AWARD NUMBER:	W81XWH-21-1-0108
TITLE:	Connexins as Potential Biomarkers and Therapeutic Targets for Vascular Malformation
PRINCIPAL INVESTIGATOR:	Jennifer S. Fang, Ph.D.
CONTRACTING ORGANIZATION:	The University of California, Irvine, CA
REPORT DATE:	March 2023
TYPE OF REPORT:	Annual
PREPARED FOR:	U.S. Army Medical Research and Development Command Fort Detrick, Maryland 21702-5012
DISTRIBUTION STATEMENT:	Approved for Public Release; Distribution Unlimited

REPORT DOCUMENTATION PAGE		Form Approved OMB No. 0704-0188

1. REPORT DATE March 2023	**2. REPORT TYPE** Annual	**3. DATES COVERED** 01Feb2022-31Jan2023

4. TITLE AND SUBTITLE

Connexins as Potential Biomarkers and Therapeutic Targets for Vascular Malformation

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-21-1-0108

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Jennifer S. Fang, Ph.D.

E-Mail: jennifer.fang@uci.edu

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

UNIVERSITY OF CALIFORNIA, IRVINE
141 INNOVATION DR STE 250
IRVINE CA 92617-3213

8. PERFORMING ORGANIZATION REPORT

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Development Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)
DOD PRMRP

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

Blood vessels form during development through coordinated signaling between vascular endothelial cells (EC) and their adjacent cell neighbors. Inherited mutations that disrupt these signaling processes can lead to vascular malformations with profound consequences on vascular organization and function. In the rare congenital disease Hereditary Hemorrhagic Telangiectasia (HHT), loss-of-function mutations affecting the Alk1 cell surface receptor drives the appearance of disorganized vascular lesions prone to sudden, serious rupture. The goal of this study is to understand how Alk1 mutation may promote vascular malformation by dysregulating connexins (Cx), constituent proteins of vascular gap junctions that support direct cell-cell signaling of small electrochemical signals. In the second year of this study, we have made significant progress towards understanding this question. We have improved our understanding of connexins are regulated by Alk1 and VEGF signaling, and how changes in Cx expression influence EC gene expression and function. Lastly, we have developed several tools and performed proof-of-concept experiments to manipulate Cxs in 2D and organ-on-a-chip platforms.

15. SUBJECT TERMS
None listed.

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** USAMRDC
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
Unclassified	Unclassified	Unclassified	Unclassified	15	

TABLE OF CONTENTS

Page

INTRODUCTION

Blood vessels form during development through coordinated signaling between vascular endothelial cells (EC) and their cell neighbors to support organized and controlled cell proliferation and migration to ensure proper organization of the resulting vascular network. Inherited mutations that disrupt these signaling processes can lead to the development of malformed vessels with profound consequences on vascular organization and function. In the rare congenital disease Hereditary Hemorrhagic Telangiectasia (HHT), loss-of-function mutations affecting the Alk1 cell surface receptor drives the appearance of disorganized vascular lesions prone to sudden, serious rupture. HHT affects 1 in 5,000 live births and has variable presentation, making both HHT and associated vascular malformations (VM) difficult to diagnose. Furthermore, although HHT symptoms often begin in childhood and progress in severity, patients typically only receive a definitive HHT diagnosis at >40 years of age. Thus, it is possible that many active and retired military personnel currently harbor undiagnosed HHT that might put them at risk for sudden and severe bleeding, as well as other major health problems. Thus, a major goal of this study is to improve our understanding of how Alk1 drives vascular malformation in HHT, and to understand whether targeted proteins might serve as potentially useful early screening tools for HHT. Specifically, our aim is to understand how Alk1 mutation may promote vascular malformation by dysregulating connexins (Cx), constituent proteins of vascular gap junctions that support direct cell-cell signaling of small electrochemical signals.

KEYWORDS

Hereditary Hemorrhagic Telangiectasia, HHT, Alk1, Vasculature, Vascular Malformation, Blood Vessels, Arteriovenous Malformation, Connexin, Gap Junction, Microphysiological Systems, Organ-on-a-Chip

ACCOMPLISHMENTS

Major Goals and Accomplishments

In the second year of this study, we made progress towards our major goals and milestones. In particular, we confirmed that Alk1 signaling regulates Cx37 and Cx40, and we identified striking dysregulation of Cx43 in vascular malformations in the HHT-on-a-chip platform. We performed several studies to understand how Cx expression profile affects EC behaviors relevant to angiogenesis. Lastly, we used next-generation sequencing to identify putative Alk1 and Cx43-regulated pathways that might be abnormal in the vessel wall of malformations.

Specific Objectives and Outcomes

<u>Specific Aim 1: Determine how abnormal Cx expression in the vessel wall contributes to VM development.</u>

Overall Progress: ~80%

Major Task 1A: Map Cx expression and localization in a developing VM.

The goal of this task is to determine how Cx expression is altered in a developing VM in HHT. We have now collected striking data linking misregulated overexpression of Cx43 with VM progression. Using qPCR, we found that BMP9-induced Alk1 signaling activation appears to downregulate expression of Cx43 at 24h, but not at 6h, suggesting that this occurs through an indirect feedback mechanism (**Figure 1A**). Next, we found that 48hour of Alk1 using silencing RNA induces a several-fold upregulation of Cx43 transcript (**Figure 1B**); further replicates are currently underway for this experiment to achieve sufficient statistical power. We further confirmed this result using an inducible short-hairpin RNA (shRNA) approach to assess the effect of prolonged Alk1 knockdown and observed even greater upregulation of Cx43 transcript at 96h (**Figure 1C**). We confirmed this result at the protein level using immunofluorescence (**Figure 1D**) and observed increased size and number of Cx43 punctae at cell-cell junctions (**Figure 1D**, inset).

