香港浸會大學
HONG KONG BAPTIST UNIVERSITY

School of 中醫藥學院
Chinese Medicine

Discovery of Novel Determinants of Endothelial Lineage: Insights from Chimeric Heterokaryons

Dr. Wong Wing Tak, Jack

Assistant Professor, Department of Cardiovascular Sciences
Institute for Academic Medicine Houston Methodist Research Institute
Department of Cardiothoracic Surgery
Weill Cornell Medical College of Cornell University

Date : 3 June 2015 (Wednesday)
Time : 4:30- 6:00 PM
Venue : SCM809
Language : English
Facilitator : Prof. Li Min

Abstract

Objectives: We aim to use induced pluripotent stem cells (iPSCs) to generate endothelial cells (iPSCS-EC) for disease modelling and therapeutic applications. Currently, there is insufficient information to efficiently or reliably create EC by directed differentiation from pluripotent cells. Using our extensive expertise in the generation of heterokaryons and in characterization of EC phenotype, we aim to identify novel determinants of differentiation to endothelial lineage and confirm the role of these novel determinants in endothelial differentiation and development.

Methods and Results: Murine embryonic stem cells (ESC) were fused with human endothelial cells in stable non-dividing heterokaryons. Using RNA-seq, it is possible to discriminate between human and mouse transcripts in these chimeric heterokaryons in ~95% of cases. Accordingly, it is possible to observe the effect upon murine ESC transcription, of the factors maintaining endothelial phenotype in the human EC. We determined the temporal pattern of gene expression in the ESC within the heterokaryons, so as to identify early acting candidate factors underlying reprogramming to an EC fate. Subsequent studies focused on those transcription factors and/or epigenetic modifiers which had not hitherto been identified as endothelial lineage factors. The role of candidate factors were then confirmed via loss-of-function studies by siRNA-mediated gene knockdown, and by gain-of-function studies using retroviral vector mediated over-expression. These LOF and GOF approaches were applied in studies of pluripotent stem cell differentiation to endothelial lineage in vitro, which included assessment of endothelial phenotype by transcriptional and immunohistochemical markers, as well endothelial function (e.g. tubulogenesis and nitric oxide production). The importance of candidate factors in endothelial development was further validated using the zebrafish model and morpholino technology.

Conclusions: We have identified novel transcriptional factors and epigenetic modifiers of EC lineage using a heterokaryon strategy combined with RNAseq. These studies provide a systematic, mechanistic approach to identifying key regulators of endothelial cell development, and will provide insights that will be useful in formulating strategies of directed differentiation of pluripotent stem cells to the endothelial lineage.

~ ALL ARE WELCOME ~