Transforming scar tissue into beating hearts: The next instalment

April 1 2012

The latest research developments to reprogram scar tissue resulting from myocardial infarction (MI) into viable heart muscle cells, were presented at the Frontiers in CardioVascular Biology (FCVB) 2012 meeting, held 30 March to 1 April at the South Kensington Campus of Imperial College in London.

In a keynote lecture Dr Deepak Srivastava outlined his approach that has been described as a "game changer" with the potential to revolutionise treatment of MI. For the first time at the FCVB meeting, Srivastava presented the results of his latest studies using viral vectors to deliver genes directly into the hearts of adult mice that had experienced an MI. The FCVB meeting was organised by the Council on Basic Cardiovascular Science (CBCS) of the European Society of Cardiology (ESC).

In his original "proof of principle" study, published August 2010 in "*Cell*"[1], Srivastava was able to show that all that was needed for the direct reprogramming of fibroblasts (a major component of scar tissue) into myocytes (heart muscle cells responsible for beating) was the delivery of three genes. The work , which took place in a Petri dish, was considered groundbreaking since it showed for the first time that unrelated adult cells could be reprogrammed from one cell type to another without having to go all the way back to a stem cell state.

"Our ultimate hope is that, during the acute period following MI, patients will be able to receive direct injections of factors that transform

the existing fibroblast cells in the "scar" into new myocytes. The resulting increase in muscle mass should help MI survivors to live more normal lives," explained Srivastava, who is director of the Gladstone Institute of Cardiovascular Disease in San Francisco, US.

Healthy heart tissue is composed of a mixture of several kinds of cells, including cardiomyocytes, which provide beating muscle, and cardiac fibroblasts, that provide architectural support to the myocytes. "When heart muscle cells become injured and die following an MI, patients have the major problem that these cells have little or no capacity for regeneration," explained Srivastava. Part of the process of remodelling that occurs following the injury is that fibroblast cells migrate to the site and create the scar.

"The process at first can be considered beneficial since without fibroblasts adding structural support damaged hearts would rupture. But later difficulties arise when the fibrotic scar doesn't contract like the muscle it has replaced. Reduced global contractility means the heart has to work much harder, and the extra stress can ultimately lead to heart failure and even death," said Srivastava.

One of the Holy Grails of cardiovascular research has been to replace these lost myocytes and return functionality to the heart. Some of the first approaches to be investigated were the introduction of stem or progenitor cells to the sites of injury.

"But many hurdles have been encountered including getting cells to integrate with neighbouring cells in the heart, and there have been concerns that residual "rogue" cells could persist with the potential to keep dividing and give rise to tumours," said Srivastava. "Harnessing the vast reservoir of fibroblasts already present in the heart, we felt, could overcome many of these issues. They've the big advantage they're already present in the organ and closely integrated with neighbouring

cells."

In the address Srivastava, a paediatric cardiologist, explained how he had first got ahead of the game by "leveraging" knowledge from his work in embryo hearts. Over the past 15 years the focus of Srivastava's lab has been to identify genetic factors responsible for the formation of embryonic hearts.

From this work the team identified 14 key genes that they felt were the major "on/off" switches for cardiac genetic programming. When the genes were "knocked out" in animal models, they disrupted normal formation of the heart.

In the 2010 *Cell* paper, working in a Petri dish, the team were able to identify three of these genes Gata4, Mef2c, and Tbx5 that could convert fibroblasts taken from the hearts of adult mice into new myocytes. "By removing each gene one at a time we were able to whittle things down to the three factors that were indispensible," explained Srivastava.

In the second part of the study, the team injected fibroblasts that already had the three genes inserted directly into the scar tissue of mice. They were able to show the fibroblasts differentiated into cardiomyocyte-like cells.

"The fibroblasts converted into cells with nice patterns of striations, typical of myocytes, and developed units that could generate force," he said.

In the latest study (currently in press), Srivastava explained, they have been able to take the process one step further by injecting a viral vector encoding the genes for Gata4, Mef2c, and Tbx5 directly into the scar tissue of mice who had just experienced an MI. "With these studies we've obtained even better results showing that the fibroblasts become

more like cardiomyocytes and functionally couple with their neighbours. They could beat in synchrony and improve the function of the heart," he said.

The team have also shown that fibroblasts taken from the skin of mice (dermal fibroblasts) can be converted into muscle-like cells. "Such data suggests fibroblasts throughout the body have the potential to be transformed, which means that similar approaches, using different factors, could be used to regenerate nerve cells for patients with spinal cord injuries and other cells for diabetic patients," said Srivastava.

For MI treatment, said Srivastava, the next step will be to test the direct injection approach in a larger animal, such as a pig, whose hearts are similar in size to humans. But a big question remains whether the same combination of genes will work in human hearts.

"Major safety issues will need to be explored, such as whether or not new cells in the area of the scar might predispose patients to rhythm disturbances," said Srivastava. "A future hope is that we'll identify small molecules that can be used to directly turn the genes on so that we no longer need the gene therapy approach."

More information: M Leda, J Dong Fu, P Delgado-Olguin, et al. Direct Reprogramming of Fibroblasts into Functional Cardiomyocytes by Defined Factors. Cell. August 6 2010, 142: 375-386 Key note lecture Deepak Srivastava (San Francisco, US). Transdifferentiation of somatic cells into cardiomyocytes. Sunday 1 April 2012 16.45-17.30. Great Hall. FPN 229

Provided by European Society of Cardiology