Because VEGF signaling appears to be required during – or permissive of – VM in HHT mice, we performed preliminary experiments to assess how VEGF might cross-talk with the Alk1 signaling pathway. We had previously observed that 6h VEGF signaling appears to upregulate Cx43 protein expression (**Figure 2A**) although

Figure 1. Cx43 is regulated by Alk1 signaling and significantly upregulated by Alk1 knockdown. A) Cx43 transcript appears to be downregulated at 24h following recombinant BMP9. **B)** Cx43 transcript appears upregulated by siRNA-mediate Alk1 knockdown at 48h. **C)** Cx43 transcript and **D)** protein is significantly upregulated by prolonged Alk1 knockdown using shRNA.

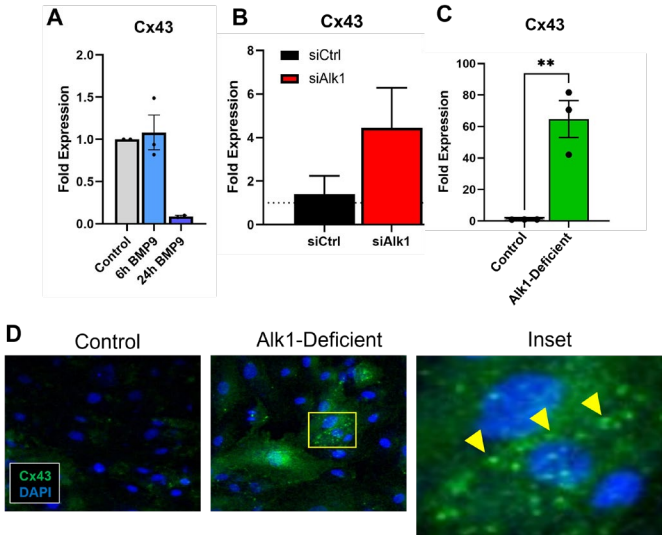
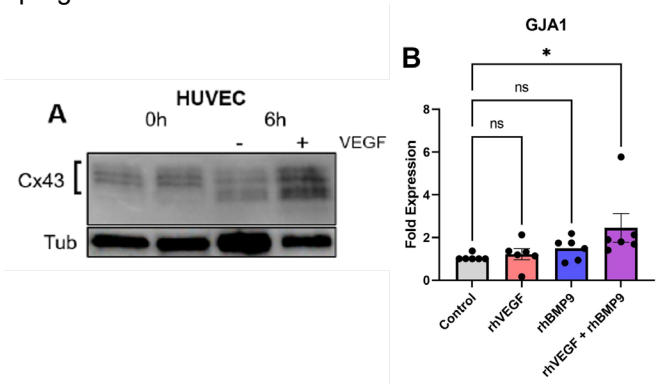
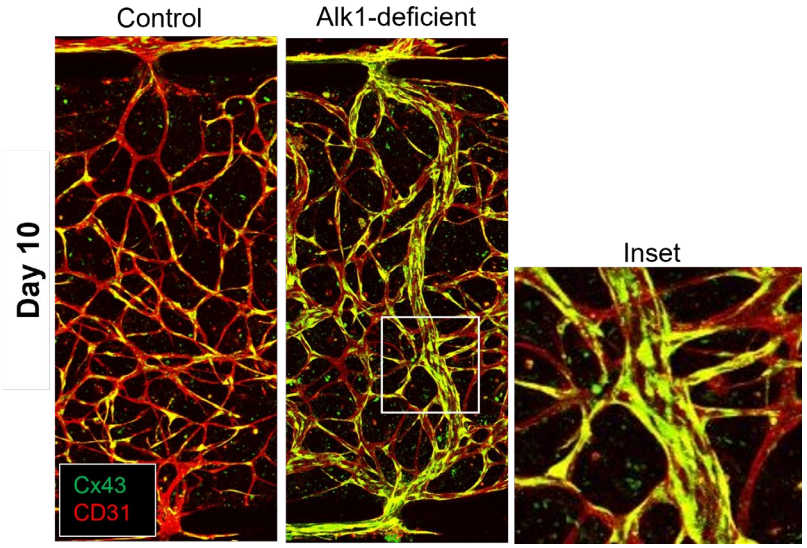


Figure 2. Cx43 is regulated by both VEGF and Alk1. A) Cx43 protein is upregulated by 6h VEGF stimulation, but **B)** no upregulation of Cx43 transcript is evident at 6h. However, combined 6h VEGF and BMP9 stimulation upregulates Cx43 mRNA.



