Review Article

Electrophysiological properties and calcium handling of embryonic stem cell-derived cardiomyocytes

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ABSTRACT

Embryonic stem cell-derived cardiomyocytes (ESC-CMs) hold great interest in many fields of research including clinical applications such as stem cell and gene therapy for cardiac repair or regeneration. ESC-CMs are also used as a platform tool for pharmacological tests or for investigations of cardiac remodeling. ESC-CMs have many different aspects of morphology, electrophysiology, calcium handling, and bioenergetics compared with adult cardiomyocytes. They are immature in morphology, similar to sinus nodal-like in the electrophysiology, higher contribution of trans-sarcolemmal Ca^{2+} influx to Ca^{2+} handling, and higher dependence on anaerobic glycolysis. Here, I review a detailed electrophysiology and Ca^{2+} handling features of ESC-CMs during differentiation into adult cardiomyocytes to gain insights into how all the developmental changes are related to each other to display cardinal features of developing cardiomyocytes.

1. Introduction

Embryonic stem cell-derived cardiomyocytes (ESC-CMs) are greatly promising for stem cell therapy against cardiovascular diseases such as myocardial infarction and potentially life-threatening arrhythmias because of their ability to differentiate into sinus–nodal, atrial, or ventricular-type of cardiomyocytes.[1,2] ESC-CMs also have a potential to be a novel pharmacological tool in, for example, high-throughput screening tests of newly developed drug for cardiotoxicity.[3] In

addition, ESC-CMs could be a powerful new model system to study mechanisms of inherited cardiomyopathies.[4]

ESC-CMs are immature in both morphological and functional aspects. They have underdeveloped contractile machinery and lack transverse-tubular (T-tubular) system.[5,6] They display diastolic potentials similar to those of adult sinus–nodal cells and slower upstroke velocity.[1,2] They have fewer mitochondria[5] and are predominantly glycolytic.[7] Ca^{2+} handling is also significantly different from that of their adult counterpart displaying slower kinetics and smaller amplitude.[8] This review will primarily focus on the

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Table 1 – Model parameter values in mathematical model[*]			
Model parameters	Nodal type (AP)	Atrial type (AP)	Ventricular type (AP)
E_{CaL}, mV	47	47	47
g_{CaL}, nS	11.0140	12.5874	12.5874
k_1	0.9	1.1	1.8
k_2	1.0	1.3	2.2
$g_{HCN,Na}$, nS	0.0529	0.0529	0.0529
$g_{HCN,K}$, nS	0.0851	0.0851	0.0851
$g_{to,UF}$, nS	0.3346	1.3382	0.6691
$g_{to,F}$, nS	0.1338	0.5352	0.2676
$g_{to,S}$, nS	0.2091	0.8364	0.4182
g_{Ks}, nS	0.0411	0.2057	0.4114
$g_{b,Ca}$, nS	0	0	0
$g_{b,Na}$, nS	0.170	0.122	0.122
$g_{b,K}$, nS	0.035	0.025	0.025
g_{CaT}, nS	0	0	0
g_{K1}, nS	0	0.6075	2.2275
g_{Kr}, nS	1.5319	0.6383	0.6383
$g_{Na,1.5}$, nS	0.0059	0.0237	0.0296
$g_{Na,1.1}$, nS	0.0059	0.0237	0.0296
g_{st}, nS	0	0	0
g_{sus}, nS	0	0	0

