HHS Public Access

Author manuscript

J Mol Cell Cardiol. Author manuscript; available in PMC 2021 April 23.

Published in final edited form as:

J Mol Cell Cardiol. 2020 April ; 141: 105–106. doi:10.1016/j.yjmcc.2020.03.010.

Response to Zhao and Huang's Commentary Letter, *"Conversion of Human Cardiac Progenitor Cells using Reprogramming Factors into Heterogeneous Cardiac Pacemaker-like Cells"*, regarding our Manuscript: *"Conversion of Human Cardiac Progenitor Cells into Cardiac Pacemaker-like Cells"*

Robert J. Schwartz[b,1], **Bradley K. McConnell**[a,1,*]

[a]Department of Pharmacological and Pharmaceutical Sciences, College of Pharmacy, University of Houston, Houston, TX 77204-5037, USA

[b]Department of Biology and Biochemistry, University of Houston, Houston, TX 77204-5001, USA

We wish to comment on *Zhao and Huang's Commentary Letter* to the *Editor* regarding our research manuscript [1] entitled, *"Conversion of Human Cardiac Progenitor Cells into Cardiac Pacemaker-like Cells"*, where we showed that the *SHOX2, HCN2*, and *TBX5* (SHT5) combination of transcription factors and channel protein can be used to reprogram human cardiac progenitor cells (CPCs) into Pacemaker-like cells as a potential stem cell therapy for sick sinus syndrome (SSS).

As described in our Raghunathan et al. [1] manuscript, we used a screening strategy to test for reprogramming factors for the conversion of human CPCs into Pacemaker-like cells. Specifically, human transcription factors *SHOX2, TBX3, TBX5, TBX18*, and the channel protein *HCN2*, were transiently induced as single factors and in trio combinations into CPCs, first being transduced with the connexin 30.2 (CX30.2) mCherry reporter. As pointed out by *Zhao and Huang*, these same single factors (*SHOX2, TBX3, TBX5, TBX18*, and *HCN2*) and combination of factors (SHT3, SHT5, and SHT18) were also used to directly reprogram human adipogenic mesenchymal stem cells (hADMSCs) into pacemaker cells and Purkinje cells, as reported in the US Patent by Alt and Karimi [2]. In both, identical reprogramming factors were used since all the viral vectors and technology in these reports were developed in collaboration in the Schwartz laboratory. However, a key difference between the methodology of these studies is that in Raghunathan et al. [1], the hADMSCs were first reprogrammed using the human transcription factors *ETS2/MESP1* for the differentiation into CPCs [3]. In fact, we found at least an order of magnitude higher conversion rates when using CPCs, rather than hADMSCs, as the starting cells to test these reprogramming factors for the conversion into Pacemaker-like cells.

*Corresponding author at: Department of Pharmacological and Pharmaceutical Sciences, University of Houston College of Pharmacy, 4849 Calhoun Road, Health-2 (H2) Building, Room 5024, Houston, TX 77204-5037, USA. bkmcconn@central.uh.edu (B.K. McConnell).

[1]Co-senior authors.

Declaration of Competing Interest

The authors declare no conflicts of interest.

In addition, as reported in Raghunathan et al. [1], we performed RTPCR and RNA sequencing of SHOX2, HCN2, and TBX5 (SHT5) mCherry + and did not observe the conversion of our CPCs into Purkinje cells, as suggested by *Zhao and Huang*. Instead, SHT5 factors upregulated pacemaker specific gene and transcriptome expression, attributing to the pacemaker phenotype of these cells. Specifically, following screens for reporter CX30.2 mCherry gene activation and FACS enrichment, we observed the definitive expression of many pacemaker specific genes; including, CX30.2, *KCNN4, HCN4, HCN3, HCN1*, and *SCN3b* [4–9]. These findings suggest that the SHT5 combination of transcription factors is a much better candidate in driving the CPCs into Pacemaker-like cells than other combinations of transcription factors or individual factors by itself. Therefore, the schematic diagram by *Zhao and Huang's* showing the conversion of CPC into Purkinje cells is not supported by Raghunathan et al. [1] The findings of our study [1] nicely show that the SHT5 combination of transcription factors and channel proteins can be used to reprogram CPCs into Pacemaker-like cells towards the construction of biological pacemakers.