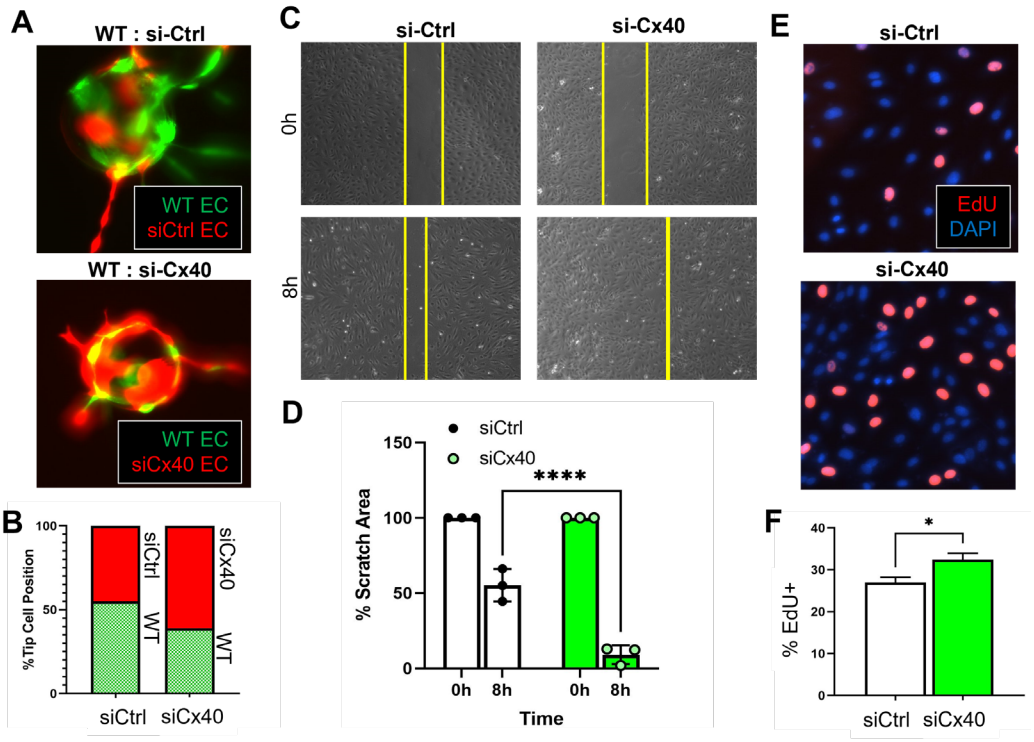
Cx43 transcript is not affected at this timepoint (**Figure 2B**). This suggests that Cx43 is targeted at the post-transcriptional level by VEGF and Alk1 signaling, perhaps via a common kinase target that phosphorylates and stabilizes the Cx43 protein at the cell-cell junction. Additional studies are now underway to explore this possibility. Interestingly, we have also collected preliminary data showing that combined BMP9 and VEGF signaling synergizes to upregulate Cx43 at the transcriptional level, suggesting that perhaps in this context, positive feedback mechanisms promote Cx43 upregulation (**Figure 2B**), perhaps to help EC overcome quiescence signaling through Alk1 and enable angiogenesis. This is a novel finding that may contribute to VEGF's requirement for VM in HHT mice. Additional studies are currently underway to further understand this observation.

Figure 3. Cx43 is aberrantly overexpressed in HHT VM. Cx43 protein was visualized by immunofluorescent staining in the HHT-on-a-chip device and was found to be strongly expressed within the vessel wall of an AVM-like shunt at day 10.



Lastly, we used the HHT-on-a-chip device to determine the relevance of our EC monoculture observations presented in **Figures 1 and 2**. We found using immunofluorescent staining that there is a profound upregulation of Cx43 in HHT VM (**Figure 3**), suggesting that Cx43 misexpression contributes to the appearance of these vascular lesions. Additional studies are now underway to understand the downstream mechanisms whereby Cx43 misexpression that might drive VM pathogenesis. Future studies to complete this major task will assess Cx37 and Cx40 expression in VM of the HHT-on-a-chip device.

Figure 4. Cx40 is anti-angiogenic. A-B) EC in which siRNA has been used to knockdown endogenous Cx40 make up a greater proportion of angiogenic sprouts when mixed at a 1:1 ratio with control EC, indicating that siCx40 EC are more angiogenic. This is associated with **C-D)** significantly increased EC migration in a 2D scratch assay, and **E-F**) increased incorporation of EdU, a marker of cell proliferation.



Milestone Progress: ~90%

Major Task 1B: Determine effect of "HHT-like" Cx expression profile on VM progression.

The "HHT-like" Cx expression profile is proposed to be high Cx43 in combination with low Cx37 and Cx40. This past year, we used a combination of *in vitro* techniques to manipulated Cx expression to reproduce this profile, and assessed resulting impact on EC. We previously showed that we have validated shRNA constructs to induce selective knockdown of endogenous Cx37, Cx40, or Cx43. We have since purchased inducible expression vectors containing those shRNA constructs and are optimizing those tools for use in the HHT-on-a-chip platform (**data not shown**).

Meanwhile, we have performed exhaustive experiments in 2D monoculture to assess the effects of individual Cx37 and Cx40 knockdown on angiogenesis. In our previous report, we showed that Cx37 knockdown produces dilated angiogenic sprouts in an *in vitro* "bead assay" model of sprouting angiogenesis. Here, we find that Cx40 knockdown via siRNA significantly increases angiogenic potential of EC: si-Cx40 (red) EC make up a greater proportion of angiogenic sprouts than do Cx40-expressing wild-type (si-Ctrl) EC (**Figure 4A-B**). Furthermore, siCx40 enhances EC migration in a 2D monoculture "scratch assay" as measured by significantly reduced scratch area at 8 hours (**Figure 4C-D**). Lastly, we saw that si-Cx40 EC are more proliferative than control EC, as measured by incorporation of EdU which marks proliferating cells. (**Figure 4E-F**). Taken together, these data show that Cx40 (like Cx37) is ordinarily anti-angiogenic, and that loss of either Cx promotes angiogenesis. Additional studies are now underway to combine these data with forced overexpression of Cx43, and to assess the impact of double-knockdown of both Cx37 and Cx40 in combination.

