ROLE OF P53 IN ANTIOXIDANT DEFENSE OF HPV-POSITIVE CERVICAL CARCINOMA CELLS FOLLOWING H2O2 EXPOSURE

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Introduction. Depending on the cell type and the context of stimuli, p53 can either induce cell growth arrest to allow repair or alternatively, triggers apoptosis to prevent DNA damage in abnormal or stress-exposed cells from becoming fixed as a mutation (1-3). To provide new insights into the possible role of endogenous p53 in the antioxidant defense of HPV-positive cervical cancer cells, we aim to investigate whether p53 could modulate intracellular ROS levels under both nonstressed and stressed conditions.

Method. In order to generate a short hairpin RNAi specific for p53, we used the pSuppressorNeo p53 plasmid. A negative control plasmid (IMG-800) with a scrambled sequence was supplied from Imegenex. Intracellular production of ROS was measured using cell-permeable fluorescent dyes, 5-(and-6)-chloromethyl-7′-ichlorodihydrofluorescein diacetate, acetyl ester, DHE and MitoSOX RED.

Results. We found that SiHa cells containing integrated HPV16 had higher expression of p53 and exhibited the greatest resistant to H2O2-induced oxidative damage, as compared with HeLa, CaSki and ME180 cell lines. Downregulation of p53 using RNAi resulted in the inhibition of p53-regulated antioxidant enzymes and elevated intracellular ROS in SiHa cells. In contrast, the ROS level was not affected in HeLa, CaSki and ME180 cell lines after inhibition of p53 protein. Under mild or sever H2O2-induced stress, p53-deficient SiHa cells exhibited much higher ROS levels than mock SiHa cells. Furthermore, we analyzed cell viability and apoptosis after H2O2
treatment and found that p53 deficiency sensitized SiHa cells to H$_2$O$_2$ damage. Inhibition of p53 resulted in excessive oxidation of DNA, and mock SiHa cells exhibited a more rapid removal of 8-oxo-7,8-dihydro-2'-deoxyguanosine from DNA than p53-deficient SiHa cells exposed to the same level of H$_2$O$_2$ challenge. (See Fig.1)

**Fig.1** Reduction of p53 expression increased DNA oxidation in SiHa cells

**Discussion.** Depletion of p53 by RNAi in SiHa cell line resulted in disintegration of the antioxidant firewall and increased oxidative damage. As ROS are also involved in cancer chemoradiotherapy, these findings may help to facilitate clinical studies of HPV-positive cervical cancer therapy.

**References.**