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REVIEW ARTICLE

Human papillomavirus and disease mechanisms: relevance to oral and cervical cancers*

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Oral squamous cell carcinoma (OSCC) is the sixth most common malignancy and is a major cause of cancer morbidity and mortality worldwide. Carcinoma of the uterine cervix is the most common female malignancy in the world. While cervical cancer is a worldwide disease, oral cancer has the highest incidence in developing countries, especially among tobacco and alcohol users and betel quid chewers. A strong association of cervical and oral cancer with high-risk human papillomavirus (HPV) 16 and 18 infections underlines the importance of the virus in the pathogenesis of these squamous cell carcinomas. Functionally high-risk HPV infection contributes to carcinogenesis and tumor progression predominantly through the actions of two viral oncogenes, E6 and E7. The E6 and E7 genes have been studied in different patient populations and a number of variants have been described. More than 40 variants have been classified and may be related to differences in progression of squamous intraepithelial lesions. The transcription factor, NFκB and its activation pathways are frequently targeted by viruses and aberrant constitutive activation of NFκB is frequently found in human tumors of diverse tissue origin. Diet–gene interactions are also likely to contribute considerably to the observed inter-individual variations in HPV associated cancer risk, in response to exposures to the nutritional factors that have the potential to promote or protect against cancer.
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Introduction

Oral squamous cell carcinoma (OSCC) is the sixth most common malignancy and is a major cause of cancer morbidity and mortality worldwide. The overall survival percentage has still not changed in recent years, despite extensive research on the biological and molecular aspects of oral SCC. Among the more pressing problems in clinical management are the lack of early detection and the high incidence of local-regional recurrence, even with aggressive surgical therapy. OSCC incidence accounts for upto 40% of all malignancies in India and South East Asia (Vokes *et al*, 1993). Carcinoma of the uterine cervix is the most common female malignancy and in India accounts for about 26% of female cancers, resulting in about 95 000 women developing the disease annually (Jayant *et al*, 1995). Thus India has one-sixth of the world's population and one-third of the world's cervical cancer burden. While cervical cancer is a worldwide disease, oral cancer has the highest incidence in developing countries, especially among tobacco and alcohol users and betel quid chewers. A strong association of cervical and oral cancer with high-risk human papillomavirus (HPV) 16 and 18 infections underlines the importance of the virus in the pathogenesis of these squamous cell carcinomas. The epithelium of the Upper Aero Digestive Tract (UADT) mucosa is similar to that of the ectocervix and vagina and hence the presence of 'genital' HPV types in both sites is not surprising. An important feature of the carcinogenic process should be accumulation of sufficient genetic damage to allow transformation to occur. Thus, for example although the entire UADT epithelium is exposed to the various carcinogens, only a few focal points actually develop into malignancy. While in the uterine cervix, the primary site is itself conducive to carcinogenesis owing to the characteristic transformation zone and associated squamous metaplasia, in the UADT development of cancer has been linked to the use of tobacco and alcohol (Jayant and Notani, 1991). We have previously explained the importance of HPV in the genesis of squamous carcinoma (Lakshmi *et al*, 1993, 1995a,b; Pillai *et al*, 1993) showing that the extended field cancerization occurring in the UADT as a result of the carcinogenic insult of alcohol, tobacco and related carcinogens may be accentuated, amplified and intensified by HPV infection. Data from our laboratories have also shown that defective apoptosis, neovascularization and cellular immortality are also significantly associated

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with HPV infection (Nair *et al*, 1999a,b, 2000; Pillai *et al*, 1999a,b). It is therefore possible that the process of HPV-mediated carcinogenesis may be a consequence of such an integrated process, which led to the hypothesis suggesting the presence of a 'condemned mucosa syndrome' developing as a result of the viral infection and associated cellular and genetic alterations (Pillai and Nair, 2000).

