Review

SIRT1/eNOS Axis as a Potential Target against Vascular Senescence, Dysfunction and Atherosclerosis

Hidetaka Ota, Masato Eto, Sumito Ogawa, Katsuya Iijima, Masahiro Akishita, and Yasuyoshi Ouchi

Department of Geriatric Medicine, Graduate School of Medicine, University of Tokyo, Tokyo, Japan

Sir2 (silent information regulator-2), an NAD⁺-dependent histone deacetylase, is highly conserved in organisms ranging from archaea to humans. Yeast Sir2 is responsible for silencing at repeated DNA sequences in mating-type loci, telomeres and rDNA, and plays critical roles in DNA repair, stress resistance and longevity.

The phenomenon of human aging is known to be a critical cardiovascular risk factor. Senescence of endothelial cells has been proposed to be involved in vascular dysfunction and atherogenesis. Recent studies have demonstrated that mammalian *Sirt1* NAD⁺-dependent protein deacetylase, the closest homologue of Sir2, regulates vascular angiogenesis, homeostasis and senescence. This review focuses on SIRT1 as a potential therapeutic target against atherosclerosis.

J Atheroscler Thromb, 2010; 17:431-435.

Key words; SIRT1, Vascular senescence, Dysfunction, Atherosclerosis

Introduction

Recent studies demonstrate that cellular senescence is involved in various pathological conditions, such as atherosclerosis. In this review, we discuss the potential protective effect of SIRT1 on vascular endothelial cells.

Sirtuins

During the last decade, aging research has progressed through the use of lower organism models, such as the budding yeast *Saccharomyces cerevisiae* and the nematode *Caenorhabditis elegans*. In *S. cerevisiae*, the Sir2 (silent information regulator-2) family of genes governs budding exhaustion and replicative life span^{1, 2}; deletions of Sir2 shorten life span and an extra copy of this gene increases life span. In addition to promoting longevity, the activity of Sir2 is enhanced by caloric restriction (CR), which extends life span in

diverse species. Sir2 has been identified as an NAD⁺-dependent histone deacetylase and is responsible for maintenance of chromatin silencing and genome stability³. Sir2 genes are conserved during evolution, and seven homologs of sirtuins (*Sirt1-7*) have been cloned in mammals. Mammalian sirtuins have diverse cellular localizations, modify multiple substrates, and affect cellular functions. SIRT1 is localized in the cytoplasm and nucleus, and SIRT6 and SIRT7 are localized in the nucleus. SIRT3, SIRT4 and SIRT5 reside in the mitochondria and SIRT2 is localized in the cytoplasm. SIRT1, SIRT2, SIRT3 and SIRT5 are NAD⁺-dependent deacetylases, whereas SIRT4 and SIRT6 are primarily mono-ADP-ribosyl transferases.

SIRT1, the closest homologue of Sir2, targets a wide range of transcriptional regulators, including p53, PML (promyelocytic leukemia protein), FoxO (forkhead box O), NF- κ B (nuclear factor κ B) and PPAR- γ (peroxisome proliferators-activated receptor- γ)⁴⁻⁸. Like yeast Sir2, SIRT1 regulates the cell cycle, senescence, apoptosis and metabolism, and might act as a longevity factor in mammals.

Endothelial Senescence Induces Vascular Dysfunction and Atherosclerosis

The phenomenon of human aging is known to

Address for correspondence: Yasuyoshi Ouchi, Department of Geriatric Medicine, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan

E-mail: youchi-tyk@umin.ac.jp

Received: August 9, 2009

Accepted for publication: November 12, 2009

be a critical cardiovascular risk factor. Minamino *et al.* proposed that the senescence of endothelial cells is involved in endothelial dysfunction and atherogenesis⁹. Histological study of human atherosclerotic lesions has demonstrated the existence of vascular cells that exhibit the morphological features of senescence¹⁰.

Moreover, it has been reported that angiogenesis becomes impaired with advancing age¹¹ and that aging reduces the antithrombotic properties of the endothelium¹². These senescent changes of vascular structure and function have been suggested to result in the increased risk of atherosclerotic cardiovascular disease in the elderly.

