Characterization of Human Aortic Endothelial Cells, Endothelial Progenitor Cells, and Cardiomyocytes

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Abstract

Despite advances in therapy, heart failure remains a significant disease burden, with poor outcomes, worldwide. Reactive Oxygen Species (ROS) damage cardiomyocytes. Endothelial progenitor cells promote the repair of the endothelium of arteries damaged by ROS. However, gene expression profiles of Human Aortic Endothelial Cells (HAECs), Endothelial Progenitor Cells (HEPCs), and Cardiomyocytes (HCMs) are unclear. In the present study, we determined the expression profiles of different genes in HAECs, HEPCs, and HCMs by performing quantitative PCR. Results showed that p53 and Cx37 were up-regulated, but VEGF, Cx43, and eNOS were down-regulated in HEPCs. Cx40 and eNOS were up-regulated in HAECs. Moreover, we determined the effect of hydrogen peroxide-derived ROS on HCMs. Results showed that Cx40, Cx45, VCAM-1, ICAM-1, p53, and p21 were up-regulated, but E-cadherin was down-regulated after high concentration of hydrogen peroxide treatment.

Keywords: Human aortic endothelial cells (HAECs); Endothelial progenitor cells (HEPCs); Cardiomyocytes (HCMs); Reactive oxygen species (ROS)

Introduction

Coronary artery disease is a large disease burden in several countries. Endothelial dysfunction caused by oxidative stress and inflammation is an essential process underlying the progression of heart failure [1-3]. Tissues engineering aims to apply the principles of engineering and life science in developing biological substitutes that maintain, restore, or improve tissues. In clinical, new drugs and vascular bypass have improved the quality of life of patients with Cardiovascular Disease (CVD) but have not decreased morbidity or mortality [4]. Tateishi-Yuyama et al. reported that autologous transplantation of bone marrow-derived progenitor cells is a potential therapy of angiogenesis for patients with limb ischemia [5]. Autologous cell therapies involving bone marrow or circulating blood-derived progenitor cells are safe and exert beneficial therapeutic effects by inducing angiogenesis/vasculogenesis in patients with ischemic diseases [6,7]. In addition, human embryonic stem cell (hESC)-derived endothelial cells could be beneficial for potential applications such as engineering of new blood vessels, endothelial cell transplantation into the heart for myocardial regeneration, and induction of angiogenesis for treating regional ischemia [8]. However, because of ethical issues associated with ESCs, peripheral blood-derived epithelial progenitor cells (EPCs) are used for cell therapy [9]. EPCs are a potential inexhaustible source of functional vascular cells that have important features of mature ECs for regenerative medicine. However, it is difficult to define EPCs generated from different sources because they lack a unifying phenotype [10]. Glaser et al. suggested that different types of EPCs include colony-forming unit Hill cells, circulating cells, and endothelial colony-forming cells [11]. Therefore, it is very important to functionally characterize EPCs.

Gap junctions form conduits between adjacent cells that are composed of connexin subunits; these conduits allow direct intercellular communication [12]. Gap junctions also promote intercellular communication in the cardiovascular system and are essential for normal vascular function [13,14]. Connexins expressed in the vascular wall include Cx37, Cx40, Cx43, and Cx45 and those expressed by endothelial cells include Cx37 and Cx40 [12,14]. However, the role of these connexins in Human Aortic Endothelial Cells (HAECs), Human Endothelial Progenitor Cells (HEPCs), and Human Cardiomyocytes (HCMs) is unclear. Nitric Oxide (NO) is very important for regulating endothelial function. Increasing in NO production is either increased by Endothelial Nitric Oxide Synthase (eNOS) enzymes [15-17] or reduced by Reactive Oxygen Species (ROS) [18]. Ischemic preconditioning causes ROS overproduction in the mitochondria under hypoxia [19,20]. However, the effect of hypoxia on Cx37, Cx40, Cx43, and Cx45 is unclear. In this study, we characterized HAECs, HEPCs, and HCMs. In addition, we examined the effect of hypoxia on the expression of the abovementioned connexins in each cell model.

Materials and Methods

Cell lines and cell culture

HAECs (PromoCell GmbH, Heidelberg, Germany) were cultured in T-25 flasks (Corning Glassworks, Corning, NY, USA) containing endothelial cell growth medium MV (PromoCell GmbH) supplemented with 0.05 ml/ml fetal calf serum, 0.004 ml/ml endothelial cell growth supplement, 10 ng/ml epidermal growth factor, 90 µg/ml heparin, and 1 µg/ml hydrocortisone at 37°C and in an atmosphere of 5% CO₂/95% air.

HEPCs (Amsbio, UK) were cultured in T-25 flasks containing EPC growth medium (Cat#Z7030073; Bio Chain Institute Inc., CA, USA) at 37°C and in an atmosphere of 5% CO₂/95% air.

HCMs (PromoCell GmbH) were cultured in T-25 flasks containing myocyte growth medium (PromoCell GmbH) supplemented with 0.05 ml/ml fetal calf serum, 0.5 ng/ml epithelial growth factor, 2 ng/ml basic fibroblast growth factor, and 5 µg/ml insulin at 37°C and in an atmosphere of 5% CO₂/95% air.

