Facilitated maturation of Ca\(^{2+}\) handling properties of human embryonic stem cell-derived cardiomyocytes by calsequestrin expression

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1Stem Cell Program and 2Department of Cell Biology and Human Anatomy, University of California Davis, Davis, California; 3Institute of Pediatric Regenerative Medicine, Shriners Hospital for Children of North America, Sacramento, California; 4Stem Cell and Regenerative Medicine Program, Heart, Brain, Hormone and Healthy Aging Research Center, and 5Cardiology Division, Department of Medicine, The University of Hong Kong, Hong Kong

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Liu J, Lieu DK, Siu CW, Fu JD, Tse HF, Li RA. Facilitated maturation of Ca\(^{2+}\) handling properties of human embryonic stem cell-derived cardiomyocytes by calsequestrin expression. Am J Physiol Cell Physiol 297: C152–C159, 2009. First published April 8, 2009; doi:10.1152/ajpcell.00060.2009.—Cardiomyocytes (CMs) are nonregenerative. Self-renewable pluripotent human embryonic stem cells (hESCs) can differentiate into CMs for cell-based therapies. We recently reported that Ca\(^{2+}\) handling, crucial to excitation-contraction coupling of hESC-derived CMs (hESC-CMs), is functional but immature. Such immature properties as smaller cytosolic Ca\(^{2+}\) transient amplitudes, slower kinetics, and reduced Ca\(^{2+}\) content of sarcoplasmic reticulum (SR) can be attributed to the differential developmental expression profiles of specific Ca\(^{2+}\) handling and regulatory proteins in hESC-CMs and their adult counterparts. In particular, calsequestrin (CSQ), the most abundant, high-capacity but low-affinity, Ca\(^{2+}\)-binding protein in the SR that is anchored to the ryanodine receptor, is robustly expressed in adult CMs but completely absent in hESC-CMs. Here we hypothesized that gene transfer of CSQ in hESC-CMs suffices to induce functional improvement of SR. Transduction of hESC-CMs by the recombinant adenovirus Ad-CMV-CSQ-IRES-GFP (Ad-CSQ) significantly increased the transient amplitude, upstroke velocity, and transient decay compared with the control Ad-CMV-GFP (Ad-GFP) and Ad-CMV-CSQ4-IRES-GFP (Ad-CSQΔ, which mediated the expression of a nonfunctional, truncated version of CSQ) groups. Ad-CSQ increased the SR Ca\(^{2+}\) content but did not alter L-type Ca\(^{2+}\) current. Pharmacologically, untransduced wild-type, Ad-GFP-, Ad-CSQ4-, and Ad-CSQ4-transduced hESC-CMs behaved similarly. Whereas ryanodine significantly reduced the Ca\(^{2+}\) transient amplitude and slowed the upstroke, thapsigargin slowed the decay. Neither triadin nor junctin was affected. We conclude that CSQ expression in hESC-CMs facilitates Ca\(^{2+}\) handling maturation. Our results shed insights into the suitability of hESC-CMs for therapies and as certain heart disease models for drug screening.

