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TITLE: Exploring and Exploiting Novel Mammalian Heart Regeneration Models

PRINCIPAL INVESTIGATOR: Guo Huang

CONTRACTING ORGANIZATION: University of California, San Francisco, CA

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14. ABSTRACT Many lower vertebrate animals and even mammals at early developmental stages possess striking abilities to restore damaged and lost heart tissues. However, most adult mammals examined so far lack robust cardiac regenerative potential. It still remains enigmatic why such a seemingly beneficial trait is lost in animal evolution, and among 6,399 mammalian species whether there are any animals that still retain significant cardiac regenerative potentials. Here we present our pilot work in searching and charactering novel mammalian models with possible but previously unknown cardiac regenerative capability. Our preliminary analyses suggest the existence of unusual cardiac regenerative capability in certain mammals such as naked mole-rats. In this proposal, we will perform analysis of cardiomyocyte ploidy, a proxy of cardiac regenerative potential, in mammalian species, especially those with low metabolic rates and body temperatures. In addition, we will examine cardiomyocyte proliferative and regenerative potential in adult naked mole-rats and even mouse-naked mole-rat interspecies chimeras. Our strategy of exploring and exploiting new organisms for the study of regenerative biology is generalizable and can be applied to discovering animal and plant species with extraordinary yet unreported physiology and capability. We envision that novel mammalian regeneration models will provide new paradigms for investigation of tissue renewal capability of various organs and appendages, and may yield unprecedented insights into the fundamental principles governing tissue regeneration in animal development and evolution.					
15. SUBJECT TERMS Evolution, heart regeneration, naked mole-rats					
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1. INTRODUCTION:

This study aims to identify transition species in evolution that retain significant cardiac regenerative potential and investigate naked mole-rats as such a candidate. Our work will provide novel insights into the mechanism underlying the lack of cardiac regenerative capacity in adult human.

2. KEYWORDS:

Evolution, heart regeneration, naked mole-rats

3. ACCOMPLISHMENTS:

What were the major goals of the project?

HYPOTHESIS Based on our preliminary data, we formulate the following central hypothesis: **mammalian species with low metabolic rates and body temperatures – such as naked mole rats (NMRs) – have abundant diploid cardiomyocytes and may retain significant cellular regenerative potential.**

Aim 1. Perform phylogenetic analysis of cardiomyocyte ploidy and decode the design principle in evolution.

Aim 2. Investigate cardiomyocyte proliferative and regenerative potential of NMRs and mouse-NMR interspecies chimeras.

What was accomplished under these goals?

1) Major activities

I have been invited to present my research related to this grant in the following events.

Conferences/symposiums/workshops

- 2022 Keystone Symposia on Heart failure: Mechanisms and Therapies, Breckenridge, CO
- 2021 23rd European Congress of Endocrinology, Prague, Czech Republic (virtual)
- 2021 Annual Meeting for Endocrinology Society, San Diego (virtual)
- 2020 Asia-Oceania Congress of Endocrinology, Seoul, Korea (virtual)
- 2020 Basic Cardiovascular Sciences 2020 Scientific Sessions (virtual)
- 2020 Annual meeting for Society of Developmental Biology (virtual)

Departmental/university seminars

- 2022 Dept of Physiology, University of Texas Southwestern Medical Center, Dallas, TX
- 2021 BACLIF forum, Santa Cruz, CA
- 2021 Frontiers in Biomedical Sciences, Chinese University of Hong Kong, Hong Kong
- 2021 Academy of Cardiovascular Research Excellent Seminar Series
- 2021 Department of Biology, Santa Clara University, CA
- 2021 Cardiovascular Research Institute, Medical College of Wisconsin, Milwaukee, WI
- 2020 Department of Developmental Biology, University of Pittsburg, Pittsburg, PA
- 2020 Cardiovascular Disease Program, National University of Singapore, Singapore
- 2020 Cardiovascular Research Institute, Baylor College of Medicine, Houston, TX
- 2019 Cell Biology Program, Memorial Sloan Kettering Cancer Center, New York, NY
- 2019 The Heart Institute, Cincinnati Children's Medical Center, Cincinnati, OH
- 2019 Distinguished Lecture Series, University of Pennsylvania, Philadelphia, PA