Human papilloma virus (HPV)

Papilloma viruses are epitheliotropic viruses present in the skin and mucosa of several animals. In humans, more than 70 types have been described (Zur Hausen, 1996). Mucosal and genital HPVs, consisting of about 30 types, are divided into low risk (HPVs 6, 11, 42, 43 and 44) and high risk (HPVs 16, 18, 31, 33, 35, 45, 51, 52 and 56), according to their presence in malignant lesions of the cervix (Lowy *et al*, 1994). Recognized initially as sexually transmitted agents, HPVs are now considered human carcinogens (Munoz, 2000). Functionally high-risk HPV infection contributes to carcinogenesis and tumor progression predominantly through the actions of two viral oncogenes, E6 and E7. These oncogenes are consistently expressed in cervical cell lines and in human cancers (Milde-Langosch *et al*, 2000; McMurray *et al*, 2001). Both of these oncogenes interact with and inhibit the activities of critical components of cell cycle regulatory systems, in particular E6 with p53 and E7 with Rb (Philips and Voudsen, 1999; Milde-Langosch *et al*, 2000; McMurray *et al*, 2001). The E7 protein interacts with pRB and inactivates this cellular protein (Dyson *et al*, 1989). As a consequence, E2F transcription factor is released from pRB-E2F complex, leading to transcriptional activation of several genes involved in cell proliferation (Sellers and Kaelin, 1997). Binding of the E6 protein to the p53 promotes the degradation of the latter through a ubiquitin-dependant proteolysis system. Also of significance is that on completion of the degradation of p53 by the ubiquitin-dependant proteolysis system, the E6 protein is free to interact again with remaining p53 molecules, leading to further degradation of the latter (Scheffner, 1998). The genome of these viruses is a double-stranded DNA molecule of about 8000 bp. Three genomic regions have been identified: a late region (L), an early region (E), and a long control region (LCR). The early genes E1 and E2 encode proteins involved in viral DNA replication and control of viral transcription (Zur Hausen, 1996). The products of genes E6 and E7 are essential in the process of HPV-induced cellular immortalization and transformation (Münger *et al*, 1989; Villa and Schlegel, 1991). The late genes L1 and L2 encode the viral capsid proteins (Zur Hausen, 1996). HPV genomes are found as episomes in the nucleus of infected cells of the normal cervix, where infective viral particles can be isolated. However, in some low-grade and in most of the high-grade lesions of the uterine cervix, including cancer, HPV genomes are found integrated into the host genome (Lehn *et al*, 1988). A disruption of the E1–E2 region is required for HPV genome integration. This event results in an increased expression and stabilization of the E6 and E7 transcripts (Jeon and Lambert, 1995).

HPV variants

Intratype sequence variations of HPV 16 have been examined in cervical carcinomas worldwide and have led to the definition of six major branches of HPV 16 variants designated European (E), Asian (As), Asian-American (AA), North American (NA1) and African (Af1 and Af2) (Yamada *et al*, 1997). The HPV 16 prototype belongs to the European (E) branch. The variants are thought to differ in their biological properties and in their contribution to carcinogenesis. It has become apparent that the prevalence of the viral variants and their influence on disease is related to the geographical areas and populations studied. The European variant T350G, resulting in an amino acid change from leucine to valine at position 83 (L83V) in the E6 protein, is frequently found in cervical intraepithelial neoplasias (CINs) and cancers and has been associated with progression to cervical cancer particularly in North European women (Zehbe *et al*, 1998, 2001; Kammer *et al*, 2002).

The E6 and E7 genes have been studied in different patient populations and a number of variants have been described (Nindl *et al*, 1999; Van Duin *et al*, 2000; Ginnoudis and Herrington, 2001; Xu *et al*, 2001). It can be classified into more than 40 variants and may be related to differences in progression of squamous intraepithelial lesions. The definition of an E6 variant is based on the departure from an original prototype first isolated from an invasive cervical carcinoma in Germany (Ginnoudis and Herrington, 2001). These variants have also been geographically mapped to different areas of the world based on sequence variations of the E6, L1, L2, and LCRs (Yamada *et al*, 1997). Studies from our laboratory provided the first report on HPV 16 E6 and E7 gene variations from India and provide evidence for the association of specific E6 gene variants with the risk of cervical cancer (Pillai *et al*, 2002). That these particular variants may contribute to increased risk and/or severity of cervical carcinoma is supported by *in vitro* studies demonstrating that differences exist in the immortalizing activity, transforming potential and rate/extent of p53 degradation of the different HPV 16 variants. Results from a study on Mexican women, with either Low Grade Squamous Intraepithelial Lesions (LGSIL) or Invasive Carcinoma (IC), indicates that the EP E6 gene variant of HPV16 was predominant since it was found in 42.5% of the samples (Gonzalez Losa *et al*, 2004). A previous study, on HPV variations in IC samples from the American continent, has reported the presence of HPV 16 EP350G in 40% of the samples from North America and in 52.2% of those from Central and South America (Yamada *et al*, 1997). The EP350G variant represents a subclass of EP, in which thymidine has been replaced by guanine at position 350. This substitution causes a change from leucine to valine at the corresponding amino acid.