According to the free-radical theory, reactive oxygen species (ROS) may be potential candidates responsible for vascular dysfunction and atherosclerosis¹³, and upon the production of high levels of ROS, the redox balance is disturbed and cells shift into a state of oxidative stress, which subsequently leads to endothelial dysfunction and senescence with shortening of telomeres¹⁴. Endothelial NO synthase (eNOS) activity is reduced in human senescent endothelial cells, accompanied by a reduction of nitric oxide (NO) production. Endothelial-derived NO regulates vascular relaxation and has athero-protective effects¹⁵. Intriguingly, endothelial NO can protect against a state of oxidative stress, and activation of eNOS and subsequent production of NO delay endothelial cellular senescence^{16, 17}.

SIRT1 Plays a Critical Role in Endothelial Homeostasis

SIRT1 likely plays a critical role in endothelial homeostasis by regulating endothelial nitric oxide synthase (eNOS). A recent study showed that levels of cGMP and eNOS are elevated in tissues of calorie-restricted mice, and production of NO by CR increases SIRT1 expression. The induction of SIRT1 expression is blunted in eNOS-deficient mice, and eNOS has been implicated in regulation of the expression of SIRT1¹⁸.

It has been reported that SIRT1 promotes endothelial-dependent vasodilation by targeting eNOS for deacetylation, leading to enhanced NO production¹⁹. Intriguingly, SIRT1 has been shown to directly bind to eNOS, which is deacetylated at lysines 496 and 506 in the calmodulin-binding domain and posttranscriptionally leads to activation of eNOS. Inhibition of SIRT1 by a deacetylase-defective mutant SIRT1 decreases NO bioavailability and inhibits endothelium-dependent vasorelaxation. Consistent with these

results, we showed that SIRT1 inhibition by sirtinol or RNAi-mediated knock down induced a premature senescent phenotype in human endothelial cells. Conversely, overexpression of SIRT1 prevented oxidative stress-induced endothelial senescence²⁰. Intriguingly, a micro RNA (miR-217) was recently identified and miR-217 induced endothelial senescence through direct inhibition of SIRT1²¹.

In addition to endothelial protection, SIRT1 regulates the angiogenic activity of endothelial cells. It has been reported by Potente *et al.* that SIRT1 deacetylase activity plays a critical role in the angiogenesis of endothelial cells²². Knockdown of SIRT1, but not SIRT2-7, was uniquely associated with loss of sprouting angiogenesis *in vitro*. Moreover, *Sirt1* mutant mice, which have genetic deletion of SIRT1 activity in the endothelium postnatally, have impaired formation of new vessels in response to angiogenic signals such as ischemic stress.

Cilostazol Inhibits Oxidative Stress-Induced Premature Senescence via Up-Regulation of SIRT1 in Human Endothelial Cells

A PDE3 inhibitor, cilostazol, is used as a vasodilating anti-platelet drug for treating intermittent claudication, and in preclinical studies was shown to have a protective effect on endothelial cells by increasing eNOS activity²³. Cilostazol increases intracellular cAMP content accordingly and activates protein kinase A (PKA) and PI3K/Akt signaling²⁴. We found that treatment with cilostazol inhibited the senescent phenotype. Cilostazol increased eNOS activity, expression of eNOS and the phosphorylation of eNOS at Ser¹¹⁷⁷ in parallel with the phosphorylation of Akt at Ser⁴⁷³. These results suggest that the protective effect against a senescent phenotype may be attributable to an increase in NO via eNOS activation by cilostazol²⁵.

To explore the mechanism by which cilostazol prevents endothelial senescence, we considered that an increase in NO production could promote the longevity gene, SIRT1. We found that cilostazol significantly increased SIRT1 mRNA and protein in a concentration-dependent manner. In contrast, SIRT1 inhibition abrogated the effect of cilostazol on specific senescent changes. Although NO is known to be involved in reducing oxidative stress and the progression of atherosclerosis, we suggest that the NO-mediated prevention of senescence is attributable to SIRT1 function (**Fig. 1**). These findings implicate the eNOS-NO-SIRT1 axis as one of the fundamental determinants of endothelial senescence, and the role of SIRT1 as a driver of cellular stress resistance and longevity is note-

Endothelial cell senescence / Arteriosclerosis

Fig. 1. Signaling pathway of inhibition of endothelial senescence, dysfunction and atherosclerosis by cilostazol treatment.

worthy in the context of its expression profile (**Fig. 2**).