HUMAN EMBRYONIC STEM CELLS (hESCs), isolated from the inner cell mass of blastocysts, can self-renew while maintaining their pluripotency to differentiate into all cell types (33), including cardiomyocytes (CMs) (7, 16, 19, 24, 37). Therefore, hESCs may provide an unlimited ex vivo source of CMs for cell-based heart therapies. Although hESC-derived CMs (hESC-CMs) have been reported to improve cardiac function in several animal myocardial infarct models (7, 16), numerous hurdles need to be overcome before their clinical applications. For instance, we (19) and others (10, 26) have recently reported that although Ca\(^{2+}\) handling is functional in hESC-CMs, such Ca\(^{2+}\) transient properties as smaller peak amplitude, slower rise, and decay kinetics are indeed immature relative to the adult form. A number of crucial Ca\(^{2+}\)-handling proteins are differentially expressed in hESC-, fetal, and adult CMs (19). During an action potential of adult CMs, Ca\(^{2+}\) entry into the cytosol through sarcoendoplasmal L-type Ca\(^{2+}\) current (ICa,L or Cav1.2) channels triggers the release of Ca\(^{2+}\) from the intracellular Ca\(^{2+}\) stores (also known as sarcoplasmic reticulum, SR) via the ryanodine receptor (RyR). This process, the so-called Ca\(^{2+}\)-induced Ca\(^{2+}\)-release (CICR) (4), escalates the cytosolic Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]\(_i\)) to activate the contractile apparatus for contraction. For relaxation, elevated [Ca\(^{2+}\)]\(_i\) gets pumped back into the SR by the sarco/endoplasmic reticulum Ca\(^{2+}\)-ATPase (SERCA) and extruded by the Na\(^+\)/Ca\(^{2+}\) exchanger (NCX) to return to the resting [Ca\(^{2+}\)]\(_i\) level. Such a rise and subsequent decay of [Ca\(^{2+}\)]\(_i\) is known as Ca\(^{2+}\) transient. Both the contractile force (inotropic) and frequency (chronotropic) of CMs are dependent on the amplitude and kinetic properties of Ca\(^{2+}\) transients. Given the central importance of CICR in cardiac excitation-contraction coupling, proper Ca\(^{2+}\)-handling properties of hESC-CMs are therefore crucial for their successful functional integration with the recipient heart after transplantation. Indeed, abnormal Ca\(^{2+}\) handling, as in the case of heart failure, can even be arrhythmogenic (e.g., to cause delayed after depolarization) (4, 34).

Ca\(^{2+}\) homeostasis is dependent on such Ca\(^{2+}\)-handling proteins as ICa,L channels, RyR, SERCA, and NCX. RyRs are arranged in large organized arrays (up to 200 nm in diameter with more than 100 RyRs) at the junctions between the SR and sarcosome (i.e., t-tubules) beneath ICa,L channels. These arrays constitute a large functional Ca\(^{2+}\) release complex. RyRs are also coupled to other proteins at the luminal SR surface such as triadin, junctin, and calsequestrin (CSQ). As the most abundant, high-capacity but low-infinity Ca\(^{2+}\)-binding protein in the SR, the cardiac isoform CSQ2 can store up to 20 mM Ca\(^{2+}\) while buffering the free SR [Ca\(^{2+}\)] at ~1 mM. This allows repetitive muscle contractions without rundown. Although CSQ is robustly expressed in adult CMs, we have previously shown that CSQ is completely absent in hESC-CMs (19). Indeed, the role of CSQ in the Ca\(^{2+}\)-handling properties of hESC-CMs is completely unknown. Here we tested the hypothesis that CSQ plays a pivotal role in the development of Ca\(^{2+}\)-handling properties of hESC-CMs via somatic gene transfer. We conclude that expression of the missing protein CSQ in hESC-CMs facilitates maturation in the levels of SR calcium transients; ryanodine receptor; adenovirus

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activity by increasing the Ca\(^{2+}\) load and enhancing RyR-mediated Ca\(^{2+}\) release. A better basic understanding will help evaluate the suitability and use of hESC-CMs for therapies and as certain heart disease models for drug screening.

MATERIALS AND METHODS

**hESC culturing and differentiation.** H1 cells (33) (WiCells, Madison, WI) were grown on irradiated mouse embryonic fibroblasts from 13.5-day embryos of CF-1 mice and propagated as previously described (19, 35, 37). The culture medium consisted of 80% Dulbecco’s modified Eagle’s medium, 20% knockout serum replacement, 4 ng/ml basic fibroblast growth factor (b-FGF), 1 mmol/l L-glutamine, 0.1 mmol/l β-mercaptoethanol, and 1% nonessential amino acid solution (all from Invitrogen). To induce the formation of embryoid bodies (EBs), H1 cells were detached using 1 mg/ml type IV collagenase and transferred to petri dishes containing 20% fetal bovine serum defined (HyClone, Logan, UT), 1 mmol/l L-glutamine, and 1% nonessential amino acid stock in the absence of b-FGF. The aggregates were cultured in suspension for 7 days followed by being plated on gelatin-coated (0.1%; Sigma-Aldrich, St. Louis, MO), six-well plates to form hESC-CMs.

