Review Article

Prospective In Vitro Models of Channelopathies and Cardiomyopathies

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An in vitro heart disease model is a promising model used for identifying the genes responsible for the disease, evaluating the effects of drugs, and regenerative medicine. We were interested in disease models using a patient-induced pluripotent stem (iPS) cell-derived cardiomyocytes because of their similarity to a patient’s tissues. However, as these studies have just begun, we would like to review the literature in this and other related fields and discuss the path for future models of molecular biology that can help to diagnose and cure diseases, and its involvement in regenerative medicine. The heterogeneity of iPS cells and/or differentiated cardiomyocytes has been recognized as a problem. An in vitro heart disease model should be evaluated using molecular biological analyses, such as mRNA and micro-RNA expression profiles and proteomic analysis.

1. Introduction

Most of the genes responsible for congenital heart diseases have been identified with genetic studies, where healthy individuals and patients’ genes sequences were compared to find mutations. The responsible genes were then subjected to functional analyses, using knock-out mouse and/or other animals to make a disease model which possessed the mutated genes [1, 2].

Since their establishment [3–5], iPS cells have been used to make in vitro disease models because of the difficulty in using a patient’s cells or tissues, especially from the heart [6–9]. Transfection of mutated genes into a normal parent cell prior to formation of iPS cells has also been used to make an in vitro disease model. Thus, iPS cells or differentiated cells containing the mutated gene can be compared with parent cells that do not have the mutated gene. ES cells and iPS cells differentiate into heart cells more easily than adult cardiac stem cells in both mice and humans because of their multipotency and pluripotency characteristics. Therefore, these cells have been used in regenerative medicine studies [10–12]. Although cardiac stem cells have advantage for in vivo regenerative medicine [13, 14], heterogeneity was observed in long-term cultures in our in vitro cultures [15]. A previous report showed that immature cardiomyocytes were obtained in vitro differentiation [16], suggesting the limitations of using adult stem cells as a cell source for in vitro disease model. Taken together, ES cells/iPS cells provide a better cell source of cardiomyocytes required for in vitro disease models.

In heart disease, iPS cells from Long-QT-syndrome-(LQTS-)type1 [17] and LQTS-type2 [18] patients were made and differentiated cardiomyocytes were obtained from these iPS cells. These cardiomyocytes worked as in vitro heart disease models since they possessed similar characters to patients’ cardiomyocytes. LQT1 and LQT2 are caused by missense mutations of the KCNH1 and KCNH2 gene, respectively. These mutations in potassium channels lead to QT interval prolongation [19]. Interestingly, the differentiated cardiomyocytes also showed marked arrhythmogenicity and early afterdepolarizations [18]. Potassium channel activators, such as PD118057, cured prolonged action potentials of LQT2-hiPS cell-derived cardiomyocytes [20]. Cardiomyocytes derived from patients’ fibroblasts, or other somatic cells, are gaining attention as promising models to discover drug targets for disease.
The differentiated cardiomyocytes from murine iPS cells, mutated with the LQT3 gene (Scn5aΔ/Δ), showed prolonged action potentials because of a Na channel dysfunction mutation in an LQTS-type3 patient [21], suggesting even murine iPS-derived cardiomyocytes can be used for an in vitro disease model. iPS cell-derived cardiomyocytes from Timothy syndrome showed irregular contractility consistent with the disease phenotype [22]. At least 13 LQTS genes have been reported so far, and similar abnormalities in iPS-derived cardiomyocytes from patients can be anticipated.

Channelopathies have been currently used as in vitro disease models because of the development of systematic current measurements. Another recent model from channelopathy was catecholaminergic polymorphic ventricular tachycardia (CPVT), carrying a novel mutation (S406L) of the ryanodine receptor (RYR) 2 which reduced sarcoplasmic reticular (SR) Ca\(^{2+}\) content to levels lower than control myocytes. In this case, Dantrolene is a drug rescued arrhythmogenic phenotype [23]. LEOPARD (lentigines, electrocardiographic abnormalities, ocular hypertelorism, pulmonary valve stenosis, abnormal genitalia, retardation of growth, and deafness) syndrome is caused by a different missense mutation of the PTPN11 gene (T468M and Y279C are the most recurrent). Differentiated cardiomyocytes from these patients were larger than wt-iPS-cell- or ES-cell-derived cardiomyocytes, which correspond to the disease phenotype of LEOPARD cardiac hypertrophy [24].

Another attractive method can be direct programming into stem cells/progenitors/cardiomyocytes from patients’ somatic cells. iPS cells induced from adult neural stem cells with only one transcription factor (TF), Oct4, were similar to ES cells [25]; therefore, primitive cells may be more suitable than differentiated cells to make iPS cells with only one factor introduction. Transient introduction of Yamanaka 4 factors (Oct3/4, Sox2, Klf4, and c-myc) and immediate growth factors, mainly bone morphogenetic protein 4 (BMP4), to cultured cells adequately directed cardiomyogenesis [26]. Interestingly, direct reprogramming from fibroblasts into cardiomyocytes was successful using 3 TFs, which are associated with cardiomyogenesis [27], which is another possible method of producing cardiomyocytes.