* See glossary of Kharche et al's[9] model for detailed explanation of each channel.
Note. Adapted from "Dual modulation of the mitochondrial permeability transition pore and redox signaling synergistically promotes cardiomyocyte differentiation from pluripotent stem cells," by S.W. Cho, J.S. Park, H.J. Heo, S.W. Park, S. Song, I. Kim et al., 2014, *J Am Heart Assoc*, 3, e000693. Copyright 2014 The Authors. Reprinted with permission.
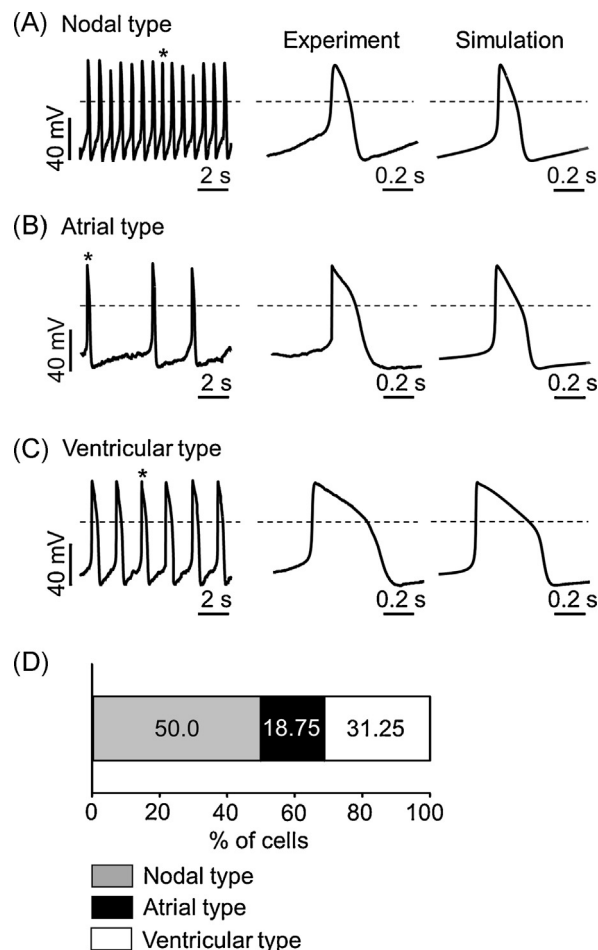AP; action potential.

Fig. 1 – **Representative action potential (AP) morphologies from patch clamp recordings and mathematical modelling. (A) Nodal type, (B) atrial type, and (C) ventricular type APs recorded from different embryonic stem cell-derived cardiomyocytes in a current–clamp mode. Each AP trace in the middle panel corresponds to an expanded trace of single AP denoted with an asterisk in the left panel. Each AP trace in the right panel corresponds to a simulated AP trace. Dotted lines indicate zero voltage level. (D) Percentile distribution of three different types of APs in embryonic stem cell-derived cardiomyocytes. See Table 1 for model parameters used in the mathematical model.**
Note. **Fig. 1D is from "Dual modulation of the mitochondrial permeability transition pore and redox signaling synergistically promotes cardiomyocyte differentiation from pluripotent stem cells," by S.W. Cho, J.S. Park, H.J. Heo, S.W. Park, S. Song, I. Kim et al., 2014, *J Am Heart Assoc*, 3, e000693. Copyright 2014 The Authors. Reprinted with permission.**

electrophysiological properties and Ca^{2+} handling features of ESC-CMs during differentiation into adult cardiomyocytes.

2. Electrophysiological properties of ESC-CMs

Early studies on the differentiation of pluripotent mouse embryonic stem cells (ESCs) into adult cardiomyocytes revealed that the rhythmic action potentials (APs) of early differentiated cardiomyocytes are very similar to those of sinus–node cells.[1,2] Terminally differentiated cardiomyocytes generated APs similar to those of adult sinus–nodal, atrial, and ventricular myocytes, suggesting that there should be developmental changes in cardiac ion channels and calcium handling properties along with differentiation into adult cardiomyocytes. Representative traces of nodal-like, atrial-like, and ventricular-like APs from patch–clamp recording and mathematical modelling are demonstrated in Fig. 1. The mathematical model was based on the Kharche's model[9] and modified parameters are summarized in Table 1. In the following, functional changes in electrophysiological properties during differentiation are described in detail.

2.1. Capacitance

In one early study on mouse ESC-CMs, membrane capacitance was found to steadily increase from 24.5 pF to 50.0 pF during differentiation.[10] As the specific membrane capacitance was calculated to be 0.85–0.86 μF/cm^2, it was concluded that the T-tubular system apparently was not developed during differentiation.[10] T-tubule formation was not observed at the ultrastructural level in human pluripotent stem cell-derived cardiomyocytes, when electron microscopy or membrane staining by Di-8-ANNEPPS were used.[6]

2.2. L-type Ca^{2+} current

L-type Ca^{2+} current (I$_{CaL}$) is the most prominent inward current at the early stage of differentiation in mouse ESC-CMs and is found throughout the entire differentiation period.[10] The maximum current density (pA/pF) occurred near 0 mV and were apparently 13 pA/pF at early stage and 25 pA/pF at terminally differentiated stage, respectively. I$_{CaL}$ is thought to synchronize multiple local Ca^{2+} releases (LCRs) to induce global Ca^{2+} release.[11]

The mRNA of *CACNA1C*, a gene encoding one subtype of L-type Ca^{2+} channel, was detected in undifferentiated human ESCs and at all stages of differentiation by using reverse transcription–polymerase chain reaction (RT-PCR) analysis.[12] I$_{CaL}$ was recorded in both human ESCs and ESC-CMs at all stages of differentiation. Maximum current densities were 0.9 pA/pF in human ESCs and 5.8 pA/pF in human ESC-CMs, respectively.[12]

As for the pharmacology, there is a report[13] that human ESC-CMs have an altered pharmacology for activators of I$_{CaL}$ while the pharmacology for antagonist is preserved.

