Stem Cell Reports
Article

ISSCR

OPEN ACCESS

Fibroblast Growth Factors and Vascular Endothelial Growth Factor Promote Cardiac Reprogramming under Defined Conditions

Hiroyuki Yamakawa,[1] Naoto Muraoka,[1] Kazutaka Miyamoto,[1] Taketaro Sadahiro,[1] Mari Isomi,[1] Sho Haginiwa,[1] Hidenori Kojima,[1] Tomohiko Umei,[1] Mizuha Akiyama,[1] Yuki Kuishi,[1] Junko Kurokawa,[2] Tetsushi Furukawa,[2] Keiichi Fukuda,[1] and Masaki Ieda[1,3,*]

[1]Department of Cardiology, Keio University School of Medicine, Tokyo 160-8582, Japan
[2]Department of Bio-Informational Pharmacology, Medical Research Institute, Tokyo Medical Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8510, Japan
[3]JST, CREST, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan
*Correspondence: mieda@z8.keio.jp
http://dx.doi.org/10.1016/j.stemcr.2015.10.019

SUMMARY

Fibroblasts can be directly reprogrammed into cardiomyocyte-like cells (iCMs) by overexpression of cardiac transcription factors, including *Gata4*, *Mef2c*, and *Tbx5*; however, this process is inefficient under serum-based culture conditions, in which conversion of partially reprogrammed cells into fully reprogrammed functional iCMs has been a major hurdle. Here, we report that a combination of fibroblast growth factor (FGF) 2, FGF10, and vascular endothelial growth factor (VEGF), termed FFV, promoted cardiac reprogramming under defined serum-free conditions, increasing spontaneously beating iCMs by 100-fold compared with those under conventional serum-based conditions. Mechanistically, FFV activated multiple cardiac transcriptional regulators and converted partially reprogrammed cells into functional iCMs through the p38 mitogen-activated protein kinase and phosphoinositol 3-kinase/AKT pathways. Moreover, FFV enabled cardiac reprogramming with only *Mef2c* and *Tbx5* through the induction of cardiac reprogramming factors, including *Gata4*. Thus, defined culture conditions promoted the quality of cardiac reprogramming, and this finding provides new insight into the mechanism of cardiac reprogramming.

INTRODUCTION

Direct reprogramming generates the desired cell types from fibroblasts without passing through a stem cell state by overexpression of tissue-specific transcription factors. Transduction with reprogramming factors rapidly suppresses the fibroblast signature and concomitantly activates the target cell program to generate partially reprogrammed cells, which become more fully reprogrammed functional cells after prolonged cultivation (Sadahiro et al., 2015; Vierbuchen and Wernig, 2012). We and others reported the generation of cardiomyocyte-like cells (iCMs) from fibroblasts using combinations of cardiac-specific factors, including *Gata4*, *Mef2c*, and *Tbx5* (GMT) (Addis et al., 2013; Chen et al., 2012; Ieda et al., 2010; Jayawardena et al., 2012; Protze et al., 2012; Song et al., 2012). Transduction with GMT suppresses the expression of fibroblast-related genes and activates a cardiac reporter, *αMHC* promoter-driven *GFP* (αMHC-GFP), and cardiac protein expression in ~20% of fibroblasts after 1 week; however, few cells (~0.1% of the starting fibroblasts) are fully reprogrammed into functional iCMs after 4 weeks under conventional serum-based culture conditions, suggesting that most αMHC-GFP$^+$ cells remain partially reprogrammed or immature iCMs by the original method (Ieda et al., 2010; Sadahiro et al., 2015). We and others have reported that *miR-133* and inhibition of pro-fibrotic signaling promote cardiac reprogramming by silencing fibroblast signatures at the early stage of reprogramming; however, the molecular mechanisms underlying the conversion of partially reprogrammed cells into functional iCMs at the later stage remain undetermined (Muraoka et al., 2014; Zhao et al., 2015). Given that endogenous cardiac fibroblasts can be converted into more fully reprogrammed functional iCMs by in vivo reprogramming, undefined extrinsic factors may promote the quality of cardiac reprogramming (Inagawa et al., 2012; Qian et al., 2012; Song et al., 2012).

