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Hypercholesterolemia suppresses Kir channels in porcine bone marrow progenitor cells *in vivo*

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Abstract

Objective: Inwardly-rectifying K^+ (Kir) channels are responsible for maintaining membrane potentials in a variety of cell types including endothelial cells where they modulate endothelium-dependent vasorelaxation. The goal of this study is to determine the functional expression of Kir channels in porcine bone marrow-derived side population (BM-SP) cells that demonstrate phenotypes of endothelial progenitor cells (EPCs). We further asses the hypercholesterolemia sensitivity of Kir channels in BM-SP cells, which may play a key role in hypercholesterolemia-mediated regulation of EPCs.

Methods: To assess the effect of hypercholesterolemia on Kir channels in BM-SP, Kir currents were recorded in SP cells sorted from the bone marrow of healthy or hypercholesterolemic animals.

Results: We found Kir channels constitute the major conductance in porcine bone marrow-derived side population (BM-SP) cells. These cells are defined by their efficiency of Hoechst dye efflux and have been reported to differentiate into multiple cell lineages including endothelium *in vivo*. We demonstrate here that porcine BM-SP cells differentiate to an endothelial lineage ($CD31^+$, vWF^+) supporting the hypothesis that these cells are endothelial progenitor cells. Also, BM-SP cells express Kir with biophysical properties recapitulating those in mature endothelial cells, but with a much higher current density. Flow cytometric (FACS) analysis indicated that the number of SP cells was unaffected by hypercholesterolemia. However, hypercholesterolemia significantly inhibited Kir channels in BM-SP cells.

Conclusions: We successfully demonstrate that BM side population cells represent an origin of endothelial progenitor cells. This study further shows, for the fist time, that the functional expression of Kir channels in bone marrow (BM)-derived SP. Moreover, we demonstrate that hypercholesterolemia condition significantly suppresses the Kir channels in BM-SP cells, suggesting that hypercholesterolemia-mediated regulation of Kir channels may be an important factor not only in dysfunction of mature endothelium but also in dysfunction of BM-SP cells.

Keywords: Progenitor cells; Endothelium; Potassium channels; Cholesterol; Lipoproteins; Flow; Vasodilatation

Endothelial dysfunction occurs early and throughout the pathogenic course of atherosclerosis [1,2]. A growing number of studies suggest that maintenance of the endothelium and repair involves recruitment of bone marrow-derived endothelial progenitor cells (EPCs). Supporting this concept is the finding that different subsets of $CD34^+$ hematopoietic progenitor cells are capable of differentiating into endothelial cells *in vitro* [3–5] and incorporating into the sites of neovascularization *in vivo* [3,6,7]. Also, the number of circulating EPCs is reduced in patients with

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coronary artery disease (CAD) [8] and in patients with elevated serum cholesterol, hypertension, and diabetes [9] indicating a dynamic interaction between to bone marrow and endothelium. It is important to note that when these three cardiovascular risk factors were adjusted for the age of subjects, hypercholesterolemia was the most highly associated factor [9]. Conversely, a decrease in serum cholesterol by HMG-CoA reductase inhibitors (statins) increased the number of circulating EPCs [8,10] and facilitated re-endothelization of balloon-injured arterial segments in rats [10].

Atherosclerosis is associated not only with a reduction in number of EPCs, but also with impairment of EPC function. For example, Vasa et al. showed that EPCs isolated from CAD patients display an impaired migratory response [8]. Furthermore, Rauscher and colleagues demonstrated that transfusion of bone marrow stem cells from young non-atherosclerotic ApoE$^{-/-}$ mice prevents lesion progression in ApoE$^{-/-}$ recipients, while cells derived from older mice with manifest atherosclerosis did not [11]. Atherosclerosis progression is associated with a loss of the EPC repair capacity [12]. Little is known, however, about the mechanisms responsible for the functional impairment of EPCs in atherosclerosis. Our previous studies showed that hypercholesterolemia strongly suppresses inwardly-rectifying K^+ channels (Kir) in aortic endothelial cells *in vitro* [13,14] and *in vivo* [14]. Other reports indicate that suppression of Kir channels in human hematopoietic progenitor cells impairs expansion and differentiation of these cells [15,16]. These observations are consistent with the general role of K^+ channels in control of cellular proliferation and differentiation (reviewed by [17,18]).

