GERMANY/HONG KONG JOINT RESEARCH SCHEME
THE PROJECT REPORT
(for Project Completion)

Project Number: G_HK029/11

Title

Effects of Inflammatory Cytokines on the Cardiac Differentiation of Embryonic Stem Cells: Role of Reactive Oxygen Species

Particulars

	Hong Kong team	German team
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Other project team members (if any)		

Funding Period

	1st year	2nd year (if applicable)
Start Date	Jan 2012	Jan 2013
Completion Date	Dec 2012	Dec 2013

Objective(s) as per original application

1. To investigate the effects of inflammatory cytokines that are released during myocardial infarction on the cardiac differentiation of ESCs.
2. To investigate the effects of inflammatory cytokines on the production of reactive oxygen species in cardiac progenitors derived from ESCs.
3. To investigate if inflammatory cytokines can increase the cardiac differentiation of ESCs via increasing the production of reactive oxygen species.

Details of Report [Please attach relevant document(s)]

i) Outline of proposed research and results obtained

1. To investigate the effects of inflammatory cytokines that are released during myocardial infarction on the cardiac differentiation of embryonic stem cells (ESCs).

It has been reported that during myocardial infarction, cardiomyocytes will secrete a portfolio of inflammatory cytokines, including interleukin (IL)-1β, IL-6, IL-10 and IL-18 and tumor necrosis factor-α (TNF-α).
In our investigation, we found that IL-1β, IL-10, IL-18 and TNF-α upregulated the expression of cardiac structural proteins (α-myosin heavy chain, α-actinin and cardiac actin) in ESC-derived cardiomyocytes. On the other hand, IL-1α decreased while IL-6 did not affect the expression of cardiac structural proteins. Therefore, we concluded that IL-1β, IL-10, IL-18 and TNF-α increased the cardiac differentiation of ESCs.

2. To investigate the effects of inflammatory cytokines on the production of reactive oxygen species (ROS) in cardiac progenitors derived from ESCs.

ROS-treated embryoid bodies were found to have enhanced cardiac differentiation in the long run as reflected by, firstly, an earlier appearance of beating EBs, and secondly, an upregulation in cardiac structural protein expression at both mRNA and protein levels. Also, ROS upregulated the expression of several cardiac-related transcription factors, and increased the post-translationally-activated transcription factors SRF and AP-1. The results clearly indicated that ROS increased cardiac differentiation of ESCs.
By staining with a ROS sensitive dye followed by confocal microscopy, we reported that for the inflammatory cytokines (IL-1β, IL-10, IL-18) that were found to increase cardiac differentiation of ESCs (in Aim 1), they increased the ROS level in differentiating ESCs.

3. To investigate if inflammatory cytokines can increase the cardiac differentiation of ESCs via increasing the production of ROS.

Degree of cardiac differentiation was examined by immunostaining of cardiac troponin T followed by flow cytometry analysis to determine the percentage of cardiomyocytes in differentiating embryoid bodies. IL-10 and IL-18 were found to increase the percentage of cardiomyocytes formed, consistent with the western blot results on cardiac structural proteins. ROS scavenger Trolox was found to concentration-dependently reverse the cardiogenic effect of IL-10 and IL-18. The results suggested that IL-10 and IL-18 increased the cardiac differentiation of ESCs by increasing the production of ROS.

ii) Significance of research results

Myocardial infarction is the leading cause of mortality in developed countries over the past few decades. ESC-derived cardiomyocytes are potential cell source for cell replacement therapy in the treatment of myocardial infarction. On the other hand, it was reported that during myocardial infarction, cardiomyocytes will secrete a portfolio of inflammatory cytokines, including IL-1β, IL-6, IL-10 and IL-18 and TNF-α.
Since the fate of transplanted ESC-derived derivatives will ultimately determine the functional efficacy of the cell replacement therapy in the treatment of myocardial infarction, it will be of extreme importance to study the possible effects of these cytokines on the cardiac differentiation of ESCs.
The present study provided information about the effects of inflammatory cytokines that are released during myocardial infarction on the cardiac differentiation of ESCs. Therefore, the present study provided important insights for the modulations of cytokines in future ESC transplantation therapy for improved healing after myocardial infarction. In addition, the study provided novel insights into possible biochemical approaches for guiding ESCs to differentiate into cardiomyocytes.

iii) Research output

Law SK, Leung CSL, Yau KL, Tse CL, Wong CK, Leung FP, Mascheck L, Huang Y, **Sauer H** and **Tsang SY** (2013) Regulation of multiple transcription factors by reactive oxygen species and effects of pro-inflammatory cytokines released during myocardial infarction on cardiac differentiation of embryonic stem cells. *International Journal of Cardiology*. **168**:3458-72.
(please see attached paper)

iv) Potential for or impact on further research collaboration

Prof. Sauer's and Prof. Tsang's groups are interested in determining the signals for cardiac differentiation of ESCs. Specifically, Prof. Sauer's expertise is in the ROS signalling of differentiating ESCs, while Prof. Tsang's expertise is in the ion channels and biology of ESCs. The results obtained from this study revealed that multiple inflammatory cytokines regulate the cardiac differentiation of ESCs. These results would foster future collaboration on the role of ROS-sensitive calcium channels in the cardiac differentiation of ESCs.