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Culture medium was replaced every 2 days. After reaching 70–80% confluency, the cells were trypsinized and were seeded in six-well plastic dishes for performing subsequent experiments. Passages 3–6 HAECs, 3–10 HEPCs, and 3–9 HCMs were used in subsequent experiments.

RNA isolation and quantitative PCR

Total RNA was isolated from the cells by using TRIzol reagent (Invitrogen, Thermo Fisher Scientific, CA, and USA). Primer sequences and procedure used for quantitative PCR (qPCR) analysis of genes encoding vascular endothelial growth factor (VEGF), p53, p21, Cx40, Cx45, eNOS, VE-cadherin, vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and β-actin. cDNAs of genes encoding VEGF, p53, p21, Cx43, Cx44, Cx45, eNOS, VE-cadherin, VCAM-1, ICAM-1, and β-actin were synthesized using the following primer sets: 5'-TGC AGA TTA CTC ACA GCT TTC-3' and 5'-CAG TTG AAG GAT GCG GGA GTA TAT G-3' for the gene encoding VE-cadherin, 5'-CTT CC-3' and 5'-ACA GAG CTC CAC TCA CGC TC-3' for the gene encoding eNOS, 5'-CAT GAG CGA GGC GGA CAT CAC CAG GAA GAA GA-3' and 5'-CAT GAG CGA GGC GGA CAT CAC CAG GAA GAA GA-3' for the gene encoding ICAM-1, 5'-GCC ACC GTT ACC ACA GTG ATG ACA A-3' for the gene encoding VCAM-1, and 5'-TCC ACC TTC CAG CAT ATG TG-3' and 5'-GCATTGGCGGTGGACGAT-3' for the gene encoding β-actin. qPCR was performed using Platinum SYBR Green qPCR kit (Invitrogen, Thermo Fisher Scientific) in iCycler (Bio-Rad).

Statistical analysis

Data are shown as mean ± SEM. Treatment groups were compared using a two-tailed t-test by using a SAS software.

Results and Discussion

Gene profiles of HAECs, HEPCs, and HCMs

We validated the expression of genes encoding Cx37, 40, 43, 45, eNOS, VEGF, p21, and p53 by performing qPCR (Figures 1 and 2). EPCs play a critical role in neovascularization and re-endothelialization after ischemia and endothelial injury, respectively [21,22]. Interestingly, we observed that the expression of VEGF was decreased in HAECs and HEPCs (Figure 1). Previous studies have reported that VEGF is expressed in cardiac myofibroblasts, non-endothelial cells with the morphological features of fibroblasts in rat myocardial infarcts [23,24]. However, limited anti-VEGF antibody-based therapeutic approaches are available for preventing cellular senescence in patients with CVD because these approaches exert different therapeutic effects in animal experiments and clinical trials [25]. Therefore, it is important to determine the gene expression profiles of HAECs, HEPCs, and HCMs.

Effect of hypoxia on HCMs

Mitochondria play a crucial role in regulating intrinsic pathways of apoptosis or programmed cell death [26]. Mitochondria are the major source of endogenous ROS in cells because they contain the electron transport chain required for oxidative phosphorylation [27,28]. However, the effect of hypoxia on HCMs is limited. We used hydrogen peroxide (H2O2) to mimic hypoxic condition [29] and determined the gene expression profiles of HAECs, HEPCs, and HCMs under hypoxia. Our results showed that the expression of genes encoding Cx43, Cx45, VCAM-1, ICAM-1, p21, and p53 was upregulated and that of the gene encoding E-cadherin was downregulated in cells treated with high concentration of H2O2 (200 µM) (Figure 3).

ROS upregulate VCAM-1 expression in endothelial cells [30]; however, this was not observed in HCMs. H2O2 increases the secretion of ICAM-1 in canine myocytes [31], which is consistent with the results of the present study. Long et al. reported that the expression of p21/WAF-1/CIP-1, a well-characterized target of p53 transactivation, also increases under hypoxia. Furthermore, hypoxia-induced rat cardiac myocytes to apoptosis via p53 activation [32]. Thus, our results suggest that H2O2-derived ROS trigger the apoptosis of HCMs. However, further studies should be performed to confirm this hypothesis.

Interestingly, ST2 (suppression of tumor formation) is a receptor for the interleukin-33 and critical to coronary artery disease. Marzullo et al. suggested that ST2/IL-33 pathway may play a central role in the novel mechanism of plaque development and eventually rupture [33].

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References


Figure 2: Relative mRNA levels of genes encoding various connexins and endothelial nitric oxide synthase (eNOS) in human aortic endothelial cells (HAECs), human endothelial progenitor cells (HEPCs), and human cardiomyocytes (HCMs). All data are presented as mean ± SEM (n=3); *p<0.05 and **p<0.01 compared with control.

Figure 3: Effect of hydrogen peroxide (H₂O₂) on human cardiomyocytes (HCMs).