The goal of this study is to determine whether K^+ channels are expressed in bone marrow-derived EPCs and whether the activity of K^+ channels in these cells is suppressed by hypercholesterolemia. We utilize a porcine model of atherosclerosis because porcine lipoprotein profiles are similar to those in humans [19] and because hypercholesterolemic pigs develop atherosclerotic lesion within a few months after the initiation of a high-cholesterol diet [20]. However, there are limited surface markers for porcine progenitor cells. Therefore, we isolated side population (SP) cells which include a subpopulation of EPCs [21]. These SP cells are lineage marker-negative [22,21]. *In vitro* culture studies have shown that rhesus SP cells are highly enriched for long-term culture-initiating cells (LTC-ICs), an indicator of primitive hematopoietic cells. Also, SP cells can incorporate into the arterial wall where they display the characteristics of endothelial cells [21].

In this study, we demonstrate that similar to mature aortic endothelial cells, potassium membrane conductance of SP cells freshly derived from porcine bone marrow (BM) is dominated by strongly-rectifying Kir channels. We also show that the functional expression of Kir channels in bone marrow (BM)-derived SP cells is significantly higher than that in mature endothelial cells isolated from the aorta. Furthermore, differentiation of BM-SPs into EC-like cells *in vitro* is accompanied by the partial loss of the Kir current. Finally, we show that Kir current in BM-SPs is markedly suppressed in cells isolated from animals with diet-induced hypercholesterolemia. These data demonstrate that diet-induced hypercholesterolemia impairs the functional expression of Kir channels in SPs while they still reside within the bone marrow. We suggest that suppression of BM-SPs Kir contributes to the functional deficiency of EPCs in atherosclerosis.

Materials and methods

Isolation of bone marrow cells. Castrated male Yorkshire pigs 27–32 kg in weight were randomized into two groups of hypercholesterolemia ($n = 4$) and control ($n = 6$). Hypercholesterolemia was induced by administration of atherogenic diet (0.5% cholesterol, 10% lard, and 1.5% sodium cholate) for 3–6 months. Control group received standard chow diet. A lipid profile was assessed at baseline, monthly thereafter, and prior to euthanasia on all animals. The study was approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania. A sternal bone marrow aspirate was performed under sterile conditions on a series of two groups of pigs (hypercholesterolemic or control); aspirate (5–25 mL) was collected in heparinized tubes and immediately processed for flow cytometric analysis and cell sorting. The aspirate was washed with Hanks' balanced salt solution (HBSS) containing 2% fetal bovine serum and 10 mM Hepes buffer solution and then filtered through a 70 μm mesh filter. The aspirate was then centrifuged for 10 min at 1000 RPM and the supernatant aspirated. Ammonium chloride lysis was performed on the remaining pellet to remove red blood cells. Once completely lysed, the pellet was resuspended in HBSS and washed twice. The final (white) pellet was then resuspended in 10–30 mL of HBSS and the cells counted.

Hoechst staining of bone marrow cells. Cells were aliquoted into tubes of 2E6 cells and incubated in Hoechst solution (5 μg/mL) for two hours at 37 °C, following a protocol similar to that used by Goodell et al. [22]. As a control, select tubes of cells were incubated in a 200 μM solution of Verapamil for 7–10 min prior to incubation with Hoechst. After the two hour incubation with Hoechst, cells were centrifuged, the pellets combined and resuspended in 2 μg/mL propidium iodide (PI) solution at a concentration of 20E6 cells/mL and kept on ice. Typically 2–6 samples of 40E6 cells were analyzed and sorted.

Sorting of BM-SP cells. Using a Becton–Dickinson FACSDiVA cell sorter, viable (as assessed by PI exclusion), low to medium side scatter singlets were analyzed for BM-SP cells, or efflux of the Hoechst dye, and sorted for *in vitro* culture studies. Singlets were gated as the prominent cluster of cells identified from a plot of side scatter width versus forward scatter width to ensure that cell aggregates were excluded from analysis. Cells incubated with Verapamil prior to Hoechst staining were used as controls, to confirm the location and presence of SP cells.

