Tao R, Lau C-P, Tse H-F, Li G-R. Functional ion channels in mouse bone marrow mesenchymal stem cells. Am J Physiol Cell Physiol 293: C1561–C1567, 2007. First published August 15, 2007; doi:10.1152/ajpcell.00240.2007.—Bone marrow mesenchymal stem cells (MSCs) are used as a cell source for cardiomyoplasty; however, the cellular electrophysiological properties are not fully understood. The present study was to investigate the functional ion channels in undifferentiated mouse bone marrow MSCs using whole cell patch-voltage clamp technique, RT-PCR, and Western immunoblotting analysis. We found that three types of ion currents were present in mouse MSCs, including a Ca$^{2+}$-activated K$^+$ current (IC$\text{aK}$), an inwardly rectifying K$^+$ current (IK$\text{ir}$), and a chloride current (ICl). IC$\text{ir}$ was inhibited by Ba$^{2+}$, and IK$\text{ir}$ was activated by the Ca$^{2+}$ ionophore A-23187 and inhibited by the intermediate-conductance IK$\text{aK}$ channel blocker etidronate. ICl was activated by hypotonic (0.8 T) conditions and inhibited by the chloride channel blockers DIDS and NPPB. The corresponding ion channel genes and proteins, KCa3.1 for IK$\text{aK}$, Kir2.1 for IK$\text{ir}$, and Clcn3 for ICl, were confirmed by RT-PCR and Western immunoblotting analysis in mouse MSCs. These results demonstrate that three types of functional ion channel currents (i.e., IK$\text{ir}$, IK$\text{aK}$, and ICl) are present in mouse bone marrow MSCs. Inward rectifier potassium current; intermediate-conductance calcium-activated potassium current; volume-sensitive chloride current

BONE MARROW MESENCHYMAL STEM CELLS (MSCs) are pluripotent adult stem cells that can differentiate into osteoblasts, chondrocytes, adipocytes (25), and excitable cells such as neurons (36), skeletal muscle cells (7), and cardiomyocytes (29). MSCs can be isolated and expanded in vitro (27). In addition, it is believed that MSCs lack the B7 costimulatory molecules CD80 and CD86 and are nonimmunogenic upon allogeneic transplantation (12). These properties make MSCs an ideal cell source for regenerative medicine. It has been demonstrated that the implantation of MSCs to infarcted myocardium induces myocardial regeneration (10) or angiogenesis and improves cardiac function (31). Although the therapeutic effects are encouraging, the potential proarrhythmic effect of transplanted MSCs was observed in both in vitro and in vivo studies (3, 26). These reports point out an important concern that the implantation of MSCs might lead to cardiac electrical remodeling and initiate cardiac arrhythmia. These potential adverse effects could hinder the application of MSCs in clinical practice in cardiac diseases. Therefore, it is essential to understand the electrophysiological properties of MSCs before differentiation induction. Recent studies described that multiple ion channel currents were present in human, rabbit, and rat MSCs (5, 9, 15, 16). Differences in both current types and encoding genes have been observed between these species. The present study was designed to investigate the electrophysiological properties of mouse MSCs from bone marrow. We found three functional ion currents in mouse MSCs different from those observed in human and rat MSCs.

MATERIALS AND METHODS

Cell culture. Mouse bone marrow MSCs (passage 5) from C57Bl/6 mice are kindly provided by Dr. Darwin J. Prockop (Center for Gene Therapy, Tulane University, New Orleans, LA; http://www.som.tulane.edu/gene_therapy), are positive for Sca-1 and negative for CD34, CD45, and CD11B-C surface markers, and can be differentiated into adipocytes and osteocytes. The cells were cultured in Iscove’s modified Dulbecco’s medium (Sigma-Aldrich, St. Louis, MO) with 2 mM l-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin, 10% fetal bovine serum (Invitrogen), and 10% horse serum (Invitrogen) and incubated at 37°C in a humidified atmosphere of 95% air and 5% CO$_2$. When cells grew to ~70–80% confluence, they were detached for subculture or electrophysiological study by using 0.125% trypsin and 1 mM EDTA in phosphate-buffered saline solution. Cells used in electrophysiological and molecular biological studies were from the early passages 6–8 to limit the possible variations in functional ion channel currents, genes, and proteins induced by cell senescence at later passages, because it has been reported that cell senescence occurs with many passages of in vitro MSC culture (2).