2.3. T-type Ca^{2+} current

An inward current permeable for Ca^{2+} and Ba^{2+} with lower threshold for activation than that of I$_{CaL}$ was detected in an early study on differentiation of pluripotent ESCs of the mouse.[10] As it was insensitive to 1,4-dihydropyridines but was almost completely blocked by 50 μM Ni^{2+}, the T-type Ca^{2+} channel was suggested to be the candidate channel to allow the inward current.

2.4. Voltage-gated Na$^+$ current

The density of voltage-gated Na$^+$ current (I$_{Na}$) in mouse ESC-CMs was 95 pA/pF at the early differentiation stage and increased to above 300 pA/pF at the terminal differentiation stage.[10] I$_{Na}$ was predominantly observed in atrial-like and ventricular-like cells but to a lesser degree in sinus-nodal-like cells. The density of I$_{Na}$ in human ESC-CMs was 244 pA/pF at mid-stage (20–35 days postplating).[14] As the spontaneous electrical activity from cell clusters was completely abolished by perfusion of tetrodotoxin, a specific blocker of I$_{Na}$, it was suggested that the I$_{Na}$ is crucial for initiation of spontaneous excitation in cell clusters at mid-stage development. Interestingly, an AP clamp using the morphology of APs from human ESC-CMs elicited a larger early and late I$_{Na}$ than that using the morphology of APs from either the canine epicardial or endocardial ventricular myocytes in some voltage-gated Na$^+$ channel (Na$_V$1.5) variants.[15] The proposed mechanism was that the AP from human ESC-CMs displays a relatively slower upstroke velocity than those from canine ventricular myocytes, which induces increased open probability and driving force of I$_{Na}$.[15]

2.5. Voltage-gated K$^+$ current

A transient outward current (I$_{to}$) was found from the very early stage of differentiation in mouse ESC-CMs.[10] The density of I$_{to}$ was 14.9 pA/pF at the early differentiation stage and

41.5 pA/pF at the terminal differentiation stage, respectively. The 4-aminopyridine sensitive transient outward current (I$_{to}$) was also observed in the other early stage murine cardiomyocytes where the current density was 10.3 pA/pF.[16] In human ESC-CMs, expression of two isoforms, Kv1.4 and Kv4.3, was detected by RT-PCR.[12] The two isoforms were not detected in undifferentiated human ESC. A 4-aminopyridine sensitive transient outward current was also recorded in the presence of intracellular EGTA, a chelator of Ca^{2+}, indicating that it is the Ca^{2+}-independent transient outward K$^+$ current (I$_{to1}$). Its peak density increased during development from 4.2 pA/pF to 7.7 pA/pF.

The delayed rectifier K$^+$ current was not detectable at the early stage of differentiation but was seen only at the intermediate and terminal differentiation stages.[10] The density of delayed rectifier K$^+$ current was 3.5 pA/pF at the intermediate differentiation stage and 9.4 pA/pF at the terminal differentiation stage.

Gryshchenko et al[16] identified two components of rapidly activating, outwardly rectifying current in early and late stage cardiomyocytes, which were characterized by slow (IK$_{sus}$) or no inactivation (I$_{res}$), respectively, during depolarizing test pulses. I$_{res}$ was 4-aminopyridine resistant and was observed in 75% of early stage cardiomyocytes. The maximum current density was increased from 3.6 pA/pF to 8 pA/pF during differentiation into late stage cardiomyocytes. Meanwhile, I$_{Ksus}$ was observed in only 25% of early stage cardiomyocytes and its maximum density was increased from 3.5 pA/pF to 8.3 pA/pF during differentiation into late stage cardiomyocytes.