Local seminars

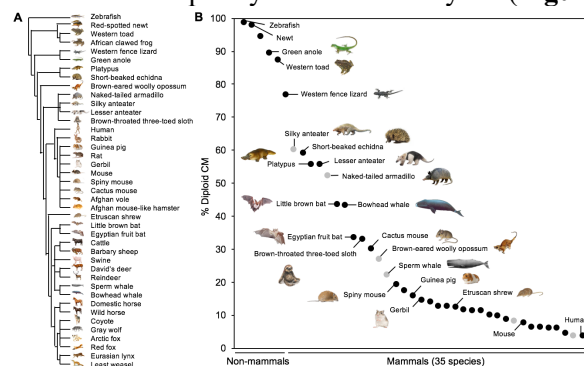
- 2022 ZSFG Dean Seminar, UCSF, San Francisco, CA
- 2022 Program in Craniofacial Biology Seminar Series, UCSF, San Francisco, CA
- 2020 UCSF Annual Bioengineering Graduate Program Research Conference
- 2020 UCSF Annual BMS Graduate Program Research Conference
- 2020 UCSF Annual TETRAD Graduate Program Research Conference
- 2019 UCSF Annual TETRAD Graduate Program Research Conference, Lake Tahoe, CA
- 2019 Parnassus Faculty Talk, UCSF, San Francisco, CA

2) Specific objectives: Not applicable.

3) Significant results or key outcomes

Aim 1. Perform phylogenetic analysis of cardiomyocyte ploidy and decode the design principle.

We have collected the hearts from the proposed monotreme, edentate, and cetacean species. CM nucleation and ploidy have been analyzed (**Figure 1**).



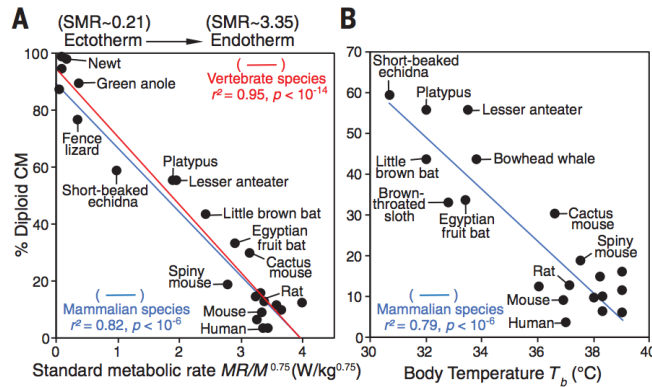


Figure 2. Diploid CM frequency inversely correlates with standard metabolic rate (A) and body temperature (B).

Our current analysis shows that the power expended by the heart (cardiac output W) in overcoming viscous forces is a size-independent fraction of metabolic rate (BMR), i.e. $W \propto BMR$.

Here W is a function of the volume rate of fluid flow (Q) and blood pressure (Δp) as $W = Q \Delta p$. It is known that BMR determines the volume rate of fluid flow (Q) that transports oxygen and nutrients for metabolism as $Q \propto BMR$. BMR scales to body mass (M) as $BMR \propto M^{3/4}$, thus $Q \propto M^{3/4}$. While blood pressure Δp is independent of body mass (both mathematically and experimentally supported), cardiac output $W = Q \Delta p \propto M^{3/4}$, which consequently means $W \propto BMR$. Thus, increases of basal metabolic rate in evolution and development would increase blood flow through the cardiovascular system as powered by increase of cardiac output.

Aim 2. Investigate naked mole rats (NMR) and bats as putative cardiac regenerative models.

We have performed myocardial infarction surgery on a batch of NMRs and mice. Although we did not observe heart functional improvement, we did observe enhanced cardiomyocyte proliferation when compared with mice (**Figure 3**). There are at least two possible interpretation. First, even though adult NMR cardiomyocytes retain significant proliferative potential, the post-injury expansion of cardiomyocytes is not robust enough to yield functional restoration. Second, other factors such as the inability to form new blood vessels in the infarcted area in NMR may limit the extent of regeneration.