Electrophysiology. Membrane ionic currents were recorded with the whole cell patch-clamp technique as described previously (16). Borosilicate glass electrodes (1.2-mm outer diameter) were pulled with a Brown-Flanninger puller (model P-97; Sutter Instrument, Novato, CA) and had tip resistances of ~2–3 MΩ when filled with pipette solution. The tip potentials were compensated before the pipette touched the cell. After a gigaohm seal was obtained by negative suction, the cell membrane was ruptured by gentle suction to establish whole cell configuration. Data were acquired with an EPC10 amplifier (Heka, Lambrecht, Germany). Membrane currents were low-pass filtered at 5 kHz and stored on the hard disk of an IBM-compatible computer. Tyrode solution contained (mM) 136 NaCl, 5.4 KCl, 1.0 MgCl$_2$, 1.8 CaCl$_2$, 3.3 Na$_2$-HPO$_4$, 10 glucose, and 10 HEPES; pH was adjusted to 7.3 with NaOH. The pipette solution contained (mM) 20 KCl, 110 K-aspartate, 1.0 MgCl$_2$, 10 HEPES, 0.05 EGTA, 0.1 GTP, 5.0 Na$_2$-phosphocreatine, and 5.0 Mg$_2$-ATP; pH was adjusted to 7.2 with KOH. The experiments were conducted at room temperature (21–22°C).

Drugs and reagents. The small-conductance Ca$^{2+}$-activated K$^+$ channel (SKCa) blocker UCL 1684 was purchased from Tocris (Bristol, UK). Rabbit polyclonal anti-Kir2.1, anti-IClCa, goat polyclonal anti-Clcn3, and goat anti-rabbit and donkey anti-goat IgG-hors eradish peroxidase antibodies were products of Santa Cruz Biotechnology (Santa Cruz, CA). Other reagents were obtained from Sigma-Aldrich.

Reverse transcription-polymerase chain reaction. Total RNA of mouse MSCs from passages 6–8 was extracted using Trizol reagent (Invitrogen) following its enclosed protocol. RNA was treated with RNase-free DNase (Invitrogen) following its enclosed protocol. The RNA was treated with DNase to deplete any possible RNA contamination. The resulting cDNA was used to perform RT-PCR. 

Address for reprint requests and other correspondence: G.-R. Li, L8-01, Laboratory Block, Faculty of Medicine Bldg., The Univ. of Hong Kong, 21 Sassoon Road, Pokfulam, Hong Kong SAR, China (e-mail: grli@hkucc.hku.hk).

http://www.ajpcell.org 0363-6143/07 $8.00 Copyright © 2007 the American Physiological Society C1561

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
DNase I (GE Healthcare) to remove genomic DNA. Reverse transcription (RT) was performed with the RT system (Promega, Madison, WI) protocol in a 20-µl reaction mixture using oligo(dT)₁₅ primers. After the RT procedure, the reaction mixture (cDNA) was used for polymerase chain reaction (PCR).

PCR primers were designed with Primer Premier 5 software (Premier Biosoft International, Palo Alto, CA) and synthesized at the Genome Research Center at the University of Hong Kong. PCR was performed with the Promega PCR Core System I. The cDNA in 2-µl aliquots was amplified using a DNA thermal cycler (Mycycler; Bio-Rad Laboratories, Hercules, CA) in a 25-µl reaction volume containing recommended concentrations of PCR components. The amplification was performed under the following conditions: the mixture was initially denatured at 95°C for 2 min, followed by 28–30 cycles amplification (denaturation, 95°C for 45 s; annealing, 58°C for 45 s; extension, 72°C for 1 min). This was followed by a final extension at 72°C (5 min) to ensure complete product extension. The PCR products were resolved through 1.5% agarose gel electrophoresis, and the amplified cDNA bands were visualized using ethidium bromide staining and imaged using a Chemi-Genius Bio Imaging System (GE Healthcare).

