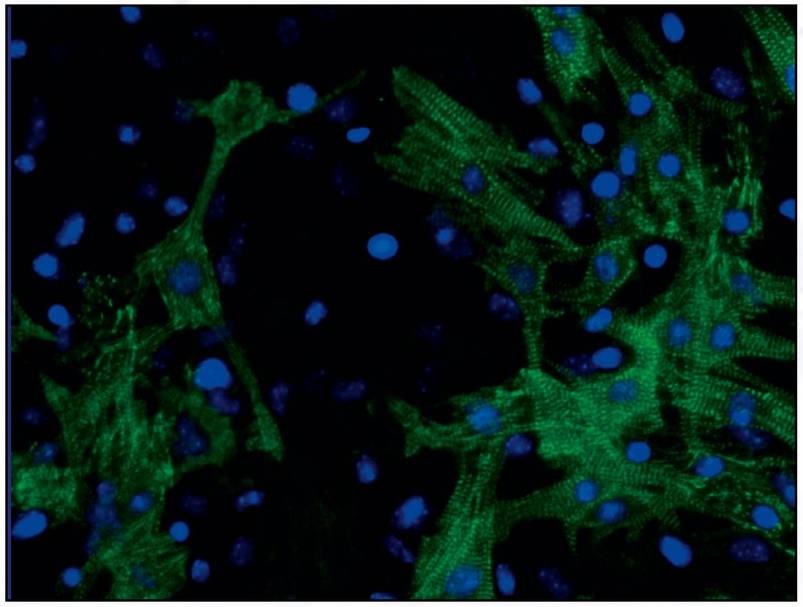

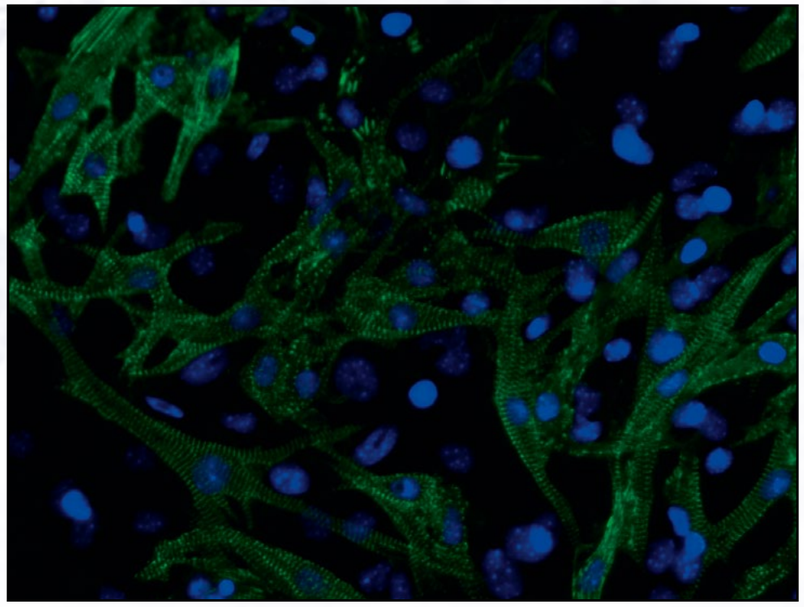

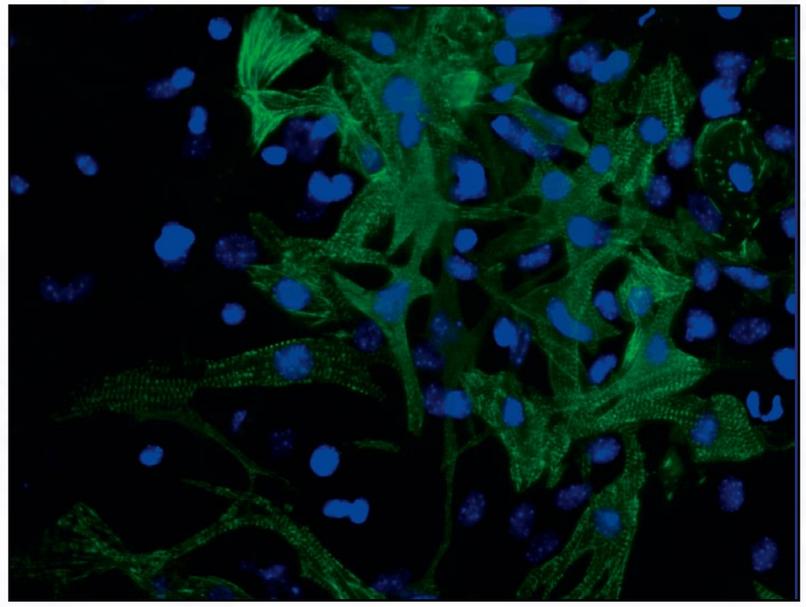

Repairing a broken heart

Dr Li Qian, Assistant Professor at the University of North Carolina School of Medicine has been fascinated by the heart since her undergraduate days. Now, her innovative technique for reprogramming resident cardiac fibroblasts in the damaged heart into functional cardiomyocytes could lead to a new treatment for heart disease, one of the Western world's biggest killers.

Heart failure – the inability of the heart to pump blood effectively around the body – is one of the leading causes of disease and death in the Western world, affecting an estimated 5.7 million people in the US alone.

Heart failure occurs when healthy heart muscle cells, known as 'cardiomyocytes', die – for instance, during a heart attack – and are replaced by scar tissue comprising a different type of cell, cardiac 'fibroblasts'. Normal fibroblasts are an important structural component of a healthy heart, but following an injury, they are produced in too great a quantity in the wrong parts of the heart –what Dr Qian terms as 'bad' fibroblasts.

The body cannot naturally regenerate lost cardiomyocytes so this change is generally considered to be irreversible. If only there were a way to convert fibroblasts back into cardiomyocytes – the award-winning Dr Qian and her coworkers are exploring a way to do just that.

REPAIR THROUGH REPROGRAMMING

In 2012, Dr Qian published ground-breaking research showing that cardiac fibroblasts in living mice could be reprogrammed to become functional, contracting cardiomyocytes, when treated with the right 'cocktail' of proteins. The three proteins – 'Gata4,' 'Mef2c,' and 'Tbx5' – are all types of 'transcription