**Construction of recombinant adenoviruses.** To mediate the expression of CSQ in hESC-CMs, the human cardiac CSQ isoform 2 (sc119365, Origene technologies) was subcloned into the adenoviral shuttle vector pAdCMV-IRES-GFP (pAd-GFP) that we described previously (38) using primers containing the Bmt and SpeI restriction sites to generate pAdCMV-CSQ-IRES-GFP (pAd-CSQ). IRES (internal ribosomal entry site) enables simultaneous translation of CSQ and green fluorescent protein (GFP) with a single transcript. A truncated CSQ mutant CSQΔ was constructed in Ad-CSQ by deleting 817 bp (53 bp–869 bp) using the two EcoM1 sites within the coding sequence of CSQ. A similar truncated CSQ created by introducing a stop codon after amino acid residue 71 has been shown to be nonfunctional (32). Adenoviral particles were generated by Cre-lox recombination of purified φ6 viral DNA and shuttle vector DNA using Cre4 cells as previously described (12). The recombinant products were plaque purified, amplified, and purified again by Vivapure Adenovirus Pack (Vivascreen), yielding concentrations of the order of 10\(^9\) plaque-forming units per milliliter.

**Isolation of hESC-CMs and adenoviral gene transfer.** For isolating hESC-CMs, beating outgrowths were microscopically dissected from hESC-derived EBs (21 to 28 days) by a glass knife, followed by incubation in collagenase II (1 mg/ml) at 37°C for 30 min. The isolated cells were incubated with Kraftbühre solution containing (in mM) 140 NaCl, 5 KCl, 2 CaCl\(_2\), 1 MgCl\(_2\), 25 HEPES, and 10 glucose (pH 7.4) at 37°C. Patch pipette solution contained (in mM) 110 K\(^{+}\) aspartate, 20 KCl, 1 MgCl\(_2\), 5 Ca\(^{2+}\), 5 Mg-ATP, 5 Na\(^{+}\)-phosphocreatine, 1 EGTA, 10 HEPES, pH adjusted to 7.3 with KOH. To elicit \(I_{Ca,L}\), cells were held at a −40 mV potential and pulsed from −40 mV to +60 mV with 10-mV increments for 2 s. \(I_{Ca,L}\) was defined as 5 nM nifedipine-sensitive currents.

**Di-8-ANEPPS staining of T-tubule.** hESC-CMs were incubated with 10 μM Di-8-ANEPPS (Invitrogen) for 5 min at room temperature. After being wash for 10 min with PBS, the midplanes of the cell height were imaged using a confocal laser-scanning microscope (C1si; Nikon, Tokyo, Japan).

**Statistical analysis.** All data were expressed as means ± SE. One-way ANOVA followed by Newman-Keuls multiple comparison tests or paired t-test was carried out to test for differences between the mean values within the same study. A difference of \(P < 0.05\) was considered significant.

RESULTS

**Ad-CSQ transduction of hESC-CMs upregulated CSQ but did not alter other Ca\(^{2+}\)-handling proteins.** As we previously reported, CSQ is completely absent in hESC-CMs (19). In the present study, we first confirmed the efficacy of our adenoviral constructs to mediate CSQ expression in hESC-CMs. Figure 1A shows that the CSQ mRNA transcript was significantly elevated in Ad-CSQ-transduced hESC-CMs compared with the Ad-GFP-transduced control group. The increased transcript level translated into robust CSQ protein expression in Ad-CSQ-transduced hESC-CMs, although it remained less than that of human adult ventricular CMs (Fig. 1B). As anticipated, Ad-CSQΔ led to detectable increase in neither the CSQ transcript nor protein, same as Ad-GFP (\(P > 0.05\)). Transduction of hESC-CMs by Ad-GFP, Ad-CSQ, or Ad-CSQΔ had no effect on the transcript levels of other Ca\(^{2+}\)-handling proteins such as RyR, triadin, junctin, SERCA, Cav1.2, and calreticulin (Fig. 1A). Consistently, the expression levels of the same Ca\(^{2+}\)-handling proteins were identical in Ad-CSQ- and Ad-CSQΔ-transduced hESC-CMs (Fig. 1C).

