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ARTICLE

Modulation of cardiac stem cell characteristics by metoprolol in hypertensive heart disease

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Abstract

Cardiac stem cells (CSCs) play a vital role in cardiac remodeling. Uncontrolled hypertension leads to cardiac hypertrophy, followed by cardiac failure. Pathological remodeling is associated with enhanced oxidative stress. Decreased cardiac stem cell efficiency is speculated in heart diseases. Maintaining a healthy stem cell population is essential for preventing progressive cardiac remodeling. Some anti-hypertensive drugs are cardioprotective. However, the effect of these drugs on CSCs has not been investigated. Metoprolol is a cardioprotective anti-hypertensive agent. To examine whether metoprolol can prevent the deterioration of CSC efficiency, spontaneously hypertensive rats (SHRs) were treated with this drug, and the effects on stem cell function were evaluated. Six-month-old male SHRs were treated with metoprolol (50 mg × kg^{-1}per day) for 2 months. The effectiveness of the treatment at reducing blood pressure and reducing hypertrophy was ensured, and the animals were killed. Cardiac stem cells were isolated from the atrial tissue, and the effect of metoprolol on stem cell migration, proliferation, differentiation, and survival was evaluated by comparing the treated SHRs with untreated SHRs and normotensive Wistar rats. Compared to the Wistar rats, the SHR rats presented with a decrease in stem cell migration and proliferation and an increase in intracellular oxidative stress and senescence. Treating SHRs with metoprolol increased CSC migration and proliferation potential and stemness retention. Cellular senescence and oxidative stress were reduced. The attributes of stem cells from the metoprolol-treated SHRs were comparable to those of the Wistar rats. The restoration of stem cell efficiency is expected to prevent hypertension-induced progressive cardiac remodeling.

Introduction

Cardiac hypertrophy and failure are common cardiac sequelae of hypertension. Stem cells have a mediatory role in progressive cardiac remodeling. Compromised stem cell function has been implicated in different cardiac ailments [1], but the efficiency of stem cells in hypertensive heart disease has not been evaluated. Protecting the resident cardiac stem cell population as a prelude to preventing cardiac failure has not received much attention. Cycling stem cells for the replenishment of lost myocytes and the unfavorable microenvironment of the pathological heart can lead to stem cell aging. A decrease in the proportion of healthy stem cells can affect the reparative capacity. The role of stem cells in tissue regeneration is further highlighted by the observation of the positive effect of stem cell transplantation on myocardial regeneration [2]. The efficiency of c-kit^+ cardiac stem cells was found to be impaired in disease models, implying the role of stem cells in maladaptive cardiac remodeling [1]. Only one report on a surgical model of hypertrophy exists in which the β-adrenergic blocker metoprolol induced the attenuation of LV remodeling in rat and was associated with an increased number of c-kit^+ cells [3]. Metoprolol was more effective than an angiotensin receptor blocker, losartan [3]. However, functional changes in stem cells were not evaluated.

Beta-adrenergic blockers are beneficial for patients with symptomatic heart failure. Oxidative stress has been implicated in the pathogenesis of cardiovascular diseases [4, 5], including heart failure [6, 7]. Beta-blockers have antioxidant potential. Carvedilol decreased the serum levels of 8-OHdG by 19% [8] and of myocardial HNE-modified proteins by 40%, in addition to ameliorating the cardiac function of patients with heart failure [9]. Antioxidant β-

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blockers such as carvedilol and nebivolol attenuated oxidation and improved Prdx2 expression as well as increased the number of endothelial progenitor cells (EPCs) in the cardiovascular system of SHRs; in contrast, compared with carvedilol and nebivolol, short-term treatment with the angiotensin-II receptor blocker telmisartan had more beneficial effects on cardiovascular protection, EPC number, and Prdx2 expression [10]. Chronic β-receptor inhibition prevented cardiac remodeling in Dahl salt-sensitive hypertensive rats as effectively as renal denervation, despite maintaining blood pressure, thereby delinking blood pressure from cardiac remodeling [11]. Metoprolol is a cardioselective β1-adrenergic blocking agent. Cardiac hypertrophy regression and an increased life span have been reported in hypertensive individuals treated with metoprolol [12]. Experimental studies have shown that the continuous administration of β-receptor antagonists prevented the development of hypertension in growing SHRs [13]. Chronic metoprolol treatment markedly attenuated both cardiac and vascular remodeling in aging SHRs, thus preventing the onset of heart failure and improving survival, independent of blood pressure reduction [14]. The cause for the transition from adaptive remodeling to cardiac decompensation is enigmatic. Stem cell attribute deterioration is one of the causative factors of progressive cardiac remodeling. Based on the premise that stem cell function is compromised in hypertensive heart disease, this study was carried out to evaluate the efficiency of metoprolol in restoring stem cell attributes in SHRs.