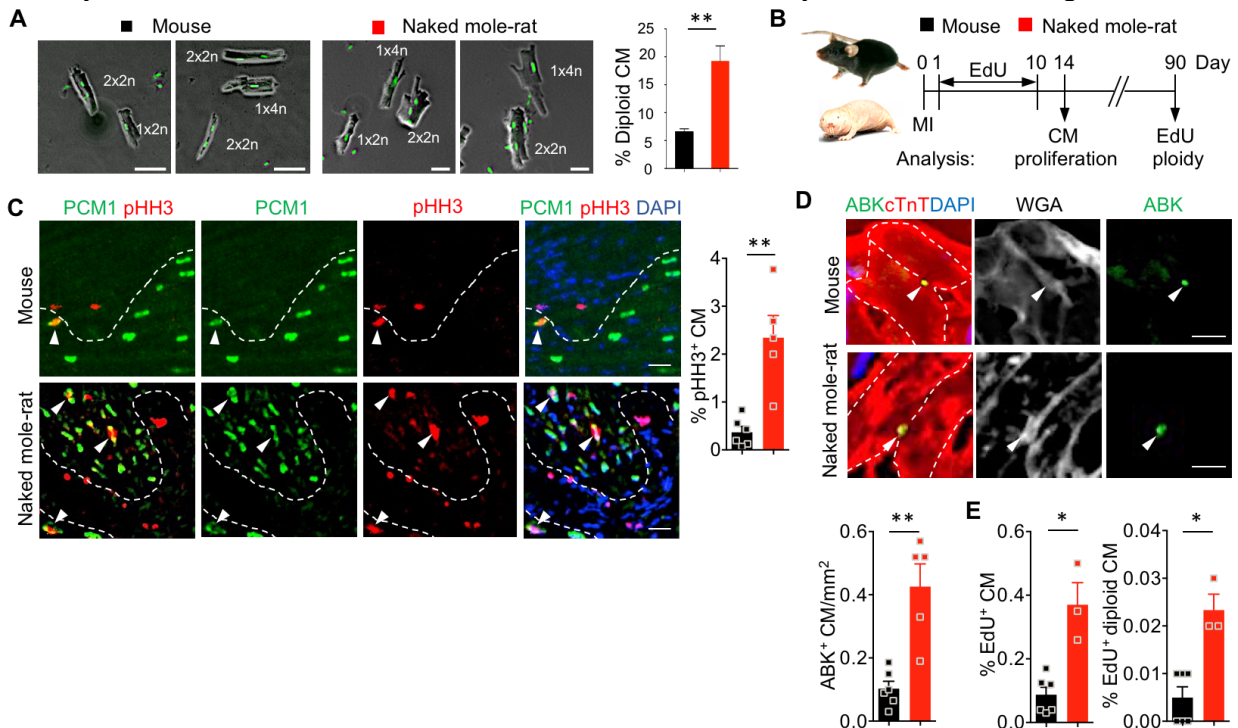


Figure 3. Naked mole-rats show more diploid cardiomyocytes (CMs) and enhanced CM regeneration following ischemic injury than mice. (A) Representative images and ploidy quantification of adult mouse and naked mole-rat CMs (n=3). **(B)** Schematic of the experimental plan to assess CM proliferation after myocardial infarction (MI). **(C)** CM proliferation analysis using phosphor-histone H3 marker (pHH3). Arrow heads indicate proliferating CMs. **(D)** Aurora B kinase (ABK) localization at the cleavage furrow showing CMs undergoing cytokinesis. **(E)** EdU incorporation and ploidy analysis of dissociated CMs. Each dot represents one animal. Values are mean \pm SEM. * P <0.05, ** P <0.01. Scale bars, 100 μ m (A), 25 μ m (B), 10 μ m (C).

4) Other achievements: none.

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

This grant provided opportunities for my trainees to attend and present in the following conferences.

Kentaro Hirose (Postdoc fellow) Oral presentation
2019 Weinstein Meeting in Cardiovascular Development and Regeneration, Indianapolis, IN

Alex Payumo (Postdoc fellow) Poster Presentation
2019 Gordon Conference on Tissue Regeneration and Repair, New London, NH

Stephen Cutie (Ph.D. student) Poster presentation
2019 Pan-American Evo-Devo Conference, Miami, FL
Oral presentation
2020 UCSF Annual Bioengineering Graduate Program Research Conference

Xiaoxin Chen (Postdoc fellow)
2020 American Heart Association Basic Cardiovascular Science Meeting (virtual)
Poster presentation
2020 UCSF Annual TETRAD Graduate Program Research Conference

Xi Chen (Postdoc fellow)
2020 American Heart Association Basic Cardiovascular Science Meeting (virtual)

How were the results disseminated to communities of interest?

Our findings in the *Science* paper (Hirose et al., 2019) have been reported by 11 news stories from 11 outlets and disseminated by 279 tweeters. <https://www.altmetric.com/details/56660658/news>

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

N/A

4. IMPACT:

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

We provide evidence for a new theory that our ability to regenerate the heart gets lost when we become warm-blooded, thus the loss of cardiac regenerative potential may be a tradeoff of our gain of the activity to generate body heat and maintain body temperature.

