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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

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

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

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

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

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**).

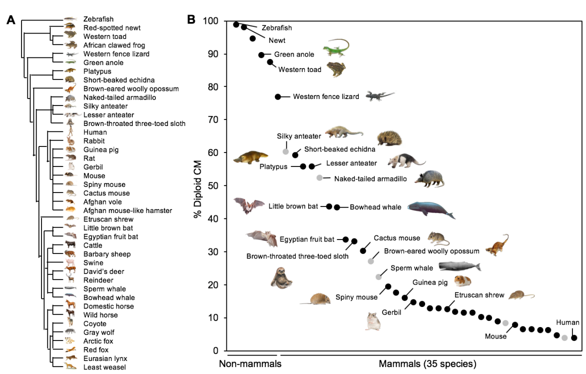

Figure 1. **Phylogenetic analysis of vertebrate cardiomyocyte (CM) nucleation and ploidy.** (A) Cladogram of species. (B) Percentages of mononucleated diploid CMs. Each dot represents the value from the adult heart(s) of one species (black: multiple samples are quantified; grey: only a single specimen is collected and analyzed). The data are listed from left to right based on the value of diploid CMs.

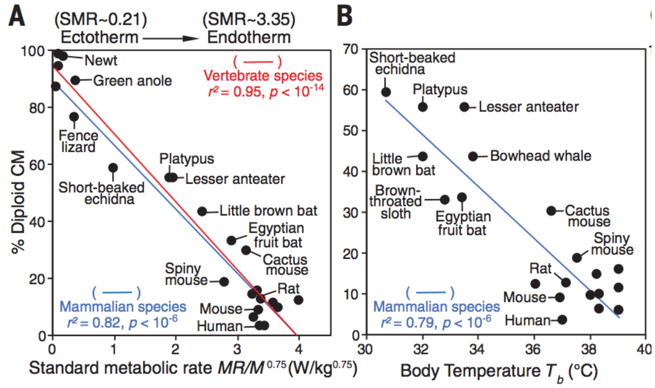

Figure 2. Diploid CM frequency inversely correlates with standard metabolic rate (A) and body temperature (B).

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.