2. Generation of iPS Cells from Patients

Although a retrovirus was originally used, recently there have been several methods of reprogramming developed to introduce Yamanaka 4 factors (Figure 1). The Sendai-virus [28, 29], transient transfection of mRNA [30, 31], is more attractive than conventional retroviral infections because of safety, which is important for regenerative medicine and also in vitro models. If reprogramming vectors are integrated into the host genome, tracking the location can be difficult. Moreover, additional artifacts are also a concern. Recent studies show that epigenetic modulators such as the histone deacetylase inhibitor, valproic acid (VPA) can affect reprogramming efficiency [32]. In this way, only two factors (Sox2 and Oct4) efficiently induce iPS cells [33]. The butyrate [34] DNA methyl transferase inhibitor, RG108 [35], improves the efficiency of skeletal myoblast reprogramming. Interestingly, cardiomyocytes differentiated from these skeletal myoblast-derived iPSs (SiPS) improved the cardiac function of an infarcted heart without tumorgenesis [35, 36]. Epigenetic studies of reprogramming and stemness have attracted the interest of many researchers [37–40]. Indeed, hot spots are investigated that are difficult to methylate [41]. Therefore,
more efficiency is expected by identifying and modifying these spots.

Congenital heart diseases, modified from the work of Ackerman et al. [42], are summarized in Table 1. The diseases of channelopathies and cardiomyopathies are listed and summarized with experts evaluation, “is recommended” or “not is recommended,” according to the present characterization of gene mutations. Currently, channelopathies have been well characterized because of systematic measurements of cardiomyocytes or beating embryonic bodies (EBs). These diseases are candidates for in vitro models from iPS cells. Recently, iPS cell-derived cardiomyocytes from Pompe disease, known as a glycogen storage disease, were established and were revealed to have higher glycogen contents than hESC and control iPS-derived cardiomyocytes [43]. The generation of iPS-derived cardiomyocytes from these patients is expected to provide important information about these diseases.

3. Generation of Cardiomyocytes from iPS Cells

The differentiation method from iPS cells into cardiomyocytes basically follows the protocol of embryonic stem (ES) cells, using embryonic bodies (EBs, see Figure 2). Yang et al. showed that KDRlow/C-KIT neg EBs differentiated into cardiomyocyte lineages and became NKX2.5, ISL1, TBX5 positive but not KDRlow/C-KITpos or KDRneg/C-KITpos [44]. The combination of activin A, BMP 4, basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), and Dickkopf homolog 1 (DKK1) in a serum-free media was necessary for cardiomyogenesis. Likewise, addition of Wnt inhibitors to BMP 4 enhanced cardiomyogenesis [45]. These activin/nodal and BMP signaling pathways promote cardiac differentiation in a stage-specific manner [46]. The role of c-kit may be different even in the embryonic stage, since c-kit high-expressing cells became cardiomyocytes and other cardiac cell lineages near birth [47]. The level and timing of c-kit expression can change its role [48]. Flk1+ cells from EB clusters are produced in ES cell cultures without LIF, and cardiac progenitors and cardiovascular cells were also formed from these EB clusters [49, 50].

Cardiomyocytes obtained from iPS cells were functionally similar to ES cells-derived cardiomyocytes [51], and multiple type action potential (nodal, atrial, and ventricular) phenotypes were observed [52]. Overall, the gene expression profiles of iPS cells were similar to ES cells, but differentiation direction and efficiency were variable [53, 54]. Overall, iPS-cell-derived cardiomyocytes have similar contractile behaviors to ES cell-derived cardiomyocytes but are significantly different from native tissues from comparable ages [52]. However, the drug effect on iPS-cell-derived cardiomyocytes is similar to cardiomyocytes derived from hES cells [55]. As a cell source, ventricular cardiomyocytes produced more cardiomyocytes than somatic cells such as tail-tip fibroblasts [56]. The variability of differentiation among the cell lines has been previously reported [57]. The heterogeneity of iPS-derived cardiomyocytes is a problem for establishing good models [58]. One of the solutions is to obtain extremely pure cardiomyocytes to eliminate heterogeneity as much as possible. Ma et al. selected highly purified iPS cell-derived cardiomyocytes using blastcindin resistance gene expression controlled from the cardiac-specific endogenous MYH6 promoter and investigated drug electrophysical properties [59]. Another method used to eliminate heterogeneity was to establish a systematic protocol which produced highly purified cardiomyocytes (more than 90%) by optimization of the culture condition [60]. Cao et al. reported that ascorbic acid robustly enhanced cardiomyogenesis of all 11 lines so that differences were smaller [61]. Ascorbic acid functioned to proliferate cardiomyocyte progenitors. Ribosomal S6 kinase [62] and mitogen-activated protein kinase (MAPK) activities [63] affected cardiomyogenesis. Some small molecules had been known to have effect on cardiomyogenesis. Previously investigated effects of 36 small molecules using ES cells were summarized [64]. In addition to that, recently, small molecule, dorsomorphin, an inhibitor of BMP signaling [65], and XAV929, an inhibitor of Wnt/β signaling [45, 66], promoted cardiomyogenesis. Cyclosporin-A [67], sulfonyl hydrazine-1 [68], and even a simple dissociation of EBs [69] enhanced cardiomyogenesis. These molecules will help to accelerate cardiomyogenesis. However, a more concise profiling of molecular signatures is necessary to evaluate maturity and function.

Recently, a unique method to purify cardiomyocytes using the high number of mitochondria within cardiomyocytes was reported [70, 71]. In this method, genetic engineering is not required, and damage to cells should be decreased. On the other hand, another method was established using the signal-regulatory protein alpha (SIRPA), which can select immature cardiomyocytes which have fewer mitochondria [72].

4. Future Model of Heart Disease Composed of the iPS-Cells-Derived Cardiomyocytes

Very recently, in a genomic mutation heterozygous for polyostotic kidney disease 1 (Pkd1), the deletion is restored by spontaneous mitotic recombination [73]. Indeed, the frequency of genetic repair events by spontaneous mitotic recombination in pluripotent stem cells is higher than that in somatic cells [74]. Interestingly, from the RT-PCR data from Cheng et al., not only wild-type iPS cells but also +/- iPS were detected [73]. These results are also important to heart diseases, especially for dominant mutation. Comparison of these (+/+ and +/-) cells can be perfect because there is no genetic background difference, since they are derived from the same person.

Currently, several multielectrode array systems for in vitro extracellular electrophysiology are available for QT prolongation screening with iPS cell-derived myocytes. In order to screen the function of mutated channels located on subcellular organelles such as the RYR2, fluctuations in intracellular Ca2+ concentrations should be measured. Development of a user-friendly detection system for stimulation and recording of such channels in patient cardiomyocytes is
### Table 1: HRS/EHRA Expert Consensus Statement on Genetic Testing (Heart Rhythm 2011; 8:1308–1339).

| Cardiac Channelopathy/Cardiomyopathy | Diagnostic implications of genetic testing | Class I “is recommended” | Class IIa “can be useful” | Class IIb “may be considered” | Class III “is not recommended” | Testing genes | Common disease genes | Genes | % of disease |
|-------------------------------------|------------------------------------------|-------------------------|--------------------------|-------------------------------|--------------------------------|----------------|----------------------|-------|-------------|
| **Long QT syndrome (LQTS)**         | Patient in whom a cardiologist has established a strong clinical index of suspicion for LQTS<br>Asymptomatic patient with QT prolongation in the absence of other clinical conditions that might prolong the QT interval on serial 12-lead ECGs defined as QTc >480 ms (prepuberty) or >500 ms (adults)<br>Asymptomatic patient with otherwise idiopathic QTc values >460 ms (prepuberty) or >480 ms (adults) on serial 12-lead ECGs<br>Family members and other appropriate relatives subsequently following the identification of the LQTS-causative mutation in an index case | ○                       | ○                        | ○                             | ○                              | KCNQ1 (LQT1) 30–35%<br>KCNH2 (LQT2) 25–40%<br>SCN5A (LQT3) 5–10%<br>LQT4-13 >5% | Mutation-specific |
| **Catecholaminergic polymorphic ventricular tachycardia (CPVT)** | Patient in whom a cardiologist has established a clinical index of suspicion for CPVT<br>Family members and appropriate relatives following identification of the CPVT-causative mutation in an index case | ○                       | ○                        | ○                             | ○                              | Comprehensive or CPVT1 and CPVT2 RYR2 (CPVT1) 60% | Mutation-specific<br>CASQ2 (CPVT2) 3–5% |
| **Brugada syndrome (BrS)**          | Family members and appropriate relatives following identification of the BrS-causative mutation in an index case<br>Patient in whom a cardiologist has established a clinical index of suspicion for BrS<br>The setting of an isolated type 2 or type 3 Brugada ECG pattern | ○                       | ○                        | ○                             | ○                              | Comprehensive or SCN5A SCN5A (BrS1) 20–30% | Mutation-specific<br>— |
| Cardiac Channelopathy/Cardiomyopathy | Diagnostic implications of genetic testing | Class I “is recommended” | Class IIa “can be useful” | Class IIb “may be considered” | Class III “is not recommended” | Testing genes | Common disease genes % of disease |
|------------------------------------------|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Progressive cardiac conduction disorders (CCD) | Family members and appropriate relatives following the identification of the CCD-causative mutation in an index case Patients with either isolated CCD or CCD with concomitant congenital heart disease, especially when there is documentation of a positive family history of CCD |  |  |  | Mutation-specific SCN5A 5%  | SCN5A and TRPM4  |  |
| Short QT syndrome (SQTS) | Family members and appropriate relatives following the identification of the SQTS-causative mutation in an index case Patient in whom a cardiologist has established a strong clinical index of suspicion for SQTS based on examination of the patient’s clinical history, family history, and electrocardiographic phenotype. |  |  |  | Mutation-specific KCNH2 (SQT1) >5%  | KCNJ2 (SQT3) >5%  | SCQT1-3 |
| Atrial fibrillation (AF) | Genetic testing is not indicated for atrial fibrillation at this time. |  |  |  | None of the known disease-associated genes has been shown to account for >5% of this disease. |  |  |
| Hypertrophic cardiomyopathy (HCM) | Patient in whom a cardiologist has established a clinical diagnosis of HCM |  |  |  | Comprehensive or targeted (MYBPC3, MYH7, TNN13, TNN2, and TPM1) MYBPC3 20–45% MYH7 15–20% TNN2 1–7% TNN13 1–7% TPM1 >5%  |  |
|  | Family members and appropriate relatives following identification of the HCM-causative mutation in an index case |  |  |  | Mutation-specific TPM1 >5%  |  |  |
| Arrhythmogenic cardiomyopathy (ACM)/Arrhythmogenic right ventricular cardiomyopathy (ARVC) | Family members and appropriate relatives following identification of the ACM/ARVC-causative mutation in an index case Patients satisfying task force diagnostic criteria for ACM/ARVC Patients with possible ACM/ARVC (1 major or 2 minor criteria) according to the 2010 task force criteria (European Heart Journal) Patients with only a single minor criterion according to the 2010 task force criteria |  |  |  | Mutation-specific PKP2 25–40%  | DSG2 5–10%  | DSC2 2–12%  |
|  |  |  |  |  | Comprehensive or targeted (DSG2, DSP, JUP, PKP2, and TMEM43) DSG2 5–10% DSP 2–12% DSC2 2–7% PKP2 25–40% TMEM43 >5%  |  |  |
| Cardiac Channelopathy/Cardiomyopathy | Diagnostic implications of genetic testing | Class I “is recommended” | Class IIa “can be useful” | Class IIb “may be considered” | Class III “is not recommended” | Testing genes | Common disease genes |
|--------------------------------------|------------------------------------------|--------------------------|--------------------------|-------------------------------|--------------------------------|-------------|---------------------|
| Dilated cardiomyopathy (DCM)         | Patients with DCM and significant cardiac conduction disease (i.e., first-, second-, or third-degree heart block) and/or a family history of premature unexpected sudden death Family members and appropriate relatives following the identification of a DCM-causative mutation in the index case Patients with familial DCM to confirm the diagnosis, to recognize those who are at highest risk of arrhythmia and syndromic features, to facilitate cascade screening within the family, and to help with family planning | ○                         |                          |                               |                                |             | LMNA SCN5A >5%       |
|                                      |                                          |                          |                          |                               |                                |             |                     |
| Left ventricular noncompaction (LVNC) | Family members and appropriate relatives following the identification of an LVNC-causative mutation in the index case Patients in whom a cardiologist has established a clinical diagnosis of LVNC | ○                         |                          |                               |                          Mutation-specific | LBD3 ~5%   | LBD3, and so forth  |
|                                      |                                          |                          |                          |                               |                                |             |                     |
| Restrictive cardiomyopathy (RCM)     | Family members and appropriate relatives following the identification of an RCM-causative mutation in the index case Patients in whom a cardiologist has established a clinical index of suspicion for RCM | ○                         |                          |                               |                          Mutation-specific | β-MHC ~5%  | MYH7 TNN13 TNN2   |
|                                      |                                          |                          |                          |                               |                                |             |                     |
| Out-of-hospital cardiac arrest survivors | The survivor of an Unexplained Out-of-Hospital Cardiac Arrest Routine genetic testing, in the absence of a clinical index of suspicion for a specific cardiomyopathy or channelopathy | ○                         |                          |                               |                          Appropriate genes following diagnosis of the survivor | RYR2 10–15% | KCNQ1 5–10% KCH2 ~5% |
|                                      |                                          |                          |                          |                               |                                |             |                     |
| For all SUDS and SIDS cases, collection of a tissue sample | ○                         |                          |                          |                               |                                |             | comprehensive or targeted (RYR2, KCNQ1, KCH2, and SCN5A) RYR2 SCN5A 3–5% |
| Postmortem genetic testing in sudden death cases (SUD/SIDS) | In the setting of autopsy negative SUDS | ○                         |                          |                               |                          |             |                    |
|                                      | Family members and other appropriate relatives following identification of a SUDS-causative mutation in the decedent | ○                         |                          |                               |                          Mutation-specific |             |                     |

HRS: the Heart Rhythm Society; EHRA: European Heart Rhythm Association. We summarized their tables with permission.
expected [75], as well as sensor techniques and bioanalytical approaches for cardiotoxicity testing [76].

Because tissues are three dimensional, 3D in vitro models can be made using scaffolds [77] or cell sheets [78, 79] in the near future. The process of tissue formation can be observed and compared with normal tissue formation. For this purpose, not only cardiomyocytes, but also other cardiac cells should be developed. Hearts contain a vascular system, which is difficult to constitute using a 2D model; however, it may be possible using a 3D model [80].

Gene expression levels [81, 82] and protein profiles [83] can be analyzed similarly to other cell culture systems. Recent progresses in the investigation of micro-RNA have provided information on the process to disease. Micro-RNA can be biomarkers for cardiovascular diseases [84] and have gained attention as regulators for cardiac injury and protection [85]. Cardiac differentiation by BMP from cardiac progenitors was mediated by micro-RNA [86]. In fact, micro-RNA is associated with cell fate decision [87]. In cardiomyocyte differentiation, miR-1 and miR-133 are upregulated, and miR-499 promotes cardiomyogenesis [88]. Thus, the state of the disease can be more precisely assessed by micro-RNA expression. Networks of mRNA and micro-RNA to determine human cardiomyocytes differentiation were investigated [89], and such attempts should be required, and analytical development is also required to fit this. Not only gene expression, but also global methylation analysis of CpG islands and the identification of non-CpG islands can be performed using next generation sequences is also useful. Other epigenetic approaches should make progress in this field [90]. Because some differences were reported between iPS-cell-derived cardiomyocytes and tissue-derived cardiomyocytes, where iPS cell-derived cardiomyocytes were more immature than tissue derived cardiomyocytes, further studies should be performed to evaluate their quality.

5. Conclusion

Using iPS cells for in vitro heart disease models is a promising method for evaluating drug effects. Many disease models should be constructed. However, further studies are necessary to evaluate cardiomyocytes in terms of heterogeneity using molecular biological analyses derived from the patient’s tissues.

Figure 2: The methodology for in vitro cardiomyocyte differentiation.

References

[1] B. Ostadal, M. Nagano, and N. S. Dhall, Eds., Genes and Cardiovascular Function, Springer, New York, NY, USA, 2011.
[2] M. A. Razzache, T. Nishizawa, Y. Komoike et al., “Germline gain-of-function mutations in RAF1 cause Noonan syndrome,” Nature Genetics, vol. 39, no. 8, pp. 1013–1017, 2007.
[3] K. Takahashi and S. Yamanaka, “Induction of pluripotent stem cells from mouse embryonic and adult fibroblasts cultures by defined factors,” Cell, vol. 126, no. 4, pp. 663–676, 2006.
[4] K. Takahashi, K. Tanabe, M. Ohnuki et al., “Induction of pluripotent stem cells from human adult fibroblasts by defined factors,” Cell, vol. 131, no. 5, pp. 861–872, 2007.
[5] I. H. Park, R. Zhao, J. A. West et al., “Reprogramming of human somatic cells to pluripotency with defined factors,” Nature, vol. 451, no. 7175, pp. 141–146, 2008.
[6] K. Narsinh, K. H. Narsinh, and J. C. Wu, “Derivation of human induced pluripotent stem cells for cardiovascular disease modeling,” Circulation Research, vol. 108, no. 9, pp. 1146–1156, 2011.
[7] S. J. Kattman, C. H. Koonce, B. J. Swanson, and B. D. Anson, “Stem cells and their derivatives: a renaissance in cardiovascular translational research,” Journal of Cardiovascular Translational Research, vol. 4, no. 1, pp. 66–72, 2011.
[8] C. Dambrot, R. Passier, D. Atsma, and C. L. Mummery, “Cardiomyocyte differentiation of pluripotent stem cells and their use as cardiac disease models,” Biochemical Journal, vol. 434, no. 1, pp. 25–35, 2011.
[9] H. C. Kang, “Disease-specific pluripotent stem cells,” Korean Journal of Pediatrics, vol. 53, no. 8, pp. 786–789, 2010.
[10] Y. Yoshida and S. Yamanaka, “IPS cells: a source of cardiac regeneration,” Journal of Molecular and Cellular Cardiology, vol. 50, no. 2, pp. 327–332, 2011.
[11] T. J. Kamp and G. E. Lyons, “On the road to IPS cell cardiovascular applications,” Circulation Research, vol. 105, no. 7, pp. 617–619, 2009.
[12] L. Qian and D. Srivastava, “Monkeying around with cardiac progenitors: hope for the future,” Journal of Clinical Investigation, vol. 120, no. 4, pp. 1034–1036, 2010.
[13] G. M. Ellison, D. Torella, I. Karakikes, and B. Nadal-Ginard, “Myocyte death and renewal: modern concepts of cardiac cellular homeostasis,” Nature Clinical Practice Cardiovascular Medicine, vol. 4, no. 1, pp. S52–S59, 2007.
[14] T. Hosoda, “C-kit positive cardiac stem cells and myocardial regeneration,” American Journal of Cardiovascular Diseases, vol. 2, no. 1, pp. 58–67, 2012.
[15] S. Miyamoto, N. Kawaguchi, G. M. Ellison et al., “Characterization of long-term cultured c-kit+ cardiac stem cells derived from an adult heart,” Circulation Research, vol. 110, no. 4, pp. 570–577, 2012.
from adult rat hearts,” Stem Cells and Development, vol. 19, no. 1, pp. 105–116, 2010.

[16] A. P. Beltrami, L. Barlucchi, D. Torella et al., “Adult cardiac stem cells are multipotent and support myocardial regeneration,” Cell, vol. 114, no. 6, pp. 763–776, 2003.

[17] A. Moretti, M. Bellin, A. Welling et al., “Patient-specific induced pluripotent stem-cell models for long-QT syndrome,” New England Journal of Medicine, vol. 363, no. 15, pp. 1397–1409, 2010.

[18] I. Itzhaki, L. Maizels, I. Huber et al., “Modelling the long QT syndrome with induced pluripotent stem cells,” Nature, vol. 471, no. 7337, pp. 225–229, 2011.

[19] A. J. Moss and R. S. Kass, “Long QT syndrome: from channels to cardiac arrhythmias,” Journal of Clinical Investigation, vol. 115, no. 8, pp. 2018–2024, 2005.

[20] E. Matsa, D. Rajamohan, E. Dick et al., “Drug evaluation in cardiomyocytes derived from human induced pluripotent stem cells carrying a long QT syndrome type 2 mutation,” European Heart Journal, vol. 32, no. 8, pp. 952–962, 2011.

[21] D. Malan, S. Friedrichs, B. K. Fleischmann, and P. Sasse, “Cardiomyocytes obtained from induced pluripotent stem cells with long-QT syndrome 3 recapitulate typical disease-specific features in vitro,” Circulation Research, vol. 109, pp. 841–847, 2011.

[22] M. Yazawa, B. Hsueh, X. Jia et al., “Using induced pluripotent stem cells to investigate cardiac phenotypes in Timothy syndrome,” Nature, vol. 471, no. 7337, pp. 230–234, 2011.

[23] C. B. Jung, A. Moretti, Y. Mederos et al., “Dantrolene rescues arrhythmogenic RYR2 defect in a patient-specific stem cell model of catecholaminergic polymorphic ventricular tachycardia,” EMBO Molecular Medicine, vol. 4, no. 3, pp. 180–191, 2012.

[24] X. Carvajal-Vergara, A. Sevilla, S. L. D’souza et al., “Patient-specific induced pluripotent stem-cell-derived models of LEOPARD syndrome,” Nature, vol. 465, no. 7299, pp. 808–812, 2010.

[25] J. B. Kim, V. Sebastiano, G. Wu et al., “Oct4-induced pluripotency in adult neural stem cells,” Cell, vol. 136, no. 3, pp. 411–419, 2009.

[26] J. A. Efe, S. Hilcove, J. Kim et al., “Conversion of mouse fibroblasts into cardiomyocytes using a direct reprograming strategy,” Nature Cell Biology, vol. 13, no. 3, pp. 215–222, 2011.

[27] M. Ieda, J. D. Fu, P. Delgado-Olguin et al., “Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors,” Cell, vol. 142, no. 3, pp. 375–386, 2010.