Recently, multiple groups showed that modification of the reprogramming factors could promote cardiac reprogramming (Sadahiro et al., 2015). Cahan et al. (2014) analyzed the gene regulatory network of the αMHC-GFP$^+$ population, in which a vast majority of the cells were partially reprogrammed iCMs, using a network biology platform, CellNet, that assesses the fidelity of engineered cells (Morris et al., 2014). The αMHC-GFP$^+$ cells were found to be exclusively classified as cardiomyocytes; however, multiple cardiac transcriptional regulators, including *Gata6*, *Hand2*, and *Nkx2.5*, were incompletely activated in the αMHC-GFP$^+$ population, and supplementation of these factors could improve cardiac reprogramming. Indeed, addition of *Hand2* to GMT (GHMT) increases generation of cells expressing cardiac proteins and spontaneously beating iCMs, and addition of *Nkx2.5* to GMT or

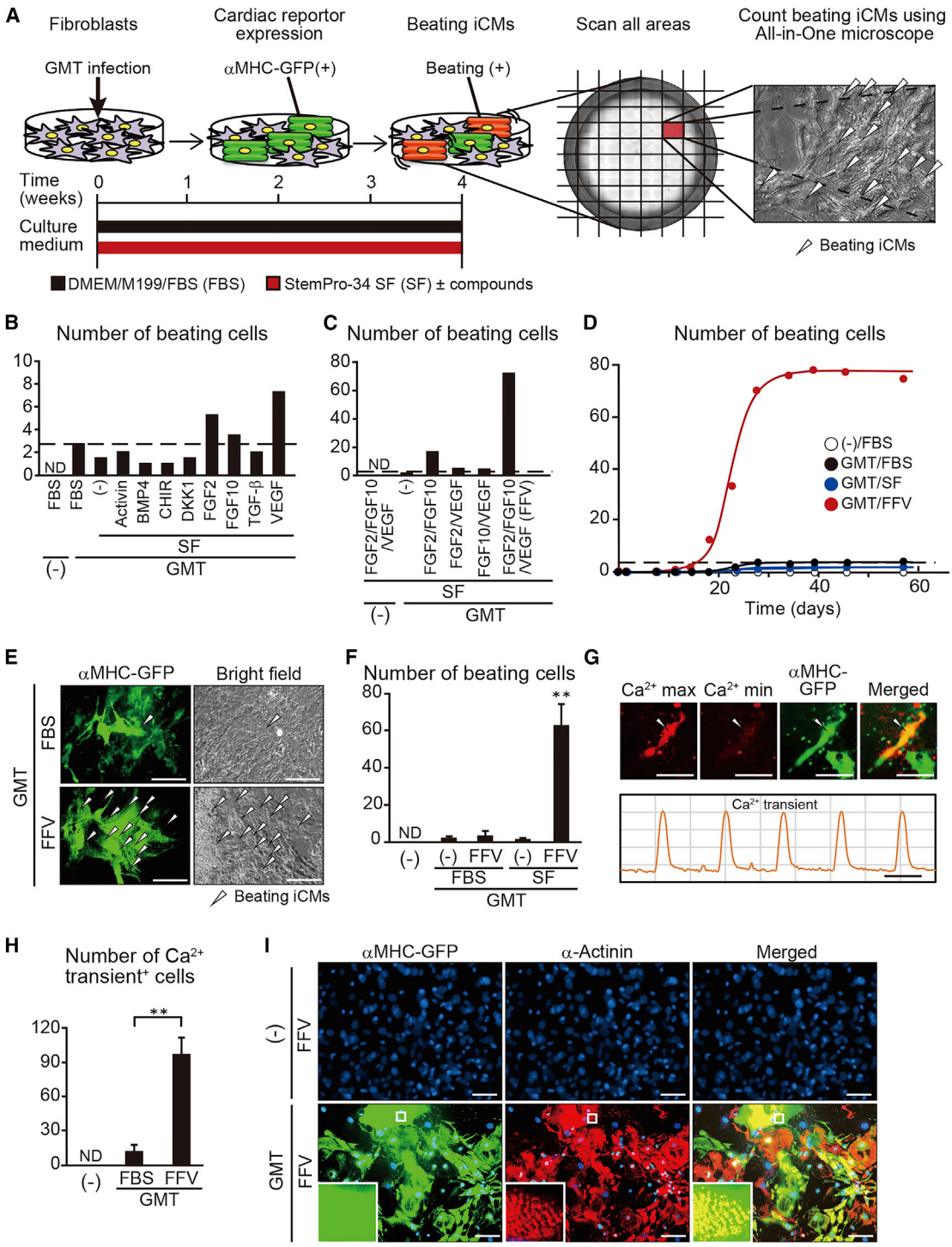

Figure 1. FFV Promoted GMT-Induced Cardiac Functional Reprogramming

(A) Schematic representation of the strategy used to test candidate compounds in SF (red bar) compared with FBS (black bar). The number of beating cells in each well was counted after 4 weeks.

(B and C) The number of spontaneously beating cells in each well after transduction of mock or GMT in MEFs. GMT-transduced cells were cultured under the indicated conditions.

(D) The number of spontaneously beating cells in each well after transduction of mock or GMT in FBS, SF, or FFV.

(legend continued on next page)