**Ad-CSQ transduction increased the SR Ca\(^{2+}\) content of hESC-CMs.** Given the known importance of CSQ in determining the SR Ca\(^{2+}\) load, we next assessed the effect of CSQ

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expression on the Ca$^{2+}$ storage capacity of SR in hESC-CMs by caffeine (10 mM) application. As anticipated from previous results (19, 26), a brief exposure to caffeine elicited robust increases of cytosolic Ca$^{2+}$ in Ad-GFP-, Ad-CSQ-, and Ad-CSQΔ-transduced hESC-CMs (Fig. 2). Interestingly, the Ca$^{2+}$ transient peak amplitude was significantly larger in Ad-CSQ- (n = 20) than that of Ad-GFP-transduced hESC-CMs (n = 12) by 32.7% (P < 0.05), indicating a substantial increase in SR Ca$^{2+}$ content presumably due to the higher Ca$^{2+}$ binding activity conferred by the expressed CSQ. The peak amplitudes of Ad-CSQΔ- and Ad-GFP-transduced hESC-CMs were not different (P > 0.05).

**Ad-CSQ transduction enhanced magnitude and kinetics of Ca$^{2+}$ transients.** To investigate whether CSQ expression in hESC-CMs facilitates the development of their Ca$^{2+}$-handling properties, electrically induced Ca$^{2+}$ transients of Ad-GFP, Ad-CSQ, and Ad-CSQΔ-transduced hESC-CMs were characterized and compared. Consistent with a larger SR load, Fig. 3 shows that Ad-CSQ-transduced hESC-CMs (n = 29) indeed generated larger Ca$^{2+}$ transients with higher upstroke and decay velocity relative to the Ad-GFP-transduced control group (n = 12, P < 0.05). When the same electrically stimulated Ca$^{2+}$ transient parameters were assessed, however, Ad-CSQΔ-transduced hESC-CMs were not different from the Ad-GFP control (P > 0.05). Previously we reported that hESC-CMs have lower cytosolic Ca$^{2+}$ than human fetal and adult CMs (19). Indeed, basal cytosolic Ca$^{2+}$ was elevated in Ad-CSQ-transduced (n = 20; P < 0.05) but not Ad-CSQΔ-transduced (n = 9; P > 0.05) compared with Ad-GFP-transduced hESC-CMs (n = 11). Taken collectively, the above results indicate that CSQ expression in hESC-CMs enhanced their SR Ca$^{2+}$ release and development of mature Ca$^{2+}$ homeostasis.

**Pharmacological responses of Ad-CSQ-transduced hESC-CMs in Ca$^{2+}$ transients.** When anchored to the RyR complex via triadin and junctin, CSQ is known to modulate RyR activity (2). To investigate the effect of CSQ expression on RyR activity of hESC-CMs, we studied and compared the responses of electrically induced Ca$^{2+}$ transients of Ad-GFP-, Ad-CSQ-, and Ad-CSQΔ-transduced cells to ryanodine and thapsigargin, known inhibitors of RyR and SERCA, respectively. Similar to our previous finding (19), Fig. 4 shows that application of 10 µM ryanodine to Ad-GFP-transduced hESC-CMs significantly (P < 0.05) decreased the electrically evoked Ca$^{2+}$ transient amplitude and slowed the upstroke velocity to 80.4 ± 2.8% and 63.8 ± 5.2% (n = 5) of the drug-free values, respectively. The inhibitory effects of ryanodine on the peak amplitude and upstroke velocity in Ad-CSQ (72.7 ± 3.0% and 60.8 ± 7.2%, respectively; n = 8)- and Ad-CSQΔ (84.2 ± 2.3% and 62.0 ± 3.2%, respectively; n = 5)-transduced hESC-CMs were identical to the Ad-GFP control (P > 0.05).