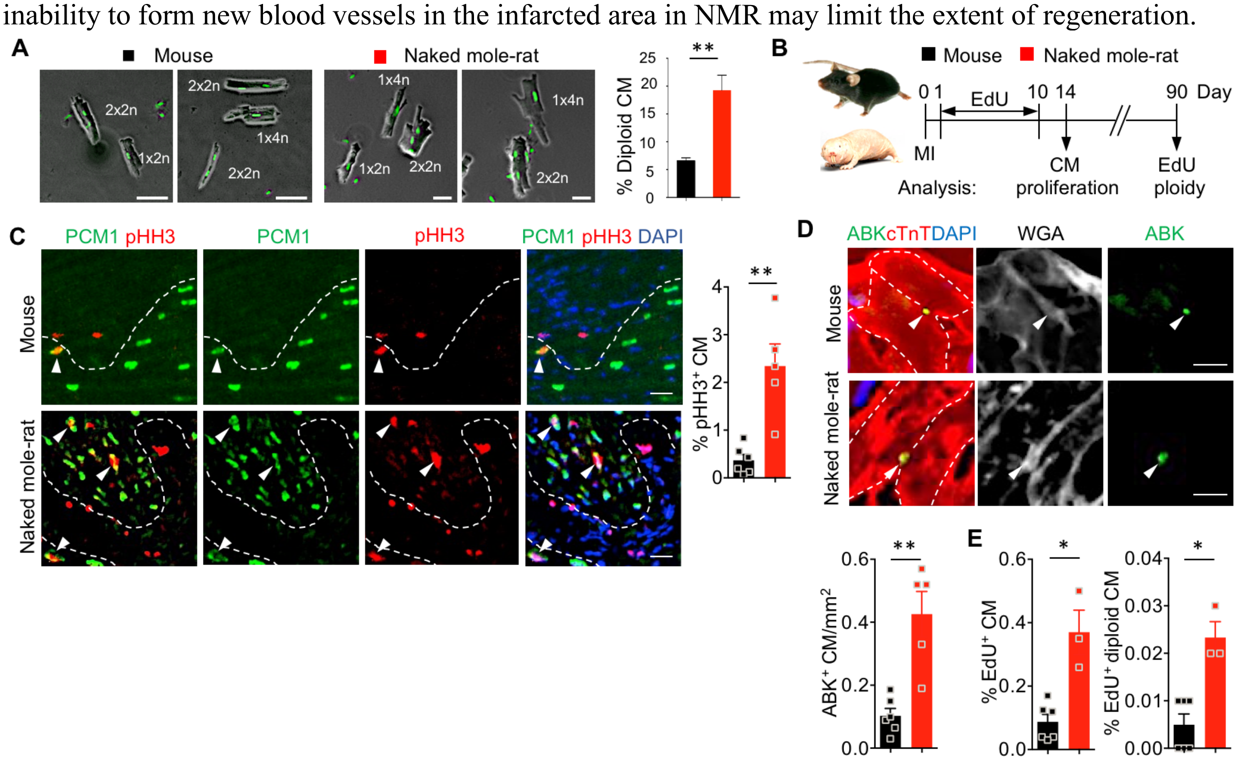

Figure 3. Naked mole-rats show more diploid cardiomyocytes (CMs) and enhanced CM regeneration following ischemic injury than mice. (**A**) Representative images and ploidy quantification of adult mouse and naked mole-rat CMs (n=3). (**B**) Schematic of the experimental plan to assess CM proliferation 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 ± SEM. $^*P<0.05$, $^{**}P<0.01$. Scale bars, 100 μm (A), 25 μm (B), 10 μm (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?

Nothing to report.

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.

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?

This provocative idea has stimulated widespread interest 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

None.

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.

None.

6. **PRODUCTS:**

- **Publications, conference papers, and presentations**

Journal publications.

We have published ten papers, in total so far, supported in part by this grant :

1. 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. PMCID:PMC6541389.
2. Payumo, A. Y. & **Huang, G. N**. (2020) Lamin B2, guardian of cardiomyocyte nuclear division. ***Dev Cell.*** 53(1): 5-7.
3. Judd, J., Lovas, J., & **Huang, G. N.** (2019) Defined factors to reactivate cell cycle activity in adult mouse cardiomyocytes. ***Scientific Reports*** 9 (1): 18830. PMCID:PMC6906479
4. 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
5. 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*** Accepted.
6. Khyeam, S., Lee, S., & **Huang, G. N**. (2021) Genetic, epigenetic, and post-transcriptional basis of divergent tissure regenerative capacities among vertebrates. ***Advanced Genetics*** e10042
7. Graham, N., Huang, G. N., & **Huang, G. N**. (2021) Endocrine influence on cardiac metabolism in development and regeneration. ***Endocrinology*** 162 (9)
8. Cutie, S., & **Huang, G. N**. (2021) Vertebrate cardiac regeneration: evolutionary and developmental perspectives. ***Cell Regeneration*** 10, 6.
9. Amram, A.V., Cutie, S., & **Huang, G. N**. (2021) Hormonal control of cardiac regenerative potential. ***Endocrine Connections*** 10, R25-R35.
10. Wong, J.C., … **Huang, G. N**. … Shannon, K. (2020) KrasP34R and KrasT58I mutations induce distinct RASopathy phenotypes in mice. ***JCI Insight*** 5, e140495.

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

None.

Other publications, conference papers and presentations.

Conferences/symposiums/workshops
2021 23rd European Congress of Endocrinology, Prague, Czech Republic (virtual)
2021 Annual Meeting for Endocrinology Society, San Diego (virtual)
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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
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

- **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?

Guo Huang, PhD – Principal Investigator: 0.6 calendar months, Orcid:0000-0002-4335-9123
Nevan Powers – Junior Specialist: 6.0 calendar months, 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

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

NEW: American Heart Association Transformational Project Award: 20TPA35500000 – 01/01/21 – 12/31/23   0.6 calendar months effort; total project costs

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS
COLLABORATIVE AWARDS:
QUAD CHARTS:

9. APPENDICES: