Analysis of the genetic damage produced in human keratinocytes transduced with the HPV-18 E5, E6, E7 virla oncogenes subjected to a hypoxic microenviroment

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Resume

Objetives. In this study, we will evaluate the levels of oxidative stress and DNA damage in a cell model of early oral carcinogenesis, subjected to a hypoxic microenvironment.

Methods. We will use human keratinocytes (HaCaT) transduced with HPV-18 oncogenes (E5/E6/E7) induced in a hypoxic microenvironment using the coverslip hypoxia. The detection of reactive oxygen species (ROS) was studied using a fluorescent probe by confocal microscopy. In addition, the levels of production of nitric oxide (NO) were measured, using the Griess assay. DNA damage generated in the control HaCaT and HaCaT E5/E6/E7-HPV18 cell lines was also evaluated, both in normoxia and hypoxia using γH2AX immunocytochemistry. In addition, we evaluated the genetic damage caused by double-strand breaks in DNA using the comet assay. Finally, we evaluated cellular and nuclear morphological differences of both cell lines in normoxia and hypoxia for 24 hours, using the Nanolive 3D Cell Explorer-Fluo high-resolution microscope.

Results. We observed significantly higher intracellular ROS levels in HaCaT cells containing the HPV-18 oncogenes compared to HaCaT cells. Regarding the NO content, it was significantly higher in HaCaT E5/E6/E7-HPV18 cultured in hypoxia compared to HaCaT cells in hypoxia. In addition, HaCaT E5/E6/E7-HPV18 cells presented greater genetic damage as measured by γH2Ax fluorescence intensity, and by the comet assay, in hypoxia compared to normoxia. Finally, we observed nuclear morphological differences, and greater compaction of chromatin in both cell lines, in hypoxia, in relation to normoxia.

Conclusions. Our results suggest that E5/E6/E7 HPV-18 oncogenes cooperate with an altered microenvironment to promote oxidative stress, genetic damage, and morphological changes that could favor the malignant transformation

Key words. Hypoxia, HPV-18, DNA damage

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