AP durations from beating embryoid bodies cultured for 40–95 days were significantly prolonged by addition of E4031, a specific blocker of rapidly activating delayed rectifier K$^+$ current (I$_{Kr}$), indicating the ESC-CMs express HERG channels.[17] Even early after-depolarizations appeared after treatment with E4031. In both undifferentiated human ESCs and ESC-CMs, the mRNA of *HERG* was detected by using RT-PCR analysis.[12] E4031-sensitive outward currents were also recorded upon depolarizing test pulses from a holding potential of –40 mV, indicating both undifferentiated human ESCs and ESC-CMs functionally express HERG channels.

An E4031-resistant but chromanol 293B-sensitive K$^+$ current was observed in human ESC-CMs.[18] Current-voltage relationship, activation kinetics, and pharmacological response all demonstrated that it is the slowly activating delayed rectifier K$^+$ current (I$_{Ks}$). The maximum current density was apparently 0.7 pA/pF as measured at the end of 2 second depolarization test pulse to 40 mV.

2.6. Inward rectifier K$^+$ current

Inward rectifier K$^+$ current (I$_{K1}$) was observed predominantly in atrial-like and ventricular-like cells at the terminal differentiation stage.[10] The maximum density of I$_{K1}$ in ventricular-like cells was slightly increased from 4.9 pA/pF to 5.4 pA/pF during differentiation into terminal stage. In a study of transgenic mouse ESCs expressing enhanced green fluorescent protein (EGFP), both the early sinus–nodal- and atrial-like cardiomyocytes expressed I$_{K1}$ but the percentage of I$_{K1}$ expressing sinus-nodal-like cardiomyocytes was very small compared with that of I$_{K1}$ expressing atrial-like cardiomyocytes.[19] The

maximum density was also lower in sinus-nodal-like cardiomyocytes (1.6 pA/pF vs. 3.6 pA/pF). The mRNA of Kir2.1, a molecular identity of I_{K1}, was detected in both undifferentiated human ESCs and ESC-CMs by using RT-PCR analysis.[12] Ba^{2+}-sensitive background K^+ currents were recorded at all stages of differentiation indicating that human ESC-CMs functionally express I_{K1}.

2.7. ATP-dependent K^+ current

Some cardiomyocytes in early differentiation were found to display a bursting behavior of APs, characterized by alternating periods of burst firing and silence.[20] The positive chronotropy by glibencamide, a blocker of ATP-dependent K^+ current ($I_{K,ATP}$) and negative chronotropy by dinitrophenol, a known activator of $I_{K,ATP}$, suggest that the ATP-dependent K^+ channels are present and play a role in bursting behavior of early stage cardiomyocytes. Perforated patch-clamp recordings in the voltage-clamp mode were conducted to isolate $I_{K,ATP}$, and the maximum current densities were 45.1 pA/pF in early stage cells and 90.9 pA/pF in late stage cells, respectively. The $I_{K,ATP}$ was suggested to mirror the metabolic oscillations and serve as a cardioprotective role under conditions of low O_2 saturation in the embryo.

2.8. Acetylcholine-dependent K^+ current

As the carbachol activated inwardly rectifying K^+ currents at the terminal differentiation stage, which was almost completely blocked by atropine, the presence of acetylcholine-dependent K^+ current was suggested.[10] In a study of transgenic mouse ESC-CMs expressing EGFP, five out of nine sinus-nodal-like cardiomyocytes completely stopped spontaneous electrical activity in response to carbachol indicating that acetylcholine-dependent K^+ current exists.[19] Meanwhile, only one out of 10 atrial-like cardiomyocytes completely stopped spontaneous electrical activity by addition of carbachol.

2.9. Hyperpolarization-activated nonselective cation current

Hyperpolarization-activated nonselective cation current (I_f) was first observed at the intermediate differentiation stage of mouse ESC-CMs.[10] Peak amplitudes of I_f were between 50 pA and 500 pA at the intermediate differentiation stage and ranged between 500 pA and 2000 pA at the terminal differentiation stage. Abi-Gerges et al[21] found that the percentage of cardiomyocytes expressing I_f slightly decreased from 65% to 45% throughout differentiation. Meanwhile, peak I_f densities increased from 11.4 pA/pF to 15.5 pA/pF throughout differentiation. They also demonstrated that the activation of I_f during early and late stages of differentiation is not mediated by direct cAMP binding but by phosphorylation via protein kinase A. The automaticity seen in atrial-like and ventricular-like cardiomyocytes during differentiation is in part due to their relatively high densities of I_f compared with those of adult atrial and ventricular cardiomyocytes. In the study of transgenic mouse ESC-CMs expressing EGFP, both the early sinus–nodal-like and atrial-like cardiomyocytes

