CDK5-mediated phosphorylation of SIRT1 at serine 47 contributes to the development of endothelial senescence

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Background−Senescence of endothelial cells precedes the occurrence of vascular dysfunction and promotes the development of atherosclerosis. SIRT1 is a NAD-dependent deacetylase possessing anti-aging activities. In senescent endothelial cells, both the activity and expression level of SIRT1 are decreased. However, mechanisms underlying this down-regulation of SIRT1 are largely uncharacterized. The present study evaluated the regulation and role of SIRT1 phosphorylation (S47) in the development of endothelial senescence. **Methods and Results**−Primary cultures of porcine aortic endothelial cells (PAECs) exhibited severe senescence phenotype after repetitive passages. Western blotting revealed that the phosphorylation of SIRT1 at S47 was significantly enhanced in senescent endothelial cells. Moreover, S47 phosphorylation was stimulated by agents promoting premature senescence and attenuated by anti-senescence drugs. Mutation of S47 to phospho-mimicking aspartic acid (S47D) abolished the anti-senescence activity of SIRT1, while replacing this residue with alanine (S47A) improved the anti-senescent and cell growth-promoting functions of this deacetylase. SIRT1 S47A showed much stronger interactions with LKB1, a senescence-promoting kinase. Pharmacological or siRNA inhibition of cyclin-dependent kinase 5 (CDK5) significantly attenuated SIRT1 phosphorylation at S47, and reduced the number of senescent PAECs. An *in vitro* phosphorylation assay confirmed that CDK5 acted as a SIRT1 kinase modulating phosphorylation at S47. P25, a truncated regulatory subunit of CDK5, accumulated in senescent PAECs. Chronic treatment with roscovitine blocked the development of senescence and atherosclerosis in aortae of hypercholesterolemic ApoE-/- mice with endothelial-specific overexpression of human SIRT1. **Conclusion**−CDK5-mediated hyperphosphorylation of SIRT1 plays a critical role in the development of endothelial senescence and atherosclerosis.