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PRINCIPAL INVESTIGATOR: Ching-Ling Lien

CONTRACTING ORGANIZATION: Children's Hospital Los Angeles

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14. ABSTRACT

Heart failure is the leading cause of death in the US and globally. There is a pressing need for novel therapeutic interventions to prevent heart failure in patients with coronary heart diseases. Tissue regeneration holds great promise of treating organ injures and chronic diseases, including coronary heart disease. We proposed to investigate the roles of cardiac lymphatic vessels in revascularization and immune modulation, two processes important for heart regeneration. Our preliminary data suggest that cardiac lymphatic vessels can carry blood and perfuse myocardium of zebrafish, an animal with remarkable capacity of heart regeneration. These cardiac lymphatic vessels form in close association with coronary arteries, and this morphology is conserved with human hearts. We also found that hearts with impaired cardiac lymphatic vessels fail to regenerate. We will continue to investigate how cardiac lymphatic vessels modulate revascularization and fibrotic scar resolution. Molecular mechanisms underlying cardiac lymphatic vessel formation after heart injury might lead to development of novel therapeutic designs for myocardial revascularization and regeneration.

15. SUBJECT TERMS

Heart Regeneration, Cardiac Lymphatic Vessels, revascularization,

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1. INTRODUCTION

The lymphatic vasculature is a specialized network of vessels that drains fluid from tissues and enables immune-cell trafficking and surveillance throughout the body. How lymphatic vessels affect tissue regeneration is not well understood. After ischemic heart injuries, myocardial edema decreases cardiac output and can cause interstitial fibrosis. In addition to fluid homeostasis and immune cell surveillance, our unexpected findings suggest that zebrafish cardiac lymphatic vessels can carry red blood cells and perfuse the myocardium in zebrafish. Zebrafish provide a unique opportunity to study the roles of lymphatic vessels after tissue injuries. They have remarkable regenerative capacity after traumatic injuries. Available forward and reverse genetic mutants and transgenic lines make detailed molecular imaging and mechanistic studies possible. Furthermore, well established injuries models for different zebrafish organs allow us to test different injury types and severities even for the same organ. Our data suggest that new cardiac lymphatic vessels form in response to cryoinjury, a model mimicking the pathogenesis of myocardial infarction. Furthermore, zebrafish with impaired cardiac lymphatic vessels in fail to regenerate their hearts after cryoinjury. We will continue to determine the **roles of lymphatic vessels in revascularization and immune cell trafficking, two processes that are important for fibrotic scar resolution and cardiac regeneration.**

2. KEYWORDS

Heart regeneration

Cardiac lymphatic vessel

revascularization

fibrotic scar resolution

Immune cell trafficking

zebrafish

3. ACCOMPLISHMENTS

What were the major goals of this project

1. To determine how cardiac lymphatic vessels affect myocardial revascularization?

We will investigate whether blood perfusion through cardiac lymphatic vessels increases in response to physiological stress or after heart amputation or cryoinjuries. These experiments will elucidate how lymphatic vessels might affect revascularization after different types of heart injuries.

2. To determine how cardiac lymphangiogenesis affect myocardial regeneration and scar resolution

We will ablate the lymphatic vessels to observe how myocardial regeneration is impacted after heart amputation or cryoinjuries. We will also utilize an *ackr3a* fish mutant that displays increased lymphatic vessels to evaluate whether they have enhanced myocardial regeneration. These experiments will shed light on how lymphatic vessels might regulate fibrotic scar resolution and myocardial regeneration after different type of injuries.

What was accomplished under these goals?

Major Activities 1 (Major Task 1 in SOW): Determine blood prefusion and revascularization via cardiac lymphatic vessels occur in response to physiological stress or heart injury (7-12 months)

Subtask 2: Revascularization by cardiac lymphatic vessels during zebrafish heart regeneration

We validated our preliminary data and use a different transgenic reporter line (*lyve1:GFP*) that marks cardiac lymphatic vessels and observed that *gata1:dsRed*+ erythrocytes are present in cardiac lymphatic vessels at one day post heart cryoinjury (dpc) (Fig. 1). These erythrocytes are absent in cardiac lymphatic vessels in uninjured hearts.

