Fluorescent protein helps scientists with heart, stem cell research

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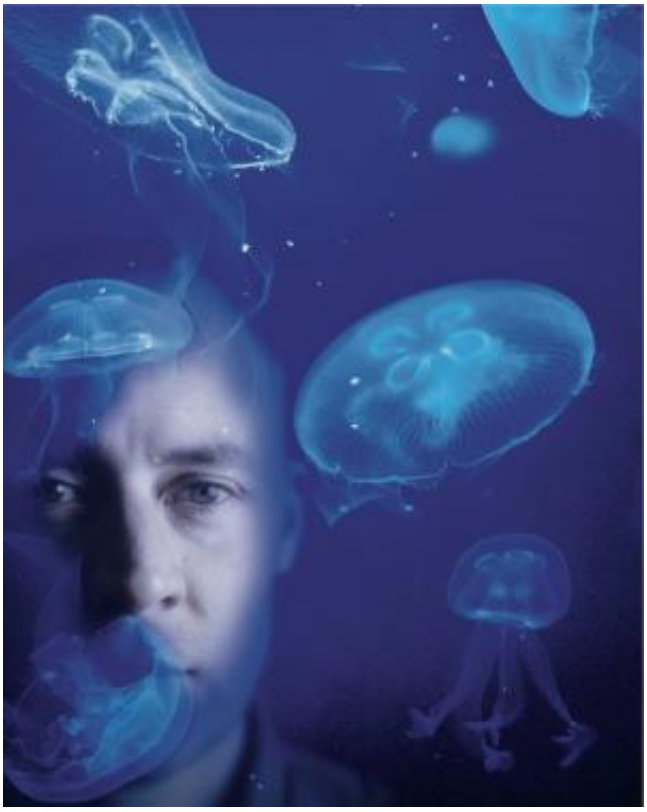

Dr David Elliott.

(Medical Xpress)—A fluorescent protein from a deep-sea jellyfish has helped scientists isolate heart cells in the laboratory, creating an invaluable aid to work on heart disease treatments and extraordinary opportunities for stem cell researchers around the world.

They arrive by FedEx courier, packages half the size of a shoebox marked 'Biological', their contents packed with styrofoam to protect the 96 Petri dishes inside. The cost of international delivery for this room-temperature package is about A$250, but for biomedical science, the value of the contents is priceless.

Each box that the research group of Dr David Elliott from Monash University sends to international collaborators contains about half a million purified human heart muscle cells. Isolated from other cell types through a remarkable new technique, they are the material through which researchers can study heart function and test therapies for the diseases that attack the engine room of the human body.

One of the keys to ultimate success in finding treatments for heart disease is a deeper understanding of how the cardiac cells within the muscular wall of the heart are both damaged and repaired. For years, scientists and drug companies have been working with unpurified cell lines, unsure of whether the effects they observe, such as cell death, are caused by a reaction of the cardiomyocytes (heart muscle cells) themselves, or by surrounding, supporting cells.

In 2011, an international team led by Dr Elliott, and which also included Monash Professors Andrew Elefanty and Ed Stanley, published a paper in *Nature Methods* outlining research that would guarantee a potentially inexhaustible supply of heart cells. It was not only the researchers who were glowing from what was lauded as a foundational advance in stem cell science – the cells also glowed.

"Researchers need to study cardiac cells in vitro to understand more about the progression of various forms of the disease," Dr Elliott says. "They need to be able to screen new drugs safely and establish whether or not they are toxic to heart tissue – but obtaining enough mature, live cardiac cells to do this has proved remarkably difficult."

Cracking the challenge was a four-year task for the 26 scientists from Monash, the Walter and Eliza Hall Institute, the Baker IDI Heart and Diabetes Institute, the Leiden University Medical Centre and the Netherlands Proteomics Centre.

They had a little help from a jellyfish – Aequorea victoria, an almost crystal-clear medusa jellyfish that haunts the Atlantic waters off North America and Europe. Since the 1990s, stem cell scientists have been using a green fluorescent protein from the jellyfish as an invaluable way to mark cells by making them glow green in ultraviolet light.

