Viruses in Human Oral Cancers

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The role of viruses in the etiology of human oral cancer is critically reviewed. Available evidences show a positive correlation for human oral cancer with human papilloma virus (HPV), herpes simplex virus (HSV) and human herpes virus type-6 (HHV-6), having strongest association with HPV. These viruses may act alone or in combination with other carcinogens in the genesis of head and neck malignancies.

Key Words: Oral cancer, Head and neck cancer, Viruses, HPV, HSV, HHV-6

Oral cancer (head and neck cancer) represents about 27% of total cancers in Southern India, where it is the most common tumor among males and the second commonest in females (1). Many etiological factors such as tobacco chewing, alcohol consumption, sexually transmitted diseases, poor oral hygiene and even malnutrition have been considered responsible for the high prevalence of oral cancer (2). Oral carcinogenesis is a multi-step process with a multi-factorial aetiology. The cellular transformation can be due to chemical, physical or biological agents, including viruses. Genetic susceptibility, immunologic responsiveness and environmental carcinogens are other important factors which contribute to viral oncogenesis. Even though viruses were known to cause many cancers in animals, the same cannot be proved conclusively in human cases (3). A virus is said to be etiologically associated with human cancer when all or many of the following criteria are satisfied: (a) High antibody titres against that virus in a particular form of cancer. (b) Expression of virally coded antigens on cancer cells, detectable by immunofluroescence or similar methods. (c) Expression of the virus particles, full or partial forms from the cancer cells, directly or when the cells are cultured or when the virus is induced under appropriate conditions. (d) The virus, or some part of the virus gene, is shown to be integrated into the host tumour DNA by means of DNA hybridisation or by Southern blot techniques. (e) The tumor extract could induce transformation in susceptible cell lines in tissue culture conditions, and these transformed cells could produce transplantable tumors in nude mice. Most of the above mentioned criteria have been satisfied in the case of Epstein-Barr virus (EBV) as an etiological agent of Burkitt's lymphoma in African children and of nasopharyngeal carcinoma in the Chinese population (4, 5). The progression of Burkitt's lymphoma is recognised to go through three stages. The first step is EBV infecting B lymphocytes, which are then immortalised, but are still dependent on the B cell growth factors. The second step is the chromosome translocation, usually from 8 to 14 and rarely from 8 to 22 or from 8 to 2 (6). This event confers independence from growth factors; but the growth potential is still limited. The third step is the activation of the c-myc oncogene, which completes the transformation. Although such a clear-cut scenario is not elucidated, it is known that some viruses are etiologically associated with oral cancers. This review attempts to critically evaluate the available evidences for the role of viruses in the production of oral (head and neck) cancers.

Herpes simplex virus (HSV) is ubiquitous in the human population. It persists in the host in a latent state, despite the presence of humoral antibodies. Many experimental evidences link HSV with human uterine cervical cancers (7-9) but only limited reports are available to show its link with oral cancers. Generally speaking, HSV-2 is related with cervical cancer, and HSV-1 with oral cancer. Hollinshead et al. (10) have found a high correlation of herpes antibodies in oral cancer patients. Others have also detected high seroprevalence of anti-HSV antibodies in head and neck cancers (11-13). HSV-1-related proteins were demonstrated in the cytoplasm, but not in the nuclei of oral

cancer cells (12). Increased concentration of HSV-related antigens has been shown in transformed cells of head and neck neoplasms (14). It seems possible that HSV acts synergistically with nitrosoamines (15) or with other chemicals (16). HSV-1 induced chromosomal aberrations and mutations were reported (17). The production of antibodies by the host may be the result of the recognition of virus-specific membrane antigens. Oral cancer patients had higher levels of circulating antibodies to HSV-associated antigens when compared to normal controls (8, 13, 18, 19). Oral cancer patients also showed increased cell mediated immune response against HSV-related antigens (20). Immunofluorescence has shown that cells infected with HSV develop new antigens which alter the antigenic specificity of the host cell membranes (21). HSV-1 antigens on oral cancer cells were demonstrated by immunofluorescent and immunoperoxidase techniques (22): Serum IgG antibodies against HSV were found in higher concentrations in smokers with oral cancers than in smokers without cancer (23). RNA complementary to HSV DNA was demonstrated in biopsy specimens from oral carcinoma (13, 24). Vasudevan et al. (25) reported the presence of HSV-1 DNA segments in the DNA of tissue samples from human oral cancer biopsies by dot-blot and in-situ hybridization techniques. A population based control study showed a high risk of oral cancer in patients with HSV infection (26).