Methods

Experimental design

Spontaneously hypertensive rats, a genetic model of hypertension and cardiac hypertrophy, were used for the experimental model and were compared with normotensive Wistar (WST) rats. Six-month-old male SHR and WST rats were housed at 22 °C, maintained on a 12 h light–dark cycle, fed with regular rat chow and given free access to drinking water. All animal procedures were approved by the Institutional Animal Ethics Committee, according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines.

Twelve male SHRs were randomly assigned to two groups of six rats each. Untreated SHRs served as the hypertensive control, and the other group received a daily oral dose of 50 mg × kg^{-1} per day of metoprolol tartrate for 2 months. Sex- and age-matched WST rat served as normotensive controls. To ensure treatment effectiveness, blood pressure and cardiac hypertrophy were assessed by echocardiography. Myocardial oxidative stress was assessed by lipid peroxidation assays. After establishing the cardioprotective and antioxidant effects of the metoprolol treatment, the cardiac stem cell response was evaluated.

Isolation and characterization of cardiac stem cells

c-kit$^+$ cardiac stem cells (CSCs) were isolated following a previously reported protocol, with minor modifications [15, 16]. The atria were washed in phosphate-buffered saline (PBS), minced into small pieces (1 mm^3) and plated onto 2% gelatin-coated culture plates. The explants were grown in Iscove's modified Dulbecco's medium (IMDM) supplemented with 10% fetal bovine serum (FBS) and antibiotics. The phase-bright cells that migrated out of the explants were dissociated by mild trypsinization, and c-kit$^+$ CSCs were sorted by immunomagnetic isolation using an Easy Sep magnet and FITC-positive selection kit (Stem Cell Technologies, Canada). The sorted cells were cultured with IMDM containing 10% FBS, 10 ng/mL bFGF, and insulin–selenium–transferrin mixture at 37 °C in a humidified atmosphere with 5% CO_2. Cells from passage 3 were used for further experiments.

Immunostaining for the identification of c-kit$^+$ stem cells was performed by incubating the cells with a rat-specific rabbit anti-c-kit (diluted 1:100, Santa Cruz Biotechnology, USA) at 4 °C overnight. The cells were then incubated with FITC-conjugated rat anti-rabbit IgG (diluted 1:250) at 25 °C for 1 h. The nuclei were stained with DAPI. The immunoreactions were observed using a fluorescence microscope.

Flow cytometric analysis for cell surface markers

Immunomagnetically isolated c-kit$^+$ cells were fixed in 4% paraformaldehyde for 15 min at room temperature and were analyzed for the expression of cell surface markers. The collected cells were stained with FITC-conjugated antibodies against c-kit, CD34, and CD45 (Invitrogen, Carlsbad, CA), indicative of cardiac stem cells and endothelial and hematopoietic markers. IgG was used as the isotype control.

Clonogenic assay

Single-cell CSC suspensions were serially diluted to 50 cells in 10 mL of complete medium and seeded onto a 96-well plate at a density of 0.5 cells per well to generate single-cell clones. After 4 h, each well containing a single cell was identified under a light microscope and was examined for growing colonies twice weekly. After 2 weeks, the number of wells with clones derived from a single cell was counted. Clonogenicity was determined

using the following formula:

$$\text{Clonal efficiency}\,(\%) = (\text{Total wells with clones}/\text{Total wells with single cell}) \times 100.$$

Growth kinetics, growth rate, and population-doubling time

To determine the growth kinetics, 10,000 cells were seeded in 35 mm culture plates. Cells were dissociated by trypsinization, and the cell density was determined using a Neubauer improved hemocytometer from a minimum of three culture plates at 48 h intervals for 10 days. The growth kinetics were plotted. Population-doubling time (PDT) and growth rate (GR) were calculated. GR was determined with the following equation: $GR = \ln(N_t/N_0)/T$, where T is the incubation time, N_0 is the cell number at the beginning of the incubation time, and N_t is the cell number at the end of the incubation time. Population-doubling time was calculated using the formula $PDT = \ln(2)/GR$.

Colony-forming unit assay

CSCs were seeded onto 60 mm cell culture plates in triplicate at a density of 500 cells per plate. Culture medium was changed every 3–4 days. After 9 days, the cultures were washed with PBS and stained with 3% crystal violet in methanol for 30 min at room temperature. The number of colonies per plate was counted.

Cell migration assay

For trans-well migration assays, 1×10^4 cells were suspended in 300 µl of serum-free IMDM and seeded onto the upper chamber of transwells with an 8 µm pore size. Cells were allowed to migrate toward the IMDM containing 10% serum for 18 h; then, the cells on the upper surface of the membrane were wiped away, and the migrated cells on the lower surface of the membrane were fixed and stained. Cells were counted from six random fields, and the mean was calculated. Experiments were repeated three times.

Senescence-associated β-galactosidase staining

Senescence-associated β-galactosidase staining (β-Gal staining) was used as a biomarker of senescence. B-Gal activity was assessed cytochemically using a staining kit (Abcam), and the percentage of senescent cells, as represented by positive staining, was calculated. The expression levels of senescence-associated proteins, $p16^{ink4a}$, and p21, were detected by western blot analysis.