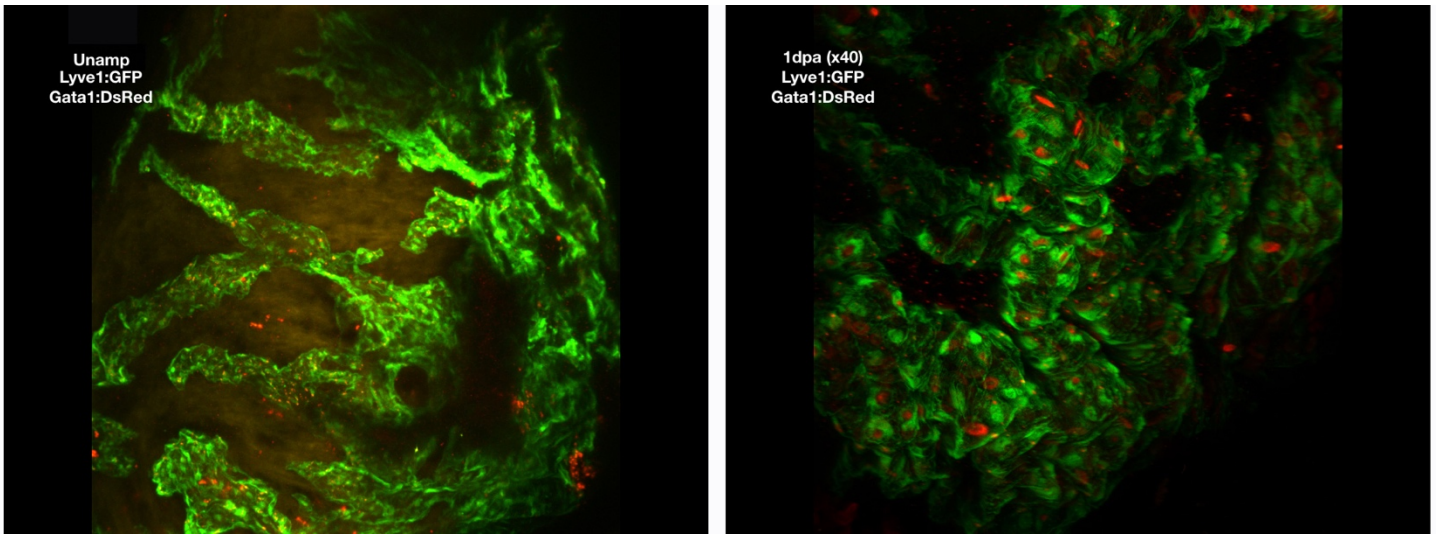

Fig. 1. Zebrafish cardiac lymphatic vessels carry erythrocytes in response to heart injuries. Erythrocytes (red blood cells) were marked with *gata1*, Red; lymphatic vessels were marked with *lyve1*, Green. Images of uninjured heart (Un-amp, left), heart at 1 day post amputation (1dpa, right)

Major Activities 2 (Major Task 2 in SOW): Determine new lymphatic vessel formation is beneficial to heart regeneration

Subtask 1: Determine the functions of blood perfusion via cardiac lymphatic vessels (8-15 months).

--How does cardiac lymphangiogenesis affect myocardial regeneration and scar resolution

Hearts without cardiac lymphatic vessels fail to regenerate.

We observed that lymphatic development is affected by the housing density and feeding conditions even in wild type fish. Fish growing up in a higher density or fed less fail to develop cardac lymphatic vessels, implicating a potential role of physiological stress. We utilize these conditions to first determine the roles of cardiac lymphatic vessels in heart regeneration and avoid the use of genetic mutants that might confound the data interpretation by inhibiting specific signaling pathways. Amputation was performed on 8-month old zebrafish hearts with *fli:GFP* and *prox1:RFP* transgenic reporter genes. The hearts were imaged by confocal fluorescence microscopy and cardiac lymphatic vessels were examined and scored at 42 dpa before performing AFOG staining. Zebrafish were divided into two groups based on whether they have cardiac lymphatic vessels or not. Compared to hearts with cardiac lymphatic vessels (A and B), hearts without cardiac lymphatic vessels had larger scars and failed to regenerate.

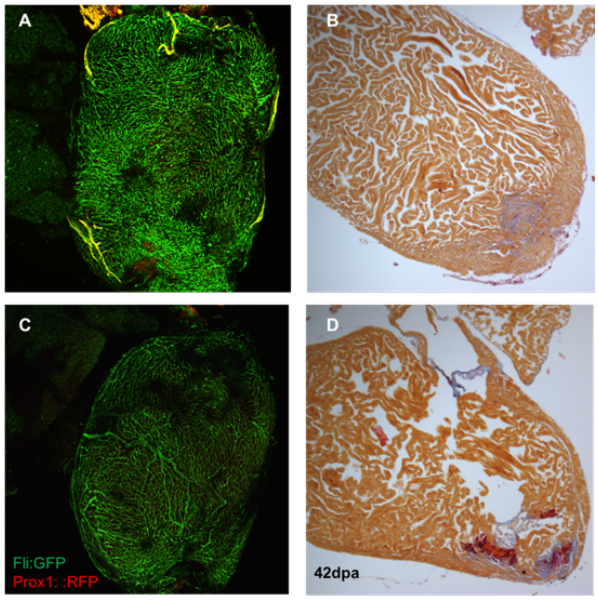

Fig. 2. Hearts without cardiac lymphatic vessels fail to regenerate. (A) A representative image showing a heart with cardiac lymphatic vessels (labeled by *prox1: RFP*) at 42 dpa. (B) AFOG staining of the heart in (A). The regeneration site can be identified with condensed cardiomyocytes at the apex. Myocardium was stained in orange, fibrin in pink, and collagen in blue (n=8). (C) A representative image showing a heart without cardiac lymphatic vessels at 42 dpa. (D) AFOG staining of heart in (C). Hearts without cardiac lymphatic vessels had bigger scar size (AFOG staining fibrin pink and scar compose of fibrils and collagen blue) and limited heart regeneration compared to hearts with cardiac lymphatic vessels (B) (n=4).