Hepatitis B surface antigen, a known etiological factor of hepatocellular carcinoma, has been reported to increase in oral and cervical cancers, especially after radiotherapy (27, 28). A few reports associating adenoviruses with human oral cancer are available (29). But other investigator did not find any significant increase either in the prevalence or in the titres of antibodies against adenoviruses in oral cancers (8, 30). Similarly, either the EBV DNA or the EBV particles could not be demonstrated in oral cancer tissues or in oral cancer cell lines (31). Although EBV DNA was detected by PCR technique in oral smears from both healthy individuals and from patients with oral cancer, this EBV infection did not appear to be directly associated with the pathogenesis of oral cancer (32).

Salahuddin et al. (33) isolated a new virus, later termed as human herpes virus type 6 (HHV-6) from lymphocytes of patients with AIDS and lymphoproliferative diseases. The HHV-6 was found to be associated with Hodgkin's disease and acute lymphatic leukemia (34). Recently, a high prevalence of serum antibodies to HHV-6 was reported in oral cancer patients when compared to their normal counterparts (35). DNA from oral cancer patients were used as template for PCR amplificatin using HHV-6 specific primers, when 67% of oral cancer tissue samples were positive for HHV-6 (36). Using monoclonal antibody, it was shown that 100% of oral cancer biopsy specimens contained HHV-6 glycoprotein, a late protein in viral replication cycle (36). The exact role of this virus in the aetiology of oral cancer has not yet been clarified.

Human papilloma virus (HPV) is known to be oncogenic for human beings. More than 66 types of HPVs are isolated from various lesions at different sites in man (37) and more than 20 HPV types are known to be associated with genital cancers (38). HPV DNA was shown in about 90% of urogenital carcinomas (39). HPV types 16 and 18 were mostly detected in cervical cancer tissues (40). HPV was shown as a significant prognostic factor in cervix (41) and bladder tumors (42), HPV virus related antigens were demonstrated in oral papilloma lesions (43).

The role of HPV in the etiology of oral cancer was pointed out by Ficarra (44). An association of HPV in pathological lesions from oral verrucous carcinoma was demonstrated by immunohistochemical methods (45, 46). HPV structural antigens were detected in squamous cell carcinoma of the oral cavity (47, 48). Using DNA hybridisation technique, HPV types 2, 4, 6, 11, 13, 32, 40 and 57 were occasionally demonstrated in oral cancers; but HPV 16 and 18 were the most frequent types (49-53). Using PCR technique, HPV sequences were detected in biopsies of the oral mucosa (54). Recently, HPV antigen was detected in 52% of oral cancer samples, while none of the normal samples were positive (55). Moreover, HPV-16 DNA was detected in 53% and HPV-18 DNA in 9% of oral cancer samples, while all the normal samples were negative (55). Further, high prevalence of HPV in oral cancers in betal quid chewers was also noted (56). Integration of HPV-16 gave a selective growth advantage of cells (57). Integration of HPV-16 at 11q22 and 18121 regions in chromosome was demonstrated in oral carcinoma (58). The expression of E6 and E7 genes of HPV may contribute to the proliferative growth phenotype of carcinoma cells. The HPV-16 E7 gene product is a small polypeptide of 98 amino acids, and can be phosphorylated. The E7 protein has marked homology to both the SV40 large T antigen and adenovirus E1A (59) and is able to bind with the retinoblastoma gene product, pRb (60). Thus, the sequestration of cellular pRb is a possible mechanism by means of which HPV derails the normal growth control. In an important study, 31% of oral cancer specimens showed HPV-16

DNA and 42% showed p53 mutations; while 10% cases showed both HPV as well as p53 mutations (61). In another study, 54% of HPV antigen positive oral cancer cases showed mutated p53 (62). It is known that E6 protein is able to complex with p53, leading to inactivation of p53 and consequent carcinogenesis (63). Inactivation of p53 by E6 has resulted in mutagenesis in cultured human cells (64). HPV-16 promoter was found to be activated by oncogene products (65). The transcriptional regulatory properties of p53 were found to be modulated by the E6 protein (66).

Although a good association of HPV with oral cancer is often reported, the etiological role of HSV or HHV-6 in the pathogenesis of oral cancer are still to be proved (67). However, HPV positivity was seen as a significant prognostic factor in squamous cell carcinoma of the oral cavity (68). Evidences suggest that synergism can occur between HSV and HPV or between HPV and benzopyrenes (69). The HPV-16 DNA was detected in 76% cases of oral carcinomas; and 82% of these patients were smokers while 52% had the habit of chewing (70). Further studies are warranted to prove whether the viruses are the etiological agents or whether they act only as co-factors.

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