**Statistical analysis.** Results are means ± SE. Paired and/or unpaired Student’s *t*-tests were used as appropriate to evaluate the statistical significance of differences between two group means, and analysis of variance was used for multiple groups. Values of *P* < 0.05 were considered to indicate statistical significance.

**Fig. 1.** Families of membrane currents in mouse mesenchymal stem cells (MSCs). A: voltage-dependent membrane current showing weak inward rectification at positive potential. Currents were elicited with the protocol shown in the inset. B: voltage-dependent current showing a property of inward rectification. Currents were recorded with 300-ms voltage steps to between −120 and 0 mV from a holding potential of −40 mV (inset). C: voltage-dependent current showing outward rectification. Currents were recorded with 300-ms voltage steps to between −120 and +60 mV from a holding potential of −40 mV (inset).

DNase I (GE Healthcare) to remove genomic DNA. Reverse transcription (RT) was performed with the RT system (Promega, Madison, WI) protocol in a 20-µl reaction mixture using oligo(dT)₁₅ primers. After the RT procedure, the reaction mixture (cDNA) was used for polymerase chain reaction (PCR).

PCR primers were designed with Primer Premier 5 software (Premier Biosoft International, Palo Alto, CA) and synthesized at the Genome Research Center at the University of Hong Kong. PCR was performed with the Promega PCR Core System I. The cDNA in 2-µl aliquots was amplified using a DNA thermal cycler (Mycycler; Bio-Rad Laboratories, Hercules, CA) in a 25-µl reaction volume containing recommended concentrations of PCR components. The amplification was performed under the following conditions: the mixture was initially denatured at 95°C for 2 min, followed by 28–30 cycles amplification (denaturation, 95°C for 45 s; annealing, 58°C for 45 s; extension, 72°C for 1 min). This was followed by a final extension at 72°C (5 min) to ensure complete product extension. The PCR products were resolved through 1.5% agarose gel electrophoresis, and the amplified cDNA bands were visualized using ethidium bromide staining and imaged using a Chemi-Genius Bio Imaging System (GE Healthcare).

**Statistical analysis.** Results are means ± SE. Paired and/or unpaired Student’s *t*-tests were used as appropriate to evaluate the statistical significance of differences between two group means, and analysis of variance was used for multiple groups. Values of *P* < 0.05 were considered to indicate statistical significance.

**Fig. 2.** Ca²⁺-activated K⁺ current (*I_{K(Ca)}*) in mouse MSCs. A: voltage-dependent current was not affected by the small-conductance *I_{K(Ca)}* blocker UCL 1684 (1 µM) and the big-conductance *I_{K(Ca)}* blocker paxilline (1 µM) but was inhibited by the intermediate-conductance *I_{K(Ca)}* blocker clotrimazole. Currents were elicited with the voltage protocol shown in the inset. B: voltage-dependent current was enhanced by the Ca²⁺ ionophore A-23187 (1 µM). A-23187-activated current was inhibited by coapplication of A-23187 and 1 µM clotrimazole (CLT); similar results were obtained in a total of 6 cells. C: current-voltage (*I-V*) relationships of membrane currents during control, in the presence of 1 µM A-23187, and in the copresence of A-23187 and 1 µM CLT. Arrows in A and B indicate the zero current level.
small- or large-conductance $I_{KCa}$, is present in mouse MSCs. In another set of experiments, the membrane current was recorded using a pipette solution containing 800 nM free Ca$^{2+}$. We found that significant $I_{KCa}$ was detected in 95% (20 of 21) of cells. The percentage of cells with $I_{KCa}$ recorded using the high concentration of free Ca$^{2+}$ in pipette solution was much higher than that with a standard low-EGTA pipette solution (95% vs. 57%, $P < 0.05$).

