

INTERVIEW

The people behind the papers – Yingxi Cao, Ken Poss and Jingli Cao

Zebrafish heart regeneration is dependent on the activation of a regenerative programme in the cells surrounding the heart, known as the epicardium. A new paper in *Development* uses genome-wide transcriptomics and chromatin accessibility profiles to identify and validate candidate enhancers linked to genes induced during regeneration in epicardial cells. To hear more about the story, we caught up with first author Yingxi Cao and senior authors Professor Ken Poss from Duke University and Jingli Cao, Assistant Professor at Weill Cornell Medicine.

Ken and Jingli, can you give us your scientific biography and the questions your labs are trying to answer?

JC: I was trained as a cell biologist in the Chinese Academy of Sciences, where I studied epithelial polarity, cell cycle regulation, and ciliogenesis. When I graduated, I was fascinated by the remarkable regenerative capacity of zebrafish heart and was lucky to join Ken's lab as a postdoctoral fellow to study the epicardium. Together with Ken and another Poss lab colleague, we contributed a few seminal findings concerning epicardial cell injury responses and the requirement of epicardium for successful heart regeneration. With this fruitful training experience, I started my independent lab at Weill Cornell in 2018. By studying heart regeneration in zebrafish, my lab aims to understand how innate tissue regeneration is regulated at the cellular and molecular levels. We plan to address how the tissue regeneration programme is activated upon an injury, how cell cycle dynamics are instructed to engage in regeneration, and how distinct cell types in a complex tissue coordinate in robust tissue regeneration. We hope our work may ultimately help form the basis for the therapeutic repair of human tissues with inadequate regenerative capacity such as the heart.

KP: My PhD thesis research was with Susumu Tonegawa at MIT, where my goal was to probe possible mechanisms of learning and memory in mice by genetics. I then joined Mark Keating's lab at the University of Utah and then at Boston Children's Hospital, where I began to study tissue regeneration in zebrafish, including establishing the heart regeneration model. I've been at Duke for 18 years, and my lab is broadly interested in how and why tissue regeneration happens. I have been very lucky to work with many talented young people, including Jingli for his postdoctoral work. He is an especially thoughtful and creative scientist! We don't have a favourite molecule, tissue or question; instead, the goal of most of our projects is to explore. Success is defined by finding new concepts or mechanisms, or new questions to think about.

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Yingxi, how did you come to work in Jingli's lab and what drives your research today?

YC: I received my PhD in Medical Genetics, focusing on human genetic diseases. Among the multiple diseases involving different systems, cardiovascular disorders and the underlying genetic mechanisms sparked my scientific curiosity. Encouraged by my PhD adviser, Dr Wu, I decided to broaden my research from clinical study to basic science to explore further the mechanisms of cardiovascular diseases. In my previous research, I had worked with animal models such as mice and *Drosophila*, but not zebrafish. When I first learned about Jingli's research regarding zebrafish heart regeneration, I found it fascinating that zebrafish can have such a remarkable regenerative capacity. It is also promising to apply the findings of zebrafish studies to understanding or even solving human health problems, from bench to bedside. When I joined Jingli's lab, I was excited to be the first lab member as a postdoc and to help establish the lab. We have so many open questions to address. With the support and guidance of Jingli, I was primarily enthusiastic about the transcriptional regulation of heart regeneration. I was inspired whenever a new transgenic line showed regeneration-specific expression patterns. I was also fortunate to meet Professor Poss right after joining Jingli's lab, which gave me the confidence and motivation to make some accomplishments in zebrafish heart development and regeneration.

Before your work, what was known about the role of enhancers in heart regeneration?

YC, KP & JC: A full answer could take up a lot of space! There has been a handful of recent studies by us and others on sequences that can direct expression after tissue injury or during repair. We call this type of regulatory sequence a tissue regeneration enhancer element (TREE), based on our initial discoveries years ago. Relevant to heart regeneration, the body of work indicates that heart regeneration requires customised changes in expression of hundreds to thousands of genes in each cardiac cell type, and that chromatin structural changes at regulatory elements like TREEs are associated with these changes. Understanding how this is orchestrated could be complex. Ultimately, the capacity for heart regeneration is likely to be determined by the instructions encoded in regulatory elements, which might be different or differently read in different species. One can try to understand regulatory components one by one and also try to capture broad themes. Each approach will help the field to understand and manipulate heart regeneration.

Can you give us the key results of the paper in a paragraph?