Carcinomas of the oropharynx and particularly of Waldeyer's tonsillar ring contain HPV DNA in more than 50%, and evidence for a casual association has been obtained by epidemiological and molecular studies (Paz *et al*, 1997; Andl *et al*, 1998; Hoffmann *et al*, 1998; Gillison *et al*, 2000; Mellin *et al*, 2000; Klussmann *et al*, 2001; Li *et al*, 2003). HPV 16 is by far (>90%) the most prevalent HPV type in HNSCC. A study on HPV 16-positive HNSCC, both primary tumors and lymph node neck metastases, have revealed by sequence analysis three different E6-E7 genotypes in the tumor specimens: the HPV 16 prototype, the T350G variant and a variant harbouring the two mutations A131G and C712A. The detection of the T350G variant in 38% of the HNSCC patients therefore indicates that this variant may also play an important role in head and neck carcinogenesis (Hoffmann *et al*, 2004). A recent report shows a novel link between HPV 16 E6 and the Mitogen Activated Protein Kinase (MAPK) signaling pathway, where the aa (amino acid) 83 variant (T350G) shows enhanced signalling through the MAPK pathway. *In vivo*, experiments in nude mice, demonstrated expression of E6 aa 83 variants results in bigger and more aggressive tumors than those with prototype E6 (Chakrabarti *et al*, 2004).

HPV and oral cancer

An association between the presence of HPV and the development of head and neck cancer has been established recently (Steenbergen *et al*, 1995; Smith *et al*, 1998; Franceschi *et al*, 2000; Gillison *et al*, 2000). The association is strengthened by the fact that the same oncogenic HPV types detected in cervical carcinomas have been identified in head and neck cancers (Paz *et al*, 1997). A critical molecular parameter supporting a causal role of HPV 16 in HNSCC is the expression of the E6 and E7 oncogenes coupled with inactivation of pRB and p53. Indeed, it has been shown that pRB protein levels are down-regulated in HPV 16-positive HNSCC, a clear indication of E7 activity (Andl *et al*, 1998; Wiest *et al*, 2002). Concerning E6 and p53, the HPV 16-positive tumors could be divided into two groups. The tumors exhibited E6 gene expression and lacked p53 mutations or, alternatively, they lacked E6 expression and carried p53 mutations (Van Houten *et al*, 2001; Wiest *et al*, 2002). Infection with oncogenic HPV types and the other major risk factors for head and neck cancer, tobacco and alcohol, may represent alternative pathways in the development of these cancers (Smith *et al*, 2004). The association of head and neck cancers with clinically significant morbidity and disfiguration makes the early detection of the diseases and biomarkers to identify individuals at high risk of great importance. One of the conclusions from a recent National Cancer Institute (NCI) workshop convened to assess viruses associated with human cancers (Wong *et al*, 2002) was that future HPV research needs to focus on developing a sensitive, validated laboratory test to detect HPV in oral exfoliated cells that would reflect HPV high risk types in head and neck tumors.