Furthermore, we perform single-cell RNA sequencing on the SHT5 activated cells to better understand the transcriptome of the individual cells within the total cell population. *Zhao and Huang* did not fully appreciate that over 500 cells SHT5 mCherry + cells were single-cell sequenced which revealed cellular enrichment of pacemaker specific markers including *SHOX2, GJD3 (CX30.2), TBX5, TBX3, BMP2, KCNN4* [10,11] the pacemaker specific transcripts for calcium and potassium channels including *KCND2, KCNK2*, *CACNB1, CACNA1A* [9] as well as the down-regulation of *NKX2.5* [12,13]; where *NKX2.5* is a specific ventricular conduction marker and highly enriched in Purkinje fibers [14]. Our Pacemaker-like cells did not display Purkinje signature markers. In fact, cells transfected with fewer than three factors did not express the pacemaker gene signature. Raghunathan *et al.* [1] did not show heterogeneity in the trio converted pacemakers. Thus, *Zhao and Huangs* argument for the SHT5 activated cells expressing Purkinje cell-specific markers is not supported by Raghunathan *et al.* [1] However, we did not test the idea that TBX factors are differentially expressed in nodes and their combinations may affect the transcriptome readout. Our plans are to test the combination of TBX factors in future studies.

Finally, we measured the *HCN* currents, characteristic of pacemaker cells, using patch-clamp recordings of the SHT5 activated cells and thus demonstrating the functional efficiency of these Pacemaker-like cells. Biological pacemaker cells are also sensitive to hormone regulation whereby β-adrenergic receptor (β-AR) stimulated cAMP binds to the *HCN* channel and thus contributing to the *HCN* pacemaker currents [15] . In fact, we demonstrated that the SHT5 cells are also sensitive to β-AR stimulation and thus further supporting the functional competence of these cells.

Overall, we demonstrated in Raghunathan et al. [1] that the SHT5 cocktail of transcription factors and channel protein reprogrammed CPCs into Pacemaker-like cells. The SHT5 factors resulted in the up-regulation of pacemaker specific gene expression and transcriptome expression, attributing the pacemaker phenotype to the cells. The SHT5 mCherry + cells also exhibited the funny current via HCN4 channels, attributing the functional characteristic of pacemaker cells. Thus, the findings of this study show that the SHT5 combination of transcription factors and channel protein can be used to reprogram

CPCs into Pacemaker-like cells thus providing a strategy for the construction of a biological pacemaker to restore function of the failing sinoatrial node (SAN) and treat sick sinus syndrome (SSS) as well as for other cardiac conduction diseases.

Funding

Research reported in this paper was supported in part by the National Heart, Lung, and Blood Institute (NHLBI) of the National Institutes of Health (NIH) [R15HL141963 (B.K.M.) and R15HL124458 (B.K.M.)], the American Heart Association (AHA) [18AIREA33960175 (to B.K.M.)], and a grant from Robert J. Kleberg, Jr. and Helen C. Kleberg Foundation (to B.K.M.). The Center for Advanced Science in Space supported research on the conversion of human adipogenic mesenchymal stem cells into cardiac progenitors (R.J.S.). The funders had no role in study design, data collection, and analysis, decision to publish, or preparation of the manuscript.

Research reported in this paper was supported in part by the National Heart, Lung, and Blood Institute (NHLBI) of the National Institutes of Health (NIH) [R15HL141963 (B.K.M.) and R15HL124458 (B.K.M.)], the American Heart Association (AHA) [18AIREA33960175 (to B.K.M.)], and a grant from Robert J. Kleberg, Jr. and Helen C. Kleberg Foundation (to B.K.M.). The Center for Advanced Science in Space supported research on the conversion of human adipogenic mesenchymal stem cells into cardiac progenitors (R.J.S.). The funders had no role in study design, data collection, and analysis, decision to publish, or preparation of the manuscript.

References

[1]. Raghunathan S, Islas JF, Mistretta B, Iyer D, Shi L, Gunaratne PH, Ko G, Schwartz RJ, McConnell BK, Conversion of human cardiac progenitor cells into cardiac pacemaker-like cells, J. Mol. Cell. Cardiol 138 (2019) 12–22. [PubMed: 31678351]

[2]. Alt E, Karimi T, Induced Pacemaker and Purkinje Cells from Adult Stem Cells, Patent No.: US 9,913,865 B2 (3 13, 2018).