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

This provocative idea has stimulated widespread interests in both scientific and general public community. These findings have been reported by 11 news stories from 11 outlets (including *Scientific American*) and disseminated by 279 tweeters. <https://www.altmetric.com/details/56660658/news>

5. CHANGES/PROBLEMS:

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them

The pandemic-associated university shutdown and later density control caused a long waiting line in the rodent transgenic core for the proposed mouse-naked mole-rat interspecies chimeric study. In the end, the proposed Aim 2b (investigate cardiomyocyte proliferative and regenerative potential of mouse-NMR interspecies chimeras) could not be completed within the funding period. We plan to continue to generate and study these chimeras.

Changes that had a significant impact on expenditures

None.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

None.

Significant changes in use or care of vertebrate animals

Significant changes in use of biohazards and/or select agents

None.

6. PRODUCTS:

- **Publications, conference papers, and presentations**
Journal publications.

We have published 13 papers supported in part by this grant.

- a. Hirose, K., Payumo, A. Y., Cutie, S., Hoang, A., Zhang, H., Lunn, D., Gu, L., Bigley, R. B., Yu, H., Wang, J., Smith, M., Gillett, E., Wilson, E., Field, K. A., Reeder, D. M., Maden, M., Yarsev, M. M., Grutzner, F., Scanlan, T. S., Flamant, F., Buffenstein, R., Hu, G., Olgin, J. E., & **Huang, G. N.** (2019) Evidence for hormonal control of heart regenerative capacity during endothermy acquisition. *Science* 364:184-188. PMID:PMC6541389.
- b. Payumo, A. Y. & **Huang, G. N.** (2020) Lamin B2, guardian of cardiomyocyte nuclear division. *Dev Cell.* 53(1): 5-7.
- c. Judd, J., Lovas, J., & **Huang, G. N.** (2019) Defined factors to reactivate cell cycle activity in adult mouse cardiomyocytes. *Scientific Reports* 9 (1): 18830. PMID:PMC6906479
- d. Cutie, S., Payumo, A. Y., Lunn, D., & **Huang, G. N.** (2020) *In vitro* and *in vivo* roles of glucocorticoid and vitamin D receptors in the control of neonatal cardiomyocyte proliferative potential. *J Mol Cell Cardiol.* 142:126-134. PMID:32289320
- e. Payumo, A.Y., Chen, X., Hirose, K., Chen, X., Hoang, A., Khyeam, S., Yu, H., Wang, J., Chen, Q., Powers, N., Chen, L., Bigley, R.B., Lovas, J., Hu, G., & **Huang, G. N.** (2021) Adrenergic-thyroid hormone interactions drive postnatal thermogenesis and loss of mammalian heart regenerative capacity. *Circulation* 144:1000-1003.
- f. Khyeam, S., Lee, S., & **Huang, G. N.** (2021) Genetic, epigenetic, and post-transcriptional basis of divergent tissue regenerative capacities among vertebrates. *Advanced Genetics* e10042
- g. Graham, N., Huang, G. N., & **Huang, G. N.** (2021) Endocrine influence on cardiac metabolism in development and regeneration. *Endocrinology* 162 (9)
- h. Cutie, S., & **Huang, G. N.** (2021) Vertebrate cardiac regeneration: evolutionary and developmental perspectives. *Cell Regeneration* 10, 6.
- i. Amram, A.V., Cutie, S., & **Huang, G. N.** (2021) Hormonal control of cardiac regenerative potential. *Endocrine Connections* 10, R25-R35.
- j. Wong, J.C., ... **Huang, G. N.** ... Shannon, K. (2020) KrasP34R and KrasT58I mutations induce distinct RASopathy phenotypes in mice. *JCI Insight* 5, e140495.
- k. Zhang, M., Qian, L., Liu, C., **Huang, G. N.**, Tao, G. (2021) Cardiomyocyte Maturation: Novel Insights for Regenerative Medicine. *Front Cell Dev Biol* 9, 730622
- l. Ross, I., Omengan, D.B., **Huang, G. N.**, Payumo, A. Y. (2021) Thyroid hormone-dependent regulation of metabolism and heart regeneration. *J of Endocrinology* 1 (aop)
- m. Auchampatch, J., Han, L., **Huang, G. N.**, et al., (2022) Getting it right: Measuring cardiomyocyte cell cycle activity and proliferation in the age of heart regeneration *American Journal of Physiology-Heart and Circulatory Physiology*

Books or other non-periodical, one-time publications.

None.

Other publications, conference papers and presentations.