Consistent with the notion that SERCA mediates Ca$^{2+}$ reuptake for SR reload in hESC-CMs, 0.5 µM thapsigargin significantly decreased the decay velocity of Ad-GFP-, Ad-CSQ-, and Ad-CSQΔ-transduced hESC-CMs to 68.5 ± 7.2% (n = 6), 60.7 ± 11% (n = 7), and 65.2 ± 6.2% (n = 6), respectively (Fig. 5). However, the transient amplitudes were not affected by thapsigargin (103.6 ± 5.3%; n = 7; 99.7 ± 7.9%; n = 6; and 102.4 ± 8.4%; n = 5, respectively; P > 0.05). These responses of all three groups of hESC-CMs were not different from each other (P > 0.05).
CSQ expression in hESC-CMs did not alter \( I_{\text{Ca,L}} \) and capacitance. Since \( I_{\text{Ca,L}} \) mediates the entry of \( \text{Ca}^{2+} \) and is known to express in even early hESC-CMs (24), its activity if increased by Ad-CSQ transduction can potentially augment CICR and SR \( \text{Ca}^{2+} \) release, thereby leading to our observations presented above. To test this possibility, we examined the current-voltage relationships of \( I_{\text{Ca,L}} \) recorded from Ad-CSQ- and Ad-CSQ\( \Delta \)-transduced hESC-CMs. Figure 6, A–C, shows that the magnitudes of peak \( I_{\text{Ca,L}} \) of the two experimental groups were not different across the entire voltage range investigated (\( P > 0.05 \)), consistent with the finding that \( I_{\text{Ca,L}} \) is expressed in early hESC-CMs and not substantially changed during maturation (25). Therefore, CSQ expression did not alter \( I_{\text{Ca,L}} \). It is known that cardiac overexpression of CSQ leads to hypertrophy with increased heart mass and cell size (14, 27). Indeed, such a transgenic approach has been used as a mouse model of hypertrophy (9, 39). Therefore, we also measured the membrane capacitance to assess cell hypertrophy after CSQ expression. However, no changes could be detected (\( P > 0.05 \), Fig. 6D).

CSQ expression did not induce the formation of t-tubules. The membrane-selective lipophilic dye Di-8-ANEPPS was used to detect the presence of t-tubules, invaginations of the surface membrane of CMs. Regardless of CSQ expression, only the periphery, but not the cellular midplane, of hESC-CMs was positively stained (Fig. 7). This was different from the organized pattern of staining found in adult CMs (18, 20), indicating that CSQ expression did not induce the formation of t-tubules not otherwise expressed in hESC-CMs (17).

DISCUSSION

In the present study, we evaluated the consequences of CSQ expression in various functional and structural aspects of hESC-CMs. The major findings were that somatic gene transfer of CSQ that is otherwise absent in hESC-CMs renders their \( \text{Ca}^{2+} \)-handling properties more mature, as reflected by an increased SR load, an elevated basal cytosolic \( \text{Ca}^{2+} \), augmented \( \text{Ca}^{2+} \)-transient amplitude, and accelerated kinetics. Although our observations with hESC-CMs share a number of similarities with previous studies of CSQ in isolated adult CMs (23, 24) and transgenic models (14, 27), several differences were also noticeable. As further elaborated and discussed below, such similarities and differences can be attributed to their common identities as CMs and different developmental stages, respectively.

CSQ2 coordinates the rates of SR \( \text{Ca}^{2+} \) release and load by modulating RyR activities. Indeed, the SR \( \text{Ca}^{2+} \) content is also known to affect the amount of \( \text{Ca}^{2+} \) released via CICR. For a given \( I_{\text{Ca,L}} \) trigger, a high SR \( \text{Ca}^{2+} \) load enhances the open probability of RyRs while directly providing more \( \text{Ca}^{2+} \) available for release. This is achieved via direct \( \text{Ca}^{2+} \) binding to the
channel protein or by acting on the CSQ-RyR complex (8, 11). By contrast, \( I_{\text{Ca,L}} \) can no longer cause CICR when the SR Ca\(^{2+}\) content is sufficiently low. Mechanistically, CSQ senses the levels of luminal Ca\(^{2+}\) and affects RyRs via triadin and junctin. When SR Ca\(^{2+}\) declines (such as that during Ca\(^{2+}\) release), the increased level of Ca\(^{2+}\)-free CSQ deactivates RyRs by binding via triadin and junctin. Alternatively, SR Ca\(^{2+}\) reload (e.g., upon relaxation when CICR terminates) relieves the CSQ-mediated inhibition of RyRs. Thus, not only does CSQ buffer to increase the SR capacity to hold luminal Ca\(^{2+}\), but it also augments the sensitivity of RyR to changes in Ca\(^{2+}\) (1).