A previous study by our group (15) showed that the Ca$^{2+}$-ionophore ionomycin increased $I_{KCa}$ in rat MSCs, and the

**RESULTS**

Families of membrane ion currents. Figure 1 illustrates families of membrane currents recorded in undifferentiated mouse MSCs using a standard pipette solution. Three types of membrane currents were observed in mouse MSCs (in a total of 87 cells). One current was activated by depolarization voltages to between −70 and +60 from −80 mV, showing a rapid activation at potentials −70 and +10 mV and a weak inward rectification at +20 to +60 mV (Fig. 1A). These features suggest this current is likely an intermediate-conductance Ca$^{2+}$-activated K$^+$ channel current ($I_{KCa}$). The current was observed in 57% (50 of 87) of cells. Another current was activated by voltage steps to between −120 and 0 mV from −40 mV, showing a property typical of inwardly rectifying K$^+$ current ($I_{Kir}$) (Fig. 1B). $I_{Kir}$ was observed in 16% (14 of 87) of cells. A third current was elicited by voltage steps to between −100 and +60 from −40 mV, showing a small inward current and a large outward current with outward rectification (Fig. 1C). This type of current was observed in 34% (30 of 87) of mouse MSCs. $I_{Kir}$ and the third type of current were copresent in a small population (8%, 7 of 87) of mouse MSCs.

Ca$^{2+}$-activated K$^+$ currents. Figure 2A shows that the SKCa blocker UCL 1684 (1 μM) and the big-conductance $I_{KCa}$ (BKCa) blocker paxilline (1 μM) did not inhibit the outward current, whereas the intermediate-conductance $I_{KCa}$ blocker clotrimazole (1 μM) dramatically inhibited the current amplitude. Similar results were obtained in a total of six cells. These results suggest that only intermediate-conductance $I_{KCa}$, not

![Image](http://ajpcell.physiology.org/)

Fig. 3. Effect of Ba$^{2+}$ on membrane current in mouse MSCs. A: voltage-dependent currents were inhibited by 0.5 mM BaCl$_2$, and Ba$^{2+}$-sensitive current was obtained by digitally subtracting the current during control by the current after application of Ba$^{2+}$. Currents were recorded with the protocol shown in the inset. Arrow indicates the zero current level. B: I-V relationships of membrane currents during control and in the presence of 0.5 mM Ba$^{2+}$, followed by the Ba$^{2+}$-sensitive current. Ba$^{2+}$ significantly inhibited the current at test potentials from −120 to −90 (n = 5, $P < 0.01$) and from −70 to −50 mV ($P < 0.05$). Ba$^{2+}$-sensitive current showed a strong inward rectification.

![Image](http://ajpcell.physiology.org/)

Fig. 4. Cl$^-$ current ($I_{Cl}$) in mouse MSCs. A: voltage-dependent currents were inhibited by the Cl$^-$ channel blocker 4,4’-dihydroxyazobenzene-2,2’-disulfonic acid (DIDS; 150 μM). Currents were elicited by 300-ms voltage steps from −40 to between −100 and +70 mV (inset). B: time course of activation of current at +60 mV upon switching from isosmotic (1T) to hypotonic (0.8T) bath solution and reduction in 0.8T solution containing low external Cl$^-$ (CL$^-$). Currents at time points a–c are shown at right. Currents were elicited by 300-ms steps to +60 from −40 mV (inset). C: voltage-dependent currents in 1T control, 0.8T solution, and 0.8T solution with 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB; 50 μM). NPPB, a blocker of $I_{Cl}$, substantially inhibited the swelling-induced current. Voltage protocol for C is shown in inset in A. D: I-V relationships for control (1.0T), 0.8T solution, and 0.8T solution containing low external Cl$^-$ (CL$^-$). Arrows in A and C indicate the zero current level.
effect was inhibited by clotrimazole. In mouse MSCs with small membrane current, a similar effect was observed. The Ca\(^{2+}\) ionophore A-23187 (1 \(\mu\)M) remarkably increased membrane conductance, and the effect was significantly inhibited by the application of 1 \(\mu\)M clotrimazole (Fig. 2B). Figure 2C shows the current-voltage (I-V) relationships of membrane current recorded using a ramp protocol in a typical experiment. The current evoked by 1 \(\mu\)M A-23187 showed a weak inward rectification and was significantly inhibited by 1 \(\mu\)M clotrimazole. These results demonstrate that intermediate conductance \(I_{\text{Ca}^2+}\) is present in mouse MSCs.

