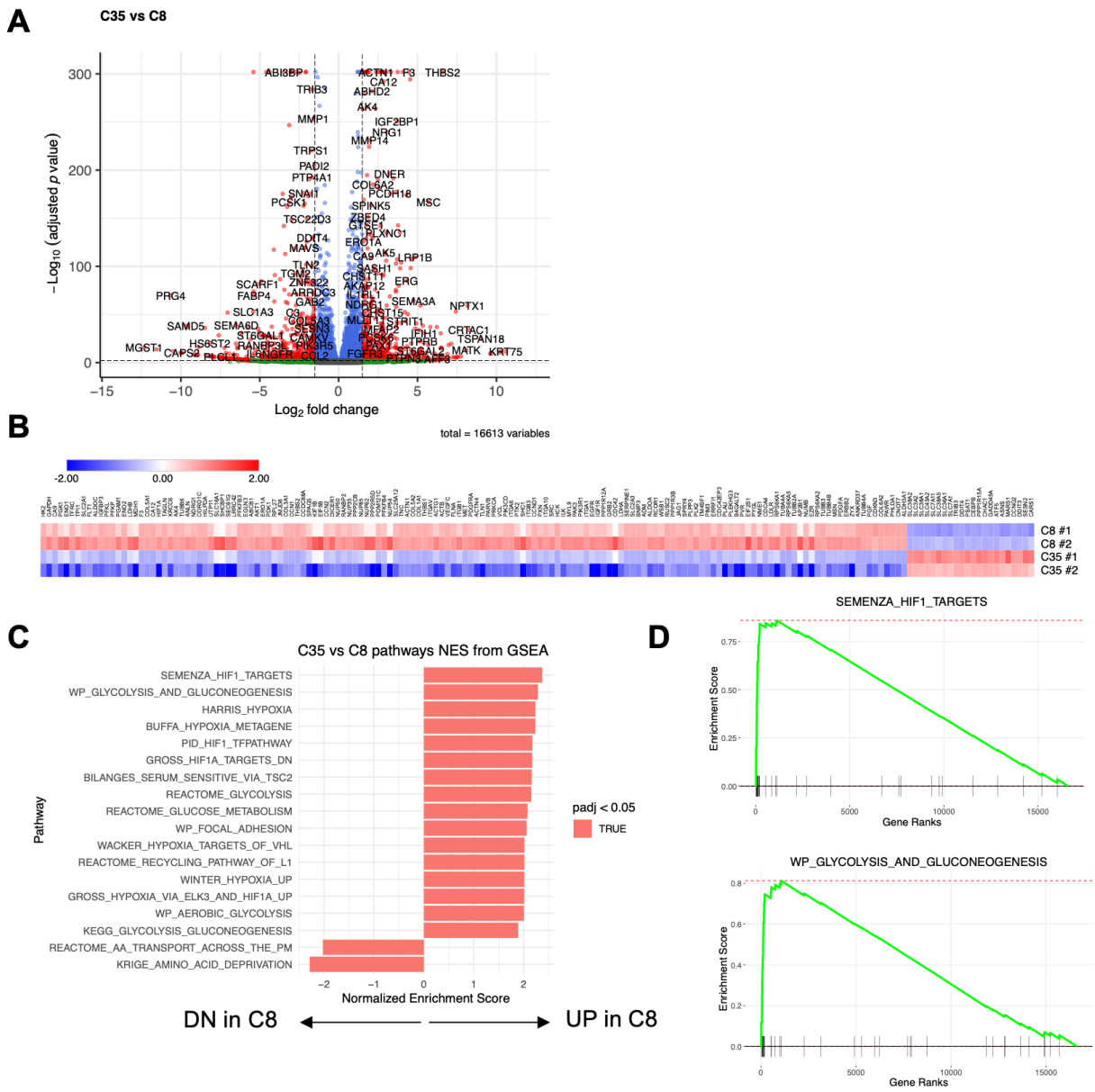

Figure 5. Different gene expression data between C8 and C35 mutant hemangiosarcoma cells. **(A)** Volcano plot depicts DEGs between C8 and C35 cells. **(B)** Heatmap visualizes key regulated genes between the mutant cells. **(C and D)** GSEA data showed the DEG-associated pathways including hypoxia and glycolysis pathways.