Milestone Progress: ~85%

Major Task 1C: Determine effect of "HHT-like" Cx expression profile on gap junctional communication.

The goal of this task is to use dual whole-cell patch clamp to understand how endothelial-endothelial and endothelial-mural gap junctional coupling is affected by Alk1-deficiency and forced "HHT-like" Cx expression profile. We previously showed in our last annual report that under control monoculture conditions, endothelial cells exhibit a Cx43-like gap junction communication profile. This work was performed by our collaborator Dr. Jose Ek Vitorin at the University of Arizona. We have since requested that Dr. Ek Vitorin be added to our study team and be sub-contracted to complete additional experiments relevant to this aim. We are currently processing this request through UCI and awaiting DOD approval to provide Dr. Ek Vitorin the subaward funds to complete this proposed work (discussed in greater detail in the **Challenges** section of this report.

Milestone Progress: ~30%

<u>Specific Aim 2: Determine how Cx expression profile may predict VM risk and severity.</u>

Overall Progress: ~20%

Major Task 2A: Determine lesion-specific changes in EC Cx expression.

The goal of this task is to assess Cx expression from HHT patient vascular lesion donor samples against adjacent healthy tissue. We are currently in the process of obtaining lesion samples for this aim.

Milestone Progress: ~15%

Major Task 2B: Determine effect of Cx SNP variants in HHT patient outcome.

The goal of this task is to obtain donor blood samples from healthy and HHT patients, and to assess Cx SNP frequencies in these two populations. We will also compare SNP frequencies against patient histories. For this task, we authored a Human Subjects Research master protocol and – after much effort – obtained initial approval for the protocol for all UCI-specific work (Protocol #20216546, approved 11/03/21) from UCI's Institutional Review Board and the Department of Defense. We have established a collaboration with UCLA as a reliant site for this IRB protocol, and have built a collaboration with Dr. Justin McWilliams, the Director of the UCLA HHT Center of Excellence. WE have also worked with Victoria Rueda, MPH, who will serve as on-site study coordinator for this study. In the past year, we have been greatly stymied in receiving UCI, UCLA, and DOD financial and regulatory approval to begin work, as is discussed further in the **Challenges** section of this report. We hope to resolve these issues as soon as possible to begin subject enrollment into this study.

Milestone Progress: ~30%

<u>Specific Aim 3: Determine whether manipulation of Cx expression in vessels can resolve or prevent VM.</u>

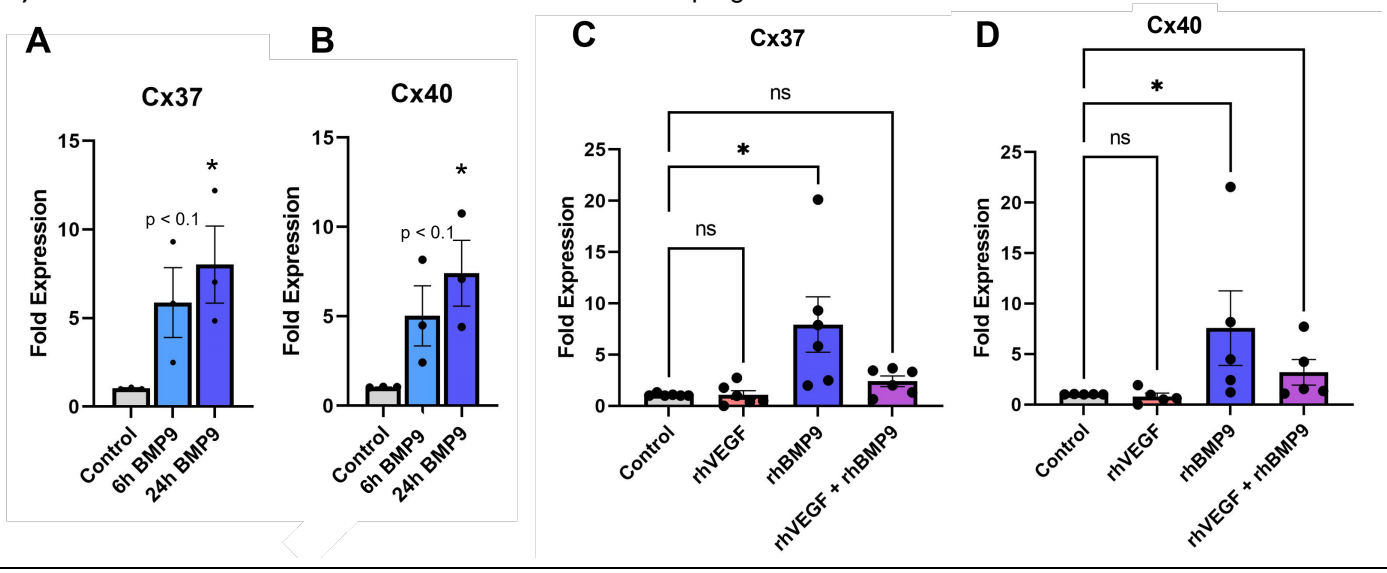
Overall Progress: ~80%

Major Task 3A: Determine effect of "Alk1-intact" Cx expression profile on VM progression.

The goal of this task is to better characterize the "Alk1-intact" Cx expression profile – which we describe as low Cx43 and high Cx37/Cx40 -- to determine how this expression profile drives proper vessel organization.