**\(Ba^{2+}\)-sensitive \(I_{\text{Kir}}\)** It is generally believed that inwardly rectifying \(K^+\) channels are sensitive to inhibition by \(Ba^{2+}\) (14). We therefore determined the effect of \(Ba^{2+}\) on \(I_{\text{Kir}}\) in mouse MSCs. Figure 3A shows original traces recorded in a representative cell using the voltage protocol shown in the inset in the absence (control) and presence of \(Ba^{2+}\). \(Ba^{2+}\) at 0.5 mM substantially reduced \(I_{\text{Kir}}\). \(Ba^{2+}\)-sensitive current was obtained by digitally subtracting currents before and after application of \(Ba^{2+}\) (Fig. 3A, right). Figure 3B displays the I-V relationships of \(I_{\text{Kir}}\) in the absence and presence of 0.5 mM \(Ba^{2+}\) and \(Ba^{2+}\)-sensitive current. \(Ba^{2+}\)-sensitive current exhibits an I-V relationship typical of an inwardly rectifying \(K^+\) current.

**\(Cl^-\) current in mouse MSCs.** The current with outward rectification shown in Fig. 1C was insensitive to inhibition of \(K^+\) channel blockers, including 5 mM tetraethylammonium (TEA), 5 mM 4-amino pyridine (4-AP), and 0.5 mM \(Ba^{2+}\) \((n = 5\) each), suggesting that the outward rectifying current is not carried by \(K^+\) ion. We then employed \(Cl^-\) channel inhibitors to determine whether the current would be carried by \(Cl^-\) ions. We found that the \(Cl^-\) channel blockers 4,4'-disothiocyanostilbene-2,2'-disulfonic acid (DIDS) and 5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB) inhibited this current. Figure 4A illustrates current traces recorded in a representative cell with the protocol shown in the inset. DIDS at 150 \(\mu\)M substantially suppressed the current, and similar results were obtained in a total of six cells with DIDS and nine cells with 50 \(\mu\)M NPPB, suggesting that the current activation is based on \(Cl^-\) channels.

To study whether the \(Cl^-\) channels are volume sensitive in mouse MSCs, we employed a 0.8 T tonic solution and recorded the membrane current using a \(K^+\)-free pipette solution as