Cell culture and immunostaining. Side population cells were plated and cultured in M-199 media containing 20% calf serum, 1% L-glutamine, 0.5% pen/strep, and 0.1 mg/mL heparin for one week before electrophysiological recordings or *in vitro* expansion. After a week of culture in growth factor-free media, adherent cells were recultured in M-199 media containing 0.03 mg/mL lyophilized endothelial cell growth supplement (Sigma) for 2 days before electrophysiological recordings or immunostainings. Expanded cells were immunostained with EC-specific anti-platelet endothelial cell adhesion molecule 1 (anti-PECAM-1) and anti-von Willebrand factor (anti-vWF) antibodies as well as smooth muscle cell-specific anti-α-actin antibody (α-SMA) [23,24].

Electrophysiology. Ionic currents of cells were measured using standard voltage-clamp techniques, as previously described [24]. Whole-cell currents were recorded during 500-ms linear voltage ramps from −160 to +60 mV at an interpulse interval of 3 s. The external solution for voltage-clamp experiments contained (in mM) 156 KCl, 10 Hepes, 1.5 $CaCl_2$, 1 $MgCl_2$

and 1 EGTA, pH 7.3. The low K^+ external solution contained (in mM) 150 NaCl, 6 KCl, 10 Hepes, 1.5 $CaCl_2$, 1 $MgCl_2$ and 1 EGTA, pH 7.3. The pipette contained (in mM) 145 KCl, 10 Hepes, 1 $MgCl_2$, 1 EGTA, and 4 ATP, pH 7.3 in all experiments. The recordings were performed at high (156 mM) extracellular K^+ to increase the amplitude of the currents [25]; as expected under this condition, all currents had a reversal potential at ~ -2 mV. We showed earlier that cholesterol sensitivity of Kir is not affected by changes in the extracellular K^+ [14].

Results

In vitro expansion and differentiation of porcine SP cells

To identify SP cells in the porcine bone marrow, whole bone marrow specimens extracted from castrated male Yorkshire pigs were incubated with Hoechst 33342 dye. Hoechst 33342 staining, when observed simultaneously at red and blue emission wavelengths, demonstrated a complex fluorescence-emission pattern indicative of cycling cell populations. A distinct population of cells with dim Hoechst staining (SP cells) was consistently observed in all experiments (data not shown). We further demonstrated that the SP population signal was specifically eliminated in the presence of the drug verapamil as previously published by Goodell et al. [26]. Verapamil-mediated ablation of porcine SP cells suggested that the low Hoechst staining was due to high activity of membrane efflux pumps of the ATP-binding cassette (ABC) transporter superfamily, a common feature across SP cells derived from hematopoietic and non-hematopoietic tissues [22,27]. The verapamil-sensitive SP subpopulation represented ~0.05% of the total viable-cell content of porcine bone marrow, consistent with previous observations in the murine model [22,27].

As shown in Fig. 1Ai, after cultured in basal media without growth factors for 7 days, adherent porcine SP cells assume a sphere-shaped morphology; however, cell type-specific phenotypes such as endothelial cells, smooth muscle cells or neurons were not observed in culture. After 7 days of culture in growth factor-free media, non-adherent cells were removed and adherent SP cells were recultured in media supplemented with Endothelial Cell Growth Factor (ECGF). After 2 days of culture in growth factor-containing media, adherent SP cells demonstrated an elongated spindle-like shape, as shown in Fig. 1Aii. The cell lineage of the spindle-like cells was defined by staining these cells with antibodies for the endothelial cell markers platelet-endothelial cell adhesion molecule (PECAM) and von Willebrand factor (vWF) (Fig. 1B), as well as the smooth muscle cell-specific marker, smooth muscle α-actin (Fig. 1C). Positive staining for PECAM and wWF was observed along with the absence of smooth muscle α-actin staining indicating that the SP cells differentiated into cells with endothelial characteristics. These data are consistent with a previous study showing SP cells displayed the characteristics of differentiated endothelial cells *in vivo* [21]. Indeed, we believe this is the first study to demonstrate *in vitro* differentiation of BM-SP cells into endothelial cells or endothelial-like cells.

