



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

Ching-Hung Chen¹ and Chan-Yen Kuo^{2*}

¹Department of Anesthesiology, Show Chwan Memorial Hospital, Changhua, Taiwan

²Graduate Institute of Systems Biology and Bioinformatics, National Central University, Chung-li, Taiwan

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 epidermal 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.

***Corresponding author:** Chan-Yen Kuo, Graduate Institute of Systems Biology and Bioinformatics, National Central University, Chung-li, Taiwan, Tel: +886-3-4227151; Fax: +886-3-4226062; E-mail: cykuo@thu.edu.tw

Received November 30, 2016; **Accepted** December 09, 2016; **Published** December 14, 2016

Citation: Chen CH, Kuo CY (2017) Characterization of Human Aortic Endothelial Cells, Endothelial Progenitor Cells, and Cardiomyocytes. J Tradit Med Clin Natur 6: 203. doi: [10.4172/2573-4555.1000203](https://doi.org/10.4172/2573-4555.1000203)

Copyright: © 2016 Chen CH, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

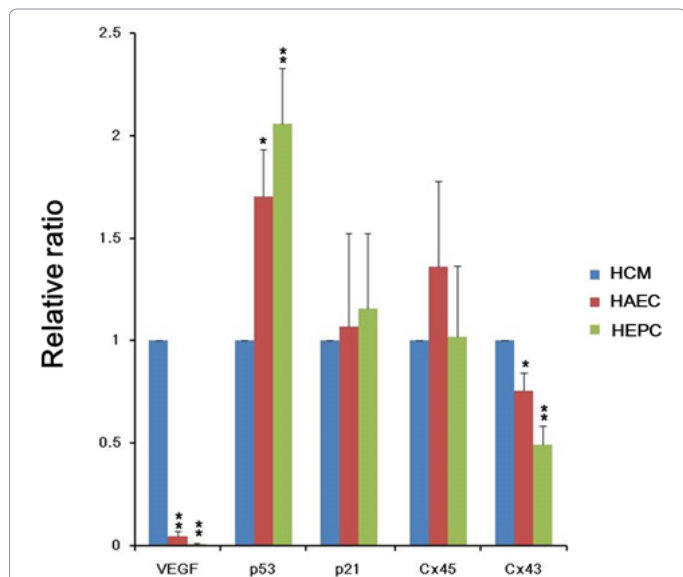


Figure 1: Relative mRNA levels of genes encoding different connexins, vascular endothelial growth factor (VEGF), p21, and p53 in human aortic endothelial cells (HAECs), human endothelial progenitor cells (HEPCs), and human cardiomyocytes (HCMs). All data are presented as mean \pm SEM (n=3); *p<0.05 and **p<0.01 compared with control.

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, Cx37, Cx40, Cx43, 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, Cx37, Cx40, Cx43, Cx45, eNOS, VE-cadherin, VCAM-1, ICAM-1, and β -actin were synthesized using the following primer sets: 5'-TGC AGA TTA TGC GGA TCA AAC C-3' and 5'-TGC ATT CAC ATT TGT TGT GCT GTA G-3' for the gene encoding VEGF, 5'-GCC CAA CAA CAC CAG CTCC T-3' and 5'-CCT GGG CAT CCT TGA GTT CC-3' for the gene encoding p53, 5'-GAG GCC GGG ATG AGT TGG GAG GAG-3' and 5'-CAG CCG GCG TTT GGA GTG GTA GAA-3' for the gene encoding p21, 5'-GTT GCT GGA CCA GGT CCA GG-3' and 5'-GGA TGC GCA GGC CAC CAT CT-3' for the gene encoding Cx37, 5'-GTA CAC AAG CAC TCG ACC GT -3' and 5'-GCA GGG TGG TCA GGA AGA TT-3' for the gene encoding Cx40, 5'-CAA TCA CTT GGC GTG ACT TC-3' and 5'-GTT TGG GCA ACC TTG AGT TC-3' for the gene encoding Cx43, 5'-GGA GCT TTC TGA CTC GCC TG-3' and 5'-CGG CCA TCA TGC TTA GGT TT-3' for the gene encoding Cx45, 5'-CGG CAT CAC CAG GAA GAA GA-3' and 5'-CAT GAG CGA GGC GGA GAT-3' for the gene encoding eNOS, 5'-CAT GAG CCT CTG CAT CTT CC-3' and 5'-ACA GAG CTC CAC TCA CGC TC-3' for the gene encoding VE-cadherin, 5'-GAT ACA ACC GTC TTG GTC AGC CC-3' and 5'-CAG TTG AAG GAT GCG GGA GTA TAT G-3' for the gene encoding VCAM-1, 5'-CGA TGA CCA TCT ACA GCT TTC