expressed I_f and peak densities were about 11 pA/pF and 8 pA/pF, respectively.[19] The expression of HCN, a molecular identity of I_f, were detected in mouse ESC-CMs and were significantly decreased at Day 23.5 of cell culture compared with that at Day 9.5.[22] Functional expression of I_f was also observed in human ESC-CMs.[12,14] The mRNA level for HCN1 isoform, which has the fastest activation kinetics among four isoforms,[23] was not detected in adult heart but was detected in undifferentiated human ESCs.[12] The expression of HCN1 was then significantly reduced during differentiation and maturation. By contrast, the mRNA level for HCN2 isoform with slower kinetics was persistent throughout the differentiation process.

2.10. Ca^{2+} activated K^+ current

In a study on the pacemaking mechanism of human ESC-CMs,[24] a previously unrecognized Ca^{2+}-activated K^+ channel (KCa3.1, SK4, or IK_{Ca} channel) was identified by using the patch–clamp technique, real-time PCR, western blotting, and immunocytochemistry. Pharmacological inhibition of IK_{Ca} by using clotrimazole or 1-[(2-chlorophenyl)diphenylmethyl]-1H-pyrazole resulted in the depolarization of maximum diastolic potential and slowing of AP beating rate. It was suggested that IK_{Ca} contributes to shaping of diastolic potential and pacemaker activity via fine tuning the balance between I_f and Na^+/Ca^{2+} exchanger current (I_{NCX}).

2.11. Background current

Background currents with a linear current–voltage relationship and an apparent reversal potential near 0 mV were observed.[10] The canonical transient receptor potential isoform 3 (TRPC3) channel, one possible molecular identity of the background inward current,[25] was recently identified in mouse ESC-CMs based on immunocytochemistry and patch clamp recordings.[26] Immunocytochemistry revealed that TRPC3 is located at the T-tubules. A Pyr3-sensitive TRPC3 current was recorded by whole-cell patch clamp technique. The block of TRPC3 decreased frequency and amplitude of spontaneous Ca^{2+} transients. The most prominent change in spontaneous AP by Pyr3 was seen in Phase 4 diastolic depolarization which was significantly delayed. As TRPC3 permeates both Ca^{2+} and Na^+, activation of TRPC3 current is considered to depolarize the membrane.[27] In addition, Ca^{2+} influx through TRPC3 could induce LCRs, which subsequently activates the forward mode of Na^+/Ca^{2+} exchanger (NCX).[28]

2.12. I_{NCX}

RT-PCR analyses of transcripts were conducted on mouse ESC-CMs and NCX1 was clearly detectable from preplating stage.[29] The role of NCX was evaluated by using Na^+ free solution, which inhibits NCX resulting in a decreased removal of Ca^{2+} from cytosol. In early stage mouse ESC-CMs, basal level of $[Ca^{2+}]_i$ was found to be regulated by NCX, whereas spontaneous Ca^{2+} transients were not. In late stage ESC-CMs, both basal level of $[Ca^{2+}]_i$ and amplitude of Ca^{2+} transients were affected by NCX. In human ESC-CMs, NCX was expressed at a higher level than the adult counterpart. The

Ni^{2+}-sensitive inward current was recorded in ventricular-like human ESC-CMs.[30] I_{NCX} density was 3.6 pA/pF at 60 mV in 40-day ventricular-like ESC-CMs and increased to 7.9 pA/pF in 90-day. NCX was suggested to translate a sporadic generation of LCRs into the diastolic depolarization, which in turn activates L-type Ca^{2+} currents to generate APs in late-stage mouse ESC-CMs.[11]

3. Calcium handling

3.1. $[Ca^{2+}]_i$ oscillation drives spontaneous contraction in early stage ESC-CMs