[28] N. Fusaki, H. Ban, A. Nishiyama, K. Saeki, and M. Hasegawa, “Efficient induction of transgene-free human pluripotent stem cells using a vector based on Sendai virus, an RNA virus that does not integrate into the host genome,” Proceedings of the Japan Academy Series B, Physical and Biological Sciences, vol. 85, no. 8, pp. 348–362, 2009.

[29] H. Ban, N. Nishihata, N. Fusaki et al., “Efficient generation of transgene-free human pluripotent stem cells (iPSCs) by temperature sensitive Sendai virus vectors,” Proceedings of the National Academy of Sciences of the United States of America, vol. 108, no. 34, pp. 14234–14239, 2011.

[30] E. Yakubov, G. Rechavi, S. Rozenblatt, and D. Givol, “Reprogramming of human fibroblasts to pluripotent stem cells using mRNA of four transcription factors,” Biochemical and Biophysical Research Communications, vol. 394, no. 1, pp. 189–193, 2010.

[31] J. R. Plews, J. Li, M. Jones et al., “Activation of pluripotency genes in human fibroblasts by a novel mRNA based approach,” PLoS One, vol. 5, no. 12, Article ID e14397, 2010.

[32] D. Huangfu, R. Maeher, W. Guo et al., “Induction of pluripotent stem cells by defined factors is greatly improved by small-molecule compounds,” Nature Biotechnology, vol. 26, no. 7, pp. 795–797, 2008.

[33] D. Huangfu, K. Osafune, R. Maeher et al., “Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2,” Nature Biotechnology, vol. 26, no. 11, pp. 1269–1275, 2008.

[34] G. Liang, O. Taranova, K. Xia, and Y. Zhang, “Butyrate promotes induced pluripotent stem cell generation,” Journal of Biological Chemistry, vol. 285, no. 33, pp. 25516–25521, 2010.

[35] Z. Pasha, H. K. Haider, and M. Ashraf, “Efficient non-viral reprogramming of myoblasts to stemness with a single small molecule to generate cardiac progenitor cells,” PLoS One, vol. 6, no. 8, Article ID e23667, 2011.

[36] R. P. H. Ahmed, H. K. Haider, S. Buccini, L. Li et al., “Reprogramming of skeletal myoblasts for induction of pluripotency for tumor-free cardiomyogenesis in the infarcted heart,” Circulation Research, vol. 109, no. 1, pp. 60–70, 2011.

[37] T. S. Mikkelsen, M. Ku, D. B. Jaffe et al., “Genome-wide maps of chromatin state in pluripotent and lineage-committed cells,” Nature, vol. 474, no. 7353, pp. 553–560, 2007.

[38] A. Meissner, T. S. Mikkelsen, H. Gu et al., “Genome-scale DNA methylation maps of pluripotent and differentiated cells,” Nature, vol. 454, no. 7205, pp. 766–770, 2008.

[39] P. Collas, “Epigenetic states in stem cells,” Biochimica et Biophysica Acta, vol. 1790, no. 9, pp. 900–905, 2009.

[40] A. Meissner, “Epigenetic modifications in pluripotent and differentiated cells,” Nature Biotechnology, vol. 28, no. 10, pp. 1079–1088, 2010.

[41] R. Lister, M. Pelizzola, Y. S. Kida et al., “Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells,” Nature, vol. 471, no. 7336, pp. 68–73, 2011.

[42] M. J. Ackerman, S. G. Priori, S. Willems et al., “HRS/EHRA expert consensus statement on the state of genetic testing for the channelopathies and cardiomyopathies: this document was developed as a partnership between the Heart Rhythm Society (HRS) and the European Heart Rhythm Association (EHRA),” Heart Rhythm, vol. 8, no. 8, pp. 1308–1339, 2011.

[43] H. F. Huang, P. H. Chen, W. L. Hwu et al., “Human Pompe disease-induced pluripotent stem cells for pathogenesis modeling, drug testing and disease marker identification,” Human Molecular Genetics, vol. 20, no. 24, pp. 4851–4864, 2011.

[44] L. Yang, M. H. Soonpaa, E. D. Adler et al., “Human cardiovascular progenitor cells develop from a KDR+ embryonic-stem-cell-derived population,” Nature, vol. 453, no. 7194, pp. 524–528, 2008.

[45] Y. Ren, M. Y. Lee, S. Schlifke et al., “Small molecule Wnt inhibitors enhance the efficiency of BMP-4-directed cardiac differentiation of human pluripotent stem cells,” Journal of Molecular and Cellular Cardiology, vol. 51, no. 3, pp. 280–287, 2011.

[46] S. J. Kattman, A. D. Witty, M. Gagliardi et al., “Stage-specific optimization of activin/nodal and BMP signaling promotes cardiac differentiation of mouse and human pluripotent stem cell lines,” Cell Stem Cell, vol. 8, no. 2, pp. 228–240, 2011.

[47] Y. N. Tallini, K. S. Greene, M. Craven et al., “c-kit expression identifies cardiovascular precursors in the neonatal heart,” Proceedings of the National Academy of Sciences of the United States of America, vol. 106, no. 6, pp. 1808–1813, 2009.

[48] N. Kawaguchi, “Stem cells for cardiac regeneration and possible roles of the transforming growth factor-β superfamily,” Biomolecular Concepts, vol. 3, no. 1, pp. 99–106, 2012.
[49] C. Freund and C. L. Mummary, “Prospects for pluripotent stem cell-derived cardiomyocytes in cardiac cell therapy and as disease models,” *Journal of Cellular Biochemistry*, vol. 107, no. 4, pp. 592–599, 2009.

[50] J. K. Yamashita, “ES and iPSC cell research for cardiovascular regeneration,” *Experimental Cell Research*, vol. 316, no. 16, pp. 2555–2559, 2010.