Using 2D EC monoculture, we confirmed that Cx37 and Cx40 mRNA are several-fold directly upregulated at 6h and 24h following recombinant BMP9 stimulation (**Figure 5A-B**). We further found that this upregulation was abolished by the presence of VEGF (**Figure 5C-D**). Taken together alone with the data presented in **Figure 2**, these data suggest that VEGF signaling is able to circumvent Alk1 signaling in the context of normal angiogenesis to induce an "HHT-like" Cx expression profile, which perhaps in this context is required to activate EC. However, this implies that in HHT VM, it is excessive or prolonged expression of high Cx43 and low Cx37/Cx40 that perhaps promotes aberrant pro-angiogenic signaling leading to VM. Additional studies are underway to understand the interplay of VEGF and Alk1 signaling to manipulate EC Cx expression profile.

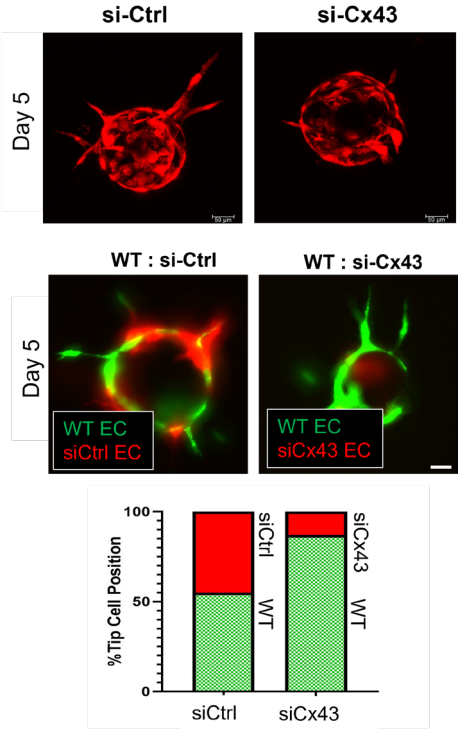
Figure 5. Cx37 and Cx40 are upregulated by BMP9/Alk1 signaling activation, but this upregulation is lost in the presence of VEGF signaling activation. A) Cx37 and **B)** Cx40 mRNA are several-fold upregulated, but that **C-D)** combined BMP9 and VEGF stimulation abolishes this upregulation.



As discussed previously, we have validated shRNA tools to manipulate Cx43 expression in an inducible manner and we are optimizing these constructs for use in healthy and HHT vessel-on-a-chip platforms; separately, are also validating our Cx37 and Cx40 forced expression constructs. In the meanwhile, we have used siRNA to selectively knockdown Cx43 to better understand its role in pro-angiogenic signaling in healthy and HHT vasculature. We previously showed that si-Cx43 EC are poor at undergoing angiogenic sprouting in the "bead assay". We have since replicated these findings and found that si-Cx43 EC are significantly outcompeted by control EC to contribute to the vessel wall of nascent angiogenic sprouts. (**Figure 6**) Additional studies are currently underway to identify whether this is associated with increased EC proliferation (as is expected based on published work) or migration.

Milestone Progress: ~75%

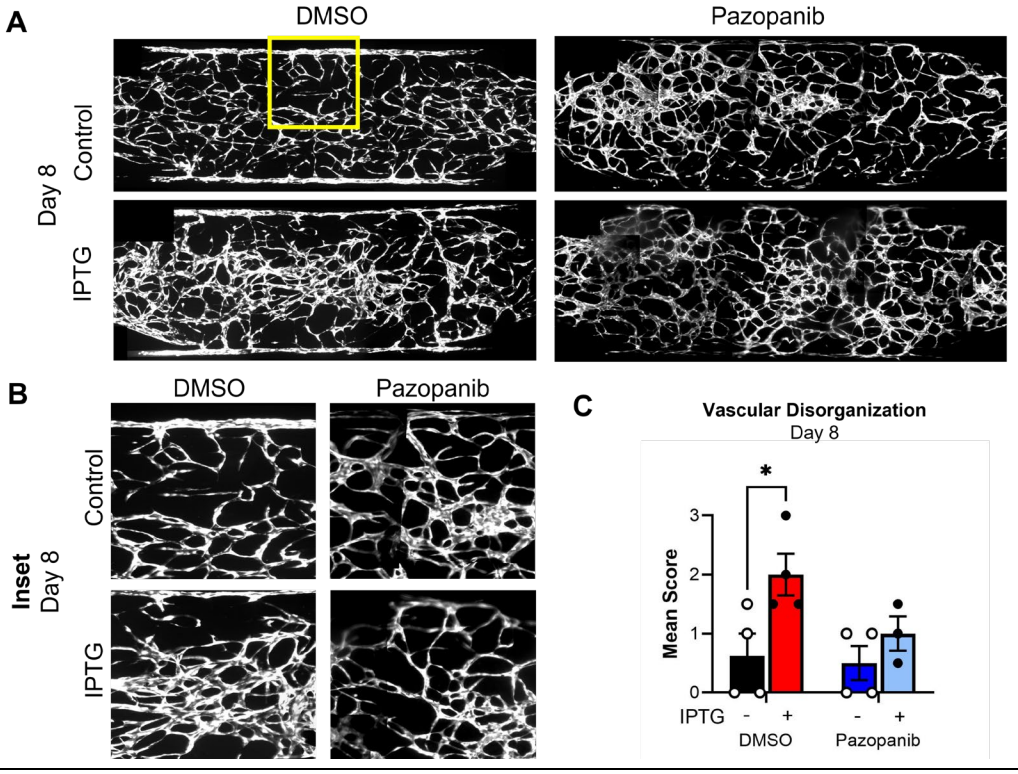
Figure 6. Cx43 is pro-angiogenic. si-Cx43 EC are less likely to form angiogenic sprouts, and are instead outcompeted by wild-type cells when both are mixed in a 1:1 ratio in the sprouting angiogenesis bead assay.