**Table 1. Mouse gene-specific primers for RT-PCR**

<table>
<thead>
<tr>
<th>Name</th>
<th>Accession No.</th>
<th>Primer Sequence (5'-3')</th>
<th>Product Size, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>SK1</td>
<td>AF357239</td>
<td>Forward: GTCGACTGTCTATTTGCTTG</td>
<td>499</td>
</tr>
<tr>
<td>SK2</td>
<td>AF357240</td>
<td>Reverse: GTCATCCTGCTGGTTATCG</td>
<td>448</td>
</tr>
<tr>
<td>SK3</td>
<td>AF357241</td>
<td>Forward: ACACACGCGTCAAGAATG</td>
<td>449</td>
</tr>
<tr>
<td>IK1</td>
<td>AF072884</td>
<td>Reverse: AATGGTCGTCTGGGTCTCC</td>
<td>315</td>
</tr>
<tr>
<td>Slo</td>
<td>NM-010610</td>
<td>Reverse: CCTCCAGGGATGCTCTTAC</td>
<td>402</td>
</tr>
<tr>
<td>Kir1.1</td>
<td>NM-019659</td>
<td>Reverse: CCTCCAGGGATGCTCTTAC</td>
<td>402</td>
</tr>
<tr>
<td>Kir2.1</td>
<td>NM-008425</td>
<td>Reverse: CCTCCAGGGATGCTCTTAC</td>
<td>402</td>
</tr>
<tr>
<td>Kir2.2</td>
<td>NM-010603</td>
<td>Reverse: CCTCCAGGGATGCTCTTAC</td>
<td>402</td>
</tr>
<tr>
<td>Kir2.3</td>
<td>NM-008427</td>
<td>Reverse: CCTCCAGGGATGCTCTTAC</td>
<td>402</td>
</tr>
<tr>
<td>Kir2.4</td>
<td>NM-145963</td>
<td>Reverse: CCTCCAGGGATGCTCTTAC</td>
<td>402</td>
</tr>
<tr>
<td>Kir3.1</td>
<td>NM-008426</td>
<td>Reverse: CCTCCAGGGATGCTCTTAC</td>
<td>402</td>
</tr>
<tr>
<td>Clcn2</td>
<td>NM-009900</td>
<td>Reverse: CTCATGATGATGAGCTTGC</td>
<td>341</td>
</tr>
<tr>
<td>Clcn3</td>
<td>BC057133</td>
<td>Reverse: GAGAATTAAGTACCTGAGT</td>
<td>341</td>
</tr>
<tr>
<td>Clca1</td>
<td>NM-009899</td>
<td>Reverse: GAGAATTAAGTACCTGAGT</td>
<td>341</td>
</tr>
<tr>
<td>Clca2</td>
<td>NM-017474</td>
<td>Reverse: GAGAATTAAGTACCTGAGT</td>
<td>341</td>
</tr>
<tr>
<td>Clca4</td>
<td>NM-139148</td>
<td>Reverse: GAGAATTAAGTACCTGAGT</td>
<td>341</td>
</tr>
<tr>
<td>Clca5</td>
<td>NM-178697</td>
<td>Reverse: GAGAATTAAGTACCTGAGT</td>
<td>341</td>
</tr>
<tr>
<td>Clca6</td>
<td>NM-207208</td>
<td>Reverse: GAGAATTAAGTACCTGAGT</td>
<td>341</td>
</tr>
<tr>
<td>GAPDH</td>
<td>NM-001001303</td>
<td>Reverse: GAGAATTAAGTACCTGAGT</td>
<td>470</td>
</tr>
</tbody>
</table>

**SK** (i.e., \(KCa_{2.2}\)), small-conductance \(Ca^{2+}\)-activated \(K^+\) channel; \(IK1\) (i.e., \(KCa_{3.1}\)), intermediate-conductance \(Ca^{2+}\)-activated \(K^+\) channel; \(Slo\) (i.e., \(KCa_{1.1}\)), big-conductance \(Ca^{2+}\)-activated \(K^+\) channel; \(Kir\), inward rectifier \(K^+\) channel; \(Clcn\), \(Cl^+\) channel; \(Clca\), \(Ca^{2+}\)-activated \(Cl^-\) channel; \(GAPDH\), glyceraldehyde-3-phosphate dehydrogenase.
described previously (6). Figure 4B illustrates the time course of swelling-induced changes in membrane current at +60 mV in a mouse MSC when bath solution osmolarity (T) was switched from isosmotic (1T) to hyposmotic (0.8T) solution and then to 0.8T with low external Cl− (10 mM). Membrane current in 0.8T gradually increased to a relatively steady-state level and remarkably decreased after exposure to low external Cl−. Similar results were obtained in 85.7% (7 of 8) of cells. The swelling-induced current is volume-sensitive Icy, as described previously in human atrial myocytes (6). Figure 4C displays the currents elicited by voltage steps to between −100 and +70 mV from −40 mV in 1T, 0.8T, and 0.8T with 50 μM NPPB. Similar results were observed in 85.7% (12 of 14) of cells. Figure 4D shows the I-V relationships of the swelling-induced current before and after exposure to NPPB (n = 5). The swelling-induced current outwardly rectified and reversed at −27 mV (−37 mV after correction for the liquid junction potential), near the predicted Cl− equilibrium potential, −35 mV. NPPB remarkably inhibited both inward and the outward currents. These results suggest that volume-sensitive Icy is present in mouse MSCs.