Specific Aim 2: To develop in vitro tumor models to define molecular mechanisms that regulate convergent oncogenic pathways in angiosarcomas

Major Task 1: Gene engineering and differentiation in iPSCs

Subtask 1: Generation of iPSCs and preparation of reagents; commercially available iPSC cell lines (iPS12-10 and BYS-0110)
- Partially completed (75% of completion) and ongoing
The experimental protocol was adapted to the PI's new lab. Since the BYS-0110 cell line is not currently available from a commercial vendor, as an alternative, the HYS0103 iPSC line and respective reagents will be obtained from ATCC.

Subtask 2: Engineering gene mutation (*TP53, PIK3CA, TP53/PIK3CA*) in iPSCs
- Partially completed (40% of completion) and deferred

Subtask 3: Differentiation of hemangioblasts from engineered iPSCs
- Partially completed (50% of completion) and deferred

Subtask 4: Functional validation of engineered cells
- Partially completed (50% of completion) and ongoing
We analyzed flow cytometry data obtained from the differentiation of iPSC into hemangioblast. To determine whether thee differentiated cells expressed endothelial and hematopoietic markers, we examined surface markers, including CD31, CD34, CD144, CD43, CXCR4, and CD73 (**Figure 6**). Our findings revealed that a subpopulation of putative hemangioblasts differentiated from iPSCs exhibited expression of endothelial cell markers CD31, CD34, and CD144. Hematopoietic stem and progenitor markers CXCR4 and CD73 were also detected with minimal expression of CD43. No difference in these marker expressions were found between WT and *TP53*-mutant cells.

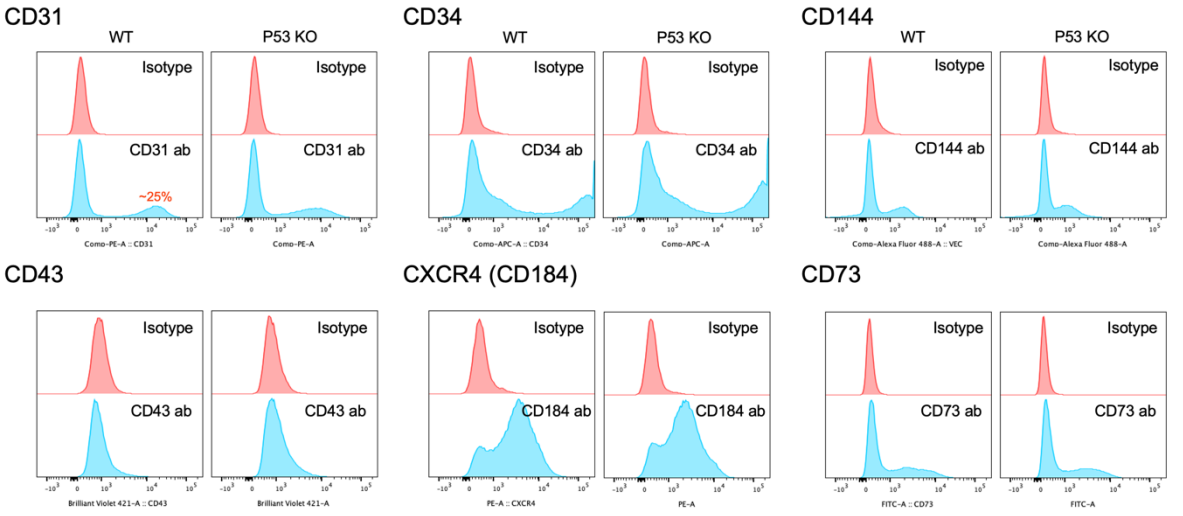

Figure 6. Expression of surface protein markers for hemangioblast between cells differentiated from WT and TP53-mutant iPSCs. Histogram plots depict the expression of surface markers on iPSC-derived cells at differentiation day 5.