Based on the finding that the early stage ESC-CMs continue beating in high extracellular K^+ solution, not transmembrane ion currents but $[Ca^{2+}]_i$ oscillations were suggested as the mechanism to drive spontaneous contractions of cardiomyocytes in the early stage of differentiation into adult cardiomyocytes.[31] Interestingly, many small fluctuations of membrane potential were superimposed with fewer number of APs in the early stage cardiomyocytes. 50 nM nisoldipine, a blocker of I_{CaL}, halted APs but not the membrane potential fluctuations and accompanying spontaneous contractions. The small fluctuations of membrane potential were completely blocked by the addition of thapsigargin, a sarcoplasmic Ca^{2+}-ATPase inhibitor. Therefore, it was concluded that the $[Ca^{2+}]_i$ oscillations primarily drive small fluctuations of membrane potential and that if the fluctuations are big enough to activate L-type Ca^{2+} channels, the APs are generated. Recently, NCX was suggested to translate spontaneous Ca^{2+} oscillations into fluctuations of membrane potential.[32] NCX was also suggested to translate spontaneous Ca^{2+} oscillations into spontaneous inward currents in some voltage clamp conditions.[33] In addition, the $[Ca^{2+}]_i$ oscillations in early stage embryonic stem cell-derived cardiomyocytes are thought to be crucial for structural maturation of the cardiomyocytes.[34] The appearance of spontaneous contractions under high extracellular K^+ solution was not observed in terminally differentiated cardiomyocytes indicating that calcium handling properties change with maturation (Fig. 2). In the following, Ca^{2+} handling in mouse and human ESC-CMs are described in more detail.

3.2. Ca^{2+} handling in mouse ESC-CMs

Ca^{2+} sparks, which represent Ca^{2+} release from the opening of clustered ryanodine receptor channels (RyRs) within restricted sarcoplasmic reticulum (SR) junctional regions,[35] were not observed in very early developmental stage.[36] By contrast, global $[Ca^{2+}]_i$ fluctuations occurred from the very early developmental stage. Both the Ca^{2+} sparks and global $[Ca^{2+}]_i$ fluctuations were markedly increased in amplitude and frequency during differentiation into late stage cardiomyocytes. Perfusion of either 10 mM caffeine or high extracellular K^+ (140 mM) solution to the cardiomyocytes in very early developmental stage resulted in Ca^{2+} transients indicating that RyRs and L-type Ca^{2+} channels are present and work from the beginning of differentiation. The presence of RyRs in early stage cardiomyocytes was further confirmed by the finding that the

heart rate was markedly depressed in mouse ESC-CMs with a functional knockout of the RyR2.[37,38] The absence of Ca^{2+} spark in spite of functional RyRs and L-type Ca^{2+} channels in the very early developmental stage indicates that they are not yet in close apposition in the very early stage of differentiation. Indeed, it was found that there is no colocalization of L-type Ca^{2+} channels and RyRs in mouse and human ESC-CMs.[39]

Although Ca^{2+} sparks were not observed in RyR2 knockout cells, spontaneous Ca^{2+} transients were still observable but with significantly slower kinetics.[37] The residual source of Ca^{2+} increase in RyR2 KO cells is possibly reverse mode NCX, L-type Ca^{2+} channels, or inositol-1,4,5-trisphosphate receptor (IP_3R) channels. Indeed, 2-aminoethoxydipheylborate, an inhibitor of store-operated channels or inositol-1,4,5-trisphosphate (IP_3)-induced Ca^{2+} release,[40] significantly reduced amplitude of Ca^{2+} transients. The inhibitory effect of 2-aminoethoxydipheylborate was significantly attenuated at late stage of differentiation indicating the contribution of IP_3Rs to total SR Ca^{2+} release is downregulated during differentiation.[37,41] Furthermore, there is strong evidence that IP_3R and NCX are spatially coupled to turn Ca^{2+} oscillations into membrane potential fluctuations in early stage of differentiation.[32]

Nifedipine, a blocker of L-type Ca^{2+} channels, also reduced amplitude of Ca^{2+} transients in electrically stimulated cardiomyocytes.[29] It was suggested that the L-type Ca^{2+} channel is the predominant contributor to spontaneous Ca^{2+} transients in early differentiation and then Ca^{2+}-induced Ca^{2+} release takes over the role of major contributor to Ca^{2+} transients in late stage differentiation.[41] Frequency and amplitude of Ca^{2+} transients were both significantly increased from early to late stage differentiation.[38]

As the exposure of mouse ESC-CMs to caffeine elicited a bigger size of Ca^{2+} transients during differentiation, the effects of differentiation on SR were proposed to be increasing its volume and Ca^{2+} content.[38]

As for the Ca^{2+}-removal mechanism, NCX was suggested to be the main contributor in the early stage of differentiation whereas sarcoplasmic reticulum Ca^{2+}-ATPase (SERCA) takes over the main role in the late stage of differentiation.[41] In a later study,[42] however, SERCA was evaluated to be responsible for ~76% of total Ca^{2+} removal, while NCX was responsible for only ~21% at early differentiation stage of mouse ESC-CMs. At late differentiation stage, the contribution of SERCA to Ca^{2+} removal was further increased to ~88% while that of NCX was decreased to ~10%, indicating that the trend of chronological changes in contribution of two Ca^{2+} removal mechanisms is the same between these two studies.

