ASSOCIATION OF VIRUSES WITH ORAL CANCER

DR. D. M. VASUDEVAN
KMC, MANGALORE.

SUMMARY :

Evidences obtained from the investigations in recent years have implicated different types of herpes simplex virus (HSV) and human papilloma virus (HPV) in a wide range of epithelial malignancies. A causative role of HPVs in cervical carcinoma has been well established. A few studies so far undertaken abroad have provided some evidence for the association of certain HPV types with squamous cell carcinomas of the human oral cavity. We have found that about 53% of the oral cancer tissue samples were positive for HPV-16 DNA and 9% positive for HPV-18 DNA.

REVIEW OF LITERATURE

Oral cancer is reported to be the most common cancer among men and the second most prevalent cancer among women in India (Padmanabhan and Vasudevan, 1982) Epidemiological studies have attributed squamous cell carcinoma of the oral cavity to abuse of tobacco and consumption of alcohol (Gupta et al 1986), but a multifactorial aetiology for this disease is more probable. Compelling evidences, obtained through investigations during the past two decades, suggest the association of human papilloma viruses (HPV) with a wide range of epithelial malignancies of the anogenital tract (zur Hausen, 1977) There is a close similarity between oral and genital mucosa. Like the cervical mucosa, oral mucosa is aiso exposed to frequent viral infections. In view of these factors, the possibility of an aetiological role for HPVs in human oral squamous epithelial carcinoma appears reasonable.

HUMAN PAPILLOMA VIRUSES AND CANCER :

More than 66 genotypes of HPV have been isolated and characterised from pathological lesions in skin, genital mucosa and laryngeal mucosa (Yeudall. 1992), The first evidence for a consistent association of HPV with a cancer came from the studies of Orth et al (1980) on skin cancers arising in epidermodysplasia verruciformis patients Subsequently, other workers have also reported HPV DNA in carcinomas and metastatic lesions (Pfister et al, 1983). HPV-6 and HPV-11 have been demonstrated in laryngeal papillomas in children (Mounts et al, 1982)

De villiers et al (1981) isolated the first genital HPV type (HPV-6), and characterised it by cloning its DNA. At present, more than 20 HPV types are known to be associated with anogenital tract lesions HPV-6, 11 and 42 are usually found in warts (condylomata accuminata). But HPV types belonging to 16, 18, 31, 33, 35, 39, 45, 51, 52 and 56 are found in cases of cervical dysplasia and in invasive cervical carcinomas, and therefore, belong to the "high risk" group (Howley 1991). Studies in recent years have detected HPV DNA in about 90% of genital carcinomas. HPV-16 and HPV-18 are the most common genotypes. A study in India

has domonstrated HPV-16 DNA in about 64% of the cervical cancers (Das et al, 1992) Recently, association of HPV with penile squamous cell carcinoma (Gregoire et al, 1995. renal carcinoma (Furihata et al, 1995), vulva (van Beurden et al, 1995) and anus (Heino) et al, 1995) have also been reported. By PCR technique, HPV DNA was detected in a large percentage of cervical carcinomas (Karisen et al, 1995). HPV was shown as a significant prognostic factor in carcinomas of cervix (Kjaer et al, 1996), bladder (Lcpez-Beltran, 1996), lungs (Nuorva et al, 1995) and prostate (Moyret-Lalle, 1995).

MECHANISM OF ONCOGENESIS BY HPV :

HPV DNA usually occurs in an integrated state, either as single copy or as multiple tandom repeated structures. The viral DNA is extrachromosomal in premalignant lesions (Durst et al, 1985). In some cell lines, the integration has been found in the vicinity of known oncogens (Durst et al, 1987). This disrupts the E2 coding sequence and removes E2 mediated repression (Romanizuk et al, 1990), leading to the higher levels of transcription of the E6 and E7 open reading frames.

HPV AND ONCOGENES :

HPV-16 and 18 have been shown to immortalise human foreskin and cervical keratinocytes in tissue culture (Kaur et al, 1988). Matlashewski et al (1988) observed that co-transfection of HPV-16 DNA and activation of ras oncogene produces transformation of primary human fibroblasts, but none of them alone can cause transformation, indicating a synergism of HPV with oncogenes. Couturier et al (1991) reported the integration of HPV DNA close to structurally altered c-myc gene in genital carcinomas.

HPV E1 and E2 proteins are essential for the initiation of viral DNA replication (Muller and Sapp 1996) The expression of E6 and E7 genes of HPV contributed to proliferative growth phenotype of cervical carcinoma cells (von Knebel et al, 1988) HPV-18 E6-E7 mRNA is detected in carcinoma of lung (Kinoshita et al, 1995) and cervical cancers (Czegledy et al, 1994). Nurnberg et al (1995) found that HPV-16 and 18 DNA were detected in 60% specimens, fos and jun oncogene activation in 100% specimens and myc activation in 40% specimens of cervical carcinoma. They suggested that deregulation of nuclear proto-oncogne expression may contribute to an over-expression of HPV derived oncogenic proteins E6 and E7. HPV-16 DNA was shown in 35% cases and p53 mutations in 32% cases of cervical cancers (Jiko et al, 1994).