Major Task 3B: Determine effect of gap junction blockers on VM progression.

The goal of this task is to determine whether pharmacological gap junction inhibition can resolve or prevent vascular lesions in the HHT-on-a-chip platform. We previously discussed howe we developed a well-plate format version of the HHT-on-a-chip platform to significantly increase experimental capacity and now

Figure 7. Pazopanib prevents vascular lesions in the HHT-on-a-chip platform. A-B) Pazopanib was added to circulating media beginning on day 3, and vessel architecture was observed on day 8. **C)** Pazopanib prevented the appearance of malformed vessels.



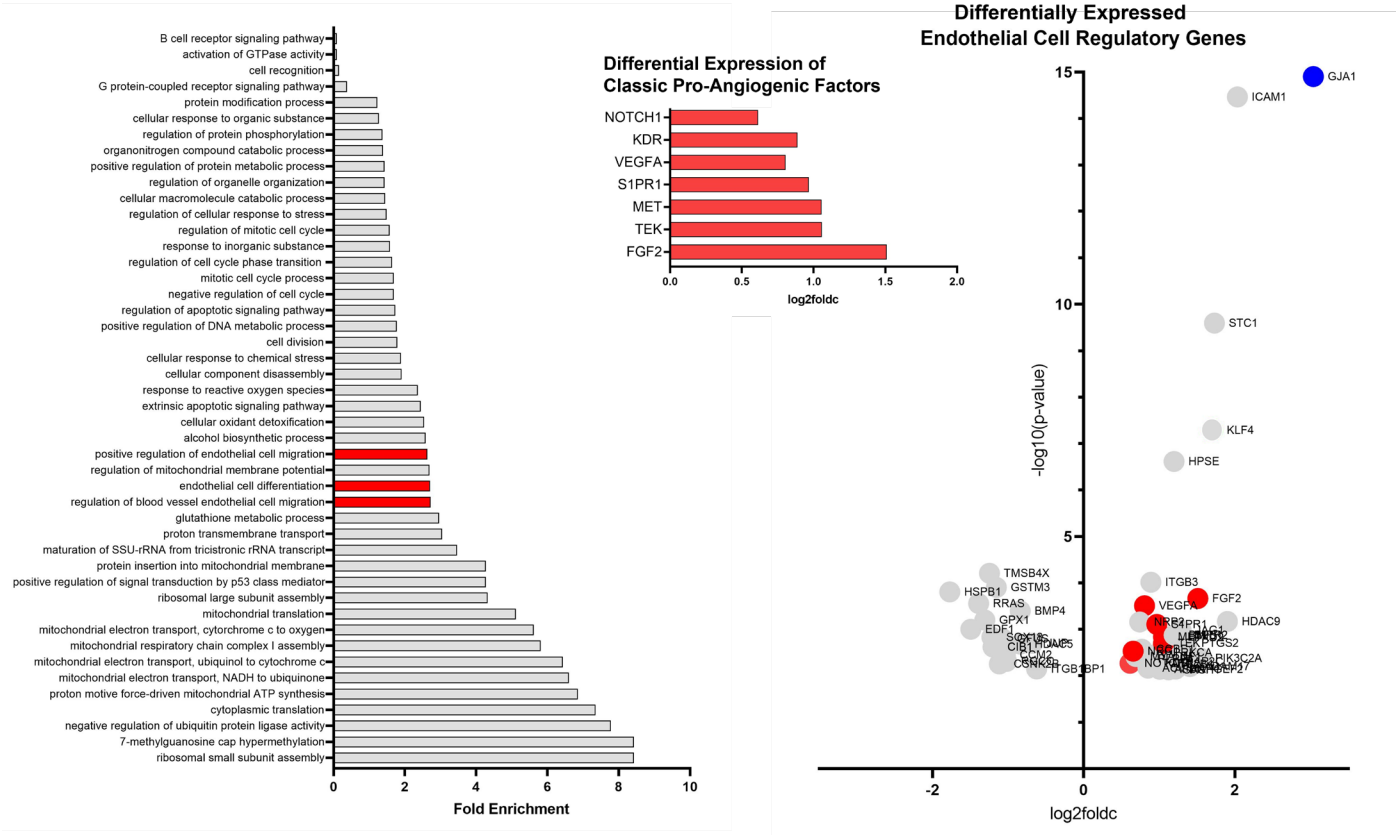
enables us to perform sophisticated dose response drug screening studies. In the past year, we used this model to determine the appropriate timing for addition of putative HHT drugs to resolve the lesion phenotype in the HHT-on-a-chip platform, as well as to optimize conditions for using the device to screen for novel drugs. We found that knockdown of Alk1 beginning at day 0 supports network formation (as expected and previously shown) and that addition of the candidate HHT drug pazopanib beginning on day 3 into circulating media prevents the appearance of malformed vessels in the HHT-on-a-chip platform (**Figure 7**). With these data in-hand, studies are currently underway with both the pan-gap junction blocker carbenoxolone, as well as the Cx43-specific mimetic peptide SRPTEKT-Hdc (gift from J. Burt and S. Boitano, The University of Arizona), to determine effect of both gap junction blockers on lesion formation in the HHT-on-a-chip.