**Messenger RNAs of functional ion channels and Western blotting analysis.** To explore the molecular identities of the functional ionic currents, we examined gene expression of various ionic channels in mouse MSCs with RT-PCR, using the specific primers for KCa, Kir, Clcn, and Clca ion channel families as shown in Table 1. The primers of Kv channels were employed as described previously (34). Figure 5A displays the images of RT-PCR products corresponding to gene expression of KCa3.1 (IKca), Kir2.1 (IKir), and Clcn3 (ICl) in mouse MSCs. Weak expression of Clcn2 and no expression of Kv families were found in mouse MSCs. Western immunoblot analysis confirmed the protein expressions of KCa3.1, Kir2.1, and Clcn3 as unveiled by gene expression. Figure 5B shows images of KCa3.1, Kir2.1, and Clcn3 proteins (n = 3).

**DISCUSSION**

In the present study, we demonstrated that three types of ionic currents (IKca, IKir, and ICl) were present in undifferentiated mouse bone marrow MSCs. IKca was inhibited by the intermediate-conductance inhibitor clotrimazole, IKir was blocked by Ba2+, and ICl was inhibited by DIDS or NPPB. The currents’ corresponding channels (KCa3.1 for IKca, Kir2.1 for IKir, and Clcn3 for ICl) were confirmed by RT-PCR for gene expression and Western immunoblotting in protein levels.

IKca, IKir, and ICl were identified in 57, 16, and 34% of mouse MSCs, respectively, with the use of a standard pipette solution (Fig. 1). However, a high percentage (95%) of cells with IKca was identified when free Ca2+ was increased in the pipette solution, suggesting that intermediate IKca is the dominant current in mouse MSCs in maintaining membrane potential and desirable intracellular ion concentrations. On the other hand, the percentage of cells with ICl was increased to 88% when 0.8T external solution was employed, indicating that activation of ICl is dependent on cell volume and/or size of the cells related to cell cycling (18).

The electrophysiological properties of MSCs were initially studied by Kawano et al. (11). They demonstrated that the dominant BKCa current was present in most human MSCs, and L-type Ca2+ current was present in a small population of cells. The activity of BKCa (i.e., KCa1.1) channel was regulated by the spontaneous Ca2+ oscillation, resulting in fluctuations of membrane currents and potentials. Our group (16) and others (9) provided additional information that multiple ion channels were expressed in human MSCs, including nifedipine-sensitive L-type Ca2+ current (ICaL), transient outward K+ current (Ito),

![Fig. 5. RT-PCR and Western blotting analysis for detecting ion channels expressed in mouse MSCs. A: images show that genes for KCa3.1 (IK1), Kir2.1, and Clcn3 are expressed in mouse MSCs and that Kv channel genes are absent. A weak Clcn2 gene was also detected, although no functional current was observed. B: ion channel proteins KCa3.1, Kir2.1, and Clcn3 were detected by Western immunoblotting at 50 (KCa3.1), 48 (Kir2.1), and ~80–90 kDa (Clcn3) in mouse MSCs. SK, small-conductance Ca2+-activated K+ channel; IK1, intermediate-conductance Ca2+-activated K+ channel; Slo, big-conductance Ca2+-activated K+ channel; Kir, inward rectifier K+ channel; Clcn, Cl− channel; Clca, Ca2+-activated Cl− channel; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.](http://ajpcell.physiology.org/)}
tetrodotoxin-sensitive Na+ current (I\textsubscript{Na,TTX}), and a delayed rectifier K+ current (I\textsubscript{K\textsubscript{ir}}) (9, 16). The present and previous studies by our group demonstrated the heterogeneity of electrophysiological properties of MSCs present in different species. We found that human and rat MSCs expressed functional I\textsubscript{Ca,L}, I\textsubscript{Kir}, and I\textsubscript{Na,TTX}, albeit in a small portion of cells. These currents were not present in MSCs from rabbit and mouse. I\textsubscript{Kir} current was observed in rabbit and mouse MSCs but not in human and rat MSCs. In addition, I\textsubscript{Ca} was observed in mouse MSCs (Fig. 4) but not in rat, rabbit, and human MSCs (5, 14, 15). Although functional I\textsubscript{Ca} was detected by its corresponding Clcn3 gene expression in mouse MSCs, Clcn3 in other species needs to be further clarified.