Mechanisms of HPV mediated carcinogenesis: the role of E6 and p53

The influence of viruses and tumor suppressor gene inactivation are of major importance in oral cancer, HPVs are small oncogenic viruses, which are implicated, in epithelial carcinogenesis, and p53 is a tumor suppressor gene with a central role in the prevention of genomic injury. There are reports indicating that HPV and p53 protein alterations frequently coexist in oral lesions and suggest that p53 mutation may be an early genetic event in oral carcinogenesis. Moreover, this coexistance reveals that other environmental carcinogens have a more prominent role in oral carcinogenesis, one that overrides the action of HPV (Aggelopoulou *et al*, 1998). On the contrary, some reports suggest a stronger association between HPV infection and activation of the H-ras gene in oral verrucous carcinomas. These results continue to confirm the multihit hypothesis of tumorigenesis and suggest that in some cases of oral cancer at least two of these events are H-ras gene mutation and HPV infection (Anderson *et al*, 1994). The high prevalence of HPV in the oral cancers of Indian patients suggests that viral infection is an important etiological component, with betel quid probably causing additional mutagenic steps in the carcinogenic process (Balaram *et al*, 1995). The viral prevalence found in cancerous lesions reinforces the concept of heterogenic natures of oral cancer. HPV is a circumstance that increases the probability of malignancy, and when reducing, diminishes the frequency of cancer (Bustos *et al*, 1999). A role for HPV in the progression of oral cancer would be more plausible if the viral transforming genes were likely to be overexpressed in oral cancer cells. Several mutations were found in the LCRs isolated from oral cancer cells and HPV-immortalized oral epithelial cells. The promoter activity of the mutated LCRs was significantly higher than that of the equivalent wild-type LCRs in oral cancer cells that contained the same HPV type. These results imply that mutations in the LCR of HPVs in oral cancer could lead to increased expression of HPV-transforming proteins, which might contribute to the carcinogenic process (Chen *et al*, 1997). When examined whether the p53 gene, whose function is abrogated by the product of the HPV gene E6, would be mutated in those oral cancers that were free of HPV DNA, point mutations were found at known hot spots for mutational alteration of p53 in 4 of 23 lesions (Heinzel *et al*, 1996). The E6 oncoproteins of these high risk HPVs are known to bind and induce degradation of p53 tumor suppressor protein through the ubiquitin pathways. This degradation is controlled by a common polymorphism of the p53 gene encoding either a proline or an arginine at its codon 72 in exon 4. The interaction between HPV oncoproteins and the p53 gene polymorphism specifically, homozygous arginine at codon 72 appears to play no role in the development of either cervical or oral cancer and also it cannot serve as a biomarker for early identification of cervical, oral or breast cancer (Katiyar *et al*, 2003). A striking reduction in Pro/Pro allele frequency has been found in HPV positive cases, indicating Arg/Arg

genotype to be more susceptible to HPV infection and oral carcinogenesis (Nagpal *et al*, 2002).

Another group of investigators found that HPV E7 mRNA was present in 90% of patients with oral neoplasia and 100% of patients with cervical neoplasia (Ke *et al*, 1999). Estimation of the risk of HPV detection in normal oral mucosa, precancerous oral tissue and oral carcinoma using meta-analysis indicated that HPV is detected with increased frequency in oral dysplastic and carcinomatous epithelium in comparison with normal oral mucosa. The findings provide further quantitative evidence that oral infection with HPV, particularly with high-risk genotypes, is a significant independent risk factor for OSCC (Miller and Johnstone, 2001).

Mechanisms of HPV mediated carcinogenesis: role of Xenobiotic Metabolizing Enzyme gene polymorphisms

Polymorphisms of genes involved in metabolism of various endogenous and exogenous carcinogens are relatively common in most populations. Generally carcinogens are oxidized to reactive intermediates by phase I enzymes (e.g. CYPs), while phase II enzymes like glutathione S-transferases (GST) generally mediate the conjugation of water soluble moieties (such as glutathione) to these reactive metabolites, rendering them harmless (Miller *et al*, 2001).

P450 cytochromes (CYP) are enzymes, which catalyse the insertion of one atom of molecular oxygen into a substrate. This is a typical reaction of activation (phase I), which converts indirect carcinogens into active electrophiles capable of interacting with the biological macromolecules DNA, RNA and proteins. CYPs are coded by genes of the CYP super family (Pavanello and Clonfero, 2000). Glutathione S-transferases are one of the major groups of detoxifying enzymes. Each GST has distinct catalytic properties: conjugation with glutathione, peroxidation and isomerization. The cytosolic GSTs known until now belong to five different classes, are coded by atleast five gene families and according to their primary amino acid sequence, are called GST, classes α, μ, π, σ and θ (Hayes and Pulford, 1995).