factors', molecules that can switch genes on and off, thus controlling a cell's function. Unlike previous cell regeneration studies, Dr Qian found that, using her cocktail, cardiac fibroblasts did not need to first be converted to stem cells in order to be reprogrammed into cardiomyocytes.

In mice with induced heart attacks, treatment with the cocktail caused heart function to improve after eight weeks, and continue recovering for over three months. Dr Qian's team showed that this was due to the conversion of cardiac fibroblasts into cardiomyocytes, which successfully integrated with the healthy heart tissue, while reducing scar size. Essentially, the once 'irreversible' damage caused by heart attacks could be reversed and healthy heart tissue regenerated – a potential game changer for the millions of people who suffer heart attacks each year.

These findings – which were ranked second in the American Heart Association's 'Top 10 Advances in Heart Disease and Stroke Research' in 2012 – together with her own laboratory's recent work identifying molecular barriers of reprogramming, gained Dr Qian many awards, including the prestigious 2016 BoyaLife, *Science* and *Science Translational Medicine* Award in Stem Cell and Regenerative Medicine.

Born and educated in China, Dr Qian moved to the US as a PhD student, to pursue her dream of carrying out basic science with real-world applications. She hopes that her discovery will eventually lead to a novel treatment for human heart disease patients, which she believes could be in

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clinical use within a decade. However, the slow rate of cell reprogramming and limited understanding of the mechanisms underlying it has hampered the transition to pre-clinical trials.

