



Abstract

# The Senescence Marker p16Ink4a—A Player of Liver Endothelial Cells Physiology <sup>†</sup>

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**Abstract:** P16INK4A is a tumor suppressor and cell cycle regulator that has been linked to aging and senescence. In development, a potential role of p21 and of p19ARF has been postulated, but little is known about p16. Our previous results revealed a highly dynamic expression pattern of p16 in development and in different organs and cell types assessed by qRT-PCR and immunohistochemistry (IHC). In addition, we also noticed through IHC observation that p16 expression in old liver is mainly in the endothelial cells (ECs) compared to parenchymal cells. Therefore, we aimed at better understanding the role of p16 in biological processes of liver ECs, such as proliferation, migration, apoptosis, and tube formation. We also performed RNA sequencing to identify genes differentially expressed between young and old ECs. We used small hairpin (shRNA) constructs and a p16 cDNA-GFP vector to knockdown and overexpress p16 in vitro, in two types of liver ECs, CD31+ vascular ECs and CD146+ sinusoidal endothelial cells. Afterwards, we assessed p16’s down- and up-regulation effect on ECs function. Brdu incorporation assays showed that p16 upregulation was associated with slower proliferation compared to control cells, whereas its downregulation induced higher proliferation compared to control cells. Scratch assay and transwell migration assays showed attenuated migration in p16 overexpressed cells compared to baseline expression, while only transwell assays showed the ameliorated migration of p16 knockdown cells compared to controls. Similar migration between p16 knockdown and control was observed in scratch assays. We also observed in  $\beta$ -gal staining, a marker of senescence, a higher number of stained cells in p16 overexpression conditions compared to controls, while less cells were stained in the case of knockdown. Additional experiments that aim to further decipher p16’s effect in ECs’ tube formation, apoptosis, and telomeres shortening are ongoing, which might contribute to the invention of more specialized anti-aging therapies.

**Keywords:** aging; development; endothelial cells; liver; p16; senescence



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