Milestone Progress: ~75%

Major Task 3C: Use next-generation sequencing to identify Cx-regulated downstream targets.

The goal of this task is to use RNASeq to better understand how Cx37, Cx40 and Cx43 regulate downstream genes, and to identify those that might be involved in driving VM. We elected to pursue this aim using siRNA approaches, since they were most developed and could provide early candidate targets for further analysis. We transfected primary EC with either siCx43, siCx37, or siCx40 (as well as si-Ctrl for control conditions) and performed RNAseq in triplicate to identify differently-expressed genes. We have completed our analysis of the siCtrl vs. siCx43 datasets, and found a striking pro-angiogenic signature associated with intact (or high) Cx43 compared to our knockdown condition (**Figure 8A**). This included increased a correlation of expression of numerous

Figure 8. Intact Cx43 expression is associated with pro-angiogenic signaling. GO term analysis of siCtrl vs. siCx43 datasets reveals several differentially-expressed genes that are associated with EC proliferation and angiogenesis. Volcano plot analysis of significantly-altered genes reveals that intact Cx43 is associated with upregulation of several class pro-angiogenic factors. Increased expression of Cx43 (blue) vs. knockdown cells was confirmed in our Cx43-intact sample.



classic pro-angiogenic signals (red)involved in VEGF, FGF, and S1P signaling (**Figure 8**, red) with intact Cx43. Additional studies are now underway to further analyze the candidate Cx43-regulated genes in this dataset against other HHT-on-a-chip next-generation sequencing datasets we have generated to identify genes regulated by Cx43 and important for HHT. Separately, bioinformatics analysis of our siCx37 and siCx40 datasets is currently underway.

Milestone progress: ~90%

IMPACT

Impact on the Principal Discipline(s):

Findings from this past reporting period have clearly demonstrated that Cx37 and Cx40 are regulated by Alk1 whereas Cx43 misexpression in Alk1-deficient EC is associated with vascular malformation. We have also found, surprisingly, that VEGF signaling – even in the context of Alk1 activation – drives a more “HHT-like” profile, suggesting that it is the interplay between these two signaling pathways that govern EC Cx expression. Our ongoing studies should further confirm and expand upon these initial findings, and reveal the mechanisms by which vascular connexins control healthy vs. disorganized (i.e. HHT) vessel growth and remodeling.

Impact on Other Discipline(s):

Work during this and the previous reporting period to progressively optimize the HHT-on-a-chip platform for higher-throughput drug screening studies are likely to have profound impact not just on HHT translational research using this model, but more broadly for several related models in the lab that will also be able to take advantage of this technology to efficiently perform large-scale drug screening studies. The next-generation sequencing datasets generated in this past period are also likely to be relevant to multiple disciplines in which connexin expression has been found to be impactful.

Impact on Society:

Nothing to report for this period.

CHANGES / PROBLEMS

Changes in Experimental Approach

We have not made significant changes to our experimental approach. Thus, we have nothing to report for this section.

Actual or Anticipated Problems or Delay

We encountered three major challenges that delayed our work in this reporting period, and we outline steps we have taken to address these obstacles below.

First, persistent supply chain issues (still related to COVID19) have resulted in delayed shipment of many of the materials needed for HHT-on-a-chip device fabrication; this has been specifically impactful with regard to PDMS, the plastic from which all chips are built. We have resorted to back-ordering PDMS from numerous suppliers, and borrowing from other labs where possible. We are hopeful that this issue will continue to resolve with further time from the nationwide COVID19 shutdown.

Second, we were significantly delayed due to persistent difficulties communicating between the UCI and UCLA sites and the DOD office. In particular, several requests for protocol change approvals needed to establish a subaward between UCI and UCLA in order to initiate the study in Aim 2 were unanswered for several months in 2022. Separately, our efforts to finalize our IRB study documents with DOD approval were also unanswered. This resulted in IRB approval delays between UCI and UCLA, since we missed several key windows within which we could establish our single IRB reliance between our two study sites. We later learned from our science officer that this was due to an office-wide email server issue at the DOD which resulted in all of our emails being lost over that time period. As a result, we were forced to request a no-cost extension for this project in order to provide sufficient time to finalize all required approvals; this also resulted in a lapse in our IRB protocol as we tried to sort through the miscommunications. Currently, email responsiveness between UCI, UCLA, and DOD has improved significantly, and we have received approval for a no-cost extension for this project. Additional requests for subaward approval is currently in submission to the DOD. We intend to use this extra time to establish the subcontract between UCI and UCLA so we can initiate (and hopefully complete) study enrollment for Aim 2, and to establish and complete a sub-contracted work with the University of Arizona.

Lastly, we encountered delays related to PI Jennifer Fang's transition from UCI to Tulane, where she accepted a tenure-track faculty position in July 2022 and has now established an independent lab in the Department of Cell and Molecular Biology. Dr. Fang retains research affiliate status with UCI to continue work on this project, and a request to transfer PI status of this project to Dr. Christopher Hughes (UCI) for the upcoming extension period of this project is underway. In addition, we may seek a subaward to Tulane to help complete this project within the upcoming extension period, although we are hopeful that this will not be necessary.