3.3. Ca^{2+} handling in human ESC-CMs

In a study of human ESC-CMs, ryanodine, thapsigargin, and caffeine did not affect Ca^{2+} transients and contraction indicating that SR Ca^{2+} release does not significantly contribute to Ca^{2+} handling in early stage of differentiation.[43] Instead, trans-sarcolemmal Ca^{2+} influx was suggested to contribute predominantly to Ca^{2+} handling in human ESC-CMs based on the finding that verapamil, a blocker of L-type Ca^{2+} channel, completely blocked contraction. Later studies, however, revealed that there are caffeine-responsive human ESC-CMs

Eearly stage ESC-CMs
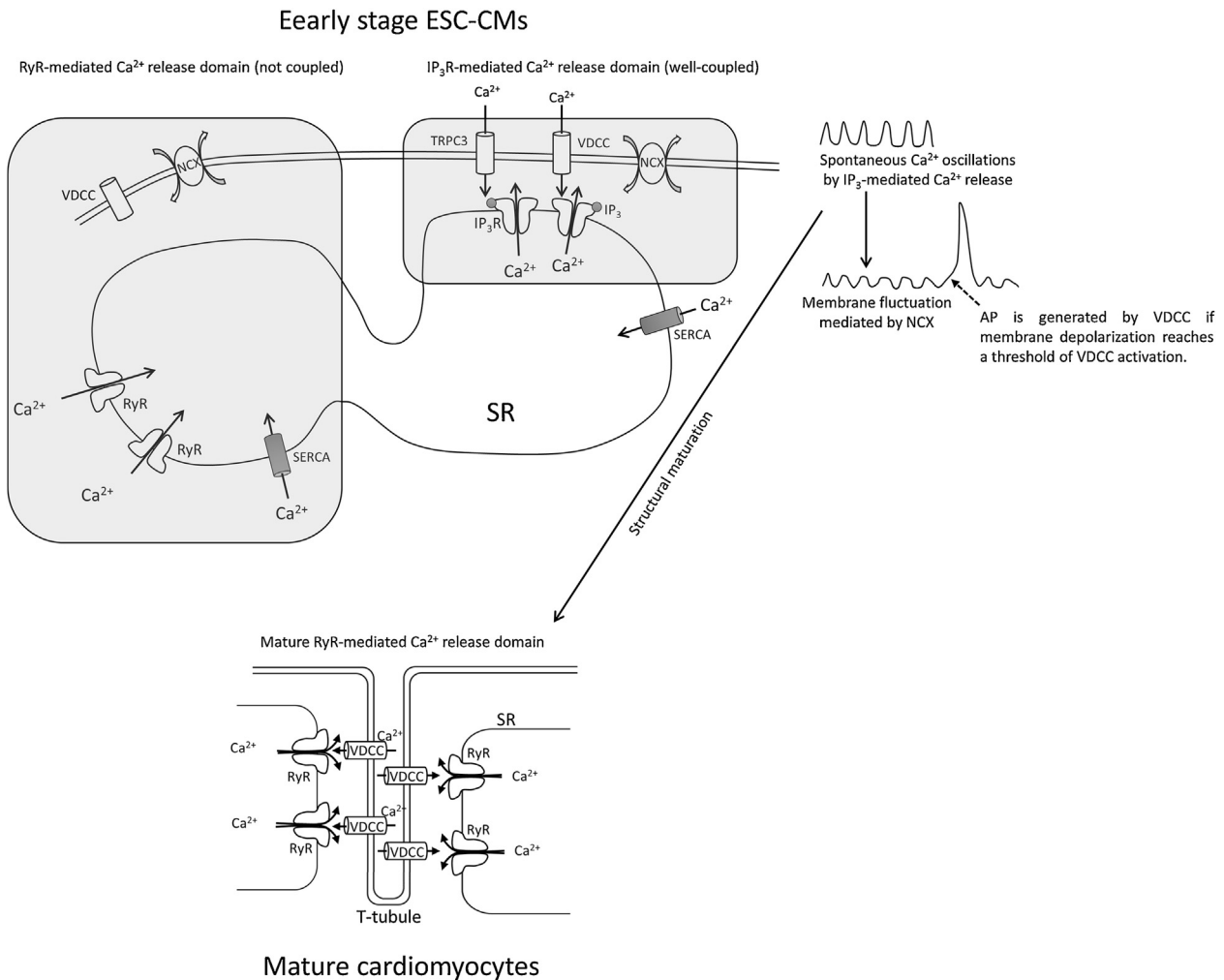

Mature cardiomyocytes

Fig. 2 – Schematic model of Ca^{2+} handling and AP generation in early stage ESC-CMs and mature cardiomyocytes. In early stage ESC-CMs, T-tubules are not developed and RyRs are poorly coupled to VDCC. Instead, IP_3R is well coupled to NCX and the IP_3R-mediated Ca^{2+} release predominantly drives spontaneous Ca^{2+} oscillations which subsequently cause spontaneous beating even under high extracellular K^+ condition.[31] Trans-sarcolemmal pathways such as TRPC3 and VDCC also significantly contribute to IP_3R-mediated Ca^{2+} release. Ca^{2+} removal depends on both NCX and SERCA in the SR. NCX is believed to translate the spontaneous Ca^{2+} oscillations into membrane potential fluctuations.[32] If the fluctuation of membrane depolarization reaches a threshold for VDCC, an AP is generated. Ca^{2+} oscillation is essential for maturation of T-tubule and RyR-VDCC coupling.[34] Therefore, the RyR-mediated Ca^{2+} release is dominant in mature cardiomyocytes. AP, action potential; ESC-CMs, embryonic stem cell-derived cardiomyocytes; IP_3, inositol-1,4,5-trisphosphate; IP_3R, inositol-1,4,5-trisphosphate receptor; NCX, Na^+/Ca^{2+} exchanger; RyRs, ryanodine receptor channels; SERCA, sarcoplasmic reticulum Ca^{2+}-ATPase; SR, sarcoplasmic reticulum; T-tubule, transverse tubule; VDCC, voltage-dependent L-type Ca^{2+} channel.