Table 1 Effect of metoprolol on blood pressure

	WST	SHR	Metoprolol
Systolic blood pressure (mmHg)	122 ± 16	188 ± 12**	133 ± 21$^{\#\#}$
Diastolic blood pressure (mmHg)	89 ± 9	119 ± 11**	94 ± 8$^{\#\#}$

Data presented as mean ± SD. Variation was analyzed by one-way ANOVA ($p < 0.01$) followed by Student's t test. (**$p < 0.01$ and SHR vs WST; $^{\#\#}p < 0.01$ SHR vs metoprolol)

Cell differentiation assay

CSCs were seeded onto six-well plates. Cell differentiation was induced in semiconfluent cultures using azacytidine (IMDM, 10% FCS and 10 µM 5-azacytidine) for 2 weeks. The differentiation potential was determined by analyzing the expression of cell-specific markers (cardiac troponin I and smooth muscle a×ctin).

Western blotting analysis

Protein concentrations were determined by the Bradford assay. Equal amounts of protein were electrophoresed on 10% polyacrylamide SDS gels. Proteins were transferred onto nitrocellulose membranes and blocked for 1 h with 5% skimmed milk in Tris-buffered saline containing 0.1% Tween 20. Proteins were incubated with primary antibodies, followed by appropriate secondary antibodies. Protein bands were visualized by chemiluminescence, and images were quantified using ImageJ software. The expression of target proteins was normalized to the respective beta actin level.

Statistical analysis

Values are presented as the means ± SD. For comparisons between groups, ANOVA was followed by Student's t test (two-tailed). Results were considered statistically significant when p values were <0.05.

Results

Cardiovascular response to treatment with metoprolol

Both the systolic and diastolic blood pressure of SHRs were significantly reduced in response to the metoprolol treatment (Table 1). Hypertrophy regression was apparent from the decreased relative wall thickness and lateral wall thickness. The reduced isovolumic relaxation time indicates functional improvement (Table 2). The decreased myocardial lipid peroxidation indicated a reduction in oxidative

stress (Table 3). The values of treated SHRs were comparable to those of WST rats.

CSC isolation, culture, and characterization

After metoprolol treatment for 60 days, animals were killed, and the hearts were dissected under aseptic conditions. For isolating cardiac stem cells, the atria were separated from the ventricular tissue and were cultured as explants. Within 14 days, small, phase-bright cells that migrated from the explants were seen loosely attached over a layer of fibroblast-like cells. These cells were trypsinized and subjected to immunomagnetic isolation to sort the c-kit^{+} CSCs. Sorted cells were cultured in IMDM containing supplements and antibiotics. The purity of the cultured CSCs was confirmed in passage 3 by flow cytometry and immunocytochemistry. The analysis revealed that >90% of the cells were positive for c-kit and negative for the hematopoietic and endothelial markers CD45 and CD34 (Fig. 1). Culture stemness was further confirmed by clonogenicity assays, where $94 \pm 4\%$ of the cells formed single-cell colonies. Cultured CSCs from the third passage were used for further experiments.

Table 2 Effect of metoprolol on LV function as assessed by 2D echocardiography

	WST	SHR	Metoprolol
RWT	0.721 ± 0.04	0.876 ± 0.05**	$0.767 \pm 0.04^{\#\#}$
IVRT (s)	17 ± 2.3	26 ± 2.7**	$20 \pm 2.1^{\#\#}$
Lateral wall thickness (mm)	1.5 ± 0.03	2.1 ± 0.044*	$1.8 \pm 0.03^{\#}$

Data presented as mean ± SD. Variation was analyzed by one-way ANOVA ($p < 0.01$) followed by Student's t test. (**$p < 0.01$ and *$p < 0.05$ SHR vs WST; $^{\#\#}p < 0.01$ and $^{\#}p < 0.05$ SHR vs metoprolol)

RWT relative wall thickness, *IVRT* isovolumetric relaxation time

Table 3 Effect of metoprolol on myocardial oxidative stress

Lipid peroxidation assay	WST	SHR	Metorpolol
nmoles of TBARS per mg of protein	10.12 ± 1.25	29.34 ± 3.56**	$16.33 \pm 2.56^{\#\#}$

Data presented as mean ± SD. Variation was analyzed by one-way ANOVA ($p < 0.01$) followed by Student's t test. (**$p < 0.01$ SHR vs WST; $^{\#\#}p < 0.01$ SHR vs metoprolol)

Effect of metoprolol on CSC self-renewal capacity

Colony formation was significantly lower in CSCs from SHRs than in WST rat CSCs (Fig. 2a). However, after treatment with metoprolol, the ability of SHR CSCs to form colonies was significantly increased ($p < 0.01$ compared to the untreated control) and was comparable with that of WST rat CSCs ($p = 0.2$).