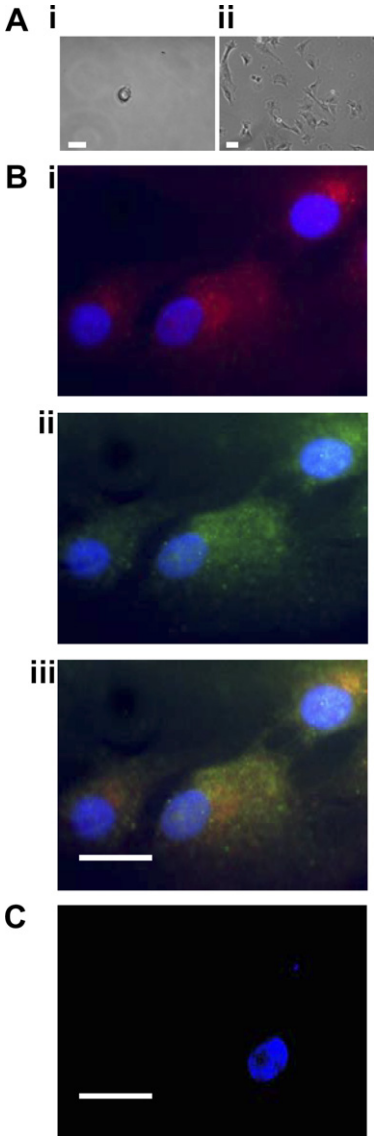

Fig. 1. *In vitro* expansion and differentiation of porcine SP cells. (A) Bright-field microscopic picture of porcine bone marrow SP cells cultured in basal media for 7 days (i) or SP cells with extended culture in growth factor-containing media (ii). (B) Immunofluorescence imaging of expanded SP cells (cells shown in Aii) stained with vWF (i), CD31 (ii), or vWF/CD31 (iii). (C) Immunofluorescence imaging of expanded SP cells stained with smooth muscle α-actin. The nuclei of the cells were stained purple by Hoechst throughout the immunostained images. Bar = 30 μm.

Functional expression of Kir channels in porcine SP cells

To examine the functional expression of potassium channels in porcine SP cells, the cells were isolated form the bone marrow, sorted and then maintained for 7–14 days in M-199 medium supplemented with 20% fetal bovine serum and the currents were recorded on days 7 and 14 (a minimum of 7 days was required for the cells to attach preventing the recordings to be made at earlier time points, no difference was observed between the days 7 and 14). Under resting conditions, all SP cells showed a pronounced inwardly-rectifying K^+ current (Fig. 2A) with

