Sirtuin 5 regulates arterial thrombosis by modulating endothelial plasminogen activator inhibitor-1

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Topic(s):
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Citation:
Introduction: Arterial thrombosis as a result of plaque rupture or erosion is a crucial event in myocardial infarction and stroke. Oxidative stress and inflammation promote endothelial dysfunction and play a pivotal role in destabilization of the atherosclerotic plaque. Sirtuin 5 (SIRT5) is a member of the sirtuin protein family with function as a NAD+-dependent protein desuccinylase and demalonylase. Being implicated in the regulation of different pathophysiological processes among which production of reactive oxygen species and transcription of inflammatory mediators, SIRT5 plays a role in the development of several cardiovascular diseases such as myocardial infarction and stroke. To date, the possible involvement of SIRT5 as a mediator of arterial thrombosis remains to be investigated.

Purpose: In this study we investigate the putative role of this protein in arterial thrombosis by using an established in vivo mouse model. The translational value of animal findings as well as the molecular mechanism underlying the observed effect will be investigated also in primary human aortic endothelial cells (HAECs).

Methods: SIRT5 knockout (KO) as well as SIRT5 transgenic (TG) animals were used for in vivo experiments. HAECs treated with SIRT5 silencing RNA (si-SIRT5) and stimulated with tumor necrosis factor (TNF)-a were used for in vitro assays.

Results: When compared to WT animals, SIRT5 KO mice display blunted carotid artery thrombus formation as underlined by delayed time to occlusion in a photochemical injury model. Oppositely, in SIRT5 TG mice the formation of an occlusive thrombus is accelerated (Fig 1). Mechanistically, SIRT5 KO and WT animals show no difference in terms of vascular tissue factor (TF) activity, TF concentration in plasma and expression of TF pathway inhibitor (TFPI) in the aorta. In line with the observed reduced thrombogenicity, SIRT5 KO animal express reduced level of the pro-thrombotic plasminogen activator-1 (PAI-1), as assessed by western blot in aorta lysate. Of interest, SIRT5 genetic deletion does not affect platelet aggregation, as assessed by ex-vivo collagen-induced aggregometry. In HAECs, SIRT5-silencing inhibits PAI-1 expression in response to TNF-a. Real-time polymerase chain reaction revealed that inhibition of PAI-1 expression occurs at the mRNA level. This effect is mediated by reduced activation of the MAP kinase Erk 1/2, but not JNK (Fig 1).

Conclusions: SIRT5 mediates arterial thrombosis by increasing endothelial PAI-1 expression. Hence, SIRT5 may be an effective therapeutic target in the context of atherothrombosis.
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Conclusions: SIRT5 mediates arterial thrombosis by increasing endothelial PAI-1 expression. Hence, SIRT5 may be an effective therapeutic target in the context of atherothrombosis.

Figure 1. A. SIRT5 KO mice show delayed time to thrombotic occlusion, as compared to WT ones. B. On the other hand, in SIRT5 overexpressing animals the time to thrombus formation is lower than in WT ones. C-E. SIRT5 KO and WT animals show similar levels of TF concentration in plasma, tissue factor (TF) activity and TF pathway inhibitor (TFPI) concentration in aorta lysate. F. SIRT5 KO animals display reduced levels of plasminogen activator-1 (PAI-1) in aorta lysate, as compared with WT mice. G. Treatment with TNF-α induces PAI-1 protein expression in scramble silencing RNA (siSCR)-treated human aortic endothelial cells (HAECs) but not in siSIRT5-treated ones. G. Treatment with TNF-α (10 ng/mL) increases the transcription of PAI-1 in siSCR-treated HAECs but not in SIRT5 silencing RNA (siSIRT5)-treated ones. D-E. siSIRT5-treated cells display reduced activation of the MAP kinase Erk 1/2, but not JNK in response to TNF-α (10 ng/mL).