CGG-3' and 5'-GCT GCT ACC ACA GTG ATG ATG ACA A-3' for the gene encoding ICAM-1, and 5'-TCC ACC TTC CAG CAG ATG TG-3' and 5'-GCATTTGCGGTGGACGAT-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 \pm 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 myofibroblasts isolated from heart 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 (H_2O_2) 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 Cx40, 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 H_2O_2 (200 μ M) (Figure 3).

ROS upregulate VCAM-1 expression in endothelial cells [30]; however, this was not observed in HCMs. H_2O_2 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 H_2O_2 -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].

Acknowledgement

This study was supported by grant RD105056 from Show Chwan Memorial Hospital, Changhua, Taiwan.

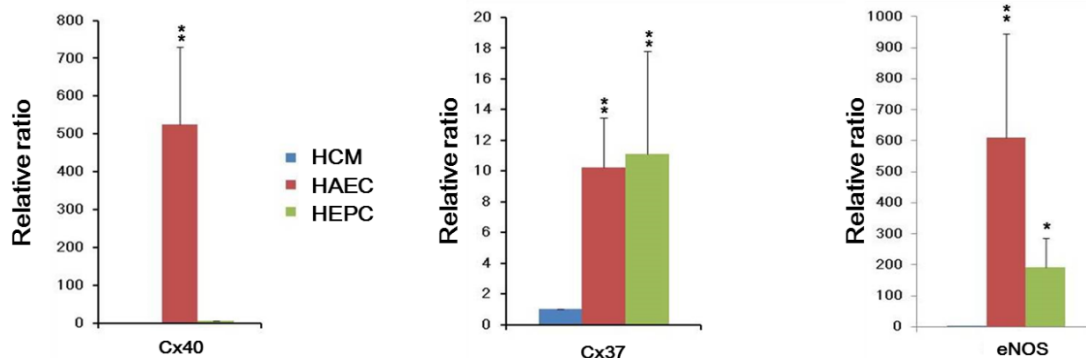


Figure 2: Relative mRNA levels of genes encoding various connexins and endothelial nitric oxide synthase (eNOS) in human aortic endothelial cells (HAECs), human endothelial progenitor cells (HEPCs), and human cardiomyocytes (HCMs). All data are presented as mean \pm SEM (n=3); * p <0.05 and ** p <0.01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