In addition to these results, we found that drugs utilized for drug-eluting stents (DES), including paclitaxel and limus family members (e.g. sirolimus, everolimus), inhibit the growth of endothelial cells and lead to endothelial senescence caused by delayed re-endothelialization²⁶. We showed that the development of endothelial senescence induced by sirolimus and everolimus is SIRT1-dependent, whereas paclitaxel acts through a SIRT1-independent pathway. Because the effects of sirolimus and everolimus involve SIRT1 modulation, cilostazol reverses sirolimus- or everolimus-induced senescence. Our results could have the interesting clinical implication that triple anti-platelet therapy may have more beneficial effects on endothelial senescence than standard dual therapy with sirolimus- or everolimus-eluting stents.

Activation of SIRT1, a Potential Therapeutic Target against Atherosclerosis

CR extends life span in diverse species. A recent study by Colman *et al.* at Wisconsin National Primate Center (WNPRC) reported that calorie-restricted Rhesus macaques showed a lower incidence of age-related diseases, such as cancer, diabetes, and cardiovascular disease, and lower age-related mortality²⁷. Resveratrol, a CR mimetic, is a polyphenolic activator

Fig. 2. Possible mechanisms of prevention of vascular senescence, dysfunction and atherosclerosis.

of SIRT1. Resveratrol increases mitochondrial biogenesis in endothelial cells via the activation of eNOS and SIRT1²⁸. Resveratrol has also been shown to increase the expression of eNOS, and a combination of resveratrol with an HMG-CoA reductase inhibitor (statin) increased the activation of eNOS, resulting in increased functional recovery in a model of acute myocardial infarction²⁹. Therefore, we suggest that increased NO bioavailability by other pharmaceutical products, such as statins, or agents with phytoestrogenic properties, such as resveratrol, may exert a protective effect against endothelial senescence via up-regulation of eNOS and SIRT1, and this possibility deserves further investigation. Our results and the findings by other laboratories indicate that micromolar levels of resveratrol are sufficient to exert vasculoprotective effects^{26, 30}. Considering that each gram of fresh grape skin contains 50–100 μg resveratrol and it is found mainly in high-quality red wine at a concentration of 20 to 60 $\mu\text{mol/L}$ as previously reported³¹, it becomes apparent that effective concentrations are unlikely to be reached in plasma *in vivo*. However, resveratrol is a lipophilic substance, and exhibits higher bioavailability and slower clearance, and has been shown to accumulate in tissues such as the heart, liver, and kidney³². By daily consumption of grapes, berries, red wine or dietary supplements containing resveratrol, an effective concentration of resveratrol may be achievable *in vivo*. Recently, novel small molecule activators of Sirt1 (SRTs), even 1000-fold more potent than resveratrol, have been identified (Sirtris Pharmaceuticals Inc., Boston, USA)³³. SRTs induce many of the beneficial metabolic changes observed with CR/resveratrol treat-

ment³⁴). We propose that using these chemical agents to activate SIRT1 may be a new attractive therapy for protection against vascular senescence, dysfunction and atherosclerosis.

Conclusions

SIRT1 is likely to play an important role in the prevention of human cardiovascular disease, including atherosclerosis. There is some evidence that SIRT1 interacts with the vascular eNOS/NO system. Just as the French paradox stands out as an excellent example of a reduced incidence of cardiovascular disease, activation of SIRT1 may have a beneficial effect on vascular senescence, dysfunction and atherosclerosis.

Acknowledgments

This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, Culture and Sports of Japan (20249041, 18590801, 18890056).

