Simvastatin (SIM) induces apoptosis and cell cycle arrest in several types of malignant tumors. The aim of this work was to evaluate the effects of simvastatin on cellular senescence of WM9 metastatic melanoma cells, and elucidate its action mechanism. Cell viability was measured upon treatment with SIM at different concentrations (0.05 to 1μmol/L) for different time points. We observed 24% reduction in cell viability when WM9 cells were treated with SIM (1μmol/L) for 72h, which is correlated with increased positive staining for Annexin-V. These data suggest that, at higher concentrations, SIM induces apoptosis. Measurements of ROS showed that simvastatin (1μmol/L, 72h) increased cellular ROS content by 51%. Cell cycle analysis showed an increase in the number of WM9 cells into G1 phase of the cell cycle already after treatment with a low dose of SIM (0.25μmol/L). In agreement with these results, senescence-associated β-galactosidase staining was evident in WM9 cells treated with SIM at both 0.25 and 1μmol/L. In addition, the mRNA expression levels of senescence markers and antioxidant enzymes were evaluated by RT-qPCR. Increased expression of p53, p21, p16, Catalase and Peroxiredoxin-1 mRNA levels were observed. Also, Western Blotting assays confirmed the augmented expression of phospho-p53 in WM9 cells treated with 0.25 and 1μmol/L of SIM. Finally, the effects of SIM on positive staining for β-Gal assay and increased ROS levels were abrogated when WM9 cells were pre-treated with pifithrin-α (a p53 inhibitor). Together, our results indicate that simvastatin treatment affects proliferation of human WM9 melanoma cells, inducing the senescent state. Also, our results showed that simvastatin effects on senescence may be mediated by increased levels of ROS and p53 activation.

Keywords: simvastatin, senescence, melanoma