Effect of metoprolol on CSC growth kinetics, population-doubling time, and growth rate

Following 10 days in culture, the CSC yield from WST rats ($126 \pm 13 \times 10^4$ cells) was significantly greater than that obtained from SHRs ($72 \pm 5.5 \times 10^4$ cells). Treatment with metoprolol stimulated CSC proliferation, and the cell yield was comparable with that of WST rats (Fig. 2b). Growth

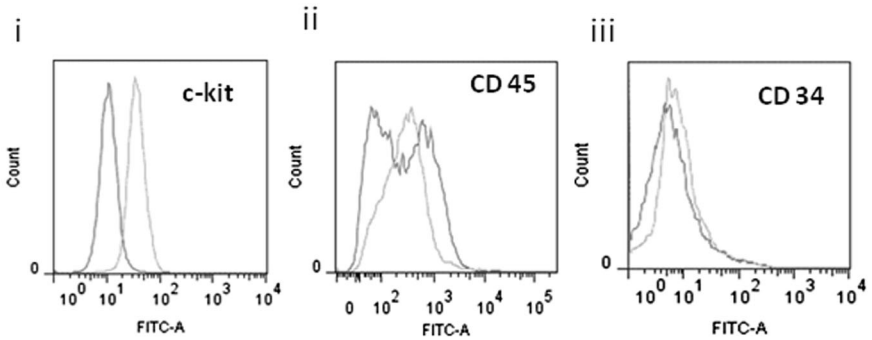

Fig. 1 Representative FACS data of the expression pattern of cell surface markers. (i) c-kit, (ii) CD45, and (iii) CD34. The marker expression percentage is given in the table

Percentage distribution of the surface markers

c-kit	CD 45	CD 34
91.5±5%	-	-

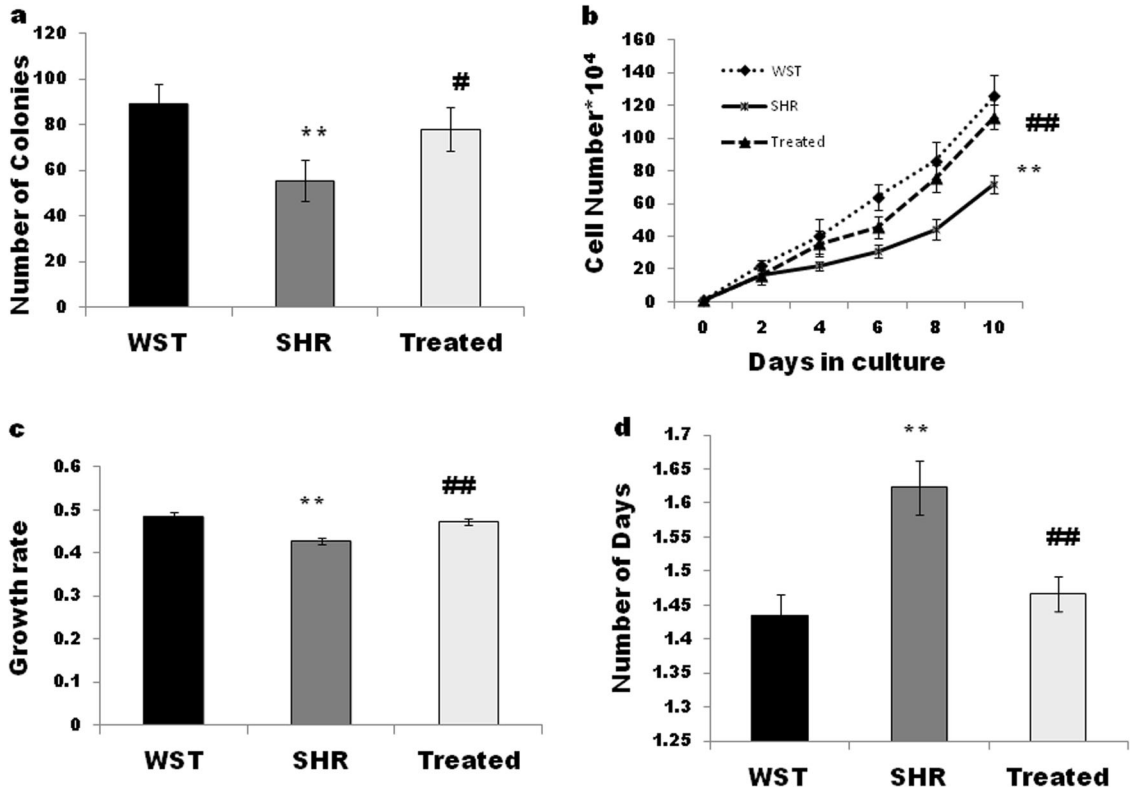

Fig. 2 Effect of metoprolol on self-renewal capacity, growth kinetics, growth rate, and population-doubling time of cardiac stem cells. **a** Age-associated variation in colony-forming units, presented as the number of colonies per culture plate. **b** Growth kinetics of CSCs, presented as cell number $\times 10^4$. **c** Age-associated variation in the growth rate of CSCs, calculated as the LogN of the ratio of the cell number at the two fixed time points. **d** Age-associated variation in the population-doubling time of CSCs, presented as the number of days. Data are presented as the means ± SD. Variation was analyzed by one-way ANOVA, followed by Student's *t* test. $^{**}p < 0.01$ SHR vs WST; $^{\#\#}p < 0.01$ SHR vs metoprolol; one-way ANOVA $p < 0.01$ ($n = 6$)

rate and PDT, both of which were negatively affected in SHRs, were restored by the beta-blocker treatment (Fig. 2c, d).