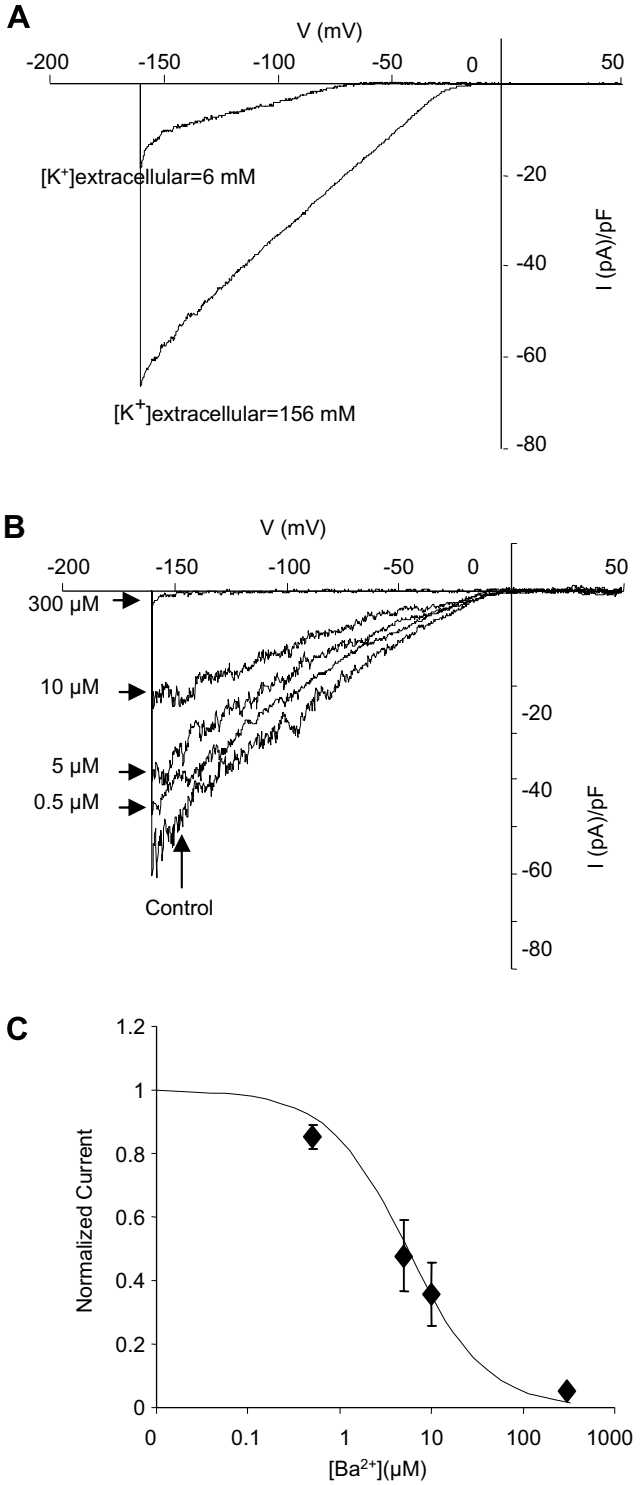

Fig. 2. Basic properties and Ba^{2+} sensitivity of endogenous Kir current in porcine BM-SP cells. (A) Comparison of Kir currents recorded at low (6 mM) and high (156 mM) extracellular $[K^+]$ solutions in the same cell. Note a shift in the reversal potential from −79 to −2 mV. (B) Kir current recorded in a single SP cell at 0, 0.5, 5, 10, and 300 μM extracellular Ba^{2+}. (C) Concentration dependence of Ba^{2+} block. Fractional block was determined at −100 mV and calculated as $(I_{control} - I_{Ba}^{2+})/I_{control}$, where $I_{control}$ is the current recorded before application of Ba^{2+} and I_{Ba}^{2+} is the current recorded at a specific Ba^{2+} concentration. Each point is the mean ± SE ($n = 5$). The data were fitted with the function $I_{Ba}^{2+}/I_{control} = 1/(1 + [Ba^{2+}]/K_d)$ where K_d is the dissociation constant.

the current–voltage (I–V) relationship similar to the I–V relationship of currents carried by Kir2.x subunits in porcine aortic endothelial cells, human aortic endothelial cells [24], and CHO cells over-expressing Kir2.x channels [28]. The reversal potential of the current was -79 ± 2 mV and -2 ± 1 mV for low and high extracellular K^+ recording solutions respectively, close to the theoretical reversal potentials of a K^+-specific current under these recording conditions (−80 and −2 mV). Thus, shift in the reversal potential of the current was in agreement with the reversal potential for potassium ions (E_K), theoretically predicted by the Nernst equation [29]. Furthermore, we demonstrated that BM-SP K^+ currents were sensitive to extracellular Ba^{2+} with a calculated IC_{50} of 5.4 μM Ba^{2+}, which is virtually identical to the IC_{50} of the Ba^{2+} block (3.42 μM) of Kir2 channels underlying K^+ conductance in mature aortic endothelial cells (Fig. 2B and C) [24]. Taken together, these data suggest that the current is carried by the channels belonging to the Kir2 family of inward rectifiers. This is the first study to describe functional expression of Kir channels in SP stem cells.

Decline of Kir channel activities in differentiated porcine BM-SP cells

It is important to note that the current density of Kir in BM-SP cells observed in this study is several-fold higher than the currents described previously in low-passage or freshly isolated mature aortic endothelial cells [14,24]. The average Kir density in BM-SP at −155 mV, 156 mM extracellular K^+ was ~80 pA/pF, whereas the currents in mature aortic ECs ranged between 10 and 20 pA/pF under the same experimental conditions. We therefore examined whether inducing the differentiation of the SP cells by exposing them to the endothelial growth factors, as described above, is sufficient to decrease Kir current density. As shown in Fig. 3, upon the differentiation, Kir current density decreased from 79 ± 9 pA/pF to 9 ± 2 pA/pF. Furthermore, we observed that Kir density in BM-SPs that underwent differentiation into endothelial-like spindle-shaped cells was nearly identical to Kir currents recorded from mature aortic endothelial isolated from the same animals.

Hypercholesterolemia has no effect on the number of BM-SP cells but induces suppression of Kir channels in BM-SP cells

Since the association between risk factors for atherosclerosis and low circulating levels of BM-derived EPCs has been established [9], we examined the hypothesis that hypercholesterolemic animals exhibit lower levels of BM-derived side population cells, which in turn, leads to lower numbers of circulating EPC. However, no significant differences were observed in the number of SP cells (0.02–0.1% of viable cells) collected for hypercholesterolemic and control pigs (Fig. 4A) suggesting that hypercholesterolemia did not affect the number of bone marrow-derived SP cells.