Reactive metabolites that are not detoxified may react with DNA to form DNA adducts which, if not required, may eventually produce somatic mutations and cancer. Of great interest in the study of xenobiotic metabolism is the existence of polymorphisms in animal model systems and humans in which a large percentage of the alleles of a particular gene are inactive (Gonzalez and Idle, 1994). Polymorphisms have been found in the P450s and many phase II enzymes. In humans, P450 polymorphisms are known to affect drug therapy. Polymorphisms in the carcinogen-metabolizing enzymes are thought to play a role in cancer susceptibility in humans. Associations of polymorphisms with cancer risk will be especially important in cases where there are known exposures to chemical carcinogens such as with tobacco smoking, high intake of food mutagens and industrial exposures (Caporaso and Goldstein, 1995). It has been reported that HPV infection, through the modulation of cellular xenobiotic metabolizing enzymes (XMEs), may play a role in the ability of cells to handle environmental carcinogens (Chen and Nirunsuksiri, 1999). Two genetic polymorphisms of the CYP1A1 gene have been reported to be associated with differences in the activity of the enzyme aryl-hydrocarbon hydroxylase (AHH) activity (Autrup, 2000) – an isoleucine to valine substitution in exon 7 at the *Nco*I restriction site (m2 polymorphism) and a thymine/cytosine point mutation in the Msp1 restriction site (m1 polymorphism). The m2 (valine variant) displays a twofold higher catalytic activity compared with the wild type enzyme (Autrup, 2000). The significance of these polymorphisms in carcinogenesis is still unclear. The homozygous m2 polymorphism has been shown to strongly correlate to lung cancer incidence among Japanese although such dramatic results were not obtained for Caucasians (Marchand *et al*, 1998). We had earlier reported increased frequency of the m2 polymorphism in oral cancer patients with a long history of tobacco use (Sreelekha *et al*, 2001). Two previous studies on cervical cancer have shown the importance of CYP1A1 polymorphisms. Women from Hawaii who were homozygous for the CYP1A1 *Msp* variant allele (m1) had an odds ratio of 3.4 of having cervical intraepithelial lesions compared to women homozygous for the wild allele (Goodman *et al*, 2001a,b). However, Kim and colleagues did not find this association in Korean women (Kim *et al*, 2000). Women with m1 and m2 CYP1A1 polymorphisms and with prolonged exposure to firewood smoke, tobacco smoke or tobacco products will therefore have higher levels of reactive metabolites capable of causing DNA damage, in addition to a pre-existing HPV infection. Studies conducted from our laboratory show that subjects who were HPV 16 positive had an odds ratio of 3.0 (95% confidence interval = 1.8–4.8) and 2.9 (95% confidence interval = 1.8–4.6) of having a m1 and m2 polymorphism respectively (MR Pillai 2004, unpublished data). A recent report has provided epidemiological evidence for the significant increased risk of cervical carcinoma in HPV infected women having prolonged exposure to firewood smoke (Velema *et al*, 2002). We have also observed that deletion of both GSTM1 and GSTT1 was significant in cases compared with controls. Unlike in the case of the CYP1A1 m1 and m2 variants, there was a moderate risk of GSTM1 deletion in relation to age (odds ratio = 1.8, 95% confidence interval = 1.17–2.77). No such association was evident in the case of GSTT1. There was also an elevated risk for women who were HPV positive of having GSTM1 and GSTT1 deletions (odds ratio = 1.6, 95% confidence interval = 1.1–2.5 for GSTM1 and odds ratio = 1.7, 95% confidence interval = 0.9–2.9).

Mechanisms of HPV mediated carcinogenesis: role of Nuclear Factor kappa B

Nuclear Factor kappa B (NFκB), is a member of the Rel family of transcription factors, which includes NFκB1 (p50), NFκB2 (p52), Rel A (p65), Rel B and c-Rel. A number of the products of ionizing radiation, including reactive oxygen intermediates and DNA damage, can modulate activation of signal transduction pathways

that induce NFκB/Rel A (p50/p65) (Prasad *et al*, 1994; Wang *et al*, 1996; Li and Karin, 1998). NFκB/Rel A (p50/p65) is activated by ionizing radiation through signal-induced phosphorylation and degradation of inhibitor κB protein α (IκBα) (Li and Karin, 1998), which complexes NFκB/Rel A in an inactive form in the cytoplasm (Duffey *et al*, 1999; Ondrey *et al*, 1999). NFκB controls the expression of a number of growth promoting cytokines (Rayet and Gelinas, 1999), and the DNA binding activity of NFκB is induced during the G_0–G_1 transition (Baldwin *et al*, 1991). NFκB also activates the expression of genes important for invasion and metastasis (Newton *et al*, 1999), IκBα expression in tumor cell decreases the frequency of metastases (Baldwin, 2001). Mutations in the IκBα gene have been detected in Hodgkin's lymphoma and are suggested to render NFκB constitutively active in Hodgkin's cells, consistent with a role for IκB as a tumor suppressor (Cabannes *et al*, 1999). Inhibition of NFκB activity potentiates cell killing of human breast cancer and fibrosarcoma cell lines by TNFα (Tumor Necrosis Factor), ionizing radiation and daunorubicin (Beg and Baltimore, 1996; Van Antwerp *et al*, 1996; Wang *et al*, 1996). NFκB inhibition also led to sensitization of tumors to chemotherapeutic compounds CPT-11-mediated cell killing *in vivo* (Cusack *et al*, 2001). NFκB directly causes increased expression of proteins that contribute to the survival of tumor cells such as inhibitors of apoptotic proteins (Chu *et al*, 1997; You *et al*, 1997). Consistent with a role for NFκB in oncogenesis and survival, aberrant constitutive activation of NFκB/Rel is frequently found in human tumors of diverse tissue origin (Rayet and Gelinas, 1999; Karin *et al*, 2002).