[51] J. Zhang, G. F. Wilson, A. G. Soerens et al., “Functional cardiomyocytes derived from human induced pluripotent stem cells,” *Circulation Research*, vol. 104, no. 4, pp. e30–e41, 2009.

[52] J. Xi, M. Khalil, N. Shishechian et al., “Comparison of contractile behavior of native murine ventricular tissue and cardiomyocytes derived from embryonic or induced pluripotent stem cells,” *FASEB Journal*, vol. 24, no. 8, pp. 2739–2751, 2010.

[53] G. Bock, E. Kiskinis, G. Verstappen et al., “Reference maps of human ES and iPSC cell variation enable high-throughput characterization of pluripotent cell lines,” *Cell*, vol. 144, no. 3, pp. 439–452, 2011.

[54] M. K. Gupta, D. I. Illich, A. Gaarz et al., “Global transcriptional profiles of beating clusters derived from human induced pluripotent stem cells and embryonic stem cells are highly similar,” *BMC Developmental Biology*, vol. 10, article no. 98, 2010.

[55] N. Yokoo, S. Baba, S. Kaichi et al., “The effects of cardiovascular drugs on cardiomyocytes derived from human induced pluripotent stem cells,” *Biochemical and Biophysical Research Communications*, vol. 387, no. 3, pp. 482–488, 2009.

[56] H. Xu, B. A. Yi, H. Wu et al., “Highly efficient derivation of ventricular cardiomyocytes from induced pluripotent stem cells with a distinct epigenetic signature,” *Cell Research*, vol. 22, no. 1, pp. 142–154, 2012.

[57] S. Kaichi, K. Hasegawa, T. Takaya et al., “Cell line-dependent differentiation of induced pluripotent stem cells into cardiomyocytes in mice,” *Cardiovascular Research*, vol. 88, no. 2, pp. 314–323, 2010.

[58] S. G. Priori, “Induced pluripotent stem cell-derived cardiomyocytes and long QT syndrome: is personalized medicine ready for prime time?” *Circulation Research*, vol. 109, pp. 822–824, 2011.

[59] J. Ma, L. Guo, S. J. Fiene et al., “High purity human-induced pluripotent stem cell-derived cardiomyocytes: electrophysiological properties of action potentials and ionic currents,” *American Journal of Physiology and Heart Circulatory Physiology*, vol. 301, no. 5, pp. H2006–H2017, 2011.

[60] P. W. Burridge, S. Thompson, M. A. Mill福德 et al., “A universal system for highly efficient cardiac differentiation of human induced pluripotent stem cells that eliminates interline variability,” *PLoS One*, vol. 6, no. 4, Article ID e18293, 2011.

[61] N. Cao, Z. Liu, Z. Chen et al., “Ascorbic acid enhances the cardiac differentiation of induced pluripotent stem cells through promoting the proliferation of cardiac progenitor cells,” *Cell Research*, vol. 22, no. 6, pp. 219–236, 2012.

[62] L. Li, S.M. Larabee, S. Chen et al., “Novel STOP miRNAs regulated by ribosomal S6 kinase are important for cardiomyocyte development: S6 kinase suppression limits cardiac differentiation and promotes pluripotent cells towards a neural lineage,” *Stem Cells and Development*, vol. 21, no. 9, pp. 1538–1548, 2012.

[63] H. Kempf, M. Lecina, S. Ting et al., “Distinct regulation of mitogen-activated protein kinase activities is coupled with enhanced cardiac differentiation of human embryonic stem cells,” *Stem Cell Research*, vol. 7, no. 3, pp. 198–209, 2011.

[64] A. Sachinidis, S. Schwengberg, R. Hippler-Altenburg et al., “Identification of small signalling molecules promoting cardiac-specific differentiation of mouse embryonic stem cells,” *Cellular Physiology and Biochemistry*, vol. 18, no. 6, pp. 303–314, 2006.

[65] J. Hao, M. A. Daleo, C. K. Murphy et al., “Dorsomorphin, a selective small molecule inhibitor of BMP signaling, promotes cardiomyogenesis in embryonic stem cells,” *PLoS One*, vol. 3, no. 8, Article ID e2904, 2008.

[66] H. Wang, J. Hao, and C. C. Hong, “Cardiac induction of embryonic stem cells by a small molecule inhibitor of Wnt/β-catenin signaling,” *ACS Chemical Biology*, vol. 6, no. 2, pp. 192–197, 2011.

[67] M. Fujii, P. Yan, T. G. Otsuji et al., “Induction and enhancement of cardiac cell differentiation from mouse and human induced pluripotent stem cells with cyclosporin-A,” *PLoS One*, vol. 6, no. 2, Article ID e16734, 2011.

[68] M. Quattrocelli, G. Palazzolo, I. Agnolin et al., “Synthetic sulfonyl-hydrazone-1 positively regulates cardiomyogenic microRNA expression and cardiomyocyte differentiation of induced pluripotent stem cells,” *Journal of Cellular Biochemistry*, vol. 112, no. 8, pp. 2006–2014, 2011.

[69] T. Shinozawa, H. Furukawa, E. Sato, and K. Takami, “A novel purification method of murine embryonic stem cell- and human-induced pluripotent stem cell-derived cardiomyocytes by simple manual dissociation,” *Journal of Biomolecular Screen*, vol. 17, no. 5, pp. 683–691, 2012.

[70] F. Hattori, H. Chen, H. Yamashita et al., “Nongenetic method for purifying stem cell-derived cardiomyocytes,” *Nature Methods*, vol. 7, no. 1, pp. 61–66, 2010.