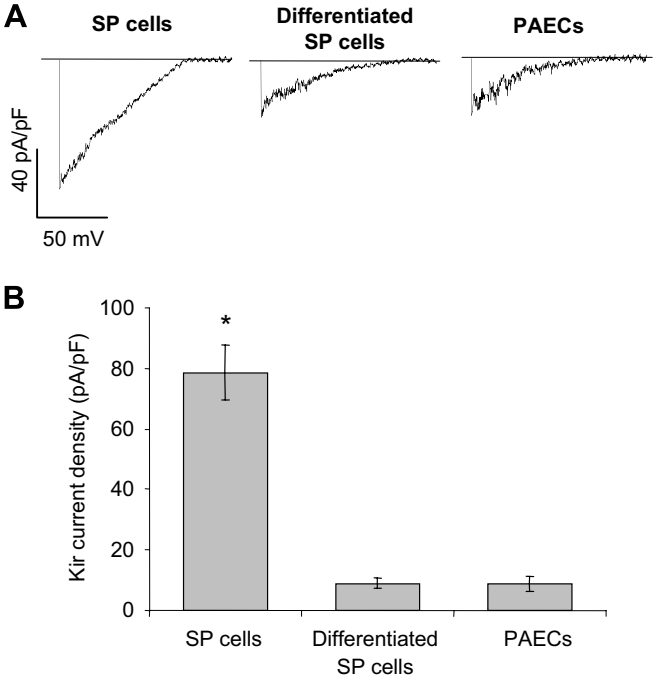

Fig. 3. Descent of Kir current in differentiated porcine BM-SP cells. (A) Representative recordings of Kir currents in porcine BM-SP cells, expanded SP cells, and freshly-isolated porcine aortic endothelial cells. (B) Average Kir peak current densities (−155 mV) recorded from the same populations (BM-SP cells, $n = 35$; expanded SP cells, $n = 7$; porcine aortic endothelial cells, $n = 25$) *indicates $p < 0.05$; SP, side population cells; and PAECs, porcine aortic endothelial cells.

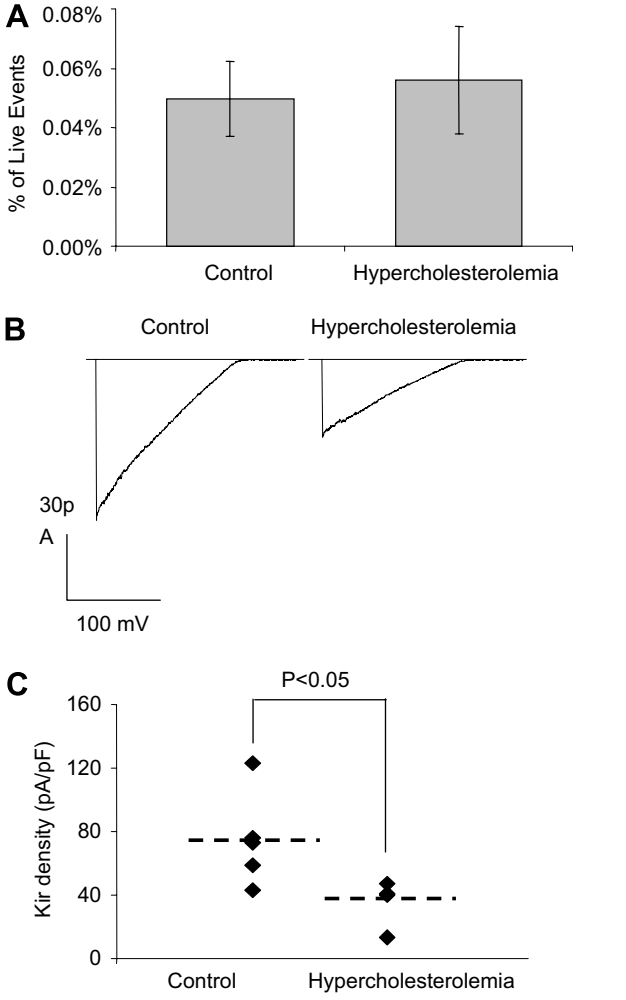

Fig. 4. Suppression of Kir channels in porcine BM-SP cells by hypercholesterolemia. (A) The percentage of side population cells in porcine bone marrow cells in control ($n = 3$) and hypercholesterolemia ($n = 4$) animals. (B) Representative recordings of Kir currents in BM-SP cells from control and hypercholesterolemic pigs. (C) Kir peak current densities (−155 mV) recorded from the same populations (control: 6 animals, 7–10 cells per animal; hypercholesterolemia: 4 animals, 7–10 cells per animal). Mean values of each group are indicated by dashed lines.