Effect of metoprolol on CSC migration potential

The migratory capacity of CSCs from SHRs was 62% lower than that of WST rats, as evidenced by the trans-well migration assays (Fig. 3). Metoprolol enhanced the ability of CSCs to migrate by 72% and was comparable with the ability of WST rat CSCs.

Effect of metoprolol on intracellular Reactive oxygen species (ROS) levels in CSCs

Oxidative stress is implicated in hypertrophy. CSCs are also influenced by the oxidative stress in the surrounding milieu, which was apparent in the H_2DCFDA fluorescence assay. CSCs from SHRs had significantly higher levels of ROS than CSCs from WST rats (Fig. 4). Metoprolol treatment decreased the ROS levels, comparable to that of normotensive rats.

Effect of metoprolol on CSC differentiation potential

The differentiation potential, as detected by the expression levels of cardiac troponin I and smooth muscle actin, were not significantly different among the three groups. CSCs from both SHRs and WSTs had comparable expression levels of both the proteins, and these levels remained unaffected by the metoprolol treatment (Fig. 5).

Effect of metoprolol on CSC senescence

The proportion of senescent CSCs was about threefold higher in SHRs than in WSTs, as identified by beta-galactosidase staining (Fig. 6a). The proportion of senescent cells was decreased in response to the treatment and was comparable with that in WSTs. Immunoblot analysis of $p16^{ink4a}$ and p21 also exhibited the same trend (Fig. 6b, c). The levels of both proteins were significantly higher in SHR CSCs than in WST CSCs. Metoprolol reduced the expression of these proteins, indicating the prevention of stem cell aging.

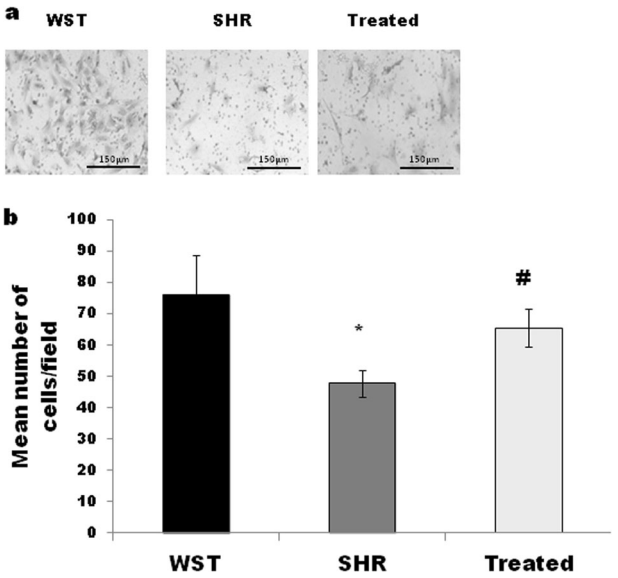

Fig. 3 Effect of metoprolol treatment on the migration potential of cardiac stem cells. **a** Representative images of the age-associated variation in the migration potential of CSCs. **b** Graphical representation of the migration potential, presented as the mean number of cells per field. Data are presented as the means ± SD. Variation was analyzed by one-way ANOVA, followed by Student's *t* test. $^{*}p < 0.05$ SHR vs WST; $^{\#}p < 0.05$ SHR vs metoprolol; one-way ANOVA $p < 0.01$ ($n = 6$)

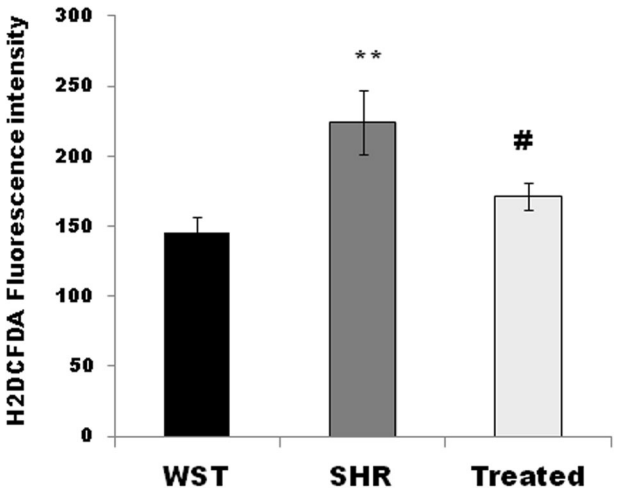

Fig. 4 Effect of metoprolol treatment on the intracellular generation of reactive oxygen species in cardiac stem cells. Reactive oxygen species level in CSCs, represented as H_2DCFDA fluorescence intensity. Data are presented as the means ± SD. Variation was analyzed by one-way ANOVA, followed by Student's *t* test. $^{**}p < 0.01$ SHR vs WST; $^{\#}p < 0.05$ SHR vs metoprolol; one-way ANOVA $p < 0.01$ ($n = 6$)