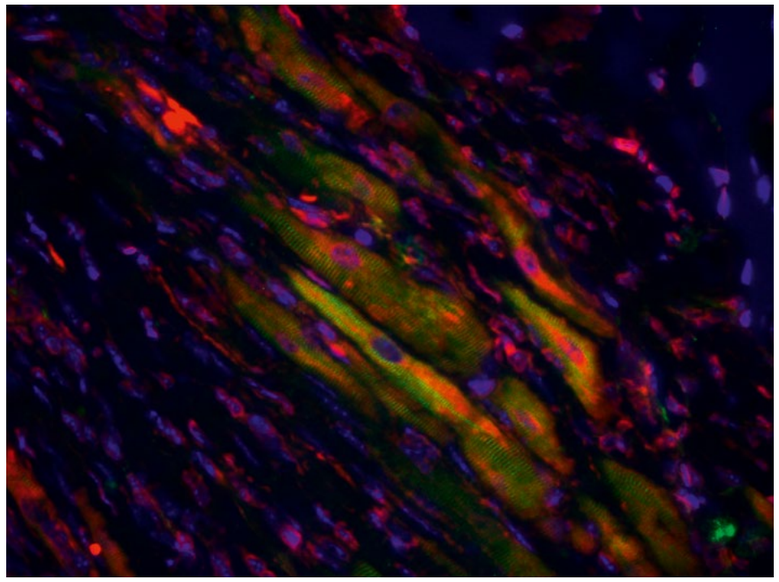
UNRAVELLING THE MECHANISMS

To resolve this stumbling-block, Dr Qian's latest project – 'Molecular Mechanisms of Direct Cardiac Reprogramming' – aims to work out exactly how fibroblasts are reprogrammed into cardiomyocytes. Normally, once a cell's role is determined it cannot be reprogrammed. Dr Qian hypothesises that the application of her transcription factor 'cocktail' – with each protein present in the right proportions and at the right time – somehow overcomes the barriers that usually prevent cells from switching type.

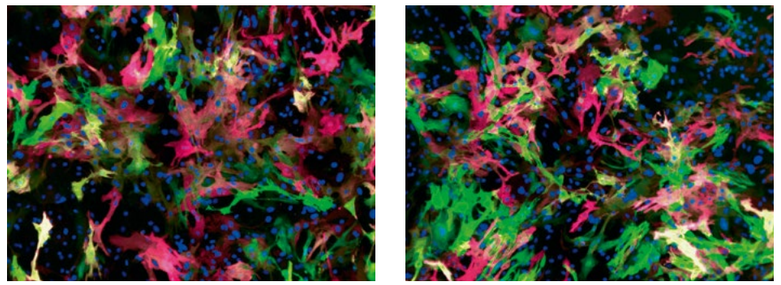
But to find the perfect cocktail – first for mice, then for pigs, and eventually for humans – she needs to understand the conditions under which heart cells are generated, both during reprogramming and from first principles – in foetal heart tissue. As Dr Qian points out, understanding the basic biology of the heart at the cellular level will provide the tools to fix it when it breaks.

The project aims, not only to determine the optimal conditions for reprogramming cardiac cells in human patients, but to provide insights into how cells' fates are determined in general, with implications for regenerative medicine of many types. Dr Qian says she was surprised that cells reprogrammed in a living heart more closely resembled endogenous adult cardiomyocytes, than cells reprogrammed *in vitro*. She thinks it likely that the microenvironment of the heart may enhance the success of reprogramming by providing growth factors, signalling molecules, and mechanical cues to cells undergoing reprogramming – all subjects that need further research.

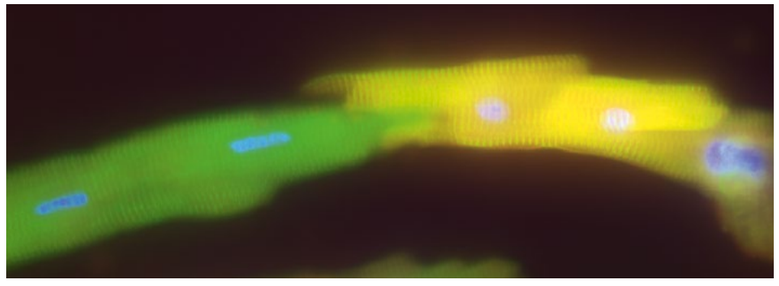
In 2016, Dr Qian made further progress towards increasing the rate of conversion from fibroblast cells to cardiomyocytes. Her team discovered – for the first time – that a protein called Bmi1 was interfering with the production of the protein cocktail in heart tissue. By blocking the gene producing Bmi1, they were able to dramatically increase the rate of reprogramming, giving a ten-fold greater rate of conversion to 'beating' heart cells.



A magnified view of a cluster of newly derived *in vivo* iCMs in a reprogrammed injured (myocardial infarction) heart. Green, cardiac marker alpha-Actinin; red, fibroblast lineage marker demonstrating the fibroblast origin; blue, DAPI labelling nuclei.