Given the important contribution of NFκB for central biological processes, this transcription factor and its activation pathways are frequently targeted by viruses. Early evidence for viral appropriation of the NFκB pathway came from the finding that the turkey retrovirus REV-T encodes the v-rel protein, an oncogene homologue to the NFκB DNA-binding subunits (Gilmore, 1992). It is frequently observed that products of a variety of viruses induce NFκB activity in order to ensure NFκB-dependant expression of viral genes (Hiscott *et al*, 2001). On the other hand, the relevance of NFκB for innate immunity and induction of apoptosis forced some viruses to evolve strategies to counteract NFκB activation. For example, the African swine fever virus encodes a functional and stable IκB protein, which is able to inhibit NFκB activity by replacement of the proteasome-degraded endogenous IκBα protein (Tait *et al*, 2000). Carcinogenesis by HPV critically depends on the virus encoded E6 and E7 oncoproteins, which stimulate proliferation by manipulating the function of a variety of host key regulatory proteins. There are reports showing that both viral proteins dose-dependently interfere with transcriptional activity of NFκB. A variety of experimental approaches revealed that a fraction of E7 proteins is found in association with the IκB kinase complex and attenuates induced kinase activity of IκB kinase α (IKKα) and IKK*β*, thus resulting in impaired IκBα phosphorylation and degradation. Indirect immunofluorescence shows that E7 impairs TNFα-induced nuclear translocation of NFκB, thus preventing NFκB from binding to its cognate DNA. While E7 obviates IKK activation in the cytoplasm, the E6 protein reduces NFκB p65-dependent transcriptional activity within the nucleus (Spitkovsky *et al*, 2002). It has been reported that NFκB can bind HPV 16 LCR and acts as a transcriptional repressor in the context of HPV 16 LCR (Fontaine *et al*, 2000).

TNFα is a cytokine that induces apoptosis in a number of cell types and is employed by cytotoxic T cells to eliminate virus infected cells. An adenovirus-mediated gene transfer of NFκB inhibitor, super-repressor IκBα has been reported to block TNF-induced NFκB activation and also sensitize oral SCC cells to TNF killing (Chen *et al*., 2002). Studies conducted on biopsy specimens by immunohistochemistry suggest that high expression levels of p65 and IKK contribute to malignant behavior and antiapoptotic activity in SCC of the oral squamous epithelium (Nakayama *et al*., 2001). A positive correlation has been reported between the level of HPV16 E7 and the nuclear localization of the p65 subunit of NFκB, in laryngeal SCC cells infected with HPV (Du *et al*., 2003). There are reports suggesting that chronic infection of keratinocytes by HPV modifies the expression of potentially important cytokines by interfering with the NFκB signal pathway and have shown that E6 modulates the NFκB signaling pathway (Havard *et al*, 2002). Analysis by cDNA microarray has shown that HPV16 E6 protein stimulates expression of multiple genes known to be inducible by NFκB and AP-1, in cervical keratinocytes (Nees *et al*., 2001). A more recent report has demonstrated that NFκB is constitutively activated during human cervical cancer progression (Nair *et al*., 2003). These results have subsequently been elaborated in our laboratories (M.R. Pillai et al., unpublished data).