Discussion

Cardiac stem cells play a critical role in pathological remodeling [1]. It is now recognized that reactive oxygen species are involved in the pathophysiology of myocardial hypertrophy and failure [17]. There is evidence for increased oxidative stress in patients with heart failure and in animal models of pressure overload [18]. The adverse microenvironment that prevails in this disease condition can have a significant role in modulating stem cell characteristics. Hypertension and hypertrophy are assumed to affect the behavior of CSCs. c-kit^{+} CSCs were isolated from atrial explants by immunomagnetic isolation and were further expanded in culture to obtain a sufficient number of CSCs for experiments. Cell purity and stemness were assessed by immunostaining, clonogenic assays, and flow cytometric analysis (Fig. 1). CSCs from the third passage were used for assessing stem cell characteristics and efficiency. Previous studies have shown that even after long-term culture, up to 40 passages, c-kit^{+} CSCs maintain their characteristics in vitro [19]. Supporting this observation, epigenetic modifications characteristic of the somatic tissue of origin are retained in cultured, induced pluripotent stem cells [20]. Even after long-term culture, DNA methylation reflects the tissue of origin. DNA methylation patterns are maintained throughout the long-term culture of human MSCs [21]. These studies reiterate the contention that in vitro cultures of CSCs maintain their characteristics. Studies have shown that metoprolol's anti-hypertensive response is mediated by downregulating the DNA methylation status [22].

Reduced hypertension was accompanied by hypertrophy regression and decreased oxidative stress (Tables 1, 2, and 3). Although the effect of oxidative stress on differentiated cells is well known, little is known regarding how stem cells respond to oxidative stress. Reactive oxygen species can alter gene expression profiles, leading to a decline in factors that promote stem cell self-renewal and maintenance.

Compared with WST rats, SHRs presented with a significant decrease in GR (Fig. 2b, c, d), colony formation (Fig. 2a), and migration potential (Fig. 3) and an increase in senescence (Fig. 6a, b, c). Oxidative stress was also significantly higher in SHR CSCs (Fig. 4). Since CSC efficiency is compromised in SHRs, decreased cardiac regeneration potential is anticipated, which, in turn, can lead to cardiac failure. A therapeutic intervention that restores the efficiency of stem cells is therefore anticipated to maintain the myocardium in a healthy state and prevent progressive remodeling. Some of the anti-hypertensive drugs prevent cardiac remodeling and reduce oxidative stress. However, the impact of these drugs on stem cell characteristics remains unexplored. Hence, we investigated the effect of a commonly used cardioprotective beta-blocker, metoprolol, on CSCs. To our knowledge, the effect of metoprolol on CSC properties in chronic pressure overload has not been studied. Our study demonstrates that in addition to reduced blood pressure and cardioprotection, metoprolol increased the CSC efficiency, which was compromised in the untreated SHRs.

