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Editorial
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On Track for Cardiac Subtype Specific Differentiation of Embryonic Stem Cells

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Editorial

The field of stem cell research has gained increasing attention in medicine and biomedical sciences over the last decade. Since researchers all over the world found out about the huge potential of stem cells in regenerating damaged organs and in generating engineered tissue with new abilities, the number of research groups using these stem cell systems grew significantly. However, even if the effort in producing fully functional tissue or organoids rose, several problems remain largely unsolved.

As cardiac diseases and dysfunctions increase with aging in modern societies, the regeneration of heart compartments or their replacements with engineered tissue is a main focus in stem cell research. The production of cardiac tissue with similar physiological and morphological properties to native human cells improved, where as the production of cells with defined phenotypes still remains challenging [1]. Postulated a technique allowing the generation of self-beating cardiac tissue in monolayers and 3D structures on feeder-free conditions. The result was an increase of tissue material quantity, which led to enlarged experimental setups and the ability for more detailed investigations of stem cell derived cardiomyocytes.

Cardiomyocytes derived by this technique showed a high amount of ventricular-like cells and low amounts of atrial- and pacemaker-like cells. With longer maturation, the amount of ventricular-like cells could be increased above 70%, leading to a sufficient model to investigate ventricular diseases. Further, these cells are well suited to test drug candidates for their cardiac efficiency and their safety [1].In contrast to ventricular-like cells, the generation of cells with clear atrial- or pacemaker-like physiology remained a challenge, until two groups reported on production of these cells in 2017.

The principle of cardiac differentiation is identical for all three cell types (ventricular-, atrial- and pacemaker-like cells). Crucial for success is the appropriate timing of activation of the WNT- and BMP-pathway by supplementing agents like CHIR9 and BMP4 to the early differentiation medium. The activation of these pathways leads to mesodermal specification of the HiPSC, detectable by early mesodermal markers as EOMES and SP5 [1]. In the second phase of differentiation, an additional specification step of the cells occurs, in which cells develop into cardiac mesoderm. For this purpose inhibition of the WNT signaling by C59 or similar detergents is required to shut-off the smad2/3 pathway, which leads to the suppression of pluripotency genes like NANOG and OCT4 [2,3]. Furthermore, cells in this phase of differentiation have the ability to either follow the atrial-like fate or stay non-specified [4].

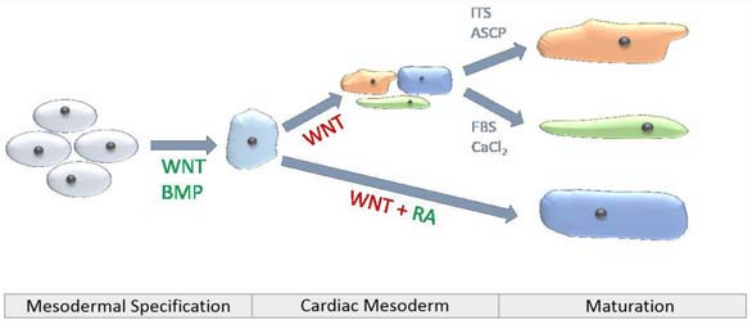

Figure: Schematic Illustration; How to produce specific cardiac cell types from induced pluripotent stem cells. An on-switch of WNT and BMP in the early differentiation is mandatory followed by an optional WNT off-switch or retinoic acid (RA) on-switch to push the cells into the desired fate. RA leads to an atrial specification, while a WNT-inhibition followed by appropriate maturation results in ventricular or pacemaker specification.

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Recently, [4,5] showed, that adding retinoic acid (RA) in early stage development of cardiac tissue, there by activating retinaldehyde dehydrogenase 2 (RALDH2), pushes HiPSC's towards an atrial fate. The addition of RA showed significant down regulation of ventricular genes like MYL2 and upregulation of the atrial-specific ion channel KCNJ3 (GIRK). The mechanism of atrial specification via RA-signaling is still unclear, but the expression of RA-receptors (RAR-α; -β and -γ) in early cardiac development indicates its importance [4]. Subsequent removal of RA after 5 days of differentiation followed by two weeks of maturation, leads to generation of cardiomyocytes expressing atrial-specific markers like KCNA5 and KCNJ3, proving the postulated differentiation protocol [4,6].

The differentiation into ventricular- or pacemaker-like cells is based on another maturation procedure. After achieving a cell layer of cardiac mesoderm, further maturation pushes cells into the desired fate. After shutdown of the WNT pathway, the result is a mixed culture of cells, consisting of different, early cardiac cell-types still open for their final fate [1,7]. Showed that further maturation in media containing insulin-transferrin-selenium (ITS) and phospho-ascorbate (ASCP) led to the enrichment of ventricular-like cells in culture, expressing typical ventricular markers like MYL2 and IRX4. The same data set also showed that long-term maturation of these ventricular-like cells of about 8 weeks led to similar marker gene expressions than in mature human heart samples, indicating that this maturation system produces cardiomyocytes in cell culture similar to adult human cardiomyocytes [7]. Clearly, these stem cell derived cardiac myocytes are not identical to adult human cardiomyocytes and interpretations always require careful re-evaluation!

The enrichment of pacemaker-like cells follows a specific maturation process aswell. While it was long-time impossible to artificially produce these cells, recent data from Schweizer et al. indicate that pacemaker generation is simpler and straighter forward than expected. After the differentiation of cardiac mesoderm, similar to ventricular maturation, cells were cultured in a medium containing high yield of FBS (10-20%) and $CaCl_2$ [8].After a maturation process of 8 weeks, the cells were analyzed for pacemaker-specific marker genes and specific ion-channels via qPCR. A highly significant upregulation of the cell specificity marker SHOX2 in combination with a down regulation of markers like NKX2.5 and Tbx5 normally promoting ventricular development indicates the versatility of this maturation method [8-10]. Further investigation of pacemaker-specific ion channels, such as HCN1 and HCN4, aswell as the specific connexin Cx45 and the sodium-calcium exchanger NCX1, which is crucial for the physiological pacemaker function, revealed an upregulation of all these proteins. The pacemaker marker-proteins reached sufficient expression levels after 8 weeks of maturation, which were well comparable to human SAN tissue. In the case of NCX1 the expression level even exceeded the level in human SAN by almost 4-fold [8]. The presence of $CaCl_2$ and high levels of FBS indicates that an increased Ca^{2+}-homeostasis and a raised energy supply support the maturation into pacemaker-like cells. However, the exact principle behind this maturation is unclear yet and therefore additional, careful investigation has to be done.

Latest investigations and recent data support the hypothesis, that the generation of cell systems of high purity is not a challenge of the future anymore. The newest differentiation protocols and the growing understanding in the importance of cell maturation made it possible to enrich the three major cardiac cell types in petri dish experimentations. While still a lot of work concerning an even higher purity, upscaling and optimizing physiological processes needs to be done, the latest findings exert huge improvements in the field of personalized medicine and drug research.

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