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‘Interaction between low-risk human papillomavirus 6 and 4-nitroquinoline-1-oxide in epithelial transformation’.

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High-risk HPV16 and 18 are the most common HPV types found in cervical cancer and play a crucial role in the pathogenesis of cervical cancer. They are associated with oropharyngeal, anal and penile cancers. Their oncogenic property is essentially due to the E6 and E7 proteins which target the two tumour suppressor proteins wtp53 and pRb for degradation resulting in perturbed cell proliferation. In contrast, low-risk HPV6 and HPV11 are associated with benign lesions of the genital tract and oral mucosa. The E6 and E7 proteins of low-risk papillomavirus do not immortalize cells due to the inability to degrade wtp53 and pRb. Low-risk papillomavirus have received little attention. However, HPV6 has occasionally been demonstrated in pre- and malignant lesions such as giant condylomata or Buschke-Löwenstein tumours or verrucous carcinoma. Interestingly, HPV6 has also been reported in squamous cell carcinomas of the esophagus and of the head and neck including tongue and tonsillar carcinoma. These studies propose a possible role for co-factors which may co-operate with low-risk papillomavirus to promote epithelial transformation. Tobacco and alcohol are, presently regarded as main risk factors in the development of head and neck cancers. We have investigated whether a chemical carcinogen - 4-NQO can act as a co-factor to confer oncogenic potential to HPV6, by two mechanisms a) enhancing the URR activity b) co-operate with HPV6E6 to perturb the key regulators of proliferation, differentiation and apoptosis. 4-NQO is the preferred carcinogen to study chemically-induced carcinogenesis in animal models. It exhibits features similar to the carcinogens present in tobacco, including generation of reactive oxygen species. 4-NQO-induced mouse oral cavity cancer resembles HNSCC in humans in that the expression of many genes related to tumorigenesis in humans are affected.

The present study provides evidence that 4-NQO can act as a co-factor in enhancing the URR activity and perturbing expression of proteins involved in proliferation, differentiation and

apoptosis. In this study, the role of 4-NQO was evident in three different *in vitro* systems which are physiologically relevant i.e. HPV16E6/E7 immortalized oral keratinocytes cell line (Oral KER16), primary human foreskin keratinocytes (HFK) and a p53-null small-cell lung carcinoma cell line H1299:

1. 4-NQO up-regulated HPV6 URR activity in Oral KER16 cells and HFK.
2. 4-NQO enhanced the expression of HPV6 E6 and E7 proteins in HFK.
3. HPV6 E6 did not exert any obvious influence on wtp53 and mutp53R248W proteins in the presence of 4-NQO in comparison to 4-NQO alone. 4-NQO treatment alone did not induce apoptosis and resulted in inhibition of cell cycle arrest, increased DNA synthesis in H1299 cells.
4. Other p53 family members e.g. TAp63α and ΔNp63α play a role in cell proliferation and differentiation. The concerted action of HPV6E6, TAp63α and 4-NQO promoted proliferation and inhibited cell cycle arrest in H1299 cells. 4-NQO inhibited HPV6E6 mediated up-regulation of full length and cleaved TAp63α in both H1299 cells and HFK. This cleavage was mediated through Caspase-3.
5. HPV6E6 up-regulated ΔNp63α through PI3-kinase pathway. This was inhibited by 4-NQO in H1299 cells.

Abnormalities in the p53 gene have been regarded as the most consistent genetic abnormalities in head and neck squamous cell carcinogenesis. We provide the evidence for a role of HPV6-E6 as a regulator of p53 family proteins i.e. p63α proteins. We demonstrated that the interplay between HPV6-E6 and 4-NQO impairs the expression of the p63α isoforms which resulted in cell proliferation. This short term *in vitro* study provides insight into the possible cross talk between low-risk HPV6 and chemical carcinogen 4-NQO in regulating important cellular factors involved in cell growth.