Changes That Had Significant Impact on Expenditures:

We have not made significant changes that have impacted our expenditures outside of what is described in our approved Budget Justification and Statement of Work. Thus, we have nothing to report for this section.

Changes in Human Subjects, Vertebrate Animals, Biohazards, and/or Select Agents

We have not made any changes that have impacted our human subjects work. We do not have any approved work with vertebrate animals, biohazards, or select agents. Thus, we have nothing to report for this section.

PRODUCTS

Publications, Conference Papers, and Presentations

- **Journal Publications:**
  1. **Fang, J.S.** and Burt, J.M. Connexin37 Regulates Cell Cycle in the Vasculature. 2022. *J. Vasc. Res.* 1-14. (review)

- **Books or other Non-Periodicals:** Nothing to report for this period.

- **Other Publications, Conference Papers, and Presentations:**

  Platform Presentations

  1. "Generation of Vascular Malformations in a Novel HHT-on-a-Chip Microphysiological Model." 2022 International Vascular Biologists' Meeting (San Francisco, CA)
  2. "An HHT-on-a-Chip Microphysiological Model that Recapitulates Vascular Lesions of Patients." 2022 International CureHHT Scientific Meeting (Cascais, Portugal)
  3. "Lessons Learned Engineering Microphysiological Systems as a Physiologist." 2022 MPS World Summit (New Orleans, LA)

  Posters

  1. Looker, E.K., **Fang, J.S.** "Investigating opposing roles of pro- and anti-angiogenic connexins in vascular malformation." 2023 Vasculata (New Orleans, LA) (upcoming)
  2. Hatch, C.J., Hachey, S.J., **Fang, J.S.**, Phan, D.T., Ewald, M., Chen, J., Lee, A.P., Hughes, C.C.W. "Cellular crosstalk conditions endothelial cells to develop organ-specific vasculature in microphysiological systems of health and disease." 2023 SynBioBeta. (Oakland, CA) (upcoming)
  3. **Fang, J.S.**, Hatch, C.J., van Trigt, W., and Hughes, C.C.W. "Vascular Malformations in a Novel HHT-on-a-Chip Microphysiological System Model." 2023 MPS World Summit. (Berlin, Germany) (upcoming)
  4. Matsumoto, S., **Fang J.S.**, Chen, Y., Lee, A.P., Hughes, C.C.W. "Recapitulating the Arteriovenous Malformations of Hereditary Hemorrhagic Telangiectasia in a microfluidic model." 2022 International Vascular Biologists' Meeting. (San Francisco, CA)
  5. **Fang, J.S.**, Hatch, C.J., McWilliams, J.P., Hughes, C.C.W. "Alk1 Controls Endothelial Cx43 to Regulate Vessel Growth." 2022 International Vascular Biologists' Meeting. (San Francisco, CA)
  6. **Fang, J.S.**, McWilliams, J.P., Hughes, C.C.W. "BMP9/Alk1 Signaling Controls Endothelial Connexins to Regulate Vessel Growth." 2022 International CureHHT Scientific Meeting. (Cascais, Portugal)
  7. Matsumoto, S., **Fang., J.S.**, Chen, Y., Lee, A.P., Hughes, C.C.W. "In Vitro Vascular Formation in a Microfluidic Device with Liver Sinusoidal Endothelial Cells." 2022 MicroTAS. (Hangzhou, China)
  8. **Fang, J.S.**, Andrejecsk, J., Matsumoto, S., Phan, D.T.T., Hughes, C.C.W. "Alk1-Deficient Endothelial Cells Drive Vascular Malformation in a Microphysiological Disease Model of Hereditary Hemorrhagic Telangiectasia." 2022 MPS World Summit. (New Orleans, LA)

Websites or Other Internet Sites

Nothing to report for this period.

Technologies or Techniques

As part of this project, we modified the HHT-on-a-chip platform for a standardized well-plate format, and we designed a custom widget to support high rates of intravascular flow and fluid shear stress. This technology will be described in the primary peer-reviewed paper associated with this project, and it will be made available to the research community upon that paper's publication.

As part of this project, we are developing and validating several Cx reporter, expression and silencing constructs. Completed tools will be described in the primary peer-reviewed paper associated with this project, and they will be made available to the research community upon that paper's publication.

Inventions, Patent Applications, and/or Licenses

Nothing to report for this period.

Other Products

Nothing to report for this period.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Individuals

The following individuals have worked at least one person month per year on this project during the reporting period.

Name	Jennifer S. Fang
Project Role	Primary Investigator
Research Identifier	ORCID ID: 0000-0001-5703-2239
Nearest Person Month Worked	10
Contribution to Project	Dr. Fang planned experiments, generated tools, collected data, and provided technical and administrative oversight and support for this project, including device fabrication and supply purchases.
Funding Support	• Department of Defense (this award) • NIH/NCATS

Changes in Active Other Support for Senior/Key Personnel

There have been no changes in active other support for senior or key personnel, thus there is nothing to report for this period.

Other Organizations as Partners

Organization Name	University of California-Los Angeles (UCLA)
Location of Organization	Los Angeles, CA
Partner's Contribution	**In-Kind Support:** Dr. Justin McWilliams will be providing his time and effort to identify prospective subjects for clinical study enrollment. **Facilities:** The facilities at the UCLA HHT Center of Excellence will be used to recruit and enroll HHT patients into the study, and to obtain and process donor samples. **Collaboration:** Staff at the UCLA HHT Center of Excellence will be involved in study coordination and subject enrollment.