Myocardial ischemic injury and protection

Project leader Professor Peipei Ping outlines how a collaborative effort to demonstrate how manipulation of the heart's molecular basis can lead to a greater understanding of how to protect this vital organ from ischemic damage

Could you explain the founding principles objectives of the project?

From its inception, our project has set out to elucidate the mechanism of cardioprotection: how a heart can be capacitated to withstand a debilitating assault (e.g., ischemic injury) by manipulation of its innate molecular machinery. We are guided by the primary belief that cardiac mitochondria mitigate and contain much of the protective signalling pathways during myocardial ischemic injury. Our aims have focused on establishing the components and dynamics of these signalling networks and how they respond to various environmental challenges when the heart is exposed to ischemic injury. This information is a tremendous step forward towards the overall objective: understanding what molecular changes and reactions are able to yield the desired alterations in mitochondrial pathways and cardiac function, thereby leading to protection of the heart against ischemic damage.

What is myocardial ischemic injury, and which people tend to be most vulnerable to it?

Myocardial ischemic injury describes a situation in which the oxygen demand of the heart exceeds the oxygen supply provided by the coronary circulation, leading to subsequent damage. The traditional risk factors include age, smoking, obesity, high blood pressure, cholesterol levels, diabetes mellitus and physical inactivity. In the long-term, we hope to achieve both pre-emptive protection and to mitigate the damage as it occurs or has occurred. On an even grander scale, our research is aimed to one day develop individualised molecular based medical treatments (Molecular Medicine for Individuals) that take each individual's unique condition into account and thus minimise the side effects.

How have your previous projects in the field of cardioprotection helped to shape your current work?

Well, the initiative to protect the heart against injury has been a Herculean task. On a scientific front, it is a tedious process teasing apart and investigating the complexities of the mitochondrial pathways and cardiac signalling networks. Each step of research, from some of our initial work concerning PKC epsilon isoform mediated cardioprotection, to VDAC and the Src Kinase targets in the mitochondria, has been integral to developing a concept of how various targets interlock and respond towards one another in the molecular pathway. Establishing a complete picture, replete with a thorough understanding of the heart's signalling components, remains the key goal of our research. This approach is essential in order to develop new therapies and drugs with high efficiency and low side effects.

How have advances in technology assisted you in your research?

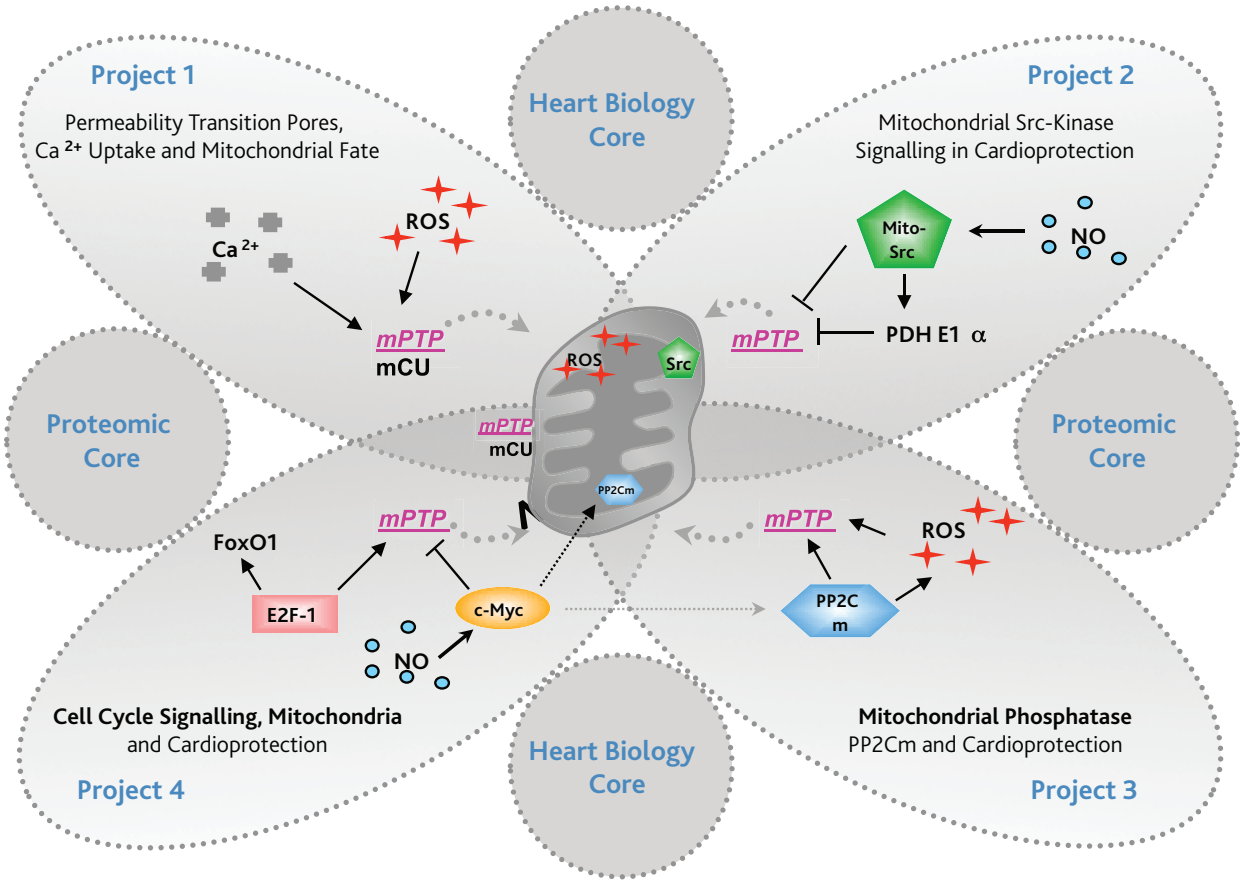
We are doing research in very exciting times. Our current work would have been impossible just a few years ago; and we are fortunate to have pursued this direction because the technology we depend on has advanced so rapidly. Amongst the more prominent technologies featured in our research are the advanced tandem mass spectrometers that allow us to screen, detect, analyse, identify and sequence the thousands of proteins and their respective posttranslational modifications in any given sample. These proteins are the essential blocks of mitochondrial pathways and cardiac signalling networks. The immense capacity and sensitivity of these instruments allow us to detect minute molecular changes in a tissue's protein compliment between conditions, while simultaneously analysing the biochemical status of each individual molecule. Where previous analyses were capable of correlating a single protein to physiological changes, we are now capable of looking at an entire functional proteome, or subsections of the proteome, to examine how different phenotypes can emerge form the synergistic properties of many proteins.