The HPV-16 E7 gene product is a small polypeptide of 98 amino acids and is a zinc-binding posphoprotein. It is phosphorylated on serine residues by casein kinase 11. (Barbosa et al 1990) Edmonds and Vousden (1989) identified 3 regions in the protein that are important for transformation. Two of the three regions in HPV-16 E7 protein have marked homology to both SV40 large T antigen and adenovirus E1A (Dyson et al, 1989). E7 is able to bind the retinoblastoma gene product, pRB. The pRB protein binding regions of E1A and T antigen are known to be responsible for transformation. Interestingly, these regions are the ones which show homology with E7 protein (Vousden

and Jat, 1989). Transformation of mammalian cells by HPV-16 requires binding of viral E7 protein to cellular pRB. Binding of the E7 to pRB inhibits association of pRB with transcription factor E2F (Patrick et al, 1994).

HPV requires both E6 and E7 for efficient immortalisation of human keratinocytes (Hudson et al, 1990). but E7 alone can do it only at a low frequency (Helbert et al, 1991) The E6 protein stimulaies the degradation of p53 via the ubiquitin dependent protease system (Scheffner et al, 1990), by which the natural cell growth control is removed. A cellular factor, E6-AP, present in normal cells, is capable of forming a stable complex with E6 in the absence of p53. This AP is shown to mediate the association of E6 with p53. (Huibregtse et al, 1991). The E6 protein functions as a transcriptional activator of various promoters to target p53 degradation (Etscheid et al, 1994). HPV-16 DNA was found in 53% of prostate cancers, but most of them had no mutations in p53 gene. Thus HPV infection could inactivate p53 through E6 (Moyret-Lalle et al, 1995). However, HPV DNA integration in uterine cervix carcinoma cells were shown to exhibit mutations in p53 (Jiko et al, 1994).

HPV AND ORAL CANCERS :

Oral cancer has traditionally been attributed to abuse of tobacco and alcohol. However, recent studies suggest that viral foctors may be involved in the aetiology. The presence of papilloma virus structural antigens in oral papillomas and other related benign lesions of the oral cavity has been reported (Syrjanen et al, 1984). Several HPV types. including HPV-2, 4, 6, 11, 13, 16, 32, 40 and 57 have been demonstrated in oral lesions by DNA hybridisation (Garlick et al 1989). HPV DNA was demonstrated in 3 out of 7 tongue cancers using Southern blot analysis (de Villiers et al, 1985). Chang et al (1989) detected HPV-16 DNA, by Southern blot analysis, in 76% of 17 oral carcinomas studied in Taiwan. Maitland et al (1987) found HPV-16 in 7 out of 15 (45%) oral cancer samples samples studied by Southern blot analysis. Yeudall and Campo (1991), demonstrated the presence of HPV-18, 4 and 16 in oral squamous cell carcinomas by Southern blot and PCR reactions. In this series, PCR allowed detection of viral DNA in samples which were found negative in Southern blots. Maitland et al (1989), by using PCR, demonstrated HPV -16 DNA in 50% of oral carcinoma biopsies. HPV-16 was detected by PCR technique in 31% and p53 mutations in 42% of oral cancers (Mao et al, 1996). In this study, 10% of oral cancers showed both HPV-16 positivity as well as p53 mutations The HPV capsid antigens were detected in oral squamous cell carninomas by immunoperoxidase techique, when 54% of HPV antigen positive cases showed mutated p53 (Mukhopadhyay et al, 1994). Integration of HPV has been shown at chromosome 11q22 and 18q21 in oral cancer (Steenbergen et al, 1995) and at the region of 10q24 in tonsillar carcinoma (Kahn et al, 1994): the latter region being the sites of photo-oncogens Hox11 end Lyt10. HPV-33 was shown to be integrated on chromosome 13q, 33-34 or to 9p 13 in cervical cancercell lines (Gilles et al, 1996).

While specific HPV types have been found to have the potential to bring about cell transformation together with co-factors, the exact role of HPV in the aetiology of oral

squamous cell carcinoma is not well understood. Synergism between HPV-16 and benzopyrene has been shown in oral carcinognesis (Park et al, 1995). Maitland et al (1989). opioned that HPV cannot be considered as the single aetiological agent responsible for human oral cancer, but detection of HPV DNA alone can be considered as a poor prognostic indication for the individuals who are at the risk of developing oral cancer. HPV was seen as a significant prognostic factor in squamous cell carcinoma of oral cavity (Chiba et al, 1996). No large scale studies have so far been undertaken to establsh the risk of HPV infection as far as oral premalignant and malignant lesions are concerned.

