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Viruses and Oral Squamous Carcinoma

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INTRODUCTION

ORAL SQUAMOUS carcinogenesis is clearly a multistep process with a multifactorial aetiology but, in view of the evidence for a viral association in malignant neoplasia of other squamous epithelia [1], viruses might contribute aetiologically in at least some cases [2–7].

Electron microscopy has not shown viral particles in oral carcinoma [8] but this clearly does not exclude a viral aetiology. More sophisticated studies over the past few years have examined more closely the possible associations with viruses, particularly with adenoviruses, herpes viruses, and human papillomaviruses (HPV). The associations with adenoviruses, and herpes viruses are discussed here: HPV are discussed elsewhere ([6, 9–11] and Yeudall, 1992 this issue).

ADENOVIRUSES

Adenovirus 12 with SV40 can induce immortality in keratinocytes *in vitro*. These cells are non-tumorigenic, but superinfection with Kirsten sarcoma virus induces tumorigenicity, possibly due to the acquisition of the Ki-*ras* oncogene [12]. Human *ras* oncogenes can complement the adenovirus EIA gene [13] and cellular *myc* gene [14] in inducing the neoplastic phenotype at least in rat fibroblasts *in vitro*. The EIA gene can also downregulate MHC class I antigen expression [15] which might facilitate tumour cell evasion of the immune system; and EIA complexes with the retinoblastoma oncosuppressor protein pRb [16] and with related proteins such as p107—interactions that could play a role in cellular transformation.

Studies of oral squamous carcinoma however, have failed to reveal adenovirus antigens in tumour explants, or a difference in titres of serum antibodies against adenoviruses 12 or 18 from those in controls [17, 18]. Furthermore, there is no evidence of raised titres of serum antibodies against adenoviruses of high oncogenicity (adenovirus 13) or other adenoviruses [19, 20] in patients with oral carcinoma. More recently, we, and others have, by *in situ* hybridisation, also discounted a role for some adenoviruses [21, 22]. A role for these human adenoviruses in oral carcinoma therefore appears unlikely from the results of these rather crude studies: more detailed analyses using sensitive detection methods such as polymerase chain reaction are indicated.

HERPES VIRUSES

Most evidence relates to possible associations between herpes simplex virus and oral carcinoma. The other herpes viruses are however, discussed first.

Epstein–Barr virus (EBV)

Though the association of EBV with anaplastic nasopharyngeal carcinoma is well established and the oncogenicity not in doubt, EBV DNA and antigens have not been demonstrated in oral carcinoma tissue [22–24] or in carcinoma cell lines [24].

Cytomegalovirus (CMV)

There is, at least on serological evidence, no association between CMV and oral carcinoma [25].

Varicella-zoster virus (VZV)

As with CMV there is, at least on serological evidence, no association between VZV and oral carcinoma [25].

Human herpes virus 6 *(HHV-6)*

A recent study has indicated a high prevalence of serum antibodies to HHV-6 in patients with oral carcinoma compared with controls, and significantly raised titres [26]. Any significance of these observations is unclear and the findings are certainly not specific.

Herpes simplex virus (HSV)

HSV-1 is capable of transforming cells *in vitro* provided cytolysis is inhibited [27, 28]. Factors which can inhibit HSV-mediated cytolysis include ultraviolet light [28] and certain chemicals [29]. In some *in vitro* systems such as SV40-transformed hamster embryo cells, HSV is more effective than some chemical carcinogens in amplifying SV40 DNA sequences [30, 31] acting via HSV-encoded DNA polymerase [31, 32]. Several reports indicate that HSV acts synergistically with chemical carcinogens in causing oncogenic transformation [33, 36] and it is now clear that HSV is synergistic with tobacco-specific nitrosamines in cell transformation [37].

Animal studies suggest that HSV may be a co-carcinogen with tobacco [38–40] or other chemicals [41, 42], and that immunisation against HSV prevents the co-carcinogenic activity of HSV with dimethylbenzanthracene [43].

In vitro HSV induces chromosomal aberration, mutations, and gene amplification [30, 44, 45], and in the hamster cheek pouch model of dimethylbenzanthracene-induced carcinogenesis enhances *erb*-B1 oncogene amplification and overexpression [42], a feature that coincides with the appearance of malignancy [46, 47]. HSV also binds to the receptor for basic fibroblast growth factor [48] and this interaction might conceivably activate *myc* and other oncogenes [6].

Substantial evidence suggests therefore, that HSV might under particular circumstances be oncogenic. However studies of the association of HSV with oral carcinoma have not proved any link. A number of studies have shown changes in levels of serum antibodies to HSV patients with oral carcinoma [25, 49–58]. For example, serum IgA antibodies to HSV-1 induced antigens may be increased in smokers, whether they have oral carcinoma or not, but the increases in smokers without tumours are to a lesser degree than in those with carcinomas [59].

Serum IgG antibodies against HSV are of higher titre in patients with head and neck cancer who smoke than in smokers without cancer, and there is higher reactivity to the HSV immediate early protein ICP4, suggesting a different course

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of an earlier herpetic infection, with a prolonged exposure to early immediate proteins of HSV as a consequence of smoking [25].

Smoking may act, at least in part, by suppressing natural killer-cell (NK) activity [60], which is involved in control of HSV [61]. Indeed, there are close relationships between NK-cell activity and antibody production to HSV in patients with carcinoma of the head and neck [62]. Systemic factors often associated with oral carcinoma, such as alcohol and liver disease, might also impair NK-activity [63].

Examination of oral carcinoma tissues for HSV viral "footprints" has given equivocal results though failure to demonstrate HSV products does not, of course, exclude a hit and run mechanism [64]. HSV antigens have been shown in carcinomas in some [20, 26, 65, 66] but not in all studies [67]. The demonstration, by *in situ* hybridisation, of RNA complementary to HSV-DNA in biopsy specimens from oral carcinoma but not from autologous, normal oral mucosa suggested an association of HSV with oral carcinoma [21, 68] and others have demonstrated HSV-1 DNA in oral carcinoma tissue [26, 58]. However, hybridisation could be revealing segments of normal host nucleic acid with homology to part of the HSV genome.

Therefore, the evidence for an association of oral carcinoma with HSV, though stronger than for other herpes viruses or adenoviruses can be seen to be not unequivocal. However, carcinogenesis is not a single step procedure with a single aetiology and it has been suggested that HSV may act synergistically with HPV in carcinogenesis [69]. With regards to cervical carcinoma, epidemiological evidence indicates that this may be possible [70] and, in experimental situations, it has been demonstrated that keratinocytes immortalised by HPV-16 DNA are tumorigenic in nude mice following transfection with HSV-DNA [71, 72].

Further studies are needed to investigate the roles of HSV and HPV in oral carcinogenesis.

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