Subtask 5: Cell line authentication and Mycoplasma screening (iPSCs-derived cells, HUVEC, fibroblasts, AS5, ISO-HAS)
- Completed for this period; we will continue cell authentication and Mycoplasma testing

Subtask 6: Project meetings
- Completed for this period

Subtask 7: Career development for Dr. Kim (acquisition of new experimental skills for iPSCs generation and genome engineering)
- Partially completed (50% of completion) and deferred
Dr. Kim has established his new lab at the UF, and he will continue to advance the experimental skills in the next project period.

Subtask 8: Career development for Dr. Kim (starting development of strategies to secure funding)
- Partially completed (75% of completion) and ongoing
Despite the inactive project period due to the incomplete award transfer, Dr. Kim continued to work towards securing funding during the project Year-3. He submitted multiple grant applications to external funding agencies, including DoD, NSF, and the Morris Animal Foundation, both as a principal investigator and co-investigator. As a result, he received two funded grants: one from FY22 DoD RCRP Concept Award for human angiosarcoma and another from the Morris Animal Foundation for canine hemangiosarcoma. This achievement in obtaining external funding will provide him with the opportunity to advance his career as comparative oncology researcher.

What opportunities for training and professional development has the project provided?
During this period, the DoD Career Development Award provided Dr. Kim with excellent training opportunities and experiences to advance his lab management skills, including the grant transfer process, financial account management, and recruitment of new staffs at his new institution, UF. The award also enabled him to present his work at the prestigious 2023 AACR Annual Meeting in Orlando, FL, which is internationally recognized as the largest cancer research conference. He attended the meeting in person and presented one poster related to this project. In the previous report, we highlighted Dr. Kim's initiation of a new collaboration with Drs. Michael Wagner and Eleanor Chen. As the PI, Dr. Kim submitted their new joint project to FY22 DoD RCRP, and they were successful in securing funding for the Concept Award. These activities further enhanced his grantsmanship, leading to the receipt of another research fund from the Morris Animal Foundation.

How were the results disseminated to communities of interest?
Some results of the project were disseminated through presentations at the 2023 AACR Annual Meeting, held in Orlando, FL, from Apr 14 to 19, 2023. The presentation was titled: "Oncogenic PIK3CA promotes hematopoietic reprogramming and cell transdifferentiation developing hematopoietic malignancy."

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

During this project period, Dr. Kim devoted most of his effort to settling down at the UF and establishing his new lab. He successfully secured two external research funds, as previously mentioned. The UF provides excellent computational and bioinformatics support, along with training opportunities, through its high-end supercomputing infrastructure, called HiPerGator. With the newly obtained research funds and the startup package, Dr. Kim intends to make full use of these research resources in his brand-new lab. His plan is to accomplish the proposed goals for this project by completing tasks and meeting the milestones outlined for the next reporting period.

4. IMPACT:

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

During this period, Dr. Kim made significant progress in enhancing his computational programming and data analysis skills, demonstrating a comprehensive understanding and proficiency in multi-disciplinary cancer research. His primary focus for this project is on angiosarcoma, a rare condition in humans, for which expertise combining various disciplines such as basic biology, pathology, disease modeling, and big data analysis is scarce. Given the scarcity of expertise across these multiple domains in the field of angiosarcoma, Dr. Kim's research project holds immense significance. It not only contributes to the advancement of knowledge in this area but also plays a pivotal role in shaping Dr. Kim's career trajectory as a leading figure in sarcoma biology.

What was the impact on other disciplines?