Our earlier studies have shown that endothelial Kir current is suppressed by an increase in cellular cholesterol concentration [13] and diet-induced hypercholesterolemia suppressed Kir currents in porcine aortic endothelial cells in a model of atherosclerosis [14]. To determine whether diet-induced hypercholesteromia modulated Kir currents in BM-SP cells, pigs were maintained on a high-cholesterol diet resulting in significant elevations in total plasma and LDL cholesterol and evidence of pre-clinical endothelial dysfunction accessed by loss of flow-induced vasodilatation [14]. BM-SP cells were isolated from control and hypercholesteromic pigs as described above and maintained in media. As shown in Fig. 4, hypercholestemia inhibited Kir currents in BM-SPs isolated from both control and hypercholesterolemic pigs. Thus, hypercholesterolemia-induced suppression of the Kir current in BM-derived SP cells develops *in vivo*. Furthermore, these observations indicate that hypercholesterolemia-induced impairment of endothelial function is not limited to vascular or even circulating endothelial cells, but may occur directly within the bone marrow.

Discussion

Identification of BM-SP cell derived endothelial cells

Side population cells are a rare cell population characterized by their typical low-stained fluorescence profiles of Hoechst red and Hoechst blue [22,27,30]. Goodell et al first described the capacity of BM-SP cells to repopulate the bone marrow in a lethally-irradiated murine model [22]. They further described the cardiomyogenic potential of BM-SP cells, showing that these cells displayed the characteristics of differentiated cardiomyocytes and endothelial cells in cardiac muscle and vessel structures [21]. However, the *in vitro* differentiation of SP cells into cells with cardiomyocyte and endothelial characteristics was not described in their studies. The current study describes a rare cell population (~0.05% viable cells) with a typical SP phenotype from porcine bone marrow aspirations demonstrating, low Hoechst fluorescence intensity and verapamil sensitivity. Furthermore, we demonstrated that in endothelial growth factor-containing media, porcine BM-SP cells expanded and more importantly, began to express endothelial-specific markers (vWF/CD31), but not smooth muscle

α-actin. The *in vitro* differentiation of BM-SP cells into a cell with an endothelial phenotype indicates that SP cells include a population of cells with the capacity to differentiate into bone marrow-derived EPCs. Thus, we conclude that BM-SP cells studied here include cells capable of differentiating into endothelial cells. It is important to note, however, that this cell population likely also includes progenitor cells capable of differentiating into other types of cells.

Expression of Kir channels in progenitor cells

Inwardly-rectifying K^+ channels are known to be ubiquitously expressed in a variety of mammalian cells and to play critical roles in the maintenance of the membrane potential and K^+ homeostasis [25,29,31]. However, expression of Kir channels in different populations of progenitor cells appears to be less universal. Significant Kir currents, identified as Kir1.1 and Kir4.3, were observed in primitive human hematopoietic progenitor cells isolated from the cord blood and characterized as $CD34^+/CD33^-$ or $CD34^+/CD38^-$ [15,16]. Furthermore, treatment of these cells with stem cell factor and interleukin-3 enhanced the expression of Kir channels and promoted cell expansion into lineage-restricted precursors [15,16]. Kir currents were also found in non-hemapotopoietic fraction of human umbilical cord blood that was expanded as a non-immortalized cell line and then further differentiated into neuron-like cells [32]. In contrast, several studies have shown that mesenchymal stem cells isolated form the bone marrow express multiple types of voltage-gated and Ca^{2+}-sensitive K^+ channels, but do not express functional Kir channels [33–35]. No Kir channels were also detected in pluripotent embryonic stem cells [36]. Our study is first to analyze functional expression of Kir channels in BM-SP cells. We show here that in contrast to BM-derived mesenchymal stem cells, BM-SP cells have strong Kir currents that clearly belong to the family of strong inward-rectifiers and dominate K^+ conductance in BM-SP cells. More specifically, Ba^{2+} sensitivity of the BM-SP Kir currents was found to be virtually identical to that in mature endothelial cells, whose K^+ conductance is regulated via Kir2.1 and Kir2.2 channels [24]. Interestingly, Kir2.2 channel mRNA is expressed in mesenchymal stem cells, but, as described above, the channels appear to be non-functional [33]. We have not observed any voltage-gated K^+ channels in the BM-SP cell population. Thus, the pattern of K^+ channel expression observed in BM-SP cells appears to differ significantly from the patterns described previously in different types of progenitor cells. We suggest, therefore, that expression of Kir channels may be one of the functional identifiers of BM-SP cell population.