Conferences/symposiums/workshops

- 2022 Keystone Symposia on Heart failure:Mechanisms andTherapies,Breckenridge,CO
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- 2020 Cardiovascular Research Institute, Baylor College of Medicine, Houston, TX
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- 2020 UCSF Annual BMS Graduate Program Research Conference
- 2020 UCSF Annual TETRAD Graduate Program Research Conference
- 2019 UCSF Annual TETRAD Graduate Program Research Conference,Lake Tahoe,CA
- 2019 Parnassus Faculty Talk, UCSF, San Francisco, CA

• **Website(s) or other Internet site(s)**

Our findings have been reported by 11 news stories from 11 outlets. Websites and details can be found here. <https://www.altmetric.com/details/56660658/news>

- **Technologies or techniques**

None.

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

None.

- **Other Products**

None.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Whole project period – 30 months

Name: Guo Huang

Project Role: PI

Researcher Identifier (e.g. ORCID ID): 0000-0002-4335-9123

Nearest person month worked: 1.5

Contribution to Project: Dr. Huang has performed phylogenetic analyses of cardiomyocyte ploidy, theory development, and supervised investigation of cardiac regenerative potential in naked mole-rats (NMRs) and interspecies chimeras.

Funding Support: .

Name: Matthew Pamenter
Project Role: Collaborator
Researcher Identifier (e.g. ORCID ID): 0000-0003-4035-9555
Nearest person month worked: as needed for project

Contribution to Project: Dr. Pamenter has maintained NMR, perform MI surgery on NMR and provide NMR hearts following surgery, and contributed to discussion and data interpretation.

Funding Support: National Geographic Explorers Grant

9/1/19 – 8/31/20

Name: Xi Chen
Project Role: Postdoctoral fellow
Researcher Identifier (e.g. ORCID ID): [0000-0002-3958-6905](https://orcid.org/0000-0002-3958-6905)
Nearest person month worked: 4.5

Contribution to Project: Dr. Chen performed image analysis and quantification of mouse and NMR hearts.

Funding Support:

Name: Xiaoxin Chen
Project Role: Postdoctoral fellow
Researcher Identifier (e.g. ORCID ID): [0000-0003-3092-7010](https://orcid.org/0000-0003-3092-7010)
Nearest person month worked: 5.5

Contribution to Project: Dr. Chen performed mouse cardiac surgery and functional measurement as well as analyzed cardiomyocyte proliferation in NMR and mouse after injury.

Funding Support:

Name: Stephen Cutie
Project Role: Graduate student
Researcher Identifier (e.g. ORCID ID): Not available
Nearest person month worked: ~1

Contribution to Project: Mr. Cutie analyzed cardiomyocyte ploidy in vertebrate species and developed a mathematic model to explain the evolutionary constraints on cardiomyocyte ploidy and regenerative potential.

Funding Support: Partially by National Institute of Health NRSA F31 fellowship.

Name: Sheamin Khyeam
Project Role: Junior Specialist
Researcher Identifier (e.g. ORCID ID): Not available
Nearest person month worked: 3

Contribution to Project: Shea has quantified naked mole-rat and mouse cardiomyocyte proliferation (Ki67, pHH3, EdU) after myocardial infarction, and helped maintain the naked mole-rat iPS cell culture. She also contributed to figure generation in our published papers.

Funding Support:

Name: Alison Hoang
Project Role: Staff Research Associate
Researcher Identifier (e.g. ORCID ID): Not available
Nearest person month worked: 3

Contribution to Project: Alison has analyzed cardiomyocyte ploidy for echidnas, platypus, brown-eared woolly opossum, silk anteater, lesser anteater, naked-tailed armadillo, brown-throated three-toed sloth, bowhead whale, sperm whale and beluga whale, and contributed to the manuscript writing and figure making of our published papers. .

Funding Support:

09/01/20 – 02/28/22

Name: Nevan Powers
Project Role: Junior Specialist
Researcher Identifier (e.g. ORCID ID): Not available
Nearest person month worked: 9

Contribution to Project: Nevan has maintained mouse colony and taken care of mice after surgical procedure. He measured the ploidy of EdU-incorporated cardiomyocytes from both mice and naked mole-rats after myocardial infarction. In addition, he analyzed and derived the mathematic relationship between endothermy and cardiomyocyte ploidy

Funding Support:

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

N/A

What other organizations were involved as partners?

Organization Name: University of Ottawa

Location of Organization: Ottawa, Canada

***Partner's contribution to the project:** Dr. Pamerter has maintained NMR, perform MI surgery on NMR and provide NMR hearts following surgery, and contributed to discussion and data interpretation.*

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: N/A

QUAD CHARTS: Uploaded

9. APPENDICES: None