"When you grow a culture of embryonic stem cells, you can encourage it to produce specialised cardiac cells using certain growth factors," Dr Elliott says. "But how do you then identify and separate these from the smooth muscle cells and other types in the culture? That was the first big challenge."

Through a genetic procedure, the team modified human embryonic stem cell cultures with the fluorescent jellyfish protein so that it would hook up exclusively to a gene called NKX2-5. This gene goes into action in the earliest stages of embryo development, when the tiny heart begins to form, and helps to shape the growing tissue into a mature organ. The researchers could therefore make potential cardiac cells reveal their identity by glowing bright green under ultraviolet light. Better still, the cells did this several days before actually maturing into beating heart cells, which meant the team could identify the fully developed cardiomyocytes as well as heart progenitor cells, which are destined to differentiate into heart cells.

But after identifying these cells, how would the researchers physically isolate them? The team solved this second challenge by identifying a pair of proteins on the surface of the glowing cardiac cells, which they were able to use as biochemical 'handles'. They grabbed them with specialised

antibodies, a cheap and efficient way to separate the cardiomyocytes from other cells.

Discovery delivers various applications

Besides helping to purify the culture of heart cells for research purposes, this technique could also allow cardiac specialists to isolate heart cells cultured from the stem cells of individual patients. Dr Elliott says that biotech companies are already investing in developing techniques for such 'personalised' medicine.
Consider this potential application. Some chemotherapy treatments cause damage to the heart muscle of somewhere between 20 and 30 per cent of patients, but at the moment there is no way of knowing whether an individual patient is susceptible to this damage. Scientists can already extract a skin sample from a patient and use it to culture stem cells, but this culture is not purified. The new technique means that heart muscle cells could be isolated from this culture: they would beat in a Petri dish, and the drug could then be applied to them to observe its impact.

Such cells could be used to test the new generation of heart drugs in the laboratory. From the Petri dish, they could provide crucial information on efficacy and safety before animal or human trials commenced, or had to be discontinued because of damaging side-effects. Years would be sliced off the process.

"For researchers, the significance is that this is the first time they have had access to pure human cardiac cells, free of contamination from animal products or the pathogens they might harbour," Dr Elliott says.

The team has applied for a provisional patent on the method, and has already shared cell lines with leading cardiac laboratories in Europe, the US and Asia.

Professors Elefanty and Stanley are international leaders in the field of stem cell manipulation and differentiation (the process by which stem cells are told how to develop into specialised cells – heart, skin and so forth). It was while working in their laboratory at the Monash Immunology and Stem Cell Laboratories (MISCL) that Dr Elliott began the cardiomyocyte research, before founding his own MISCL research group last year. These heart cells are the sixth cell line that the Elefanty–Stanley laboratory has developed. They are employing similar techniques to isolate insulin-producing cells for the treatment of diabetes, and blood cells for the treatment of leukaemia.

While the immediate value of the approach is to grow lines of experimental cells for research purposes and drug trials, Dr Elliott believes that down the track it could have direct medical application for the isolation and production of cell lines from a patient's own heart to replace those severely damaged by disease, sidestepping the need for heart transplants and the risks of tissue rejection. But this, he stresses, is still a long way off.

For the moment, their achievement provides extraordinary opportunities for stem cell researchers around the world. A group in the Netherlands is using cells from Dr Elliott's laboratory to study Long QT syndrome – a disease of the electrical circuitry that regulates heartbeat. The cell surface proteins that serve as biochemical handles are being used in laboratories worldwide to study a range of cardiac diseases.

The impact of the research is perhaps best summarised by a commentary piece in Nature Methods by Associate Professor Timothy Kamp, from the University of Wisconsin–Madison. "The promise of stem-cell research in the cardiovascular field continues to grow … The bright spotlight has been on the possibilities for revolutionary new approaches for research and therapy, but it is the key foundation-building work like the studies described here that will provide the necessary tools and

understanding to truly realise this promise."

Provided by Monash University