Bioinformatics and computational biology are rapidly gaining popularity, and related technologies are advancing at a swift pace. In the current science era, the ability to effectively utilize these new technologies and handle complex datasets is paramount for cancer researchers. This holds even greater significance in the case of rare cancers like angiosarcoma, where understanding the underlying biology is still rudimentary. Dr. Kim's work in this field has a profound impact on various research disciplines, not only shedding light on the biology of angiosarcoma and other soft tissue sarcomas but also extending to biotechnical applications. The project workflow developed by Dr. Kim has the potential to revolutionize research activities and guide new study designs specifically tailored for rare cancers. By leveraging bioinformatics, computational approaches, and iPSC-based disease modeling, Dr. Kim's contributions pave the way for a deeper comprehension of rare cancer biology, influencing the scientific community's approach to studying and tackling these challenging diseases.

What was the impact on technology transfer?

- Nothing to Report

What was the impact on society beyond science and technology?

- Nothing to Report

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

As reported previously, the PI, Dr. Kim transitioned his primary affiliation from the University of Minnesota (UMN) to the UF. His appointment at the UMN was terminated on May 13th, 2022, and his new appointment started at the UF started on May 23rd, 2022. The grant transfer process

to the new institution was successfully completed in April 2024, and the project was activated as of Apr 1st, 2024. Given this timeline, the current reporting period (07/01/2022 – 06/30/2023) encompasses only 3-month active period due to the transition of organizations and the subsequent grant transfer process.

Actual or anticipated problems or delays and actions or plans to resolve them
With the completion of the grant transfer, Dr. Kim has resumed the project activities and is committed to making progress towards achieving the stated goals and tasks of the project in the upcoming phase.

Changes that had a significant impact on expenditures
- There was no changes that had significant impact on expenditures, and there was no notable impact on the overall budget amount.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents
- There was no significant change in use or care of animals; instead, we changed timeline to perform the animal experiment due to PI's transition.

Significant changes in use or care of human subjects
- Nothing to Report

Significant changes in use or care of vertebrate animals
- Nothing to Report

Significant changes in use of biohazards and/or select agents
- Nothing to Report

6. PRODUCTS:

- **Publications, conference papers, and presentations**
  - Emma Kozurek, Erin B. Dickerson, Jong Hyuk Kim. *Oncogenic PIK3CA promotes hematopoietic reprogramming and cell transdifferentiation developing hematopoietic malignancy*. 2023 AACR Annual Meeting, Orlando, FL, Apr 14 -19, 2023.
- **Journal publications.**
- Nothing to Report

- **Books or other non-periodical, one-time publications.**
- Nothing to Report

- **Other publications, conference papers and presentations**.
- Nothing to Report

- **Website(s) or other Internet site(s)**
- Nothing to Report

- **Technologies or techniques**

- Nothing to Report

- **Inventions, patent applications, and/or licenses**

- Nothing to Report

- **Other Products**

- Nothing to Report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

There is no significant change from original SOW.

Name:	*Jong Hyuk Kim*
Project Role:	*Principal Investigator*
Researcher Identifier (e.g. ORCID ID):	*0000-0002-1645-0036 (ORCID ID)*
Nearest person month worked:	*7.8*
Contribution to Project:	*Dr. Kim has performed work in the area of bioinforamtics and iPSC-derived hemangioblast differentiationg.*
Funding Support:	*DoD Career Development Award*

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

Grants completed during the project Year-3 are listed below.

Completed Grants (07/01/2022 – 06/30/2023)

a) Title of project: Reprogramming the Tumor Immune Niche in Canine Hemangiosarcoma
b) Funding agency: AKC Canine Health Foundation
c) Goals of the project: *The goal of this project is to determine if the molecular programs which create the tumor niche are reversible in canine hemangiosarcoma, and that PI3K/AKT/mTOR pathways regulate the expression of inflammatory cytokines that support niche conditioning.*
d) Specific aims/tasks:

Specific Aim 1: To determine if activation of PI3K signaling in hemangiosarcoma cells supports expansion and differentiation of hematopoietic progenitors.