YC, KP & JC: The epicardium is a beautiful and underappreciated tissue layer of the heart. It is a key cell source and signalling hub for heart regeneration. We've known for 15 years that epicardial cells respond to injury with dynamic changes in gene expression, so-called epicardial activation. To begin to understand gene regulatory mechanisms that control some of these changes, we generated genome-wide transcriptome and chromatin accessibility profiles from epicardial cells purified from regenerating zebrafish hearts. We found many candidate enhancers linked to genes that are induced



L-R: Yingxi Cao, Ken Poss and Jingli Cao

during regeneration, and then we established stable transgenic lines to validate several sequences that can preferentially direct epicardial expression in injury contexts. Broadly, we just hope that our datasets will be a good resource for our close colleagues who think about the epicardium and about heart regeneration.

Did you find any enhancers that were specific for the injury site-restricted activation rather than organ-wide epicardial activation?

YC, KP & JC: We think so. Although we could not rule out minor activity in areas distal to the injury site, four TREEs largely directed activity to regenerating tissue. One enhancer we found could direct organ-wide epicardial activation.

Were you surprised that some of the enhancers work additively, whereas others operated redundantly?

YC, KP & JC: Actually, that is what we expected to see. Genes are commonly regulated by multiple enhancers that are redundant or direct complementary expression domains in developmental contexts. We intentionally tested multiple candidate enhancers linked to genes of interest to try to detect this. Point of emphasis: it's



Expression of an enhancer reporter (green) in a 3-day-old larva. Tcf21 expression is shown in red.

key to make transgenics and/or deletion mutants, as chromatin structure profiles of regenerating tissues are not enough on their own to make the calls.

When doing the research, did you have any particular result or eureka moment that has stuck with you?

YC: In this study, we identified hundreds of candidate TREEs. However, it is impossible to test all of them. Although we picked candidate TREEs based on rigorous bioinformatic analysis for functional tests, we had no idea whether any of them would work. Generating transgenic lines took a lot of time and so did screening them. When I first saw an injury-induced epicardial cell expression pattern in the adult zebrafish heart, I was so excited and encouraged to continue this project. I even started to think about the next step, picturing that the enhancers we identified could be applied to mammals someday.

And what about the flipside: any moments of frustration or despair?

YC: After many years of research experience, I have learned to be always prepared for challenges. I usually tell myself to pay attention to all the details, especially something unexpected. For our enhancer project, one of the first challenges was performing adult heart surgery and larval heart dissection in zebrafish, which required concentration and patience. This was especially true for the dissection of 6-day-old hearts because everything is almost transparent, fragile and tiny, even under a microscope, making even the finest forceps look huge. After perseverant practice, I was finally able to get good results with perfect resection and expression, and everything was worth it when I saw the beautiful images.

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Yingxi, what's next for you after this paper?

YC: We have identified many epicardial TREEs and their related genes. However, the transcriptional regulation of the TREEs and the functional significance of the associated genes in heart regeneration are still largely unknown. I am currently focusing on the functions of the TREEs and related genes, and I have generated several stable

knockout lines for further research. I also want to employ the TREEs to direct the expression of candidate pro-regenerative factors.

Where will this story take your labs next?

JC: Identifying these enhancers is only the first step in defining the regulatory programmes of heart regeneration. The transcriptional machinery of these enhancers is still a significant knowledge gap. We want to dissect the upstream regulators that may hold the key to systematically activating a regeneration programme. Taking it one step further, some of these TREEs would be ideal candidates for comparative studies in mammals.

KP: I am happy to see more and more TREEs being identified from different regenerating tissues and species, and I think the growing catalogue will provoke some interesting comparisons and ideas. Also, the work provides some new regulatory sequences to use as tools for ectopic expression of gene cassettes, and we plan to make use of these.

Finally, let's move outside the lab – what do you like to do in your spare time?

YC: I like drawing and photography, which allow me to capture great moments in my daily life. Meanwhile, I can similarly apply

these skills to my research, such as detail observation and imaging. I also like walking around with friends, going to Central Park, and visiting museums. New York is a nice city with a lot of places to be explored.

JC: I love photography, which is a natural hobby of imaging biologists, I guess. I am also an amateur astronomer and particularly interested in astrophotography, although New York City is not an ideal place to set up my telescopes. I spend most of my spare time playing with my 3-year-old son, the most enjoyable and relaxed moments.

KP: I'm in Durham, North Carolina, which is pretty different from Manhattan and a wonderful place to live and work. I take advantage of all of the green spaces with family or with my deerhound, and I do some fishing and gardening as hobbies.

Reference

Cao, Y., Xia, Y., Balowski, J. J., Ou, J., Song, L., Safi, A., Curtis, T., Crawford, G. E., Poss, K. D. and Cao, J. (2022). Identification of enhancer regulatory elements that direct epicardial gene expression during zebrafish heart regeneration. *Development* **149**, dev200133. doi:10.1242/dev.200133