Nutrition, nutrigenomics and HPV

Cervical cancer is associated with reproductive and food consumption behaviors. Epidemiological studies have suggested that smoking, nutrition and sexual patterns are major risk factors for cervical cancer. High intakes of vegetables, fruits, beta carotene, vitamin C, E and fiber have been associated with a lower risk of cervical cancer. A higher intake of vegetables and foods rich in vitamin E can reduce its risk (Atalah *et al*, 2001). Consumption of green vegetables and animal foods have been negatively correlated with cervical cancer mortality (Guo *et al*, 1994). Low vitamin C and carotenoid status are associated fairly consistently with both cervical cancer and precursors, whereas results for vitamin E status are less consistent. The effect of folate status may be restricted to early preneoplastic cervical lesions and not to more advanced disease (Potischman and Brinton, 1996). The role of nutritional factors in biochemical interactions that are part of an oncogenic process or inhibit free radical proliferation have attracted considerable interest in relation to molecular mechanism(s) and the natural history of human cancer. Measurable effects of dietary deficiencies of selected antioxidant

micronutrients (i.e. beta-carotene and vitamins A, C and E) and their association with known cervix cancer risk factors in the pathogenesis and potential prevention of cervix dysplasias, presumed to be the precursor lesions of cervical cancer have been identified (Romney *et al*, 1995). Nutritional parameters of patients with cervical cancer and endometrial cancer were prospectively evaluated and the study revealed that abnormal vitamin levels were more commonly present in patients with cervical cancer. When compared with control values, levels of folic acid, beta carotene, and vitamin C were significantly lower in patients with cervical cancer. Patients with endometrial cancer had significantly lower levels of beta carotene and vitamin C (Orr *et al*, 1985). A population-based case–control study conducted to assess the relation of diet, especially intake of vitamins A, C and E and of folic acid, to the risk of invasive cervical cancer found that high vitamin E intake was also related to a reduced risk and intake of preformed vitamin A and of folic acid, was not related to the risk of cervix cancer (Verreault *et al*, 1989). Women with cervical dysplasia (especially those who use oral contraceptives) have lower levels of folic acid than those who do not. In a clinical trial involving oral contraceptive users, cervical dysplasia gradually decreased in the group supplemented with folic acid (taken orally) but remained unchanged in the group given the placebo. Folic acid is known to be essential for the division of body cells, growth, the working of the nervous system and the production of substances carrying hereditary patterns (Ziegler, 1986).

A case–control study of women with cervical dysplasia and invasive cervical cancer was conducted during 1982–1983 in five US states reporting to the Comprehensive Cancer Patient Data System: Birmingham, AL; Chicago, IL; Denver,CO; Miami, FL; and Philadelphia, PA. Diet was assessed by asking about the usual adult frequency of consumption of 75 food items and the use of vitamin supplements. The major sources of the four micronutrients postulated to reduce the risk of cervical cancer: carotenoids, vitamin A, vitamin C, and folic acid, were included. The study concluded that dark green and yellow-orange vegetable consumption and consumption of multivitamin supplements were each strongly related to reduced risk (Ziegler *et al*, 1991). Another study in Hawaii specifically mentions cancer of the uterus and found that there was a positive correlation with consumption of animal fat and animal protein (Kolonel *et al*, 1981). Interestingly, many studies are indicating that a vegetarian diet helps protect against all forms of cancer because vegetarian populations have lower rates of common cancer and meat consumption is positively linked to the incidence and mortality of cancer.

Methylenetetrahydrofolate reductase (MTHFR) is a critical enzyme regulating the metabolism of folate and methionine, the important components of DNA synthesis and methylation. Two common genetic polymorphisms, causing reduced MTHFR activity, have been identified (Gerhard *et al*, 2003). Polymorphisms exist in the genes for the activation and conjugation metabolizing enzymes, and the induction of metabolizing enzyme activity by nutritional factors may result in either the activation of a carcinogen or the detoxification of a reactive intermediate metabolite. The relationship between the MTHFR gene and dietary folate is an example of a diet–gene interaction that involves a polymorphism in a vitamin metabolism gene, and the presence of the variant appears to influence both risk for cancer and folate requirements (Rock *et al*, 2000). Methylenetetrahydrofolate reductase deficiency paradoxically causes neurological problems but no megaloblastic anemia. This rare deficiency is the most common inborn error of folate metabolism. It is distinct from the very common MTHFR gene polymorphisms, mutations that cause mild to moderate reductions in MTHFR activity but no direct clinical manifestations. The MTHFR polymorphisms, especially the 677C $\rightarrow$ T mutation, may contribute to vascular and birth defect risks, while reducing the risk of certain malignancies, such as colon cancer. These polymorphisms and those of genes for other enzymes and proteins related to cobalamin, folate, and homocysteine metabolism may be important role players in frequent interactions between genes and the environment (Carmel *et al*, 2003). Low red blood cell folate levels have been associated with hypomethylation of DNA in dysplastic tissue and an increased risk for CIN in HPV-infected women. However other studies are contradictory. Gerhard *et al* (2003) looking at MTHFR variations as risk factors for invasive cervical cancer found no effect of these variants with increased parity or infection with high-risk-type HPV. Recent efforts have been made to analyze the associations between risk of esophageal cancer and hereditary sequence variations in genes involved in metabolism, DNA repair and cell cycle control. Statistically significant differences in genotype frequencies found in case–control comparisons were MTHFR C677T and A1298C polymorphisms (Xing *et al*, 2003). Results from a multiethnic case–control study to examine the association of dietary folate and MTHFR genotype with the odds ratios (ORs) for cervical dysplasia among women identified from several clinics on Oahu, Hawaii, between 1992 and 1996 suggest that the MTHFR T allele and reduced dietary folate may increase the risk for cervical SILs (Goodman *et al*, 2001a,b). Another study conducted to examine MTHFR polymorphism as a potential molecular marker of CIN susceptibility found a significantly increased CIN risk with an alanine to valine substitution at amino acid 223 of MTHFR with an odds ratio of 2.9 (95% confidence interval: 1.2–7.9, $P = 0.02$). Parity and MTHFR genotype displayed a strong interaction (Piyathilake *et al*, 2000).