as well as caffeine-insensitive human ESC-CMs.[44,45] Furthermore, the presence of SR Ca^{2+} release channels such as RyR2 and IP_3R was also demonstrated by immunostaining studies.[45] Ca^{2+} sparks were also identified by confocal Ca^{2+} imaging.[45–47] The involvement of IP_3-dependent Ca^{2+} release in intracellular Ca^{2+} transient was demonstrated in human ESC-CMs by using angiotensin II (AT-II) and endothelin 1 (ET-1), which activate the IP_3R-mediated Ca^{2+} release.[48] As Ca^{2+} exclusion pathways, SERCA2 and NCX were expressed at levels comparable to those of the adult porcine myocardium. However, other Ca^{2+}-regulatory proteins such as calsequestrin, triadin, junction, and phospholamban were not detectable in human

ESC-CMs.[43,44] The absence of calsequestrin and phospholamban is possibly the cause for the dysfunctional SR seen in Ca^{2+}-handling of human ESC-CMs. Indeed, a somatic gene-transfer of calsequestrin into human ESC-CMs rendered their Ca^{2+} handling more adult-like, as demonstrated by augmented size of Ca^{2+}-transient and accelerated kinetics.[49]

4.   Conclusion and future directions

Electrophysiological properties and Ca^{2+} handling features are significantly different between early stage ESC-CMs and

mature cardiomyocytes. The most prominent difference lies in the Ca^{2+} recruitment mechanism. Immature cardiomyocytes rely on trans-sarcolemmal Ca^{2+} influx and IP_3R-mediated Ca^{2+} release while mature cardiomyocytes rely on RyR-mediated Ca^{2+} release with well-developed T-tubular system and coupling between RyR and L-type Ca^{2+} channels. However, the role of TRPC3 in spontaneous Ca^{2+} oscillation in early stage ESC-CMs is not fully resolved. Studies on the role of IK_{Ca} in diastolic depolarization of pacemaker potential is also in very primitive stage. It is essential to integrate all the aspects of electrophysiology and Ca^{2+} handling into a comprehensive mathematical model.

Conflicts of interest

No competing financial interests exist.

Acknowledgments

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