Cell Research (2008) 18:s128.

www.nature.com/cr

Functional consequences of over-expressing the gap junction Cx43 in the cardiogenic potential of pluripotent human embryonic stem cells

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Gap junctions, encoded by the connexin (Cx) multi-gene family, underlie cell-cell communications and play an important role in the development and signaling of mouse cardiogenesis, however their role in human cardiogenesis is undefined. To explore the cardiogenic role of Cx43, we first established a genetically-modified H1 cell line that stably overexpresses Cx43 via lentivirus-mediated gene transfer. Cx43 overexpression in hESCs, confirmed by qPCR and Western blot analyses, completely suppressed the formation of spontaneously cardiomyocytecontaining beating clusters from embryoid bodies (vs. ~14% of control GFP-overexpressing hESCs). RT-PCR indicated that the transcripts of ventricular myosin light chain (MLC-2V) and cardiac troponin I (cTnI), which are important for contractile functions, were completely absent even 14 days after differentiation, unlike control GFP hESCs. Microarray analysis revealed that 985 transcripts were differentially up- or down-regulated in Cx43-overexpressed in comparison to GFP hESCs. Many of these genes were known to be involved in various molecular pathways such as transforming growth factor- and integrin-signaling pathways. To better understand the lack of cardiac differentiation seen in the hESC, a Cx43-mESC line was also generated. Although cardiac differentiation of mESC was not affected, Cx43-mESC embryoid bodies were more likely to contain quiescentyet-excitable CMs. Taken collectively, our results provide important insights into the role of Cx43 in human cardiogenesis.

***Keywords*:** human embryonic stem cells, connexin, cardiogenesis, troponin

Cell Research (2008) **18**:s128. doi: 10.1038/cr.2008.218; published online 4 August 2008

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