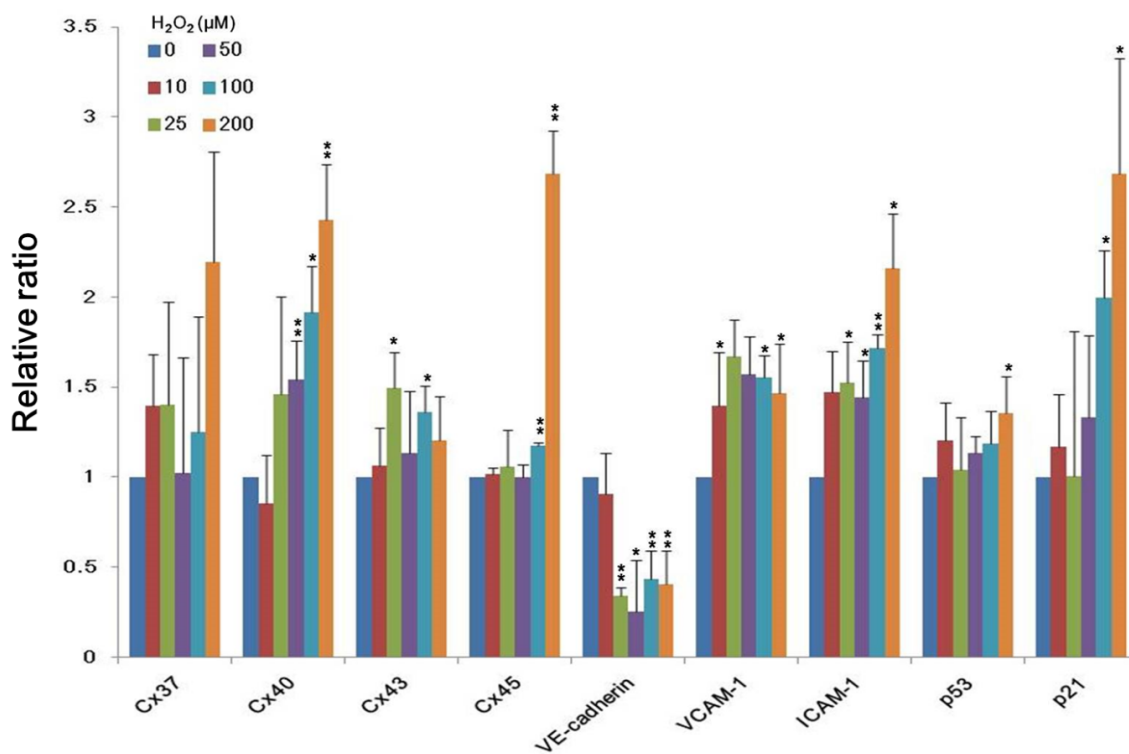


Figure 3: Effect of hydrogen peroxide (H_2O_2) on human cardiomyocytes (HCMs).

References

- Scott J (2004) Pathophysiology and biochemistry of cardiovascular disease. *Curr Opin Genet Dev* 14: 271-279.
- Sun HJ, Hou B, Wang X, Zhu XX, Li KX, et al. (2016) Endothelial dysfunction and cardiometabolic diseases: Role of long non-coding RNAs. *Life Sci*.
- Jensen HA, Mehta JL (2016) Endothelial cell dysfunction as a novel therapeutic target in atherosclerosis. *Expert Rev Cardiovas Ther* 14: 1021-1033.
- Nugent HM, Edelman ER (2003) Tissue engineering therapy for cardiovascular disease. *Circul Res* 92: 1068-1078.
- Tateishi-Yuyama E, Matsubara H, Murohara T, Ikeda U, Shintani S, et al. (2002) Therapeutic angiogenesis for patients with limb ischaemia by autologous transplantation of bone-marrow cells: a pilot study and a randomised controlled trial. *The Lancet* 360: 427-435.
- Li Z, Han Z, Wu JC (2009) Transplantation of human embryonic stem cell-derived endothelial cells for vascular diseases. *J Cell Biochem* 106: 194-199.
- Huang PP, Li SZ, Han MZ, Xiao ZJ, Yang RC, et al. (2004) Autologous transplantation of peripheral blood stem cells as an effective therapeutic approach for severe arteriosclerosis obliterans of lower extremities. *Thromb Haemost* 91: 606-609.
- Levenberg S, Golub JS, Amit M, Itskovitz-Eldor J, Langer R (2002) Endothelial cells derived from human embryonic stem cells. *Proc Natl Acad Sci* 99: 4391-4396.
- Asahara T, Murohara T, Sullivan A, Silver M, van der Zee R, et al. (1997) Isolation of putative progenitor endothelial cells for angiogenesis. *Science* 275: 964-966.
- Hirschi KK, Ingram DA, Yoder MC (2008) Assessing identity, phenotype, and fate of endothelial progenitor cells. *Arterioscler Thromb Vasc Biol* 28: 1584-1595.