WORK DONE BY THE INVESTIGATOR :

Elevated levels of circulating immune complexes in the serum samples from oral cascer patients were reported. (Vijayakumar et al, 1986 Raghunath et al 1987; Remani et al, 1988). The presence of cancer associated antigen was reported in oral cancer tissues (Abdulkader et al, 1981) and circulating immune complexes (Shanavas et al, 1991)

The etiological relationship of various viruses with human cancer has been evaluated. Initially serum samples from 111 oral can cerpatients and 100 healthy controls were investigated for the presence of antibodies against herpes simplex virus (HSV). Anti-HSV antibodies were seen in 57./· of oral canoer pationts and 38 /. of healthy controls (Kumari et al, 1982). But equally ubiquitous adenoviruses did nor show any increased prevalence rate in cancer patients. As an extension of this study, serum samples were collected from 808 oral cancer patients and 300 normal healthy, age and sex matched individuals. The HSV antibodies were seen in 71·/. of oral cancer patients, but only in 52./· normals (Kumari et al, 1985). Another 300 samples from unrelated cancers (stomach intestine) did not show any correlation with HSV antibodies. Analysis of the anti-HSV antibody showed that percentage positivity at each titre was more in oral cancer patiants and the titre values were seen highly elevated in such patients and the geometric mean titre was very high in oral cancer patients. During the follow up period, in most casses, the titre value of antl-HSV antibody has been reduced. When biopsies from 175 patients suffering from carcinoma of the oral cavity were examined for the presence of HSV-1 antigen 77% were positive by immunofluorescence and 71·/. were positive by immunoperoxidase techique (Kumari et al, 1987). There was no correlation either in the degree of flourescence or in the intensity of peroxidase staining with clinical stage of the disease. Normal oral biopsies showed 30·/. and 20% positivity in immunofluorescence and immunoperoxidase respectively (Kumari et al, 1987) HSV-1 infected Vero cells served as positive controls and non-infected Vero cells served as potive controls and non-infected Vero cells as negative controls. Radiolabelled HSV-1 probes were seen to hybridise with the DNA isolated from oral cancer tissues when studied by dot blot and in-situ hybridisation techniques (Vasudevan et al, 1991). The HSV-1 middle segment (M-A segment) probe hybridised with the DNA from 64·/. of oral cancer tissues, as against 20·/. normal tissues. HSV-1 E+K fragment hybridised with the DNA from 56% oral cancer tissues and 8 /: normal tissues. In-situ hybridisation studies using ECoRI D+1 fragments of HSV-1 showed 60·/. positivity in oral cancer tissues, but only 12·/. with normal

tissues (Vasudevan et al, 1991). The above experiments strongly suggest a strong relationship of HSV with oral cancer. In a very large number of serum samples studied, the anti-HSV antibodies were consistently higher in oral cancer patients (Kumari et al, 1982; 1985); the HSV related antigens were shown to be present on the oral cancer cells and the HSV probes were seen to be hybridised with DNA from oral cancer specimens (Vasudevan et al, 1991). These are are pioneering studies in the field of the relationship of HSV with oral cancer.

The prevailance and titre of antibodies to human herpes virus type 6 (HHV-6) were assayed in the serum samples. This is the first study on the seroprevalence of HHV-6 in India (Shanavas et al, 1992). Smears were prepared with Molt- 3 cells infected with HHV-6; incubated with sera from patients, washed and immunoperoxidase staining was done. In normal controls, 6% were positive for anti-HHV-6 antibodies, and titres ranged from 10-160, with a mean of 47. In oral cancer cases, 96% patients were positive, and titres ranged from 80-10250, with mean of 2042. Both the antibody titres and the prevalence were found highly elevated in oral cancers (Shanavas et al, 1992). Again, this is the first report on the association of HHV-6 with oral cancer. DNA from oral cancer samples were used as template for PCR amplification using HHV-6 specific primers, when 67% of oral cancer tissue samples were positive for HHV-6 (Yadav et al, 1994). Using monoclonal antibohy against HHV-6 glycoprotein, a late protein in viral replication cycle was shown in 100·/. of oral cancer biopsy specimens, but not in normal oral cells (Yadav et al, 1994).

We have also carried out some preliminary studias to detect a possible association between human papilloma viruses and oral cancer (Shanavas et al, 1994). Tissue samples from 54 histopathologically proved oral squamcus carcinomas and 20 normal buccal mucosa samples were processed for the immunohistochemical demonstration of papillomavirus structural antigens by peroxidase-antiperoxidase technique, when 52·/. oral cancer samples, but none of normal samples were positive. In dot-blot hybridisation studied on a few samples, 75 /. of oral cancer DNA samples were positive for HPV-16 and 17·/. for HPV-18; but none were positive in the normals. This is the first report from India to show a relationship of HPV with oral cancer. No large scale studies were conducted in India on the association of HPV with oral cancer.

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