Specific Aim 2: To examine if PI3K/AKT/mTOR pathways regulate the expression of inflammatory cytokines in hemangiosarcoma cells.

e) Estimated start and end date: 07/01/20 - 06/30/22 **(NCE to 03/31/2023 due to PI's transition)**
f) Level (%) of effort: 1% effort; cost-shared (0.12 person-calendar months)
g) Point of contact at the funding agency:

Andrea R. Fiumefreddo, MS
Director of Programs & Operations
Email: andrea.fiumefreddo@akcchf.org

Phone: 919-334-4022
h) Overlap: None

What other organizations were involved as partners?
- Nothing to Report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:
- Not applicable

QUAD CHARTS:
- Not applicable

9. APPENDICES:

Abstracts presented at 2023 AACR Annual Meeting, Orlando, FL, Apr 14 -19, 2023.

Oncogenic *PIK3CA* promotes hematopoietic reprogramming and cell transdifferentiation developing hematopoietic malignancy

Short title: Hematoendothelial cell fate decisions of *PIK3CA* mutant cells

Emma Kozurek[1,2,3], Erin B. Dickerson[1,2,3], Jong Hyuk Kim[4,5]

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[4] *Department of Small Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, United States*
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Section: MCB11 Oncogenes and Tumor Suppressor Genes (MCB11-06 Other)

PIK3CA mutation is one of the most common, recurrent genetic abbrations identified across multiple types of cancers. Such mutations sustain activation of PI3K/AKT/mTOR pathway, being considered reliable therapeutic targets; yet, the functional consequences still remain unclear. Angiosarcomas are a heterogenous group of soft-tissue sarcomas that form malignant endothelium with disorganized, irregular blood-filled vascular spaces, and a subset of the vascular tumors harbor *PIK3CA* mutations. In this study, we found that one of 11 canine angiosarcoma cells gave rise to vascular tumors in 3 of 3 mice in a series of xenograft experiments using a total of 86 immunodeficient BNX mice. Intriguingly, we also observed that xenografts developed lymphoproliferative tumors indicating a mouse B-cell origin in 3 of 4 mice from one case of canine patient-derived tumor xenografts: four tumor cases surgically implanted in a total of 16 BNX mice. Additionally, canine angiosarcoma cells induced splenomegaly with

expansion of Ter-119+ erythroid progenitors in NSG mice. Canine angiosarcoma cells (two cell lines; DHSA-1426 and EFB) were capable of hematopoietic expansion and cell lineage differentiaton *in vitro*. Since *PIK3CA* mutations appear to promote cellular stemness and impairment of cell lineage differentiation, we determined if the mutations play a role in hematopoietic regulations of angiosarcoma cells. DHSA-1426 angiosarcoma cells were used to induce *PIK3CA* H1047R mutations using CRISPR/Cas9. We found that *PIK3CA* mutant angiosarcoma cells dysregulated AKT, ERK, and mTOR pathway with enrichement of inflammatory cytokines such as IL-6, IL-8, and MCP-1. Single cell RNA-seq data showed that *PIK3CA* mutant angiosarcoma cells established distinct single cell clusters representing immune reactions, cellular stemness, and lineage differentiation. Furthermore, *PIK3CA* mutant angiosarcoma cells developed malignant hematopoietic tumor in one of five BNX mice, while no evidence of hematopoietic tumor formation was found in mice transplanted with non-mutant cells. Altogether, our data suggest that angiosarcoma cells have the capacity to promote hematopoietic imbalance impacting on cellular ontogeny and lineage differentiation, potentially mediated by oncogenic *PIK3CA*. Our ongoing work is determining the mechanism of the cell fate decisions using bi-potential hematoendothelial progenitors derived from induced pluripotent stem cells harboring oncogenic *PIK3CA*.