[gw22-e0822]

IDENTIFICATION AND CHARACTERISATION OF ATAXIA TELANGIECTASIA MUTATED (ATM) IN REGULATION OF OXIDATIVE STRESS-INDUCED VASCULAR ENDOTHELIAL CELLS SENESCENCE

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10.1136/heartjnl-2011-300867.94

Background Aging is known to be a major cardiovascular risk factor. Recent studies provide evidence for a critical role of cellular senescence in the process of vascular aging including hypertension and atherosclerosis. Oxidative stress regulates dysfunction and senescence of vascular endothelial cells. The DNA damage response and its main signalling pathway which involves ataxia telangiectasia mutated (ATM) have been implicated in playing a central role in mediating the actions of oxidative stress. However, the role of the ATM signalling pathway in aging and cardiovascular disease remains unclear.

Purpose To investigate pathogenic mechanisms of the vascular pathologies associated with mutated ATM, we examined the effects of ATM-mediated oxidative stress-induced senescence in vascular pathologies through actions on endothelial cells.

Methods and Results To investigate involvement of ATM in mediating oxidative stress in the vasculature, we first examined the activation and expression of ATM in cultured human umbilical vein endothelial cells (HUVECs) treated with hydrogen peroxide as an inducing agent of oxidative stress. Western blot analysis showed that hydrogen peroxide phosphorylated ATM at Ser-1981 and subsequent ATM-dependent phosphorylation of Akt at Ser-473, p53 at Ser-15 and further up-regulated downstream p21 expression which in turn induced senescence, as judged by senescence-associated β-galactosidase assay (SA-β-gal assay), in endothelial cells. These actions were suppressed in cells in which ATM was knocked-down by siRNA or blocked by specific inhibitory compounds (for example KU-55933). Moreover, the in vivo significance of this regulatory pathway was confirmed using ATM knockout mice. Age-matched ten-week-old male ATM wild-type (+/+), heterozygote (+/-), and homozygote (-/-) mice (n=6, respectively, weighing approximately 15~25g) were used. Hyperglycemia which leads to endothelial dysfunction was induced by a single intraperitoneal injection of streptozotocin (STZ) (180 mg/kg). Tail blood glucose was assayed three days after injection using glucose test strips. All diabetic animals had blood glucose values>300 mg/dl. The thoracic aortas were removed after systemic perfusion with PBS for histological examination at 10 days after treatment with STZ. SA-β-gal assay analysis showed induction of senescence in endothelial cells in the aorta in a diabetic mouse model of endothelial dysfunction and senescence was weakened in contrast to pathological changes seen in wild-type mice. Cross-sections of arteries stained with SA-β-gal showed that positive areas were mostly localised to the luminal surface which also stained positive for von Willebrand factor indicating localisation to vascular endothelial cells and not the extracellular matrix. Immunohistochemical observations demonstrated that SA-β-gal positive areas of cross-sections of thoracic aortas also stained positive for p21 and p16, which are used as senescence markers.

Conclusions Our results show that ATM through an ATM/Akt/p53/p21-dependent signalling pathway mediates an instructive role in oxidative stress-induced endothelial dysfunction and premature senescence. The major role of ATM-dependent signalling pathway in oxidative stress-induced vascular endothelial cells senescence made us to further examine the role of ATM on cardiomyopathy. We are preparing the conditional ATM-knockout mouse lines to examine the cell-type specific functions of ATM in cardiac hypertrophy and heart failure. Collectively, inhibition of ATM may be a potentially exploitable treatment strategy for vascular aging pathologies. ATM may be a new therapeutic target for cardiovascular disease.