Interestingly, in the absence of triadin and junctin, CSQ has been reported to activate purified RyRs reconstituted in lipid bilayer (3, 13). Since \( I_{\text{Ca,L}} \) was not altered by Ad-CSQ transduction, the larger electrically induced Ca\(^{2+}\) transients observed in hESC-CMs therefore did not result from a simple increase of Ca\(^{2+}\) influx via \( I_{\text{Ca,L}} \). Instead, the SR load was increased as demonstrated by our caffeine experiment. Also, the accelerated rise time of electrically induced Ca\(^{2+}\) release was most consistent with an enhanced RyR activity. CSQ-induced inhibition of RyR was not observed probably due to the absence of triadin and junctin (at both the transcript and protein levels) in Ad-CSQ-transduced hESC-CMs. Rather, the elevated basal cytosolic Ca\(^{2+}\) of Ad-CSQ-transduced hESC-CMs might contribute to the faster Ca\(^{2+}\) transient decay due to a larger Ca\(^{2+}\) gradient between cytosol and SR. Alternatively, it can also be attributed to an increased NCX activity. Further experiments will be needed to test these possibilities.

Of note, CSQ is already expressed in adult CMs but completely absent in hESC-CMs as we already reported (19). Previous studies of adult CMs and transgenic animals obtain valuable information about the biology of CSQ by altering its

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**Fig. 4. Effects of ryanodine on electrically induced Ca\(^{2+}\) transients.**

- **A:** representative tracings; amplitude (B) and maximum \( V_{\text{max,upstroke}} \) (C) after ryanodine application normalized to values recorded under control ryanodine-free conditions (dashed line i.e., 100\%); \( n = 5, 8, \) and 5 for Ad-GFP, Ad-CSQ, and Ad-CSQ\(\Delta\), respectively. **\( P < 0.01 \) vs. dashed line.

**Fig. 5. Effects of thapsigargin on electrically induced Ca\(^{2+}\) transients.**

- **A:** representative tracings; amplitude (B) and \( V_{\text{max,decay}} \) (C) normalized to values recorded under control thapsigargin-free conditions (dashed line, i.e., 100\%); \( n = 6, 7, \) and 6 for Ad-GFP, Ad-CSQ, and Ad-CSQ\(\Delta\), respectively. *\( P < 0.05, \)**\( P < 0.01 \) vs. dashed line.
expression in isolated cells or the native heart to above or below the physiological level. Our experiments, however, attempted to express the missing protein in hESC-CMs to facilitate the maturation process by rendering their Ca^{2+}-handling properties adult-like, thereby shedding insights into its developmental and functional roles. For instance, adenovirus-mediated overexpression of CSQ2 in adult rabbit ventricular CMs in vitro has been previously reported to increase the SR Ca^{2+} content while reducing the excitation-contraction coupling gain (23). Although the Ca^{2+} load is increased in transgenic mice that overexpress CSQ, however, these animals develop hypertrophy and heart failure with impaired SR Ca^{2+} release and decreased Ca^{2+} spark frequency (14, 27, 36). By contrast, CSQ2 deletion causes a striking increase in the SR volume as a compensatory response to maintain an operable SR Ca^{2+} storage (15). As a result, premature spontaneous SR Ca^{2+} release and subsequently, arrhythmias occur due to the relieved inhibition of RyRs by CSQ (15, 31). Therefore, whereas the experimental approaches are complementary in their overall goal to obtain a better understanding of CSQ in cardiac physiology, ours differs by design. In addition, species-specific properties likely contribute to the observations in this and previous studies. Despite the various experimental outcomes, it is apparent from the collective data sets that increased CSQ2 expression generally leads to an increased SR load that is crucial for CMs maturation.

