197 ENDOTHELIAL CELL SENESCENCE: THE ROLE OF SIRT6

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Introduction The loss of telomere integrity and the consequent induction of cellular senescence have been increasingly implicated in the development of vascular pathologies. Hence unravelling the mechanisms that may attenuate these processes is important to develop strategies against diseases of the cardiovascular system. Sirtuin 6 (SIRT6) belongs to a family of NAD^+-dependent deacylases that influence metabolism, stress responses and ageing, and are generally considered to fulfil beneficial functions. Whether SIRT6 has a cytoprotective role in vascular cells is unknown. Here we have investigated the expression of SIRT6 in endothelial cells from different vascular beds and explored its function as an anti-senescence gene.

Methods SIRT6 mRNA expression was measured by quantitative polymerase chain reaction. SIRT6 protein localization and levels were determined by immunohistochemistry and immunoblotting, respectively. Silencing of SIRT6 for functional studies was performed by RNA interference. The formation of DNA damage foci and telomere dysfunction-induced foci was detected by immunofluorescence microscopy using anti-gamma H2.AX antibodies. Senescence was evaluated by detection of senescence-associated β-galactosidase.

Results Generation of endothelial cells from haematopoietic progenitor cells (HPC) resulted in an increase in SIRT6 mRNA levels. Similarly, compared to HPC, mature endothelial cells derived from different vascular beds also displayed higher levels of this transcript. SIRT6 protein was readily detected in early passage cultured human umbilical vein endothelial cells (HUVEC), in aortic endothelial cells (HAEC), and in normal humna coronary artery endothelium where it displayed a nuclear localization. In cultured cells SIRT6 protein levels increased significantly upon nutrient deprivation and decreased upon senescence. Silencing of SIRT6 in both HUVEC and HAEC diminished cell proliferation, increased the fraction of senescent cells, and impaired the formation of capillary tube networks. Consistent with the induction of a senescent phenotype, SIRT6-silenced cells displayed lower expression levels of eNOS, and higher levels of ICAM-1 and PAI-1. At the molecular level, depletion of SIRT6 led to an increase in nuclear DNA damage and to the formation of telomere-dysfunction induced foci, with no evidence of an increase in intracellular oxidative stress. SIRT6-depleted HUVEC also showed evidence of activation of the p21 signalling pathway.

Conclusions This work demonstrates that in endothelial cells SIRT6 confers protection from genomic DNA damage and telomere dysfunction, preventing a decrease in replicative capacity and the onset of premature senescence. Thus, SIRT6 may be important to mantain endothelial homeostatic functions and delay vascular ageing. In this context, our findings that in endothelial cells SIRT6 levels are amenable to up-regulation, may have implications for the development of strategies to combat vascular pathologies.