References

- Guarente L: Sir2 links chromatin silencing, metabolism, and aging. *Genes Dev*, 2000; 14: 1021-1026
- Sinclair D, Mills K, Guarente L: Aging in *Saccharomyces cerevisiae*. *Annu Rev Microbiol*, 1998; 52: 533-560
- Braunstein M, Rose AB, Holmes SG, Allis CD, Broach JR: Transcriptional silencing in yeast is associated with reduced nucleosome acetylation. *Genes Dev*, 1993; 7: 592-604
- Vaziri H, Dessain SK, Ng Eaton E, Imai SI, Frye RA, Pandita TK, Guarente L, Weinberg RA: hSIR2 (SIRT1) functions as an NAD-dependent p53 deacetylase. *Cell*, 2001; 107: 149-159
- Langley E, Pearson M, Faretta M, Bauer UM, Frye RA, Minucci S, Pelicci PG, Kouzarides T: Human SIR2 deacetylates p53 and antagonizes PML/p53-induced cellular senescence. *EMBO J*, 2002; 21: 2383-2396
- Brunet A, Sweeney LB, Sturgill JF, Chua KF, Greer PL, Lin Y, Tran H, Ross SE, Mostoslavsky R, Cohen HY, Hu LS, Cheng HL, Jedrychowski MP, Gygi SP, Sinclair DA, Alt FW, Greenberg ME: Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase. *Science*, 2004; 303: 2011-2015
- Yeung F, Hoberg JE, Ramsey CS, Keller MD, Jones DR, Frye RA, Mayo MW: Modulation of NF-kappaB-dependent transcription and cell survival by the SIRT1 deacetylase. *EMBO J*, 2004; 23: 2369-2380
- Picard F, Kurtev M, Chung N, Topark-Ngarm A, Senawong T, Machado De Oliveira R, Leid M, McBurney MW, Guarente L: Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-gamma. *Nature*, 2004; 429: 771-776
- Minamino T, Miyauchi H, Yoshida T, Ishida Y, Yoshida H, Komuro I: Endothelial cell senescence in human atherosclerosis: role of telomere in endothelial dysfunction. *Circulation*, 2002; 105: 1541-1544
- Burrig KF: The endothelium of advanced arteriosclerotic plaques in humans. *Arterioscler Thromb*, 1991; 11: 1678-1689
- Rivard A, Fabre JE, Silver M, Chen D, Murohara T, Kearney M, Magner M, Asahara T, Isner JM: Age-dependent impairment of angiogenesis. *Circulation*, 1999; 99: 111-120
- Schneiderman J, Sawdey MS, Keeton MR, Bordin GM, Bernstein EF, Dilley RB, Loskutoff DJ: Increased type 1 plasminogen activator inhibitor gene expression in atherosclerotic human arteries. *Proc Natl Acad Sci U S A*, 1992; 89: 6998-7002
- Shi Y, Camici GG, Lüscher TF: Cardiovascular determinants of life span. *Pflugers Arch*, 2009 Sep 12; in press
- Kurz DJ, Decary S, Hong Y, Trivier E, Akhmedov A, Erusalimsky JD: Chronic oxidative stress compromises telomere integrity and accelerates the onset of senescence in human endothelial cells. *J Cell Sci*, 2004; 117: 2417-2426
- Oemar BS, Tschudi MR, Godoy N, Brovkovich V, Malinski T, Lüscher TF: Reduced endothelial nitric oxide synthase expression and production in human atherosclerosis. *Circulation*, 1998; 97: 2494-2498
- Hayashi T, Matsui-Hirai H, Miyazaki-Akita A, Fukatsu A, Funami J, Ding QF, Kamalanathan S, Hattori Y, Ignarro LJ, Iguchi A: Endothelial cellular senescence is inhibited by nitric oxide: implications in atherosclerosis associated with menopause and diabetes. *Proc Natl Acad Sci U S A*, 2006; 103: 17018-17023
- Vasa M, Breitschopf K, Zeiher AM, Dimmeler S: Nitric oxide activates telomerase and delays endothelial cell senescence. *Circ Res*, 2000; 87: 540-542
- Nisoli E, Tonello C, Cardile A, Cozzi V, Bracale R, Tedesco L, Falcone S, Valerio A, Cantoni O, Clementi E, Moncada S, Carruba MO: Calorie restriction promotes mitochondrial biogenesis by inducing the expression of eNOS. *Science*, 2005; 310: 314-317
- Mattagajasingh I, Kim CS, Naqvi A, Yamamori T, Hoffman TA, Jung SB, DeRico J, Kasuno K, Irani K: SIRT1 promotes endothelium-dependent vascular relaxation by activating endothelial nitric oxide synthase. *Proc Natl Acad Sci U S A*, 2007; 104: 14855-14860
- Ota H, Akishita M, Eto M, Iijima K, Kaneki M, Ouchi Y: Sirt1 modulates premature senescence-like phenotype in human endothelial cells. *J Mol Cell Cardiol*, 2007; 43: 571-579
- Menghini R, Casagrande V, Cardellini M, Martelli E, Terzoni A, Amati F, Vasa-Nicotera M, Ippoliti A, Novelli G, Melino G, Lauro R, Federici M: MicroRNA 217 Modulates Endothelial Cell Senescence via Silent Information Regulator 1. *Circulation*, 2009 Sep 28, in press
- Potente M, Ghaeni L, Baldessari D, Mostoslavsky R, Rossig L, Dequiedt F, Haendeler J, Mione M, Dejana E, Alt FW, Zeiher AM, Dimmeler S: SIRT1 controls endothelial angiogenic functions during vascular growth. *Genes Dev*, 2007; 21: 2644-2658
- Kambayashi J, Liu Y, Sun B, Shakur Y, Yoshitake M, Czer-