Heterogeneous expression of ion channels also was observed within the same species. For instance, the present study demonstrated that I\textsubscript{K\textsubscript{ir}}, I\textsubscript{Kir}, and I\textsubscript{Ca} were heterogeneously expressed in mouse MSCs. This could result from a heterogeneous cell population of the MSCs (30, 32). Consequently, a subpopulation of MSCs might display a different pattern of ion channel expression. This heterogeneity also may be explained by the fact that cultured MSCs are not synchronously at the same stage of the cell cycle. It should be noted that cells at different stages of the cell cycle might express different patterns of ion channels (20, 21, 37).

Ion channels play a role in cell cycling of proliferative cells (18, 22, 35). Kv1.3 channels and intermediate KCa channels are well known for T lymphocyte activation and its consequent proliferation upon antigen stimulation (13). Human ether-a-go-go K+ channels have been reported to have an oncogenic effect when transfected in immunodeficient mice (23), and the channel protein could interact with p38 MAPK and evoke p38 MAPK signaling, resulting in the promotion of cell proliferation (8). Moreover, I\textsubscript{Kir} has been found to participate in the proliferation of astrocytes (17) and hematopoietic progenitor cells (28). Although the underlying mechanisms for K+ channels in regulation of cell proliferation remain elusive, the involvement of K+ channels in cell proliferation is well established. In the present study we demonstrated the presence of intermediate I\textsubscript{K\textsubscript{Ca}} and I\textsubscript{K\textsubscript{r}} in mouse MSCs. Future studies are required to find out whether these two types of K+ channels contribute to MSC proliferation.

Clcn3 channel is regarded as one of the candidate channels for volume-regulated anion channels and has been shown to play an important role in cell proliferation and apoptosis (18, 19). Blockade or disruption of Clcn3 channel resulted in arrest of cell cycle and prevention of cell proliferation in several cell types (4, 33, 37). Functional Cl− current encoded by Clcn3, sensitive to cell volume, was observed in mouse MSCs (Fig. 4). Whether this I\textsubscript{Cl} contributes to mouse MSCs proliferation remains to be studied in the future.

Significant beneficial effects were observed in regenerating myocardium and improving cardiac function by implanting bone marrow MSCs into the damaged myocardium (1, 10, 31). However, potential proarrhythmia with the implantation of MSCs has been reported in clinical patients with cell implantation (24), in swine with cardiac infarction treated with MSC implantation (26), and in human MSCs cocultured with neonatal rat ventricular myocytes (3). An understanding of ion channel expression in undifferentiated MSCs in different species will help and/or facilitate possible biological solutions for these concerns and medical challenges.

In summary, the present study demonstrates that three types of functional ion channels are present in mouse bone marrow MSCs, including intermediate-conductance I\textsubscript{K\textsubscript{ir}}, I\textsubscript{Kir}, and volume-sensitive I\textsubscript{Ca}. RT-PCR and Western immunoblotting confirm gene expression and proteins of the corresponding functional ion channels. The information obtained from the present study provides a basis for future investigation of how these functional ion channels regulate biological and physiological activity of MSCs.

GRANTS
This study was supported by the Research Grant Council of Hong Kong (HKU 7347/03M). R. Tao was supported by a postgraduate studentship of the University of Hong Kong. We thank Dr. Darwin J. Prockop at the Center for Gene Therapy (supported by National Center for Research Resources Grant P40 RR017447), Tulane University, for providing the mouse MSCs.

REFERENCES