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

Dr. Michael Harrison, a postdoc associate in the lab has worked on this project. He has gained knowledge and research experience in the area of lymphatic development and heart regeneration. Dr. Harrison has completed his postdoctoral training in my laboratory and our institute and is leaving on Aug. 15, 2020 to establish his own independent laboratory at Weill Cornell Medical College.

How are the results disseminated to communities of interest?

1.The research results were presented as progress reports as weekly seminars at the Program of Developmental Biology and Regenerative Medicine, Saban Research Institute, Children's Hospital Los Angeles

2.The research results were presented at the Gordon Research Conference on Lymphatics by Dr. Ching-Ling Lien

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

Major Task 1 Milestones to be achieved: Observe blood perfusion and revascularization by cardiac lymphatic vessels after physiological stress and heart injury

1. Determine and quantify the erythrocytes present in lymphatic vessels at 3 and 7 days post heart injury to assess whether revascularization is persistent throughout heart regeneration.
2. Determine whether lymphatic vessels carry erythrocytes into the wound area

Major Task 2 Milestones to be achieved: Observe whether fibrotic scar is resolved and heart regeneration is enhanced with revascularization via new lymphatic vessel formation

1. To determine the functions of cardiac lymphatic vessels by genetically ablating the lymphatic endothelial cells.
2. Determine the consequence of gain and loss of lymphatic vessels in myocardial revascularization, hypoxia, immune cell trafficking and fibrotic scar resolution.

4. IMPACT

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

1. This project will reveal a new mechanism by which cardiac lymphatic vessels regulate immune cell clearance and fibrotic scar resolution of regenerating zebrafish hearts.

2. This project will reveal a novel mechanism by which cardiac lymphatic vessels regulate revascularization of regenerating zebrafish hearts.

3. The project might identify novel candidate genes to enhance cardiac lymphatic vessel formation.

What was the impact on other disciplines?

Nothing to report

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

Change in approach and reasons for change
Nothing to report

Actual or anticipated problems or delays and actions or plan to resolve them
The proposed experiments and milestones were delayed due to COVID-19. We have resumed research activities after our research institute re-opened and will speed up the proposed experiments.

Changes that had significant impact on expenditures
Due to COVID-19 related delays, we have not been able to place purchase requisitions required for the experiments. Therefore, we have less expenditure than proposed for this year.

Significant changes in use or care of human subjects, vertebrate animals, biohazards and/or select agents
Nothing to report

6. PRODUCTS

Publications, Conference papers, and presentation

Nothing to report

Other publications, conference paper and presentations.

The role of cardiac lymphatic vessels in zebrafish heart development and regeneration. Ching-Ling (Ellen) Lien presented at Lymphatics, Gordon Research Conference, The Growth and Function of Therapeutic Targeting During Development and Disease, March 1-6, 2020. Ventura, CA

Websites or other internet sites

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?

Name	Ching-Ling (Ellen) Lien, Ph.D.
Project Role	Project Director/Principal Investigator
Researcher Identifier (e.g. ORCID ID)	0000-0002-5100-9780
Nearest person month worked	1.2 calendar
Contribution to Project	Dr. Lien is the PI of this project and oversees the overall direction, data collection, analysis and completion of milestones of the project. She will ensure the project goals are accomplished in a rigorous and timely manner.
Funding support	NIH, TRDRP,
Name	Michael Harrison, Ph.D.
Project Role	Postdoctoral Research Associate
Researcher Identifier (e.g. ORCID ID)	0000-0003-1703-9879
Nearest person month worked	8 calendar
Contribution to Project	Dr. Harrison has performed heart injuries, tissue collection, confocal imaging and data analysis of the project
Funding support	None

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

Dr. Ching-Ling Lien has received one new grant support:

T30IP1028 (Lien, PI) 09/02/2019-08/31/2021 1.2 calendar
California Tobacco Related Disease Research Program (TRDRP) $197,220 Annual DC
Title: “Effects of tobacco and e-cigarettes on heart repair and regeneration”
The major goals of this high impact pilot project are to determine the effects of tobacco and e-cigarettes on heart repair and regeneration. The first aim will determine the effects of tobacco and e-cigarettes in zebrafish heart regeneration and cardiac lymphatic vessel functions and lymphangiogenesis. The second aim will determine the effects of tobacco and e-cigarettes on cardiac regeneration and development of heart failure in a neonatal mouse heart injury model.
There is no scientific overlap between this project and the current DoD project.
Role: PI

What other organizations were involved as partners?

None

8. SPECIAL REPORTING REQUIREMENTS

Collaborative Awards: Not Applicable

Quad Charts: Not Applicable

9.APPENDICES

Not Applicable