11. Glaser DE, Gower RM, Lauer NE, Tam K, Blancas AA, et al. (2011) Functional characterization of embryonic stem cell-derived endothelial cells. *J Vasc Res* 48: 415-428.
12. Sohl G, Willecke K (2004) Gap junctions and the connexin protein family. *Cardiovasc Res* 62: 228-232.
13. Michela P, Velia V, Aldo P, Ada P (2015) Role of connexin 43 in cardiovascular diseases. *Eur J Pharmacol* 768: 71-76.
14. Morel S (2014) Multiple roles of connexins in atherosclerosis- and restenosis-induced vascular remodelling. *J Vasc Res* 51: 149-61.
15. Alderton WK, Cooper CE, Knowles RG (2001) Nitric oxide synthases: structure, function and inhibition. *Biochem J* 357: 593-615.
16. Dudzinski DM, Igarashi J, Greif D, Michel T (2006) The regulation and pharmacology of endothelial nitric oxide synthase. *Annu Rev Pharmacol Toxicol* 46: 235-276.
17. Hambrecht R, Adams V, Erbs S, Linke A, Krankel N, et al. (2003) Regular physical activity improves endothelial function in patients with coronary artery disease by increasing phosphorylation of endothelial nitric oxide synthase. *Circulation* 107: 3152-3158.
18. Adams V, Linke A, Krankel N, Erbs S, Gielen S, et al. (2005) Impact of regular physical activity on the NAD(P)H oxidase and angiotensin receptor system in patients with coronary artery disease. *Circulation* 111: 555-562.
19. Duranteau J, Chandel NS, Kulisz A, Shao Z, Schumacker PT (1998) Intracellular signaling by reactive oxygen species during hypoxia in cardiomyocytes. *J Biol Chem* 273: 11619-11624.
20. Bagheri F, Khorrami V, Alizadeh AM, Khalighfar S, Khodayari S, et al. (2016) Reactive oxygen species-mediated cardiac-reperfusion injury: Mechanisms and therapies. *Life Sci* 165: 43-55.
21. Urbich C, Dimmeler S (2004) Endothelial progenitor cells: characterization and role in vascular biology. *Circ Res* 95: 343-53.
22. Hur J, Yoon CH, Kim HS, Choi JH, Kang HJ, et al. (2004) Characterization of two types of endothelial progenitor cells and their different contributions to neovascularization. *Arterioscler Thromb Vasc Biol* 24: 288-293.
23. Chintalgattu V, Nair DM, Katwa LC (2003) Cardiac myofibroblasts: a novel source of vascular endothelial growth factor (VEGF) and its receptors Flt-1 and KDR. *J Mol Cell Cardiol* 35: 277-286.
24. Harmey JH, Bouchier-Hayes D (2002) Vascular endothelial growth factor (VEGF), a survival factor for tumour cells: implications for anti-angiogenic therapy. *Bioessays* 24: 280-283.
25. Sanada S, Taniyama Y, Azuma J, Yuka, II, Iwabayashi M, et al. (2014) Endothelial progenitor cells in clinical settings. *J Stem Cells* 9: 117-125.
26. Green DR, Reed JC (1998) Mitochondria and apoptosis. *Science* 281: 1309-1312.
27. Murphy MP (2009) How mitochondria produce reactive oxygen species. *Biochem J* 417: 1-13.
28. Stowe DF, Camara AK (2009) Mitochondrial reactive oxygen species production in excitable cells: modulators of mitochondrial and cell function. *Antioxid Redox Signal* 11: 1373-1414.
29. Hool LC, Arthur PG (2002) Decreasing cellular hydrogen peroxide with catalase mimics the effects of hypoxia on the sensitivity of the L-type Ca²⁺ channel to beta-adrenergic receptor stimulation in cardiac myocytes. *Circ Res* 91: 601-609.
30. Cook-Mills JM, Marchese ME, Abdala-Valencia H (2011) Vascular cell adhesion molecule-1 expression and signaling during disease: regulation by reactive oxygen species and antioxidants. *Antioxid Redox Signal* 15: 1607-1638.
31. Lu H, Youker K, Ballantyne C, Entman M, Smith CW (2000) Hydrogen peroxide induces LFA-1-dependent neutrophil adherence to cardiac myocytes. *Am J Physiol Heart Circ Physiol* 278: H835-H842.
32. Long X, Boluyt MO, Hipolito ML, Lundberg MS, Zheng JS, et al. (1997) p53 and the hypoxia-induced apoptosis of cultured neonatal rat cardiac myocytes. *J Clin Invest* 99: 2635-2643.
33. Marzullo A, Ambrosi F, Inchingolo M, Manca F, Devito F, et al. (2016) ST2L Transmembrane Receptor Expression: An Immunohistochemical Study on Endarterectomy Samples. *PLoS ONE* 11: e0156315.