Hyperhomocysteinemia is a risk factor for a variety of diseases and can be reversed by a combination of non-toxic multivitamins. Since the risk of untreated hyperhomocysteinemia is high, whenever hyperhomocysteinemia is recognized, attempts are immediately made to reverse it with multivitamins. Recent studies suggest that a large percentage of Indians have hyperhomocysteinemia (Refsum *et al*, 2001). The acquisition

of folates in many cells is mediated primarily through folate receptors (FRs) that bind to physiological folates with high affinity in the nanomolar range (Antony, 1992, 1996). FRs are expressed in rapidly proliferating cells, including cervical cancer cell lines (Sun *et al*, 1995; Antony, 1996). *In vitro* studies suggest that FR, which are a target for experimental therapeutics against cancer can also be upregulated by homocysteine (Antony *et al*, 2004) and also give rise to a question as to whether the results of clinical data on expression of FR and its translational regulatory protein (heterogenous nuclear ribonucleoprotein E1, hnRNP-E1) in cervical dysplasia and invasive cancer (Pillai *et al*, 2003) have been influenced by hyperhomocysteinemia. FR and hnRNP-E1 expression was assessed semi-quantitatively, based on four grades of immunoreactivity, in our laboratory. These studies on 12 women with benign cervical histology, 22 women with LGSIL, 22 women with HGSIL and 25 women with invasive cancer identified a highly significant positive correlation between the extent of FR and hnRNP-E1 expression (Pillai *et al*, 2003).

Conclusion

The data generated from the studies described above provide a number of critical observations associated with the role of HPV in cervical and oral cancers. The cellular changes that subsequently follow HPV infection of the oral and cervical mucosa may take place in select areas of the cervical or upper aerodigestive tract mucosa. The p53 gene is a likely molecular target for the carcinogens present in tobacco. This may explain the high incidence of mutated p53 in oral lesions. At such select foci that are compromised, the virus could initiate the cellular changes described earlier. In the uterine cervix, repeated pregnancy and high metaplastic proliferation may provide the physical stimuli that permit HPV to reach basal cells. The possibly distinct step in the pathogenesis of both types of tumors may only be in the mode of p53 inactivation, mutation and inactivation of p53 in UADT and inactivation alone in the cervix. The 'condemned mucosa syndrome' could, therefore, be a possible molecular validation of this concept. The development and validation of such a molecular model has significant clinical priority. It can be used to identify target populations or individuals for intervention, to monitor the effects of intervention, and to determine which individuals or groups share inherited or acquired characteristics that place them at increased risk of injury from exposure to known toxicants. Further studies using cancer cell lines may help to identify mechanisms of activation of NFκB and clarify the use of NFκB/IκB family members as specific targets for therapeutic intervention. Diet–gene interactions are likely to contribute considerably to the observed inter-individual variations in cancer risk, in response to exposures to the nutritional factors that have the potential to promote or protect against cancer. Insights into mechanisms by which nutritional factors affect the process of carcinogenesis are provided by knowledge of the targeted gene function and enzyme activity. Increased knowledge in this area will allow a more refined approach to reducing risk for cancer, with diet interventions targeted toward individuals and subgroups that are genetically susceptible and responsive to the effects of nutritional factors.

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