Transient nature of Kir expression in BM-SP cells

In this study, we show that not only do BM-SP cells have pronounced Kir currents, but these currents are ~8-fold higher than the currents recorded in mature endothelial cells isolated from the same animals. Furthermore, inducing differentiation of BM-SP cells into endothelial-like cells down regulates Kir current to a level identical to that observed in mature endothelial cells. Interestingly, several earlier studies have demonstrated transient appearance of different types of K^+ channels, including Kir, in differentiating cells during early embryonic development implicating K^+ channels in the control of cell differentiation [37–40], as well as in adult differentiating cells [41,42]. An increase in Kir expression was shown to be essential for stem cell factor (SCF)-dependent expansion of primitive human hematopoietic progenitor cells $CD34^+33^-$ [15] and in the differentiation of an earlier and more quiescent population of primitive hematopoietic progenitor cells $CD34^+38^-$ [16]. In this regard it is noteworthy that cell cycle progression is accompanied by changes in the expression of different types of K^+ channels and that K^+ channel blockers inhibit cell proliferation in a variety of cell types (reviewed by [17,43]). However, the role of Kir channels in the regulation of cell proliferation is still controversial. While inhibition of Kir was shown to inhibit cell proliferation in human melanoma cell line [44], it was also shown to enhance proliferation of glial cells [41]. Indeed, dynamic regulation of Kir2 expression has been demonstrated to play a key role in myoblast differentiation and fusion by determining the cellular membrane potential and controlling the intracellular Ca^{2+} concentration [45]. We suggest, therefore, that the transient nature of Kir channel expression in BM-SP cells may play a critical role in regulating the differentiation of these cells. We also suggest that high levels of Kir expression may be important for the control of BM-SP proliferation.

Kir channels and vascular function

Numerous studies have shown that endothelial K^+ channels play an important role in endothelium-mediated regulation of vascular tone [46–51]. K^+ channels set the negative resting membrane potential of endothelial cells, providing the driving force for Ca^{2+} influx and regulating Ca^{2+} dependent intracellular signaling [52]. Specifically, blocking K^+ channels in aortic endothelial cells results in a decrease of Ca^{2+} influx, inhibition of bradykinin- and acetylcholine-induced release of nitric oxide (NO), a major vasorelaxing, anti-thrombotic, and anti-inflammatory factor, and a decrease in the release of the anticoagulant prostaglandin I_2 [46]. Our earlier studies have shown that hypercholesterolemia suppresses Kir channels in mature endothelial cells suggesting that inhibition of Kir may contribute to the endothelial dysfunction in early atherosclerosis [13,14]. Our current study results indicate that hypercholesterolemia impairs Kir channel function of BM-SP cells suggesting that endothelial cell function may be impaired already in endothelial progenitor cells that still reside in the bone marrow before maturation and mobilization occurs. Given the importance of Kir channels in

overall vascular function, these results suggest a new paradigm whereby cardiovascular risk factors may concomitantly affect a bone marrow insult contributing to systemic endothelial dysfunction.

Limitations and future directions

The current study is the initial description of functional expression and hypercholesterolemia sensitivity of inwardly-rectifying potassium channels in porcine BM side population cells. The significance and biological utility of Kir channels in bone marrow progenitor cells is unclear, but deserves more study given the questions raised from the current study. For example, how does hypercholesterolemia result in a decrease in the functional expression of Kir channels and do statin drugs reverse this effect? How does differential expression of Kir channels affect mobilization, differentiation, and homing of BM side population cells? The study of bone marrow progenitor cell Kir channels in normal and proatherosclerotic conditions will most likely result in a better understanding of the evolving importance of the bone marrow-systemic interaction on vascular function. The bone marrow appears an important sentinel for cardiovascular health and drug and lifestyle interventions that favorably affect bone marrow health may also reduce cardiovascular morbidity and mortality.

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