CIRCULATING ENDOTHELIAL PROGENITOR CELLS IN SMOKERS ARE DYSFUNCTIONAL DUE TO INCREASED DNA DAMAGE AND SENESCENCE

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Introduction  Cardiovascular disease (CVD) is a major cause of death in smokers, particularly in patients with chronic obstructive pulmonary disease (COPD). Circulating endothelial progenitor cells (EPC) are required for endothelial homeostasis, and their dysfunction contributes to CVD. DNA damage has also been recognized as an important contributor to CVD. Our aim was to investigate...
whether EPC from smokers and COPD patients are dysfunctional, and to investigate the role of DNA damage pathways in mediating endothelial dysfunction in these patients.

**Methods** To investigate EPC dysfunction in smokers, we isolated and expanded blood outgrowth endothelial cells (BOEC) from peripheral blood samples of healthy non-smokers, healthy smokers and COPD patients. The mononuclear fraction was placed in culture in the presence of endothelial growth factors and BOEC colonies appeared between days 7 and 24. BOEC colonies were expanded and used at passages 4 to 6 for all experiments. Endothelial senescence was measured by senescence-associated β-galactosidase (SA-β-Gal) activity. Expression of sirtuin (SIRT)-1 and of markers of senescence and DNA damage were measured by Western blotting and/or immunofluorescence confocal microscopy. SIRT1 activity was measured using a SIRT1 fluorescent activity assay kit. To investigate in vivo function, BOEC were labelled with Vybrant DiI Cell-Labelling Solution, mixed with Matrigel and injected subcutaneously into the back of NOD.CB17-Prkdcscid/NcrCrl mice. Seven days later, the mice were sacrificed and the plugs were harvested and cryosectioned.

**Results** In vitro, BOEC from smokers and COPD patients showed increased DNA double-strand breaks (measured by γ-H2AX, 53BP1) and senescence (senescence associated-β-galactosidase activity, p16 and p21 levels) compared to non-smokers. Senescence negatively correlated with sirtuin-1 (SIRT1) expression and activity, a protein deacetylase that inhibits DNA damage and cellular senescence. Inhibition of DNA damage response by silencing of ataxia telangiectasia-mutated (ATM) kinase resulted in up-regulation of SIRT1 expression and decreased senescence. Interestingly, treatment of BOEC from COPD patients with the SIRT1 activator resveratrol or a selective ATM inhibitor rescued the senescent phenotype. Using the in vivo Matrigel plug angiogenesis assay, BOEC from COPD patients displayed reduced ability to form capillary-like structures and increased DNA damage, senescence and apoptosis (measured by 53BP1, p16, TUNEL and cleaved-caspase 3 staining) compared to non-smokers.

**Conclusions** BOEC from smokers and COPD patients show reduced angiogenesis in vivo and display increased DNA damage and senescence, associated with reduced SIRT1 expression. These defects may contribute to endothelial dysfunction and cardiovascular events in smokers and COPD patients and could potentially constitute therapeutic targets for intervention.
168 CIRCULATING ENDOTHELIAL PROGENITOR CELLS IN SMOKERS ARE DYSFUNCTIONAL DUE TO INCREASED DNA DAMAGE AND SENESCENCE

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