In mature ventricular CMs, CICR is optimized by the presence of t-tubules, invaginations in the sarcolemmal membrane that concentrate Cav1.2 and bring them spatially closer to RyRs (5, 6). Fast and synchronous activation of RyR translates into a larger Ca^{2+} transient amplitude, recruitment of more actin-myosin cross-bridge cycling, and subsequent generation of greater contractile force (6, 20). Recently, our laboratory (17) and Satin et al. (26) independently reported that such a uniform, spatially and temporally synchronized Ca^{2+} propagation wavefront is not observed in hESC-CMs. Although CSQ expression in hESC-CMs increased the transient peak amplitude and hastened the kinetics, t-tubule biogenesis could not be induced, consistent with the lack of CSQ-induced cellular hypertrophy as gauged by membrane capacitance. This observation again differs from the increased heart mass and cell size reported for transgenic mice that overexpress cardiac CSQ (14, 27). With reduced contractility and Ca^{2+} transient amplitudes, these animals have indeed been employed as a model of hypertrophy (9, 39).

Fig. 6. Voltage-clamp protocol (A) used to investigate the current-voltage (I-V) curve of L-type Ca current (I_{Ca,L}) in Ad-CSQ and Ad-CSQΔ groups. B: typical tracings of I_{Ca,L}; n = 4 for Ad-CSQ and 3 for Ad-CSQΔ. D: membrane capacitance; n = 13 for Ad-CSQ and 10 for Ad-CSQΔ.
Gene transfer of calsequestrin in hESC-CMs

Terminally differentiated CMs can undergo certain compensatory changes as a result of changes in the environment, the expression or activity of certain specific gene products. For instance, the presence of thapsigargin or small interfering RNA (siRNA)-mediated genetic suppression of SERCA2A in CMs has been reported to cause remodeling of the Ca\(^{2+}\) signaling mechanisms (30). In general, these responses of adult CMs can be considered as adaptation for maintenance or homeostasis because their protein synthesis is largely determined by protein turnover rather than active synthesis (28–30). By contrast, hESC-CMs are immature cells that are robustly undergoing developmental changes with active protein synthesis. As such, it has been postulated that hESC-CMs are more functionally plastic. However, Cav1.2 and an array of Ca\(^{2+}\)-handling proteins such as junctin, triadin, RyR, SERCA, and calreticulin other than CSQ were not altered in our experiments. Furthermore, neither cellular hypertrophy nor t-tubule biogenesis was observed after Ad-CSQ transduction. Developmentally, immature CMs are known to express significantly levels of calreticulin; calreticulin decreases after birth due to posttranscriptional modification and is subsequently replaced by CSQ during SR maturation (21, 22, 31). Our results show that Ad-CSQ transduction also did not affect calreticulin. Taken together, our results indicate that CSQ expression in hESC-CMs did not induce significant compensatory response to augment or counterbalance its own gene product activity. As such, our observed functional changes can be primarily attributed to CSQ.

Of note, although signs of maturation were observed, however, it is clear that the adult level has not been reached. Indeed, factors other than CSQ such as the environments and other gene products are also likely involved in the maturation of hESC-CMs. For instance, the lack of or insufficient expression of other CSQ-related proteins (e.g., junctin and triadin) might have prevented further maturation. If so, future strategies to facilitate maturation can involve the expression of specific regulatory proteins simultaneously with CSQ. Furthermore, adenovirus-mediated gene transfer is only transient. As such, it might contribute to the lack of changes of other Ca-handling proteins that are required for global maturation. Further investigations (e.g., lentiviral gene transfer for persistent genetic modification) will be needed to shed additional insights into cardiac differentiation and maturation.

In conclusion, expression of CSQ that is absent in hESC-CMs facilitates the maturation of their Ca\(^{2+}\)-handling properties by increasing the SR load, Ca\(^{2+}\) transient amplitude, and kinetics. The results represent a first step to understand their development and maturation and may ultimately lead to effective approaches to derive adult-like stem cell-derived CMs with improved efficacy.

REFERENCES