What advantages does the combination of mouse models and proteomic platforms yield?

We would ultimately like to be able to characterise changes in the proteomes of the mitochondria and hearts representing the major disease conditions in humans, particularly when concerning ischemic injury and protection. The technology we employ and the models we use offer us an extremely unique perspective that is not afforded by any other approach. Unfortunately, there do not seem to be any other equally capable alternatives available at this time. However, combining proteomics with different mouse models affords us the unique advantage of capturing volumes of biochemical data relevant to the given model of human diseases. Since most cardiac diseases were not provoked by changes in a single molecule or an isolated pathway, it is critical that we examine this protein synergy in the context of its molecular partners and all affiliated molecular pathways in a particular disease.

What have been the major results of your research into the role of PKCε in cardioprotection?

Over the course of our research, we are finding that PKCε is a key signalling element in cardioprotection. It functions as a central node



MITOCHONDRIAL SIGNALLING NETWROKS IN ISCHEMIC INJURY AND CARDIOPROTECTION

in the signalling network, integrating signals in both the cytosol and the mitochondria of a cardiac myocyte. Much of our work has indicated that PKCε is capable of activating a variety of downstream targets with the end result of making the cardiomyocyte resistant to various environmental stressors. One notable effect demonstrated thus far is that PKCε stimulation ultimately leads to a more resilient functioning heart.

Can you outline the salient points of your study of the mitochondrial targets of Src-kinase, and the impact of your findings?

The central concept of this study revolves around the model that Src-kinase is another central node in mitochondria mediated cardioprotective signalling. We expect to find that as a signalling module, Src-kinase is capable of acting on a specific set of downstream mitochondrial targets to ameliorate mitochondria dysfunction. More specifically Src-kinase may inhibit opening of the mitochondrial permeability transition pore and preserve mitochondrial function and integrity, thus enhancing the cardiomyocytes' resistance to stress.

What are the potential clinical applications of your work?

I am a strong believer that we will soon see the translation of this discovery into daily clinical practice. Since we introduced the concept of PKCε as a pivotal signalling element of cardioprotection, numerous studies were able to show that the selective activation of PKCε provides protection against ischemic injury in human tissue. Much of this research has focused on the development of selectively designed peptide regulators, activating PKCε in an organ specific manner, and clinical studies investigating the feasibility of this revolutionary approach are on their way. Aside from ischemic heart disease sufferers, patients with other diseases, such as dementia or Parkinson's, may benefit from treatment in this area.

What are the main criteria by which you evaluate the success of your research?

Ideally, I would love to be able to gauge the success of our research by how extensively it disseminates into other fields of science and how much benefit it may provide to advance medicine. I would like to view our work as a node in the web, and if our work successfully impacts the overall direction in which this network behaves, or perhaps even in a small dimension of the capacity of the network itself, then both myself, and the team, will be very pleased. The ultimate dissemination of our work, from scientific curiosity - to medical execution, is our mission, and whether it takes months, years or decades, my unwavering confidence that we will succeed, inspires me and keeps me immeasurably excited.

What is your vision for the future of your research and development in cardioprotection?

Again, I want to restate my enthusiasm for the clinical significance and translational value demonstrated in our research programme in cardioprotection. An innate ability to protect the heart, or to protect any organ in general, is a phenomenon with tremendous promise to positively change the lives of vast swathes of the population. I see our own research as part of a concerted effort that will, within our own lifetimes, give physicians a preventative treatment option to tackle cardiovascular and other diseases affecting society.