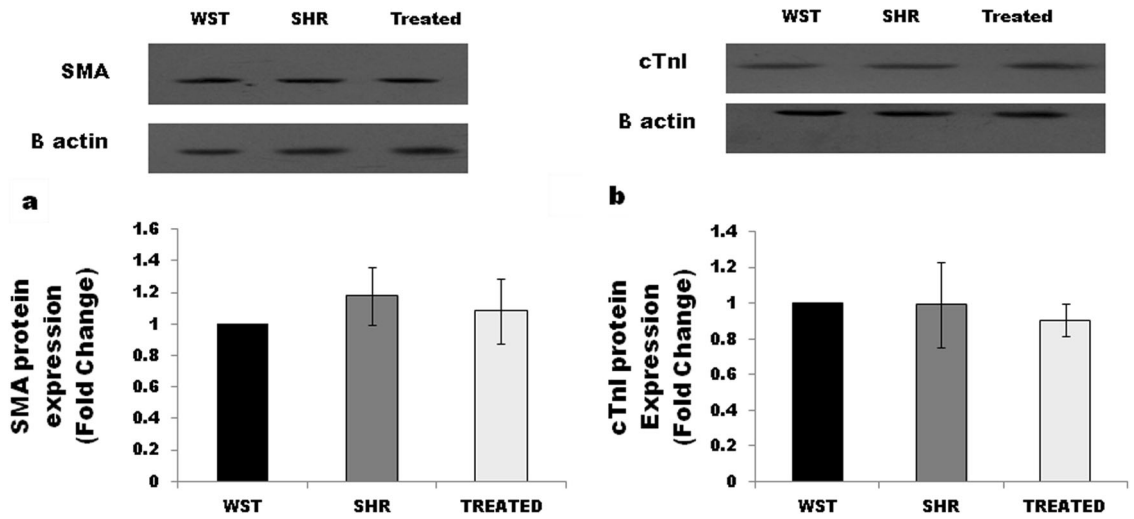

Fig. 5 Effect of metoprolol treatment on directed cardiovascular lineage differentiation. Representative blots and graphical representation of cardiac-specific protein expression in CSCs, as evaluated by western blotting: **a** smooth muscle actin and **b** cardiac troponin I protein. Data are presented as the means ± SD. Variation as analyzed by one-way ANOVA was not statistically significant ($n = 6$)

Spontaneously hypertensive rats, in the stable phase of hypertrophy, were treated with metoprolol tartrate for 2 months. In response to the treatment, reduced blood pressure, cardiac hypertrophy, and oxidative stress were confirmed (Tables 1, 2, and 3). Beta-adrenergic signaling plays an important role in cardiac adaptation following physiological demands or pathological stress [23]. Khan et al. [24] observed that selective β1-AR inhibition augmented cardiac progenitor cell survival and proliferation in the failing myocardium. However, the compromised efficiency of CSCs in hypertensive heart disease and restoration with metoprolol treatment have not been reported. Impaired growth kinetics, GRs, and PDTs were observed in SHRs (Fig. 2). After treatment with metoprolol, CSCs regained their growth potential and proliferation capacity, comparable to that of the normotensive control (Fig. 2a, b, c, d). An increase in CSC number after treatment with metoprolol has been reported in a surgical model of hypertrophy [3], which may be the consequence of increased proliferation as observed in this study. The colony-forming unit assay is a measure of the self-renewal capacity of stem cells. Colony formation was decreased 59% in the CSCs of SHRs compared to those of WST rats (Fig. 2a). Beta-blocker treatment enhanced the efficiency of CSCs to form colonies by 19%. Beta-blockers lower blood pressure and reduce oxygen consumption in the surrounding myocardium, which may help maintain the stemness/quiescent state of CSCs [25]. Hence, modifying the surrounding milieu in response to the treatment may account for the increased efficiency of stem cells.

The ability of cardiac stem cells to migrate in response to myocardial injury is a critical determinant of their efficiency. Stem cells respond to various injury-associated stimuli, such as Stromal-derived factor (SDF) release that promotes stem cell homing [26]. It is apparent that CSCs from SHRs exhibit a decreased ability to migrate as early as 6 months of age (Fig. 3). Treatment with metoprolol enhanced the migratory capacity of stem cells. A study on mouse cardiac fibroblasts reported an association between histone deacetyltransferase levels and cell migration, supporting the possibility for CSC epigenetic modification via metoprolol treatment [27]. The protective effect of metoprolol on the myocardium can therefore be attributed to the efficient availability of CSCs for repair. Directed cardiovascular lineage differentiation was unaffected in SHRs, and metoprolol also did not influence the differentiation potential (Fig. 5). Redox-based mechanisms underlie stem cell differentiation into cardiogenic lineages [28]. An increased proportion of beating cells was observed after embryonic stem cells were exposed to agents that increased the intracellular ROS levels [29]. In contrast, agents that reduce intracellular ROS levels impair cardiomyocyte formation in embryoid bodies [30, 31]. This effect possibly accounts for the relative decrease in the proportion of differentiated cells, though the difference was not statistically significant (Fig. 5). Stem cell maintenance in the undifferentiated state helps to preserve the stem cell pool. The lack of distinction between the differentiation potential of SHRs and WST rats and the insignificant response to the treatment possibly signifies that the variation in ROS levels was not beyond the limits that modulate differentiation. Embryonic stem cell exposure to H_2O_2 revealed that the enhancement and impairment of cardiomyogenic differentiation was dose- and time-dependent [30, 32]. Oxidative stress is implicated as early as 2 months of age in SHRs and can trigger left ventricular remodeling [33, 34]. Comparably,