[71] T. Egashira, S. Yuasa, and K. Fukuda, “Induced pluripotent stem cells in cardiovascular medicine,” *Stem Cells International*, vol. 2011, Article ID 348960, 7 pages, 2011.

[72] N. C. Dubois, A. M. Craft, P. Sharma et al., “SiRPA is a specific cell-surface marker for isolating cardiomyocytes derived from human pluripotent stem cells,” *Nature Biotechnology*, vol. 29, no. 11, pp. 1011–1018, 2011.

[73] L.-T. Cheng, S. Nagata, K. Harano et al., “Cure of ADPKD by selection for spontaneous genetic repair events in Pkd1-mutated iPSCs,” *PLoS One*, vol. 7, no. 2, Article ID e32018, 6 pages, 2012.

[74] T. J. Kipps and L. A. Herzenberg, “Homologous chromosome recombination generating immunoglobulin allotype and isotype switch variants,” *The EMBO Journal*, vol. 5, no. 2, pp. 263–268, 1986.

[75] M. K. Jonsson, Q. D. Wang, and B. Becker, “Impedance-based detection of beating rhythm and proarrhythmic effects of compounds on stem cell-derived cardiomyocytes,” *Assay Drug Development Technologies*, vol. 9, no. 6, pp. 589–599, 2011.

[76] C. F. Mandenius, D. Steel, F. Noor et al., “Cardiotoxicity testing using pluripotent stem cell-derived human cardiomyocytes and state-of-the-art bioanalytics: A review,” *Journal of Applied Toxicology*, vol. 31, no. 3, pp. 191–205, 2011.

[77] H. Hosseinkhani, M. Hosseinkhani, S. Hattori, R. Matsuoka, and N. Kawaguchi, “Micro and nano-scale in vitro 3D culture system for cardiac stem cells,” *Stem Cells and Development*, vol. 20, no. 11, pp. 1011–1018, 2011.

[78] M. K. Jonsson, Q. D. Wang, and B. Becker, “Impedance-based detection of beating rhythm and proarrhythmic effects of compounds on stem cell-derived cardiomyocytes,” *Assay Drug Development Technologies*, vol. 9, no. 6, pp. 589–599, 2011.

[79] C. F. Mandenius, D. Steel, F. Noor et al., “Cardiotoxicity testing using pluripotent stem cell-derived human cardiomyocytes and state-of-the-art bioanalytics: A review,” *Journal of Applied Toxicology*, vol. 31, no. 3, pp. 191–205, 2011.

[80] H. Hosseinkhani, M. Hosseinkhani, S. Hattori, R. Matsuoka, and N. Kawaguchi, “Micro and nano-scale in vitro 3D culture system for cardiac stem cells,” *Stem Cells and Development*, vol. 20, no. 11, pp. 1011–1018, 2011.

[81] M. K. Jonsson, Q. D. Wang, and B. Becker, “Impedance-based detection of beating rhythm and proarrhythmic effects of compounds on stem cell-derived cardiomyocytes,” *Assay Drug Development Technologies*, vol. 9, no. 6, pp. 589–599, 2011.
[80] N. Kawaguchi, “3D-culture system required for heart regeneration and cardiac medicine,” Submitted.

[81] N. Kawaguchi, R. Nakao, M. Yamaguchi, D. Ogawa, and R. Matsuoka, “TGF-β superfamily regulates a switch that mediates differentiation either into adipocytes or myocytes in left atrium derived pluripotent cells (LA-PCS),” *Biochemical and Biophysical Research Communications*, vol. 396, no. 3, pp. 619–625, 2010.

[82] N. Kawaguchi, “Adult cardiac-derived stem cells: differentiation regulators and survival regulators,” *Vitamins and Hormones*, vol. 87, pp. 111–125, 2011.

[83] M. Machida, Y. Takagaki, R. Matsuoka, and N. Kawaguchi, “Proteomic comparison of spherical aggregates and adherent cells of cardiac stem cells,” *International Journal of Cardiology*, vol. 153, pp. 296–305, 2011.

[84] R. C. Kukreja, C. Yin, and F. N. Salloum, “MicroRNAs: new players in cardiac injury and protection,” *Molecular Pharmacology*, vol. 80, no. 4, pp. 558–564, 2011.

[85] J. Xu, J. Zhao, G. Evan et al., “Circulating microRNAs: novel biomarkers for cardiovascular diseases,” *Journal of Molecular Medicine*, vol. 90, no. 8, pp. 865–875, 2012.

[86] J. Wang, S. B. Greene, M. Bonilla-Claudio et al., “Bmp signaling regulates myocardial differentiation from cardiac progenitors through a micro-RNA-mediated mechanism,” *Developmental Cell*, vol. 19, no. 6, pp. 903–912, 2010.

[87] K. N. Ivey and D. Srivastava, “MicroRNAs as regulators of differentiation and cell fate decisions,” *Cell stem cell*, vol. 7, no. 1, pp. 36–41, 2010.

[88] P. Jakob and U. Landmesser, “Role of microRNAs in stem/progenitor cells and cardiovascular repair,” *Cardiovascular Research*, vol. 93, no. 4, pp. 614–622, 2012.

[89] J. E. Babiarz, M. Ravon, S. Šrídhar et al., “Determination of the human cardiomyocyte mRNA and miRNA differentiation network by fine-scale profiling,” *Stem Cells and Development*, vol. 21, no. 11, pp. 1956–1965, 2012.

[90] A. Meissner, “Epigenetic modifications in pluripotent and differentiated cells,” *Nature Biotechnology*, vol. 28, no. 10, pp. 1079–1088, 2010.