MYOCARDIAL ISCHEMIC INJURY AND PROTECTION

OBJECTIVES

This research focuses on the characterisation of signalling pathways and cellular organelles in the heart, with a particular interest on alterations of subproteomes during myocardial ischemic injury

FUNDING

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PARTNERS

This is an NHLBI supported programme at UCLA; it involves 43 faculty, students, fellows, and staff members from four departments at the UCLA School of Medicine, and includes projects led by Professor Jim Weiss, Professor Ligia Toro, Professor Yibin Wang, Professor Robb MacLellan, Dr Linda Cai, Professor Enrico Stefani, Dr Thomas Vondriska, Dr Jun Zhang, Dr Julian Whitelegge, and Dr Jeff Abramson.

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PEIPEI PING is Professor of Physiology and Medicine at the University of California at Los Angeles (UCLA), School of Medicine, a position she has occupied since 2002. Her illustrious scientific career spans 25 years, over which time she has worked consistently closely with the NIH, recently serving an appointment as Chairperson of NHLBI working group on the role of mitochondria in cardiovascular diseases (2007–2008). Among numerous notable achievements, Ping has also served on the Editorial Boards of American Journal of Physiology, Circulation Research, Journal of Molecular and Cellular Cardiology, Circulation, Proteomics, and Journal of Proteome Research, and is actively involved with the Human Proteome Organization (HUPO).

Reducing Cardiac Disease

A clearer understanding of signalling pathways can lead to a significant reduction in cardiac disease. The pioneering work of UCLA, under **Professor Peipei Ping,** is investigating the role of proteins in cardioprotection

PEIPEI PING IS Professor of Physiology and Medicine at the University of California at Los Angeles (UCLA), and heads up the Cardiac Proteomics and Signaling Laboratory (CPSL). In previous years, their endeavors have focused on a handful of projects centered on the concept of cardioprotection and understanding its varied mechanisms, primarily during cell death in the course of myocardial ischemic injury. As the technology has advanced, they have sought to pursue these questions by applying the most powerful and sophisticated tools available. One of the principals of CPSL research is the use of mouse models in combination with a state-of-the-art proteomic platform to investigate the entire protein content (including post translational modifications, stoichiometry, etc.) of a signalling module, organelle, cell or tissue under a host of conditions.

THE ROLE OF PROTEIN KINASE C

Over the past few years, Ping's team has achieved a great deal of success in determining the role of PKCε (protein kinase C) in cardioprotection, specifically focusing on its role in modulating mitochondrial functionality and integrity. They have gained insight into the direct modulation of the mitochondrial electron transport chain (ETC) by PKCε, as well as investigated the interaction of PKCε with the antiapoptotic protein Bcl-2 in circumventing mitochondrial permeability transition pore (mPTP) opening. As a component of the mPTP, the voltage-dependent anion channel (VDAC) constitutes the major pathway for the entry and exit of metabolites across the outer membrane of the mitochondria and can serve as a scaffold for molecules to modulate the organelle. To clarify, the molecular mechanisms and components underlying mPTP opening, CPSL successfully crystallised murine VDAC isoform 1 at a 2.3 Å resolution. This represents an integral step in delineating the role of VDAC in myocardial cell death during myocardial ischemic injury. Additionally, they have gained insight into the protective signalling mechanisms of NO (Nitric Oxide)-induced cardioprotection and its interaction with PKCε, as well as the interaction of PKCε with VDAC and ANT (Adenine Nucleotide Translocase) in preventing mitochondrial permeability transition pore opening.

ENHANCING CARDIOPROTECTION

In recent endeavours, CPSL have identified and characterised the entire proteome of the functional murine cardiac mitochondria, consisting of functional clusters known to support oxidative phosphorylation, metabolism and biogenesis. In total, 1663 distinct proteins were identified, 624 of which had not been previously demonstrated to reside in the organelle. By combining a proteomic and metabolomic approach, Ping's team were able to demonstrate that PKCε activity plays an important role in modulating cardiac glucose metabolism, and interacts and modulates the translocation of PKCε to the mitochondria, thereby providing a possible explanation for the synergistic effects of PKCε and PKCε in cardioprotection.

UNCOVERING SRC-KINASE

Current work under Ping's leadership is following the success of CPSL previous research directions, aiming to delineate the mitochondrial targets of Src-kinase (particularly the Src-kinase/PDH module) and to characterise its capacity to systematically modulate mitochondrial function during NO-donor induced cardioprotection. A role for Src-kinase has been implicated in NO-induced cardioprotection and we have recently demonstrated mitochondrial localisation of Src-kinase, a Src-kinase mediated regulation of mitochondrial ETC activity and an increased Src-kinase activity during NO-induced cardioprotection. Ping is working to characterise the role of mitochondrial Src-kinase as a target of NO-donor induced cardioprotection and the novel targets of this kinase in the mitochondria and it is their hope that these efforts in conjunction with their past work will continue to advance our understanding of cardioprotective mechanisms.