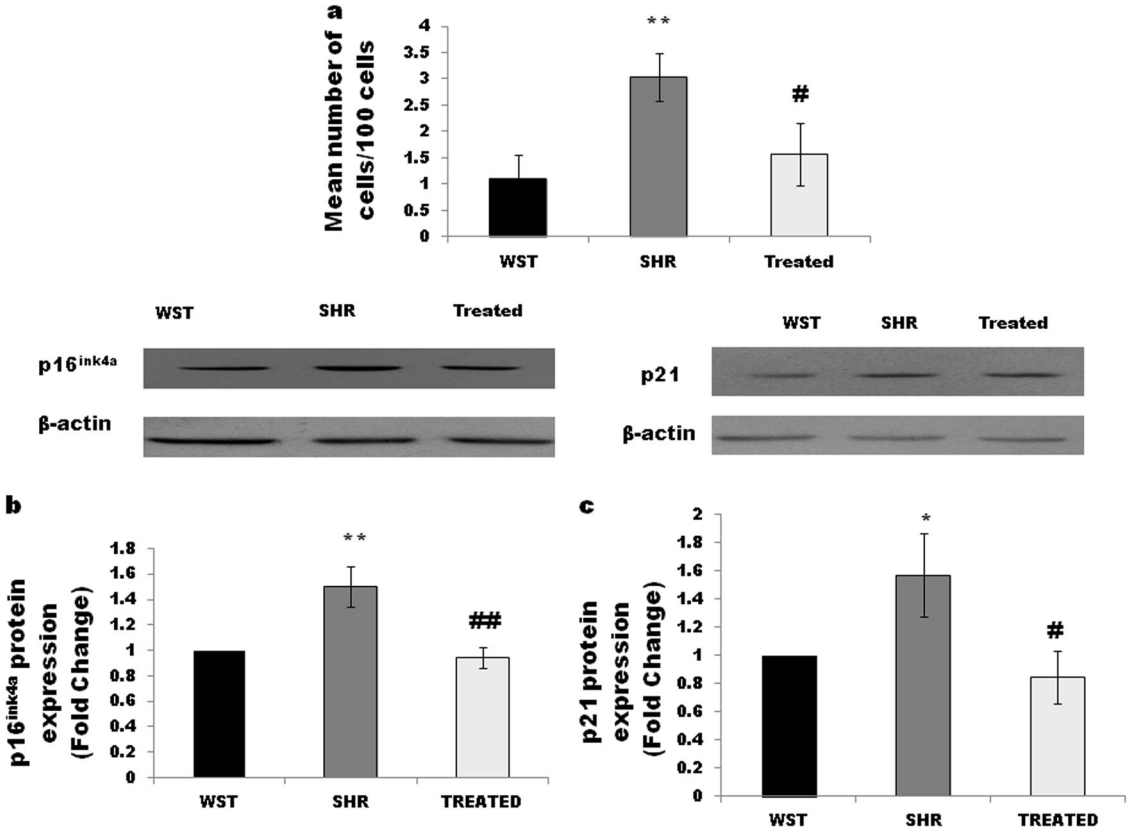

Fig. 6 Effect of metoprolol treatment on the proportion of senescent cardiac stem cells. **a** Proportion of senescent cells, presented as percentages. **b** Representative blot and graphical presentation of $p16^{ink4a}$ protein expression in CSCs, as determined by western blotting. **c** Representative blot and graphical representation of p21 protein expression, as determined by western blotting. Data are presented as the means ± SD. Variation was analyzed by one-way ANOVA, followed by Student's *t* test. $^{**}p < 0.01$ SHR vs WST; $^{\#\#}p < 0.01$ SHR vs metoprolol; one-way ANOVA $p < 0.01$ ($n = 6$)

CSCs from untreated SHRs exhibited increased intracellular ROS levels (Fig. 4). Despite the known antioxidant potential of beta-blockers, there are no reports on the impact of these drugs on the oxidative stress of CSCs. In addition to being an anti-hypertensive drug, metoprolol effectively quenches the ROS level and helps maintain a suitable atmosphere for stem cells [35]. The reduced oxidative stress in CSCs after metoprolol treatment is an interesting observation (Fig. 4). Studies using angiotensin II receptor blockers revealed that EPC dysfunction was improved through antioxidative mechanisms [36]. Studies have also shown that preconditioning human cardiac stem cells (hCSCs) with a nitric oxide adduct promotes cell survival and resistance to oxidative stress by activating cell survival pathways [37]. However, studies on the effect of antioxidants on CSCs are limited. Oxidative stress can induce cell cycle arrest or senescence in stem cells [38]. Over time, the senescent cells undergo apoptosis, thereby reducing the stem cell pool. The increased expression of senescent markers in SHR CSCs (Fig. 6) is possibly a consequence of the compromised efficiency of stem cells in the pathological setting. However, compared to the untreated animals, metoprolol-treated rats presented with an improved stem cell phenotype by retaining stemness. Receptor inhibition by metoprolol stimulates downstream pathways, thereby downregulating the expression of genes involved in senescence and apoptosis [39].

This study has demonstrated that stem cell efficiency is affected during hypertensive heart disease. In addition to being a potent anti-hypertensive drug, metoprolol modulates stem cell characteristics. Hence, the beneficial role of metoprolol in preventing progressive pathological remodeling can also be attributed to improved cardiac stem cell efficiency. These observations encourage further studies in clinical settings that validate the claim that modulating stem cell attributes prevent progressive cardiac remodeling.

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Author contributions Sherin Saheera: designed the study, performed the experiments, analyzed the data, and prepared the manuscript; Ajay Godwin Potnuri: designed and performed the animal experiments; Renuka Nair: conceived the study and edited the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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