- wiec F: Cilostazol as a unique antithrombotic agent. *Curr Pharm Des*, 2003; 9: 2289-2302
- 24) Hashimoto A, Miyakoda G, Hirose Y, Mori T: Activation of endothelial nitric oxide synthase by cilostazol via a cAMP/protein kinase A- and phosphatidylinositol 3-kinase/Akt-dependent mechanism. *Atherosclerosis*, 2006; 189: 350-357
- 25) Ota H, Eto M, Kano MR, Ogawa S, Iijima K, Akishita M, Ouchi Y: Cilostazol inhibits oxidative stress-induced premature senescence via upregulation of Sirt1 in human endothelial cells. *Arterioscler Thromb Vasc Biol*, 2008; 28: 1634-1639
- 26) Ota H, Eto M, Ako J, Ogawa S, Iijima K, Akishita M, Ouchi Y: Sirolimus and everolimus induce endothelial cellular senescence via sirtuin 1 down-regulation: therapeutic implication of cilostazol after drug-eluting stent implantation. *J Am Coll Cardiol*, 2009; 53: 2298-2305
- 27) Colman RJ, Anderson RM, Johnson SC, Kastman EK, Kosmatka KJ, Beasley TM, Allison DB, Cruzen C, Simmons HA, Kemnitz JW, Weindruch R: Caloric restriction delays disease onset and mortality in rhesus monkeys. *Science*, 2009; 325: 201-204
- 28) Csiszar A, Labinskyy N, Pinto JT, Ballabh P, Zhang H, Losonczy G, Pearson K, de Cabo R, Pacher P, Zhang C, Ungvari Z: Resveratrol induces mitochondrial biogenesis in endothelial cells. *Am J Physiol Heart Circ Physiol*, 2009; 297: H13-20
- 29) Penumathsa SV, Thirunavukkarasu M, Koneru S, Juhasz B, Zhan L, Pant R, Menon VP, Otani H, Maulik N: Statin and resveratrol in combination induces cardioprotection against myocardial infarction in hypercholesterolemic rat. *J Mol Cell Cardiol*, 2007; 42: 508-516
- 30) Ungvari Z, Orosz Z, Rivera A, Labinskyy N, Xiangmin Z, Olson S, Podlutzky A, Csiszar A: Resveratrol increases vascular oxidative stress resistance. *Am J Physiol Heart Circ Physiol*, 2007; 292: H2417-2424
- 31) Wallerath T, Deckert G, Ternes T, Anderson H, Li H, Witte K, Förstermann U: Resveratrol, a polyphenolic phytoalexin present in red wine, enhances expression and activity of endothelial nitric oxide synthase. *Circulation*, 2002; 106: 1652-1658
- 32) Bertelli A, Bertelli AA, Gozzini A, Giovannini L: Plasma and tissue resveratrol concentrations and pharmacological activity. *Drugs Exp Clin Res*, 1998; 24: 133-138
- 33) Milne JC, Lambert PD, Schenk S, Carney DP, Smith JJ, Gagne DJ, Jin L, Boss O, Perni RB, Vu CB, Bemis JE, Xie R, Disch JS, Ng PY, Nunes JJ, Lynch AV, Yang H, Galonek H, Israelian K, Choy W, Iffland A, Lavu S, Medvedik O, Sinclair DA, Olefsky JM, Jirousek MR, Elliott PJ, Westphal CH: Small molecule activators of SIRT1 as therapeutics for the treatment of type 2 diabetes. *Nature*, 2007; 450: 712-716
- 34) Feige JN, Lagouge M, Canto C, Strehle A, Houten SM, Milne JC, Lambert PD, Matakic C, Elliott PJ, Auwerx J: Specific SIRT1 activation mimics low energy levels and protects against diet-induced metabolic disorders by enhancing fat oxidation. *Cell Metab*, 2008; 8: 347-358