Facilitating Cardiac Atrial Appendage Stem Cells' Road to the Clinic: Optimizing a Cardiomyogenic Differentiation Protocol

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I. Abstract

Heart failure is primarily caused by myocardial infarction (MI). MI results in irreversible loss of massive amounts of cardiomyocytes. To date, there is no curative therapy to restore cardiac function. However, a stem cell type discovered by our research group, the cardiac atrial appendage stem cell (CASCs), presents itself as a unique candidate for myocardial regeneration (1). For the CASCs therapy to become clinically applicable *in vitro* potency testing is required for quality control purposes. To this end, the commercial STEMdiff™ Cardiomyocyte Differentiation Kit was compared to a tailor made protocol for cardiac differentiation of induced pluripotent stem cells (Burridge). Cardiac marker expression was measured using qRT-PCR and immunocytochemistry. Analysis showed that differentiation according to STEMdiff™ induced significantly higher expression of early cardiac markers transcription factor GATA-4 (1.2±0.1 vs. 0.7±0.1, p=0.01), T-box 5 (TBX5) (2.4±0.3 vs. 0.7±0.1, p=0.008), and myocyte enhancer factor 2C (MEFC2C) (3.3±0.5 vs. 1.7±0.2, p=0.008) compared to the Burridge protocol. No significant differences were observed between the expression of late cardiac markers myosin heavy chain 6 (MYH6) and troponin T (TnT) in CASCs differentiated with STEMdiff™ compared to Burridge. These results revealed the STEMdiff™ kit to be the most efficient way to differentiate CASCs *in vitro*. Immunocytochemical staining revealed the presence of early marker homeobox protein Nkx2.5 (Nkx2.5) and TnT on protein level. Furthermore, troponin I (TnI) expression, another major protein of the contractile apparatus, was also observed. Light microscopy images of CASCs differentiated with the STEMdiff™ kit showed cylinder shaped cardiomyocyte-like cells with typical cross striations. The findings of this study resulted in the establishment of a method for differentiating CASCs towards a cardiomyocyte-like genotype *in vitro*, using a chemically defined medium. Hence, these findings contribute to the development of potency testing for CASCs in a GMP setting, thereby augmenting CASCs' clinical translational value.

II. Image

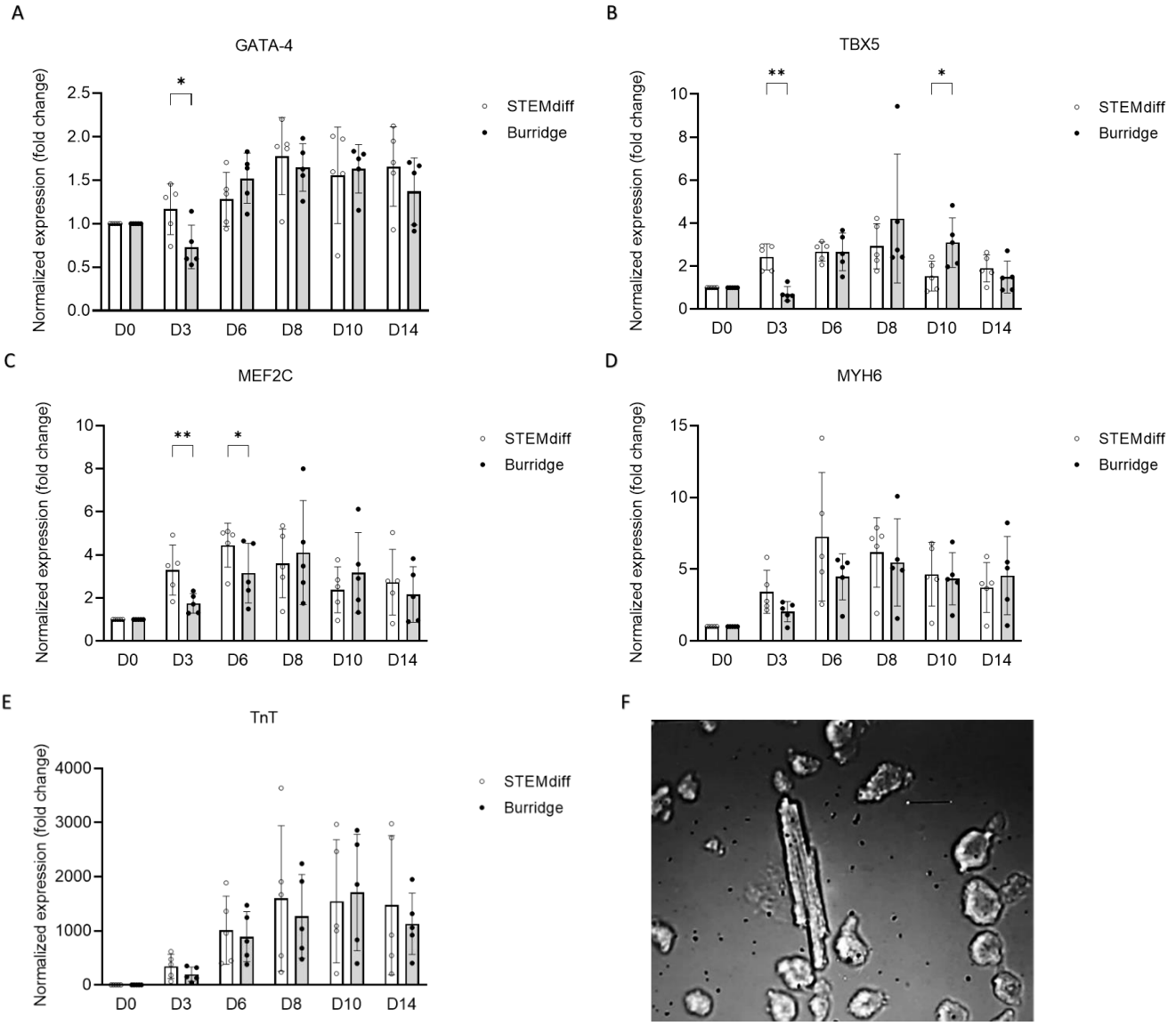


Figure 1. Comparing cardiomyocyte marker expression in CASCs differentiated according to the STEMdiff™ and Burridge protocol. qRT-PCR analysis of mRNA expression levels of (A – C) early and (D – E) late cardiac differentiation markers during CASCs differentiation with the STEMdiff™ (n=5) and Burridge (n=5) protocol. (A) GATA-4 = GATA binding protein 4; (B) TBX5 = T-box 5; (C) MEF2C = Myocyte-specific enhancer factor 2C; (D) MYH6 = Myosine heavy chain 6; (E) TnT = Troponin T. (F) = light microscopy image of CASCs differentiated according to the STEMdiff™ kit. Data are represented as mean ± SEM and were normalized to the housekeeping gene phosphoglycerate kinase 1 (PGK1). Data were analyzed using a Two-Way Anova test and subsequent Šidák's Multiple Comparisons test, * denotes $p<0.01$, ** denotes $p<0.001$.

III. References

1. Fanton Y, Robic B, Rummens JL, Daniels A, Windmolders S, Willems L, et al. Cardiac atrial appendage stem cells engraft and differentiate into cardiomyocytes in vivo: A new tool for cardiac repair after MI. Int J Cardiol. 2015;201:10-9.

IV. Biography

Ellen Heeren has obtained her master's degree in biomedical sciences from Hasselt University, Belgium. She completed an internship at the University of California, San Francisco, United States, as part of her master's thesis. Although being at the mere start of her career, she has participated in two peer reviewed articles.

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