[3]. Islas JF, Liu Y, Weng KC, Robertson MJ, Zhang S, Prejusa A, Harger J, Tikhomirova D, Chopra M, Iyer D, Mercola M, Oshima RG, Willerson JT, Potaman VN, Schwartz RJ, Transcription factors ETS2 and MESP1 transdifferentiate human dermal fibroblasts into cardiac progenitors, Proc. Natl. Acad. Sci. U. S. A 109 (32) (2012) 13016–13021. [PubMed: 22826236]

[4]. Stieber J, Herrmann S, Feil S, Loster J, Feil R, Biel M, Hofmann F, Ludwig A, The hyperpolarization-activated channel HCN4 is required for the generation of pacemaker action potentials in the embryonic heart, Proc. Natl. Acad. Sci. U. S. A 100 (25) (2003) 15235–15240. [PubMed: 14657344]

[5]. Hakim P, Brice N, Thresher R, Lawrence J, Zhang Y, Jackson AP, Grace AA, Huang CL, Scn3b knockout mice exhibit abnormal sino-atrial and cardiac conduction properties, Acta Physiol (Oxford) 198 (1) (2010) 47–59.

[6]. Kleger A, Liebau S, Calcium-activated potassium channels, cardiogenesis of pluripotent stem cells, and enrichment of pacemaker-like cells, Trends Cardiovasc. Med 21 (3) (2011) 74–83. [PubMed: 22626246]

[7]. Kleger A, Seufferlein T, Malan D, Tischendorf M, Storch A, Wolheim A, Latz S, Protze S, Porzner M, Proepper C, Brunner C, Katz SF, Varma Pusapati G, Bullinger L, Franz WM, Koehntop R, Giehl K, Spyrantis A, Wittekindt O, Lin Q, Zenke M, Fleischmann BK, Wartenberg M, Wobus AM, Boeckers TM, Liebau S, Modulation of calcium-activated potassium channels induces cardiogenesis of pluripotent stem cells and enrichment of pacemaker-like cells, Circulation 122 (18) (2010) 1823–1836. [PubMed: 20956206]

[8]. Christoffels VM, Smits GJ, Kispert A, Moorman AF, Development of the pacemaker tissues of the heart, Circ. Res 106 (2) (2010) 240–254. [PubMed: 20133910]

[9]. Bakker ML, Boukens BJ, Mommersteeg MT, Brons JF, Wakker V, Moorman AF, Christoffels VM, Transcription factor Tbx3 is required for the specification of the atrioventricular conduction system, Circ. Res 102 (11) (2008) 1340–1349. [PubMed: 18467625]

[10]. Vedantham V, New approaches to biological pacemakers: links to sinoatrial node development, Trends Mol. Med 21 (12) (2015) 749–761. [PubMed: 26611337]

[11]. Puskaric S, Schmitteckert S, Mori AD, Glaser A, Schneider KU, Bruneau BG, Blaschke RJ, Steinbeisser H, Rappold G, Shox2 mediates Tbx5 activity by regulating Bmp4 in the pacemaker region of the developing heart. Hum. Mol. Genet 19 (23) (2010) 4625–4633. [PubMed: 20858598]

[12]. Espinoza-Lewis RA, Liu H, Sun C, Chen C, Jiao K, Chen Y, Ectopic expression of Nkx2.5 suppresses the formation of the sinoatrial node in mice, Dev. Biol 356 (2) (2011) 359–369. [PubMed: 21640717]

[13]. Mommersteeg MT, Hoogaars WM, Prall OW, de Gier-de Vries C, Wiese C, Clout DE, Papaioannou VE, Brown NA, Harvey RP, Moorman AF, Christoffels VM, Molecular pathway for the localized formation of the sinoatrial node, Circ. Res 100 (3) (2007) 354–362. [PubMed: 17234970]

[14]. Maass K, Shekhar A, Lu J, Kang G, See F, Kim EE, Delgado C, Shen S, Cohen L, Fishman GI, Isolation and characterization of embryonic stem cell-derived cardiac Purkinje cells, Stem Cells 33 (4) (2015) 1102–1112. [PubMed: 25524238]

[15]. Biel M, Michalakis S, Cyclic nucleotide-gated channels, Handb. Exp. Pharmacol 191 (2009) 111–136.