Two overviews of reprogrammed iCMs from fibroblasts in a dish. Green, cardiac reporter αMHC-GFP; red, cardiac TroponinT (cTnT); blue, DAPI labelling nuclei.



A highly magnified view of two isolated cardiomyocytes from a reprogrammed heart, on the left side is an endogenous cardiomyocyte while on the right side is an induced cardiomyocyte. Green, cardiac marker alpha-Actinin; red, fibroblast lineage marker demonstrating the fibroblast origin; blue, DAPI labelling nuclei.

Understanding the basic biology of the heart at the cellular level will provide the tools to fix it when it breaks

Q&A

How did you first get into science? And how did you end up where you are now?
The "onion cell" experiment in middle school was when I got so interested in science. I was immensely excited when I first saw a cell from a prep I made by myself. I want to use "POP" to describe how I ended up where I am now: Passion, Optimism, and Perseverance. I am also very fortunate to have had strong support from my mentors throughout my career especially my PhD mentor Dr Rolf Bodmer and postdoc mentor Dr Deepak Srivastava, without which I would not be where I am now.

What is so novel and exciting about this method of reprogramming cells?
It takes advantage of the endogenous existing "bad" cells (fibroblasts becoming the scar) to turn them into healthy heart muscles.

What do you hope to achieve during the current project, looking at the molecular mechanisms underlying cell reprogramming?
The successful completion of the current project will define the molecular components and determine the optimal condition for iCM reprogramming, and thus is expected to provide the scientific basis to translate a promising laboratory finding into a workable, efficient treatment for patients suffering from heart disease.

What do you see as the challenges to converting your findings into clinical outcomes?
One is the low efficiency, which can only be solved through understanding the molecular mechanisms of this process to further remove the barriers for improved purity, efficiency and speed. The other challenge would be the delivery method: how to efficiently and precisely deliver the reprogramming factors to the cardiac fibroblasts that need to be converted? Collaboration with bio-engineers will be essential to identify the optimal way. The third challenge is the safety issue: how to minimise the side effects of this approach? Large animal experiments would be key to address this challenge.

How do you think we will be treating heart disease in twenty years' time?
Personalised treatment without open heart surgery and/or heart transplantation.

A PERSONAL APPROACH

Dr Qian calls her technique 'induced cardiomyocyte reprogramming (iCM)'. Because the body's own fibroblasts are used, there is no risk of a reaction against them as might occur with transplanted cells. And, because it does not use stem cells, there is minimal risk of uncontrolled cell growth and possible tumour formation. Ultimately, the research could extend to other organs of the body such as the liver, pancreas or nervous system.

Dr Qian also believes this is an opportunity to develop a form of 'personalised medicine.' Fibroblast cells are found not only in the heart but also in connective tissues throughout the body, such as the skin. If skin fibroblasts could also be reprogrammed into cardiomyocytes – or, potentially, any other kind of cell – under the right conditions, Dr Qian believes she could use these in the lab to screen the cells of individual patients and find the most effective drugs for them, without any risk of dangerous side effects.

Whilst moving forward towards human applications, Dr Qian believes it is important to continue studying animal models – as in the current project – to understand the underlying mechanisms she is harnessing. This will give a basic science foundation to overcome any unforeseen obstacles she may come across during the crucial move to pre-clinical trials, and beyond. Can science help repair a broken heart? With Dr Qian's help, it can.

Detail

RESEARCH OBJECTIVES

Dr Li Qian's research focuses on furthering development of a therapeutic option for heart disease. Her work is specifically aimed at reprogramming adult cells found in connective tissue, known as fibroblasts, into cardiomyocytes.

FUNDING

Dr Li Qian is supported by AHA Scientist Development Grant 13SDG17060010, the Ellison Medical Foundation (EMF) New Scholar Grant AG-NS-1064-13, NIH/NHLBI R01HL128331, and generous gifts from Dr Hugh "Chip" McAllister and Cecil Sewell.

COLLABORATORS

Dr Qian's long term collaborator Dr Jiandong Liu

BIO

Dr Li Qian studied her undergraduate degree at Fudan University in Shanghai before undertaking a PhD at the University of Michigan. Following this, she became a Postdoctoral Fellow at the Gladstone Institute for Cardiovascular Disease before, in 2012, becoming an Assistant Professor at the University of North Carolina.

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